Proceedings
SI'ETY-SECOND
ANNUAL MEETING
UNITED STATES LIVESTOCK
SANITARY ASSOCIBITION
DEAUVILLE HOTEL
Miami Beach, Florida
November 4-5-6-7, 1958
FRIDAY MORNING, NOVEMBER 7

8:30 Report of the Committee on Public Relations—C. L. Campbell, et al., Tallahassee, Florida


9:00 Feral Swine as a Reservoir of Vesicular Stomatitis Virus in Southeastern United States—R. P. Hanson, Ph.D., Lars Harstad, D.V.M., M.S., Madison, Wisconsin

9:20 Discussion


10:05 New Research Results With Systemic Insecticides—R. C. Bushland, Kerrville, Texas

10:20 Report of Committee on Stockyards, Markets, and Transportation—R. Cuff, et al., Kansas City, Missouri


11:20 Recent Studies of Properties of a Non-Virulent Hog Cholera Vaccine—J. W. Newberne, D.V.M., Indianapolis, Indiana


11:50 Report of the Committee on Transmissible Diseases of Swine

12:00 Discussion

12:10 New Business

12:20 Report of the Nominating Committee—Nominations from the floor, Election and Installation of Officers

CONFERENCE OF VETERINARY LABORATORY DIAGNOSTICIANS

NOVEMBER 4, 1958

MORNING

Paul Bennett, President

Wm. L. Sippel, Secretary

9:00 A Recommended Immunization Program for Veterinary Laboratory Personnel

9:20 Symposium on Laboratory Forms:

The General Accession Form—P. Bennett, Iowa

The Poultry Form—J. W. Newberne, Indiana

Departmental Forms—J. W. Newberne, Indiana

Reports to the Veterinarian and Owner—H. Elliott, Louisiana

Discussion

10:20 Anaerobic Bacteriological Techniques at Georgia Coastal Plain Experiment Station—E. M. Ellis

11:00 Differential Diagnosis of the Mucosal Complex Diseases, Autopsy and Laboratory—F. K. Ramsey, Iowa

11:40 The Laboratory Diagnosis of Equine Infectious Anemia—V. R. Saurino, Florida

AFTERNOON

1:20 The Diagnostic Laboratory and Parasitologic Diagnosis

2:00 Report of Meetings of Regional Associations

2:30 A Diagnostic Service for Diseases of Dogs—J. A. Baker, New York

3:10 Techniques for Methods of Evaluating Poultry Vaccines

3:40 Differential Diagnosis of Avian Hepatitids—R. A. Bankowski, California

4:20 Business Meeting—Election of Officers

5:00 Adjournment
PROGRAM

THURSDAY, NOVEMBER 4, 1958

9:00 A. M. to 5:00 P. M. Preconvention Registration. Fee $5.00

Committee Meetings. See Bulletin Board

Meeting Conference of Veterinary Laboratory Diagnosticians. See Program, Back Page

Meeting of National Association of Federal Veterinarians. See Bulletin Board

WEDNESDAY MORNING, NOVEMBER 5

8:30 Registration

9:00 Report of the Secretary-Treasurer—R. A. Hendershot, Trenton, New Jersey


9:25 Report of Representative to Annual Meeting of Association of State Departments of Agriculture—A. A. Ermann, Madison, Wisconsin


10:00 Anaplasmosis Paper—J. E. Christensen, Ph.D., D.V.M., Davis, California

10:25 Discussion


10:40 Address of Welcome—Mr. Jay B. Starkey, Chairman, Florida Livestock Board, Tallahassee, Florida

11:00 Response—J. E. Stuart, Sacramento, California

11:10 Memorial Service—M. N. Riemenschneider, Oklahoma City, Oklahoma

11:20 President’s Address—J. G. Milligan, Montgomery, Alabama

11:50 Presentation of Key to President Milligan—R. A. Hendershot

THURSDAY MORNING, NOVEMBER 6

9:00 Report of the Auditing Committee

9:05 Infectious Synovitis Control—N. O. Olson, D.V.M., and D. C. Shelton, Ph.D., Morgantown, West Virginia

9:30 Discussion

9:35 Report of the Committee on Transmissible Diseases of Poultry—R. A. Bankowski, et al., Davis, California

9:50 Animal Reservoirs of Leptospira—H. E. Goldstein, D.V.M., Reynoldsburg, Ohio

10:20 Current Diagnostic Problems in Leptospirosis—E. V. Morse, D.V.M., Ph.D., Ames, Iowa

THURSDAY AFTERNOON, NOVEMBER 6 (Continued)


11:05 Ornithosis, A Public Health Problem—K. F. Meyer, M.D., San Francisco, California

11:40 Discussion

11:45 Report of the Committee on Public Health—R. Schroeder, et al., Los Angeles, California

THURSDAY MORNING, NOVEMBER 7

1:30 Infectious Bovine Bovine Encephalomyelitis in California—J. E. Bunting, Ph.D., Davis, California


2:45 Discussion


3:05 Swine Brucellosis—S. H. McNutt, D.V.M., Madison, Wisconsin


3:45 Discussion


4:00 A Comparison of Various Diagnostic Tests With Microscopic Findings in John’s Disease—A. R. Largent, D.V.M., M.S., T. H. Rishman, D.V.M., M.S., Auburn, Alabama


5:00 Executive Committee Meeting
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NEW YORK STATE BRUCELLOSIS RING TEST PROGRAM

The State Department of Agriculture and Markets cooperating with the U. S. Department of Agriculture is now making use of the milk ring test as a major part of the Brucellosis Eradication Program. Milk ring samples are taken at four to six months' intervals by technicians at milk plants. In this way all herds in the State from which milk is shipped will be ring tested at least twice each year. Notice of the results of this test will be given owners through a letter card --

1. The white card will indicate that the herd, on the particular day that the milk samples were collected, was apparently free from brucellosis.

2. The salmon-colored card will indicate that there is a possibility brucellosis infected cattle were producing milk on the day milk samples were collected.

In the future, blood testing will be limited to tests of the herd showing suspicious reaction to the ring test, provided previous reactors have been removed. Clean herds will be blood tested on a three-year basis.

Herd owners are reminded that they must have brucellosis free herds in order to maintain markets in New Jersey after April 1, 1958 and in New York State by July 1, 1959.

NOW IS THE TIME TO PLAN FOR A BRUCELLOSIS FREE HERD. YOU SHOULD WORK TOWARDS OBTAINING A WHITE MILK RING TEST CARD.
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Proceedings

SIXTY-SECOND
ANNUAL MEETING

of the
UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

DEAUVILLE HOTEL
Miami Beach, Florida
November 4-5-6-7, 1958
CONTENTS

Officers for 1958-1959 .......................................................... vii
Committees 1959 ..................................................................... ix
Record of Previous Meetings ................................................... xiv
Introduction of Jay B. Starkey ................................................ 1
Address of Welcome, J. B. Starkey ......................................... 2
Response to Welcome, R. W. Smith ........................................ 7
President's Address, J. G. Milligan ......................................... 10
Presentation of Key to President Milligan, R. A. Hendershott ...... 15
Report of the Secretary-Treasurer, R. A. Hendershott .............. 16
Memorial Service, M. N. Riemenschneider .............................. 19
Report of Advisory Committee to A. R. S ................................. 22
Report of Representative to Annual Meeting of National Association of States
  Department of Agriculture ................................................... 24
Report of Committee on Laws and Regulations, F. L. Schneider et al. 27
Report of Committee on Public Relations, C. L. Campbell et al. 31
Report of Committee on Regulatory Education, W. T. S. Thorp .... 32
Report of Committee on Revision of Constitution and By-Laws, R. L. West et al. 34
Proposed Amendment to Constitution and By-Laws, R. W. Smith .... 35
Veterinary Medicine in the U. S. R. C. D. Van Houweling ........ 38

BIOLOGICS AND PHARMACEUTICALS

Simplified Standards for Live Virus Vaccines, D. S. Robson and J. A. Baker .... 43
Report of Committee on Biologics and Pharmaceuticals, A. A. Creamer et al. 53

CATTLE

Anaplasmosis

The Incidence of Latent Anaplasma Marginale Infection in Wild Deer in an Area
  Where Anaplasmosis is Enzootic in Cattle, J. F. Christensen et al. .... 59
Anaplasmosis Control by Test and Subsequent Treatment With Chlortetracycline.
  W. E. Brock et al. .................................................................. 66
Report of the Committee on Anaplasmosis, K. J. Peterson et al. ....... 71

Brucellosis

Status of State-Federal Cooperative Brucellosis Eradication, C. K. Mingle .... 73
Swine Brucellosis Control, S. H. McNutt and Earl Hubbard ............. 83
Report of Committee on Brucellosis, R. W. Smith et al. .............. 90

Leptospirosis

A Leptospira Wildlife Survey in Ohio, H. E. Goldstein et al. .............. 104
Current Diagnostic Problems in Leptospirosis, E. V. Morse .......... 109
Report of Committee on Leptospirosis, C. J. York et al. ............. 116
CONTENTS

Virus Diseases of Cattle

The Relationship of Infectious Pustular Vulvovaginitis Virus to Infectious Bovine Rhinotracheitis Virus. J. H. Gillespie et al. .......................... 119
Sporadic Bovine Encephalomyelitis in California. J. B. Enright et al. ....... 127
Current Status of the Newer Virus Diseases of Cattle. D. C. McKercher et al. ... 136
Report of Committee on Infectious Diseases of Cattle. J. E. Stuart et al. ....... 159

Tuberculosis

A Comparison of Various Diagnostic Tests With Microscopic Post-Mortem Findings in Cattle Infected With Johnes's Disease. A. B. Larsen and T. H. Vardaman ....... 163
Status of Federal-State Cooperative Tuberculosis Eradication. A. F. Ranney ...... 167
Report of Committee on Tuberculosis. R. L. West et al. ........................ 175

Parasitic Diseases

Screwworm Eradication Program in the Southeastern United States. R. S. Sharman 183
New Research Results with Septemic Insecticides. R. C. Bushland ............... 192
Report of Committee on Stockyards, Markets and Transportation. R. Cuff et al. 198

Poultry Diseases

Infectious Synovitis Control 10: Chlortetracycline in Chicks Inoculated at One Day of Age. N. O. Olson and D. C. Shelton .......................... 201
Report of the Committee on Transmissible Diseases of Poultry. R. A. Bankowski et al. 210

Public Health

Public Health Aspects of Anthrax. M. N. Riemenschneider ........................ 227
Ornithosis: A Public Health Problem. K. F. Meyers ............................ 230
Report of the Committee on Public Health. R. J. Schroeder et al. ............ 244
Present Status of Bat Rabies in the United States. E. S. Tierkel and P. Arnstein 248
Report of the Committee on Rabies. E. S. Tierkel et al. ........................ 253

Sheep Diseases

Ovine Virus Abortion. E. A. Tunnicliff .......................... 261

Swine Diseases

Crystal Violet Vaccine Experiment Progress Report II. J. P. Torrey et al. .... 271
Recent Studies on the Properties of a Nonvirulent Living Hog Cholera Vaccine. J. W. Newberne et al. .......................... 278
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R. A. Hendershott  R. H. Singer
## Record of Previous Meetings

<table>
<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
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<tbody>
<tr>
<td>1. Sept. 27-28, 1897‡</td>
<td>Forth Worth, Texas</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. D. O. Lively, Fort Worth, Texas</td>
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<td>2. Oct. 11-12, 1898</td>
<td>Omaha, Nebraska</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. Taylor Riddle, Kansas</td>
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<td>5. Oct. 8-9, 1901</td>
<td>Buffalo, New York</td>
<td>*Dr. E. P. Niles, Virginia</td>
<td>*Dr. F. T. Eisenman, Louisville, Ky.</td>
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<tr>
<td>26. Dec. 6-7-8, 1922</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. J. Butler, Helena, Montana</td>
<td>*Mr. J. J. Ferguson, Columbus, Ohio</td>
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<td>32. Dec. 5-6-7, 1928</td>
<td>Chicago, Ill.</td>
<td>*Dr. Chas. G. Lamb, Denver, Colo.</td>
<td>*Mr. O. E. Dyson, Kansas City, Mo.</td>
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<td>33. Dec. 4-5-6, 1929</td>
<td>Chicago, Ill.</td>
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<td>*Mr. O. E. Dyson, Kansas City, Mo.</td>
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<tr>
<td>36</td>
<td>Nov. 30–Dec. 1–2, 1932</td>
<td>Chicago, Ill.</td>
<td>*Dr. Peter Malcolm, Des Moines, Iowa</td>
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<tr>
<td>37</td>
<td>Dec. 6–7–8, 1933</td>
<td>Chicago, Ill.</td>
<td>*Dr. E. T. Faulder, Albany, N. Y.</td>
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<tr>
<td>38</td>
<td>Dec. 5–6–7, 1934</td>
<td>Chicago, Ill.</td>
<td>*Dr. T. E. Robinson, Providence, R. I.</td>
</tr>
<tr>
<td>39</td>
<td>Dec. 4–5–6, 1935</td>
<td>Chicago, Ill.</td>
<td>Dr. Edward Records, Reno, Nevada</td>
</tr>
<tr>
<td>41</td>
<td>Dec. 1–2–3, 1937</td>
<td>Chicago, Ill.</td>
<td>Dr. R. W. Smith, Concord, N. H.</td>
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<tr>
<td>43</td>
<td>Dec. 6–7–8, 1939</td>
<td>Chicago, Ill.</td>
<td>*Dr. J. L. Axby, Indianapolis, Ind.</td>
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<td>44</td>
<td>Dec. 4–5–6, 1940</td>
<td>Chicago, Ill.</td>
<td>*Dr. H. D. Port, Cheyenne, Wyoming</td>
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<tr>
<td>46</td>
<td>Dec. 2–3–4, 1942</td>
<td>Chicago, Ill.</td>
<td>*Dr. I. S. McAdory, Auburn, Alabama</td>
</tr>
<tr>
<td>47</td>
<td>Dec. 1–2–3, 1943</td>
<td>Chicago, Ill.</td>
<td>Dr. W. H. Hendricks, Salt Lake City, Utah</td>
</tr>
<tr>
<td>48</td>
<td>Dec. 6–7–8, 1944</td>
<td>Chicago, Ill.</td>
<td>Dr. J. M. Sutton, Atlanta, Ga.</td>
</tr>
<tr>
<td>50</td>
<td>Dec. 4–5–6, 1946</td>
<td>Chicago, Ill.</td>
<td>*Dr. William Moore, Raleigh, N. Carolina</td>
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<tr>
<td>51</td>
<td>Dec. 3–4–5, 1947</td>
<td>Chicago, Ill.</td>
<td>*Mr. Will J. Miller, Topeka, Kansas</td>
</tr>
<tr>
<td>53</td>
<td>Oct. 12–13–14, 1949</td>
<td>Columbus, Ohio</td>
<td>Dr. T. O. Brandenburg, Bismarck, N. D.</td>
</tr>
<tr>
<td>57</td>
<td>Sept. 23–24–25, 1953</td>
<td>Atlantic City, N. J.</td>
<td>Dr. T. Childs, Ottawa, Canada</td>
</tr>
<tr>
<td>58</td>
<td>Nov. 10–11–12, 1954</td>
<td>Omaha, Neb.</td>
<td>Dr. T. C. Green, Charleston, W. Va.</td>
</tr>
<tr>
<td>59</td>
<td>Nov. 16–17–18, 1955</td>
<td>New Orleans, La.</td>
<td>Dr. H. F. Wilkins, Helena, Montana</td>
</tr>
<tr>
<td>60</td>
<td>Nov. 28–29–30, 1956</td>
<td>Chicago, Ill.</td>
<td>Dr. A. L. Brueckner, Baltimore, Md.</td>
</tr>
<tr>
<td>61</td>
<td>Nov. 13–14–15, 1957</td>
<td>St. Louis, Mo.</td>
<td>Dr. G. H. Good, Cheyenne, Wyoming</td>
</tr>
<tr>
<td>62</td>
<td>Nov. 4–5–6–7, 1958</td>
<td>Miami Beach, Florida</td>
<td>Dr. John G. Milligan, Montgomery, Alabama</td>
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</table>

*Deceased.  
†This was the last meeting of the Interstate Association of Livestock Sanitary Boards.  
‡Reprinted in 54th Annual Report.
INTRODUCTION OF JAY B. STARKEY

DR. C. L. CAMPBELL: Mr. President, members of the United States Livestock Sanitary Association, guests: Jay B. Starkey, who has served as the Chairman of the Florida Livestock Board since 1953, has a livestock background which dates back several decades. Although in recent years his livestock grazing activities have been confined primarily to the field of beef cattle, for many years the Olnerton Ranch, which he founded shortly after World War I, near Largo, or St. Petersburg, Florida, had the largest herd of purebred Hampshire hogs in the entire south.

In reviewing Jay’s history, I found it particularly noteworthy that back in the early 20’s, when Florida ranchers raised very little feed, Olnerton Ranch put up about 160 tons of silage per year. I found also that in the up-grading of Florida beef cattle, Mr. Starkey brought into Pinellas County the first registered Angus, Hereford, Shorthorn, and Brahman bulls and Brahman cows.

Among his other achievements are: Director of the First National Bank of St. Petersburg, President of the Florida Cattlemen’s Association in 1955, and Pinellas County tax collector from 1937 to 1949.

I understand that following one of his early campaign addresses, in which he had requested his listeners to vote for him, a man from his opponent’s camp angrily jumped to his feet and shouted, “I’d rather vote for the devil!”

“Perhaps so,” Starkey replied with a smile. “But in case your friend refuses to run, can I count on you for your support?” [Laughter.]

This may explain the fact that Mr. Starkey is the only Democrat to retire undefeated in Republican-strong Pinellas County. This, I understand from yesterday’s election results, is probably a continuing trend.

It is a pleasure to introduce to you one of my nine bosses on the Florida Livestock Board, Mr. Jay B. Starkey.
ADDRESS OF WELCOME

MR. JAY B. STARKEY, Chairman,
Florida Livestock Board

[Designated as Item No. 8]

MR. STARKEY: Thank you, Doctor Campbell.
Mr. President and gentlemen of the United States Livestock Sanitary Association: Doctor Campbell gave me a very generous introduction. There was just one other thing that he might have said that would have probably covered my background a little more. He failed to tell you that I was the only man who ever served as President of the freshman class in St. Petersburg three consecutive years. [Laughter.]

In 1953, the present Florida Livestock Board was created by an Act of the Florida Legislature. Prior to that time, and for a great many years, we had had what was known as the Florida Livestock Sanitary Board. I do not know which came first, whether it was the elimination of the word “sanitary,” or the elimination of the Board, but any way, when you look over the present personnel of the Florida Livestock Board, you will see why they dropped the word “sanitary.” That is why, probably, our Board is little different than most of the Boards that you gentlemen represent. We are now the Florida Livestock Board. The “sanitary” has been eliminated.

When the members of the Florida Livestock Board were selected by the Governor in 1953, he selected these men from the cattle, dairy, swine, and poultry industry. There were eight men selected from the eight Congressional Districts and one from the state at large. I happened to be the member who was selected from the state at large, and I was not selected on my ability to speak. I am a Florida cowman. I have known a lot of them. I never knew one yet who knew what he wanted to say. If he did, he did not know how to say it. I am no exception to the rule.

I know in your organization, in many parts of the country, there are large cattlemen’s associations, and I have attended a few, and I know that that does not hold true in all the states; but in the State of Florida, I have known a lot of cattlemen, and I do not think there is too much difference in us when it comes to public speaking.

I am kind of like the little boy that got up to make a recitation in school, and he forgot his subject. And he thought a little bit, and he said, “When I got up here, God and I only knew what I was going to say. Now God only knows.” [Laughter.]

And that is about my position when I get on my feet.

I was asked this morning if I had an address. I have never written an address in my life. I do not know that I ever will. I am not capable of writing one. I probably could not read it if I did. I have made a few remarks
ADDRESS OF WELCOME

on such occasions as this. Sometimes they border on the intelligent, and
sometimes not quite so intelligent.

We are grateful to you for selecting Florida as your meeting place this
year. We are very glad you took a chance on coming to this state. I am
glad you are not like the incident I heard of—the little boy in the hen house.
He dropped his bubble gum. He looked around a little bit and shook his
head and walked out. He was scared to take a chance. [Laughter.]

I am glad you fellows were not scared to take a chance on coming to
Florida. [Laughter.]

Florida does not represent the livestock industry like a lot of states that
you folks are from, I am sure. We rank fourteenth in number of beef cattle
and about twenty-third in the number of all cattle; about thirty-first in the
number of dairy cattle, I believe. However, since 1940, our cattle in Florida
have increased by better than 140 percent. We have increased faster than
any other state.

Our industry, of course, the cattle industry of Florida, is 430 years old.
In making a talk over at the Rotary Club in St. Petersburg one time I made
that remark, and after the meeting was over an old tourist came over to me.

I told him that Ponce de Leon brought cattle here 430 years ago, and that
was back prior to the time we had open range and there were cattle on the
highways all over Florida. And this old tourist said to me, “I agree with
what you have said, Mr. Starkey. I’ve seen some of those cattle on the road.”
[Laughter.]

Our cattle in the last 20 years have changed more than in almost any other
state. We were 100 years behind the rest of the nation up to about the
middle 30’s, at least. So we have made a lot of progress; but we have got
a long ways to go.

We have a lot of cattlemen come here from other states, many from the
Western states, a good many from the north. Some come here to get in the
cattle business to make money, and some come here to get into it to spend
money. And we have been here trying to help them out. [Laughter.]

We in Florida have a number of large ranches. I believe there have only
been about 500 cattle ranches in the entire United States that have over
2,000 head of cattle on them. Florida has quite a number of those ranches.
We have ranches in Florida that comprise up to 400,000 acres of land. We
have a county in this state that has more than 100,000 head of cattle.

One of the great western organizations in Utah has bought within this
state in excess of 300,000 acres of land. They have a program going on to
clear and seed in the improved pastures 10,000 acres of ground in the next
five years. They recently bought 65,000 pounds of grass seed. The Mormon
Church, I believe it is.

You people who are seeing the fabulous Gold Coast of Florida for the first
time—I hope that you do not think that all of Florida is quite as fabulous
as what you have seen since you have been in Miami. We do, however, in
Florida, have some things I wish you were able to see while you are here—
the improvements on ranches and farms. We have some dairymen in this
ADDRESS OF WELCOME

group. I do not know what size dairies they have in most parts of the
country, but there are dairies in Florida milking 5,000 and more head of
cattle. There are large dairies here, and very modern dairies. I hope that
while you folks are here you will be able to see more of it, more of Florida,
than you will see right here.

We have had lots of adversities, lots of trouble. Over a period of years,
without climate and our close proximity to the South American and West
Indies countries, we have probably had as many kinds of diseases from in-
fecions as almost any other section of this country. We have eliminated
the tick. I do not know how many of you fellows will have the opportunity
to see the screwworm laboratory, the large animal diagnostic laboratory in
Kissimmee, and the pilot test area for hog cholera eradication in north
Florida.

Under the direction of Dr. C. L. Campbell, the hog cholera test, pilot test
area, I think is one of the greatest steps forward we have made in this state,
and I know you folks are interested in it. While we do not produce swine
in proportion to what you do in some of your mid-western states, we have
some thousand people in north Florida engaged in swine production.

We have gone through a lot of trouble. Once in a while you will hear
some fellow, some old man my age or a little older, say he would like to
have the good old days back when we had cattle on the open range. I went
with cattlemen every day when I was a boy, and we did have a lot of fun,
but I do not much care to have those days back. I am a little bit like the
man who died over in my county a few years ago, a young man who had
only been married a couple of years. He was taken sick, and he died very
suddenly. His widow—it looked like she could not ever reconcile herself
to his death. She cried for practically three weeks, and in about the third
week the life insurance man walked in one morning and handed her a
$50,000 check. She looked at it, wiped her eyes, sniffed a couple more times,
and said, “I would give $20,000 of this to have him back.” [Laughter.]

I would not give $20,000 to have those days back.

We enjoy a lot of people here in the wintertime from all over the nation.
We have a lot of people ask us lots of questions, probably more so than
most anyone else. I understand a man who was to follow me on this platform
was from California. I understand he is not to be here. I am a little sorry.
I had a story or two I wanted to tell him. But I understand I am going to
be followed by a man from New Hampshire.

That man from New Hampshire is one of the Smith brothers, I think.
I do not think he is in here right now. I was 18 years old before I found out
that damn Yankee was two words. But anyway, we are glad to have him here.
They have done a lot to make Florida what it is today. Without the Yankees
we would not have made the progress, we would not have the people coming
here, we would not have the interest in Florida, probably greater than in
any other state, with the possible exception of California.

Our growth is greater than that of any other state, except possibly California
or maybe Arizona in the last few years. And the damn Yankees have cer-
tainly had their part in it.
We are mighty glad to have them here. We want them to keep on coming.

In the wintertime, we people on the ranch have a lot of people driving around and asking questions and seeing what they can find out about Florida.

That reminds me of another incident. Over here on the west coast last year, there was a young man from the north and his family, wife and two or three children, just cruising around one afternoon, seeing what they could see. They got off on kind of a by-road and went on down past an old rancher’s place, along in February or March. We happened to have a little cool weather here in the winter that time, and a lot of our cattle got in pretty bad shape. A lot of them had heifers dropping their first calves, and a good many of them died. The old rancher was out riding his herd, and he saw a three-year-old heifer trying to deliver a calf and having a lot of trouble.

He put the rope on this calf’s head. He was trying to get the rope on the calf’s head to pull it. This young city slicker from the north, riding along, saw this old fellow and did not know what he was doing. He stopped and watched him a little bit and finally crawled through the fence and went over to where he was.

He said, “Mister, can I help you?”

This old rancher said, “Yes, you sure can. You take hold of this rope here now until I get it on this calf’s head, and you wrap it around the saddle horn and step this horse up.”

He did, and jerked the calf. The city slicker stood around and watched him a little bit. And he could not understand that. Finally the calf was delivered. The old fellow got his rope off his horse and saddle and he says, “Young man, I sure appreciate your help. What do I owe you?”

This city slicker said, “You don’t owe me a thing, but I would like to ask you a question.”

“All right, what is it?”

He said, “How fast was that calf going when he hit that cow?” [Laughter.]

We have a lot of tourists down here, and we have lots of funny questions.

I told you when I got up here that I would not make an address. I know the Committee could have selected a lot of people in Florida to do a little better job of welcoming you folks. On behalf of the Livestock Board, on behalf of the livestock industry, on behalf of the entire people of Florida, I want to welcome you here. I hope that you will come back. I hope you will stay as long as you can while you are here, and try to see as much of Florida as you can.

Florida is not the biggest state, but we have a lot of things to see. From Key West, Florida, to Pensacola, Florida, by road is a greater distance than it is from Jacksonville, Florida, to Cleveland, Ohio. We have a long state.

People ask you what you can do in Florida, or how many cattle you can keep on an acre of ground, or what you can do in this or that. I have told a good many of them that a man would be more qualified to say what you can do in Georgia or Maine than in Florida. We have that variety of climate and land and what-have-you in the State of Florida.
We have every kind of land there is, good, bad, and indifferent. I thought possibly that we had most of the poorest land in Florida, but I happened to go to California last month and stopped by Las Vegas on the way back. And I want to tell you fellows—that is a poor place for a cowman to settle, in more ways than one.

When I got up here, I know some of you wondered how long I was going to talk and wondered if this thing here [indicating microphone] was working, which unfortunately it was.

It makes me think of another incident that happened in my town. We had a young fellow come there, a Baptist preacher, for his first assignment. You Baptists know what I mean. That was his first church. He got up the first morning. In that town there was a newly married couple. They had only been married a short time. The girl was an Episcopalian, and the boy was a Baptist. They had agreed before they were married that they would alternate at each other's churches, go to one on one Sunday and to the other the next.

The first Sunday they went to Episcopalian church, and this girl's husband would nudge her and ask what the different formalities were. The Episcopalian church had quite a few more formalities, and he would nudge her and ask her what the preacher was going to do or what he had done.

The next week they went to the Baptist church, where there was a young Baptist minister. He had on a vest and a big watch—a watch in this pocket, and a chain fastened over here. And he spent quite a bit of time with this big watch his father had given him, a closed face watch. He spent quite a bit of time when he got up there. One of the first things he did was to unbutton his coat, pull out his watch, unfasten it over on this side, open the face up, and lay it up in front of him. And the girl had never seen anything like that. She nudged her husband and said, "Honey, what does that mean?"

He said, "It don't mean a damn thing!" [Laughter.]

I have tried to make you feel this is the place for you to come to. We hope you come back.

In the words of a Florida Cracker way back out in the woods years ago—he would have told you: "We are proud to have you. Glad you-all come."

Thank you. [Applause.]
RESPONSE

DR. R. W. SMITH

DOCTOR SMITH: Mr. Chairman, members of the Association: I do not know just what I can say after listening to that wonderful address.

When Mr. Starkey started in, he stated that he was president of the freshman class in his college for three years, that he never wrote an address, and that he could not speak, and so forth and so on. And then he proceeded to deliver one of the finest addresses of welcome that I have had the privilege of hearing in the 38 years that I have been coming to this convention. [Applause.]

Therefore, I do not know just how we will classify you, Mr. Starkey. At least I think you are a little untruthful. And from there on, we will give it to you.

But I do want you to know I mean every word I have said, and I think all the boys out front here agree with me.

You covered all of the subject in the State of Florida quite thoroughly. And when you sprang that "president of the freshman class for three years," you reminded me of a rancher in Oregon that I had the privilege of being entertained by at his house some 10 or 12 years ago. He carried three or four thousand heifers.

He was a particular friend of a friend of mine, and just before we arrived, my friend says: "Doc, ask this man any question you want to about the livestock industry, disease eradication, and you will find him very interesting. He knows what he is talking about. But don't, for God's sake, mention how much money is made."

So I didn't. And then he says, "I want to tell you this. He is a member of our Board of Higher Education." It seems in the State of Oregon they have a Board of Higher Education. I believe there are nine members, and they are appointed for nine years.

And he said, "A few years back, the Governor of our state decided to appoint this man as a member of that Board." The people down at the college and those interested in higher education got together, and they did not like it too well, appointing a rancher on that Board. So they appointed a committee to wait on the Governor.

And this was what took place in the Governor's office. They said, "Governor, we understand that this gentleman you propose to appoint is a very fine business man and a very fine man, but we don't think he should be appointed to the Board of Higher Education. Did you know that he never completed the fourth grade?"

The Governor replied, "Yes, I heard that. And I had it investigated and found out it was so. But," he says, "did you know I never got out of the fifth grade?"
RESPONSE

So, you see, it does not matter.

You have given us, Mr. Starkey, a very good history and information along the agricultural line and your State of Florida in general.

We have known for a long time that you were progressive down here, because of the representative that you have sent to our meetings year after year in the person of Doctor Campbell. He has contributed a lot to this organization in the way of promoting disease eradication among our domestic animals on a national basis.

He told me some things a few minutes ago, when I was asked to come here and reply to your address, that I did not know, about the State of Florida. And you have told us a lot more; and one of those is that the State of Florida is the second state east of the Mississippi River in beef cattle population. That is quite a record. And of course I know, as many others know, that one of the contributing factors to the great growth of the beef industry in this state was the eradication of the Texas fever tick some 20 years ago.

It is important for some of us to remember, and very interesting, I believe, that it was this same fever tick some 60 years ago that was responsible for the creation and organization of the United States Livestock Sanitary Association. And we are meeting here in Florida to discuss present-day problems because of a bug that was responsible for our organization and played a very important part in the growth of the beef industry in this great State of Florida.

I also am informed—and I believe you bore that out in your remarks—that you are the thirteenth state in the United States in beef cattle population. I am not going to review the others; you know more about them than I, and we have just heard about it; but the State of Florida certainly is a wonderful state in a wonderful country.

We up north, as you state, have contributed, through the persons of the people who come down here. I have been down two or three times. But up there in the wintertime, you know, everybody of importance goes to Florida. And we have been led to believe, and probably rightfully so, by the great influx of your tourist business down here, your recreational business—because we hear more about that than we do any other—that that is about all you have down here. But that is not so. I am informed that the agriculture of the State of Florida, economically, in dollars and cents, fluctuates with your resort business. Some years one is on top, and other years the other is on top.

So let us not forget that while you are furnishing the north, the frozen north, as we call it, a great place to come during the winter months, you are also contributing to this nation in the agricultural field an equal amount of energy and prosperity.

You have done a lot, too, in other lines. You mentioned the screwworm. That project that is being developed and carried on here right now in the State of Florida is going to revolutionize in some sections of the southeast the cattle raising industry, or at least, it is going to do a whole lot to make it more prosperous.
And not only that. A few years back—I believe it grew out of this organization—there was the idea and the project to eradicate hog cholera; which to my mind is one of the most costly diseases that we have here in the United States.

I am not smart enough to sit down with a pencil and even write the figures out, of the expense that that is costing the swine industry, here, to control, never mind eradicate, the disease. And here in the State of Florida, with the state cooperating, I believe, with the Federal Government, you are conducting a pilot test, which, if successful—and I believe it will be, when it is carried out to completion—will do a great deal to eradicate this costly and needless disease among the swine industry of the United States.

When our Secretary told us that we were coming here to Miami Beach, some of us were a little leery. Miami Beach, you know, as we hear about it, is a resort. And I guess perhaps that is so. But when we had to travel the length of time it took to get from the airport out here, I came to believe it was something besides a pleasure resort.

But we have been highly entertained and very nicely taken care of in this beautiful hotel, and southern hospitality has been extended to us on every side. And I personally hope that it will not be too many years before we will decide to come back here.

With these closing remarks, I want, on behalf of the United States Livestock Sanitary Association, to thank you, Mr. Starkey, for the information you have given us, the warm welcome that you have given us, and to assure you that we appreciate everything that the state has contributed to our programs and is doing for us this week in our stay here in Miami Beach.
PRESIDENT'S ADDRESS

J. G. MILLIGAN

Montgomery, Alabama

Members of the United States Livestock Sanitary Association, distinguished guests, ladies and gentlemen: It is indeed an honor for me to have the privilege to welcome you here to the Sixty-second Meeting of the United States Livestock Sanitary Association. To the new members and visitors who are here for the first time, the workings of this organization may seem somewhat puzzling for a time, but as the program progresses and the pieces fall into place, you will realize the full meaning of this gathering. This is the meeting where we get together to discuss the progress and the mistakes of the disease control and eradication programs of the past year and to plan better programs for the coming year.

Dr. R. A. Hendershott, the secretary of this organization, is to be congratulated on getting together the talent that is listed on the program. All of us will leave here better informed than when we came. The various committees of this organization are living up to their reputation of the past. The extra day of committee meetings before the program begins, now makes the work of these committees much less tiresome and much more can be accomplished. I deeply appreciate the fine spirit that has been shown by the many members of these various committees in their work this past year. It is the work of these committees that makes this organization worthwhile.

All phases of our work have been and are still being discussed in the various committee meetings. You will be informed of these discussions as well as the recommendations of these committees as each committee gives its report. For that reason I am not going to consume your time giving my views and opinions on all the various phases of our work. There are a few subjects that I would like to discuss with you briefly, and if they are included in committee reports, I am sure our views on the subjects will not vary too much.

The hog industry in this nation is one of the most important phases of our agricultural economy. The average annual death loss in hogs from infections and contagious diseases is enormous. There is need in the Agricultural Research Service for a swine disease section to cooperate with the various states in swine disease control. This has been recommended by the United States Livestock Sanitary Association in the past and should be followed up until such a section is established and financed sufficiently to operate. Once a swine disease section is established within the Agricultural Research Service, we will be able to begin a much needed hog cholera eradication program. The swine industry is eager to begin the eradication of hog cholera and is only waiting for leadership in its undertaking. Other countries have eradicated the disease. It is past time that we stop debating the merits
of this or that method of vaccination and get on with the job of eradicating hog cholera.

The scrapie program in sheep has caused some dissension among sheep owners and livestock sanitary officials during the year. Some of the actions taken have reached the point of becoming a serious threat to all disease control programs. Action has been taken to correct this misunderstanding by the appointment of a study committee that will hold a series of meetings throughout the United States and that will recommend a solution to the United States Department of Agriculture. There is no doubt but what the committee will find a satisfactory solution to the scrapie problem.

The increase in the number of animals reacting to the tuberculin test makes us more fully aware of our duties and responsibilities as they relate to the complete eradication of tuberculosis. Several factors have entered into the picture recently that tend to complicate the program more than was true in past years. The no gross lesion cases that became a problem as the percentage of reactors dropped in the initial eradication has recently increased to greater numbers than was noticed when the disease was at its peak. No satisfactory explanation has yet been found to fully answer the question of why these cases have increased to such an alarming extent in some areas. This answer must be found if our present tools are to completely eradicate the disease. Therefore, our efforts to find this cause or causes of these no gross lesion reactors must be stepped up. Tuberculosis itself has increased in some areas to the extent that it is now apparent that we must re-evaluate the entire testing program and concentrate our efforts in those areas where the increase is the greatest. Veterinary personnel must be given more training and instructions on the use of the test. Many of our present field force know so little about the application of the test because they have come along since complete area testing was conducted. Schools for the training on the use of the test are now being conducted by the Agricultural Research Service and should help in correcting this condition. Many problems still confront us in our efforts to completely eradicate tuberculosis.

The brucellosis eradication program has progressed remarkably during the year and the number of modified-certified states and counties have increased to the point that now more than 50 percent of all counties have become certified. However, this should not leave us with the feeling that the job is more than half done. The last county may be the hardest to complete, and the job of complete eradication is only just begun when the last county is declared a modified-certified brucellosis free area. The Committee on Brucellosis is one of the hardest working committees of this organization. Their report has always been a most constructive report, and I am sure that this year’s report will be no exception. There is a feeling among many of us that the eradication of swine brucellosis should be included in our brucellosis program and that before complete eradication is accomplished in cattle, brucellosis must also be eradicated from other species of animals. The brucellosis committee has been enlarged to include members whose major interests are those of swine disease control. It is hoped that the committee
can now recommend a swine brucellosis program that will be acceptable and will accomplish the desired results.

The funds available at the federal level for brucellosis eradication are always in danger of being reduced. This organization must continue to exert all of its efforts to see that these funds are kept up so that the job of complete eradication can be completed without further delays. Funds at state levels have been increased as a whole, but many states still find it hard to raise the necessary funds to match the federal budgets. It is hoped that the various states can increase their appropriations sufficiently to carry their part of the load.

The revisions made in Title 9 giving the Agricultural Research Service supervision over the interstate movement of cattle has been in effect long enough now that a fair evaluation of its merits can be made. There is no doubt that it has been of some value but if it is to be of great help a great deal more time and effort must be given toward its enforcement. So far most convictions have been against violators who have moved known reactors across state lines, and in most instances these cases have entailed a long, drawn-out process getting them through federal court. Little has been done about the movement of animals not known to be infected and about trucking firms that move such animals. Some states find it hard to enforce their own regulations when federal regulations are also violated and no immediate action is taken by federal authorities. A thorough review should be made of the results of these regulations and corrective measures made so that the regulations are enforced with speed at the Federal level. The inspection service at stockyards and packing plants that are approved for the interstate movement of animals under Title 9 seems to be a mixture of everybody’s ideas with no definite set of rules to be followed by anyone. Some states are trying to follow the regulations under which the terminal yards have operated in the past while in other states the rules are made as yards become approved. In states where meat inspection service is not supervised by the livestock sanitary officials, the packing plants, once they are approved to receive cattle interstate, operate as they please until caught. There is not now available a complete list of packing plants, stockyards, and certified counties that can be easily referred to by officials when approving the movement of animals to these destinations. These and other points in the regulations need clearing up at once or the whole matter of the interstate movement of cattle should be turned back to the states.

Some of you visited the screwworm laboratory at Sebring last Sunday, and to those who have not seen this plant I would advise that they take the time to do so before you leave Florida. The time spent in taking a trip to Sebring to see that operation will be most rewarding. This plant and the entire screwworm eradication program is a glowing example of what can be done with a cooperative program between the Agricultural Research Service and state officials. The entire operation sounds too fantastic to believe yet the results obtained are far greater than were hoped for in the beginning. Doctor Sharman will give us a report of these activities Friday morning. I only
wish he could have been allotted more time so that he could explain the entire program in detail, thereby impressing on all of us what can be accomplished in any disease eradication program when full cooperation is received from everyone involved in the program. If you will pardon an example in which I am personally involved, a report of some of the screw-worm activities in Alabama will give you some idea of the splendid cooperation given by everyone in this undertaking. On Saturday, September 6, specimens were mailed from Alabama to Sebring. Tuesday, September 9, these specimens were classified as screwworms. On Wednesday, September 10, planes from Florida were dropping sterile flies over the infested area and a veterinarian had driven all night in a station wagon to arrive in Montgomery Wednesday morning to assist in setting up an eradication program. The program was well under way by Wednesday night. The state of Florida and the Agricultural Research Service demonstrated in this instance what is really meant by the word “cooperation” in a disease control program.

The programs of disease control mentioned here are only a few of the programs that confront livestock sanitary officials daily. Many others are just as important as the ones named and are just as worthy of discussion, but I have promised not to consume your time by going into all these programs. They will be left for the committee reports.

Most of you know that livestock sanitary work in Alabama is just on the verge of recovering from the most vicious form of political attack, and for that reason I feel that I must say at least a few words about politics and disease control. There are those who feel that politics and disease control are not compatible to any degree, but since all appropriations of funds for disease control must be made by politicians and all laws pertaining to disease control must be passed by politicians there is no way to conduct a disease control program that is completely immune to politics. There is also no way to compromise with a disease so through necessity all of the results obtained in conducting a disease control program are accomplished by maintaining a delicate balance of politics and scientific know-how. When this balance is disturbed, too much on either side, radical organizational changes usually take place and in most instances many years of hard work are required before the balance can again be found. Even the Bureau of Animal Industry, an organization with a world-wide reputation for efficient and effective disease control backed by a scientific knowledge second to none, was doomed when it ceased to wield a political influence. There appears now to be a tendency in many places to allow politics to enter too much into the various programs and if extreme care is not taken, the balance could easily swing too far to the political side. We agree too readily with the Bureau of the Budget and with the department heads in Washington when the budget is trimmed to agree with their opinions. The same is also true in some instances at the state level. When programs fall through from the lack of finances or from the lack of enforcement of regulations, the livestock sanitary official is the person who receives all the blame and the true cause of the failure may never be known. We must insist that politics be left out of
disease control as far as possible but be included to the extent that the pro-
grams are properly financed and backed by sufficient laws to carry the
program through.

The combining of the offices of State Veterinarian and Veterinarian in
Charge has taken on a new light during the year. This should be viewed
with concern by this organization and every other organization interested
in animal disease control. These offices have served their separate function
well throughout the years, and have cooperated in most instances without
difficulty when working on joint programs. No one can serve two masters
well, and the joining of these offices throughout the country can lead only
to one end, that is the complete domination and usurpation of state authority
by the Federal Government. It is true that at the present time this may seem
to be an improvement over some of the states' organizations. These conditions
could best be corrected by the states involved. America is not now ready for
a centralized government in Washington. Two states have had their offices
combined during the year, and at least one more has been approached. The
control of animal diseases is a tremendous undertaking and can best be
accomplished by close cooperation of the individual states and the Federal
Government. There is a definite need for both offices.

I want to thank you for allowing me to serve as your president for the
past year, and again I want to thank Doctor Hendershott and all the others
who have worked so hard in preparing for this meeting.
PRESENTATION OF KEY TO PRESIDENT MILLIGAN

R. A. HENDERSHOTT

DOCTOR HENDERSHOTT: This is one of the most pleasant duties of this Association.

Some 10 or 12 years ago, it was decided that we should in some manner honor the men who serve our Association as its president. During the last few years, we have presented to our retiring President a key symbolizing our appreciation of the fact that he has devoted considerable time and effort to guiding the programs and work of this Association.

I think if you read the proceedings and the list of the men who have served this organization as President, you will find that we have been very, very fortunate in the selection of our officers. I know of no instance where we have not improved annually over the groups that we have had or the men that we have had serving, and this year has been no exception to that general trend. I do not know when we will reach the zenith of perfection in this particular regard, but it seems always there is some improvement annually from one President to the next.

Doctor Milligan, it is my pleasure to present to you at this time this key, as token of our appreciation of your service to the United States Livestock Sanitary Association as its President during 1957-58.

Thanks for a job well done.

PRESIDENT MILLIGAN: I want to assure all of you that I will always cherish this key.

I was a little bit taken aback here when Ralph came out with some of his remarks, because I know in my own mind I will never reach the peak that was reached by some of the Presidents in the past. I particularly have in mind one of the great Presidents of the past, whom I served for a number of years, and that was Dr. C. A. Cary. I know and you know that I will never be reaching the heights that Doctor Cary did reach, and many of your other Presidents.

I have been working with this Association, with some of the Presidents, recently, and was very closely associated with the President last year, and I certainly know that I am no match for those men.

Nevertheless, I want to thank you for this, and again I say that I always will cherish this memento.
REPORT OF THE SECRETARY-TREASURER

R. A. HENDERSHOTT
Trenton, New Jersey

Mr. President, ladies and gentlemen: This year has witnessed two important innovations as far as our Annual Meeting is concerned. In place of spending long evening hours in committee meetings, we have set aside a full day for them. While this change will not entirely eliminate evening meetings for all in attendance at this our sixty-second meeting, it has demonstrated the wisdom of the change. In future meetings it is expected that all Committee work will be accomplished through correspondence and a daytime meeting prior to our regular program.

A second and to my mind a more important addition to our Annual Meeting was the inauguration of the meeting of Conference of Veterinary Laboratory Diagnosticians. This group under the leadership of Doctors Paul Bennett, of Iowa, and William Sippel, of Florida, held its first annual meeting yesterday.

Having personally headed the diagnostic laboratory for 10 years for the Division of Animal Industry of New Jersey, I have long realized the real need for diagnosticians to get together and exchange ideas. I trust that many of you availed yourselves of the opportunity to listen in on the excellent papers and discussions. If you did not then better make a mental note to attend their second annual meeting at San Francisco in December 1959.

It shall be our privilege to publish the excellent papers of this group as an addendum to the report of this our sixty-second annual meeting.

In most every way this has been a good year, we had a good meeting in St. Louis, although the attendance was not up to expectation. The 61st Annual Proceeding was in the mail two months ahead of recent years. Our finances are in good condition and we have been able to maintain a satisfactory balance.

You will note that our individual membership has fallen slightly this year; however, we have added Alaska to our official family.

For the past decade we have held out a welcoming hand to the livestock and poultry farmer to take an active part in our Association to meet with us and assist us in the preparation of disease control programs.

At this very time the swine breeders are holding their initial meeting at Lafayette, Indiana. This is indeed unfortunate and we regret that they are not holding their meeting here in conjunction with our meeting.

It is to be hoped that livestock and poultry farmers will attend our meetings and assist us in the preparation of sound practical programs. We need them on our committees, because we fellows in regulatory medicine oftentimes are in a rather narrow alleyway. We get thinking our own thoughts relative to animal disease control and at times lose sight of the practical application
of some of our programs. We need these men in here to sort of guide us, as it were, and say, "Well, now, this is the practical thing that you can do, but these other things are idealistic, and let's hope that in the future we may be able to attain that kind of goal, but let's work it out in easy stages."

It is that type of contact that we miss greatly in this Association, believe me. Certainly the producers can do a great deal of good. I think that you men that are here from industry that have attended the Committee meetings that were held yesterday on brucellosis, tuberculosis and leptospirosis certainly ought to be imbued with the idea that such meetings constitute a democratic way of arriving at a committee report.

Believe me, those Committee reports, when completed and approved by the Animal Disease Control Officials in the United States Department of Agriculture, become the law of the land and the program that is applied across this nation in animal disease control. This procedure has been eminently successful in the past, reducing the incidence of tuberculosis and brucellosis, bringing about the control of contagious pleural pneumonia and other diseases of livestock. Dourine is no longer a disease that we have to conjure with, nor is glanders. Shortly, we hope that brucellosis will be one of those that will be relegated to textbooks. Though we have not achieved the ultimate in tuberculosis eradication, certainly we deserve credit for the remarkable reduction of the incidence of this disease in livestock.

So we still need counsel, and we invite producers and any other interested persons to come into the open meeting of the various committees and express themselves and help guide the members of the Committee in the formation of sound disease control program for that particular disease.

A lot of you men in your respective areas can do much to cement relationship between the farm people of the nation and this Association. This should be their Association. This is where their problems should be aired and where they should be discussing them with the regulatory and research people.

So I would exhort you to do what you can to encourage membership among the farm people, the producers, in this Association, and to increase our membership so that when we speak in Congressional halls we speak with considerable background of support out at the farm.

I hope that each one of you is satisfied with the accommodations that we have here at the Deauville. I think they are going all out to really entertain us and to make us welcome in this garden spot and playground of the world.
REPORT OF THE AUDITING COMMITTEE

DR. R. W. SMITH: Mr. President and members of the organization: Our President has told you that last year we made the change-over; and as I was a member of the Auditing Committee, I believe, last year, I want to say here that this change-over was made at the suggestion of our Secretary and Treasurer when we were laboring, the three of us, who knew nothing, or little or nothing, about auditing books.

It was suggested, and, as you know, the Executive Committee voted then to have it done in a professional way, and I am simply bringing to you the report that has been mailed down here.

I am not going to read to you the report. That was read by our Treasurer here yesterday morning before the convention.

It is the regular order of business that this be referred to the Executive Committee for endorsement? I will turn it over to the gentleman here.

To the Officers and Members,
United States Livestock Sanitary Association,
33 Oak Lane,
Trenton, N. J.

I have examined the Statement of Receipts and Expenditures of the UNITED STATES LIVESTOCK SANITARY ASSOCIATION for the period of 1957-1958, and the books and records relative thereto, including the checking and verification of all deposits, and the examination of the endorsements on all cancelled checks, for the period under audit.

In my opinion, the Statement of Receipts and Expenditures attached hereto, presents fairly the financial condition and the results of the operations of the Association for the 1957-1958 period audited and examined by me.

ANDREW J. GROVE, Examiner,
Department of Banking and Insurance,
State of New Jersey.

October 25, 1958.
MEMORIAL SERVICE

M. N. RIEMENSCHNEIDER

Mr. President, members of the Association, ladies and gentlemen: To the best of my information the following members have passed away since our last meeting, and it is out of respect to them, for what they did and for what they were that we conduct this service.

ROBERT L. ANDERES

Dr. Robert L. Anderes (K. S. C. '34), 56, editor and publisher of *Veterinary Medicine*, died suddenly of a cardiac seizure, on July 21, 1958.

Doctor Anderes was born in Kansas City, Kansas, and obtained his B.S. and D.V.M. Degrees from Kansas State College, the latter in 1934, with honors. He engaged in small animal practice at Evanston, Illinois, for a time and then became editor and advertising manager for Jensen-Salsbery Laboratories, Kansas City, Missouri. He served in this capacity until called to service in the Veterinary Corps in World War II, where he attained the rank of colonel.

Following military service, Doctor Anderes returned to small animal practice in Kansas City, Missouri. In 1949, he purchased *Veterinary Medicine*. Under his able guidance, this national veterinary journal took on new dimensions of appearance and readership.

Throughout his professional life, Doctor Anderes’ primary interest was the advancement of his chosen profession. During the twenty-four years of his active professional life he unselfishly donated thousands of hours to association work, committee activities and studies of professional problems. In 1957, he received the Distinguished Service Award from Kansas State College.

FORREST R. CAMERON

Dr. Forrest R. Cameron (MCK '18), 63, Hawley, Minnesota, died January 12, 1958. He graduated from McKillip Veterinary College, Chicago, Illinois in 1918. Doctor Cameron had served with Agricultural Research Service, United States Department of Agriculture.

R. J. HIGHT

Dr. R. J. Hight (CVC '07), 73, Phoenix, Arizona, died in his home after a lengthy illness. Born in Vienna, Illinois, Doctor Hight received his degree in Veterinary Medicine in 1907 from the Chicago Veterinary College. He resided in Temple, Arizona, for 44 years before moving to Phoenix. In Temple he was engaged in farming, dairying and in cattle raising and feeding. Doctor Hight was active in public life in his community and state. He had served as State Veterinarian for Arizona.
MEMORIAL SERVICE

WALTER K. LEWIS

Dr. Walter K. Lewis (MCK '00), 82, Hartsville, South Carolina, died April 4, 1958, following an illness of several weeks.

Doctor Lewis entered government service after graduation, moving to Columbia, South Carolina in 1914, where he was Veterinarian in Charge of the Bureau of Animal Industry, United States Department of Agriculture, and also State Veterinarian stationed at Clemson College for 32 years. Retiring in 1946, he resided for the past 12 years in Hartsville.

THOMAS W. MUNCE

Dr. Thomas W. Munce (UP '15), 66, Sioux City, Iowa, former director of Pitman-Moore Company's biological laboratories and retired official of the firm's parent company, Allied Laboratories, Inc., died on April 21, 1958, following a heart attack.

Doctor Munce was born at Washington, Pennsylvania, and a graduate of W. & J. Academy University of Pennsylvania veterinary school from which he received his V.M.D. in 1915. Following graduation, he practiced in Ohio for a year, then was on the staff of the state veterinarian of New Jersey before being commissioned in the Army Veterinary Corps in World War I.

Doctor Munce joined Pitman-Moore in 1919, engaging in production and research at the Zionsville, Indiana, laboratories, later became president of the Sioux City division, vice-president of Allied Laboratories, and a member of its board of directors. He retired in 1957. He was a recognized authority on swine diseases and their control and author of many published papers on the subject.

KENNETH L. RITCHIE

Dr. Kenneth L. Ritchie (ISC '33), 45, West Union, Iowa, passed away in December, 1957. Doctor Ritchie received his degree in Veterinary Medicine from the Iowa State College in 1933. He served with the Agricultural Research Service, United States Department of Agriculture.

WILLIAM G. STEVENSON

Dr. William G. Stevenson (ONT '37), 44, Guelph, Ontario, died suddenly following a heart attack on February 1, 1958. Doctor Stevenson was born at Forester's Falls, Ontario, and shortly after graduation from Ontario Veterinary College became associated with Ayerst, McKenna and Harrison, Ltd., pharmaceutical manufacturers in Montreal.

At the time of his death, he was senior member of the firm of Stevenson, Turner and Boyce, Ltd., of Guelph, producers and distributors of ethical pharmaceutical products.

R. S. SUGG

Dr. R. S. Sugg (API '15), 64, dean of the School of Veterinary Medicine, Alabama Polytechnic Institute since 1940, died January 4, 1958 following a brief illness. Doctor Sugg received a BS Degree in animal husbandry from
Alabama Polytechnic Institute in 1914 and a DVM Degree in 1915. He returned to North Carolina and practiced for about a year before returning to his alma mater where he was successively an instructor in veterinary bacteriology and pathology, assistant professor of bacteriology and then professor. From 1928 to 1930, and again from 1931 to 1940, he served as extension animal husbandman for the Alabama Agricultural Extension Service.

In 1940, Dr. Sugg was appointed State Veterinarian, and dean of the School of Veterinary Medicine. It was in the latter capacity that he began his untiring and successful work in behalf of regional education in the southern states; the groundwork for this program was laid in 1947, and largely due to his efforts, an agreement was reached by the governors of several southeastern states for regional education in medicine, veterinary medicine and dentistry. This achievement set the pattern for other regional education developments.

Since 1951, he had served full-time as dean. Interspersed in this busy professional career was active service in the Army Veterinary Corps in both World Wars. Doctor Sugg was always active in other professional affairs and received numerous honors from his colleagues for his work in their behalf, including the presidency of the Alabama V.M.A. and the Southern V.M.A. In 1951, he was awarded the Twelfth International Veterinary Congress Prize by the A.V.M.A. in recognition of his contributions to regional veterinary education.

Believing in the Fatherhood of God and the Brotherhood of man, may I humbly request all present to arise and remain standing for a moment of silent prayer for the peaceful repose of the souls of our departed colleagues.

Thank you.

I am certain that no words which may be said here this day can adequately express the keen sense of personal loss all of us feel on this occasion. It is comforting to recall the lesson that Jesus taught when he said: “Blessed are they that mourn; for they shall be comforted.”

As we bid farewell to these colleagues who have gone on before, let us be thankful for the opportunity of having had the privilege of knowing them; working with them; for the contributions they have made in their respective fields of endeavor and the ideals they have passed on to us. Let us cherish these memories and honor them with good lives, taking up the tasks that they have passed on to us, and dedicate ourselves to those principles so emulated by their lives.

In closing let us memorialize these men with the thought so ably expressed by the words of Thomas Guthrie.

“I live for those who love me,
For those who know me true,
For the heaven that bends above me,
and the good that I can do;
For the wrongs that need resistance,
For the cause that lacks resistance,
For the future in the distance,
and the good that I can do.”
REPORT OF THE UNITED STATES LIVESTOCK SANITARY ASSOCIATION ADVISORY COMMITTEE TO THE AGRICULTURAL RESEARCH SERVICE

FRANCIS G. BUZZELL, Chairman, Augusta, Maine; R. A. HENDERSHOTT, Trenton, New Jersey; W. L. BENDIX, Richmond, Virginia; R. L. WEST, St. Paul, Minnesota; J. E. STUART, Sacramento, California.

The Advisory Committee to the Agricultural Research Service has held three meetings, two in Washington, one in Philadelphia. The budget needs of the Agricultural Research Service, its present programs, and future plans have been discussed, I hope, to the mutual benefit of this Association and the Agricultural Research Service. The Committee wishes to express its thanks to the members of the Agricultural Research Service for their cooperation, not only at the meetings of the Committee, but for information furnished on pending legislation, and other matters of interest throughout the year.

At the time of the January meeting it looked as though the Brucellosis program would have to be seriously reduced. However, reasonably adequate appropriations have been made, thanks to the efforts of the commissioners, directors and secretaries of agriculture.

Tuberculosis necessarily came in for discussion at these meetings, with indications that tuberculosis in some areas of the country is actually becoming more of a problem. The Committee feels that every practical method should be employed to spot infection, but that there is a need for a greatly increased volume of well supervised and conducted testing if we are to meet the situation. The advisability of delaying this increased volume of testing four or five years, until after the accelerated Brucellosis program is nearer completion, was discussed. However, the Tuberculosis Committee at this meeting will give consideration to all phases of this problem. This Committee feels that short cuts are not the answer to tuberculosis eradication.

Sheep scabies still exists in about half of our states, but not in the range states where it was once so common. The Committee recommends that eradication be started in those infected states which are east of the clean states in the western part of our country, and continue eastward until the job is completed.

We warn against complacency in regard to vesicular exanthema, and wish to point out that even before general cooking of garbage, we sometimes went as long as three years without an outbreak.

Meat inspection has its problems, even with increased appropriations, which have been enough to just about take care of the number of plants now having inspection, to say nothing of new plants that would like to participate. Sufficient funds should be made available so that the quality of inspection could be maintained or improved, and would be available to all plants that
ADVISORY

This program not only protects the health of the people, but has a tremendous impact on the livestock industry. The Committee feels that serious considerations should be given by the Department in recognition of State inspection, providing that the quality of inspection can be maintained, and that Federal funds are not available.

With the completion of Plum Island, and the Ames Laboratory well under way towards completion in 1960, we must reconsider our recommendations of the past, because facilities for any volume of research have been lacking. We want to congratulate the Department on making a start in acquiring personnel, and realize that this must continue if we are to make the best use of new facilities. We all realize that research in the animal and poultry fields has not kept pace with the actual needs. We urge the Department to begin making plans for a permanent staff for Ames so that research can be provided on specific projects, especially Brucellosis, vibriosis, mastitis, leptospirosis, erysipelas, hog cholera, bluetongue, poultry diseases, and many others.

We need to have studies made on many new biologics and chemicals to determine their value and possible danger to livestock production.

We also recommend that plans be started to reconvert the facilities at Beltsville into a parasite research facility, as this phase of livestock production has received little consideration in relation to its importance.

With Plum Island as an exotic disease station, Ames as a disease research and diagnostic center, and with a parasite research center at Beltsville, we should come nearer meeting the demands and needs of the industry.

We believe that the Department should continue some research projects in foreign lands, that we might have a better knowledge of these diseases should we be confronted with them.

Inspection and Quarantine. We recommend that the Department give consideration to improving inspection facilities for animals imported on the Canadian border, and if working agreements with Canada can be worked out, that facilities be jointly used. The opening of the St. Lawrence Seaway will greatly increase the work of this Division, and new personnel and facilities will have to be made available, especially in ports on the Great Lakes.

The production, distribution and testing of biologics under Federal license has long been of concern to livestock disease control officials, as well as to livestock producers. The manufacture and use of biologics have greatly expanded during the past few years, far faster than the supervision over their manufacture and distribution. We feel that for the protection of the livestock industry this Division should provide a more adequate service. We believe that for many of these products procedures should be set up whereby the Department could have complete control over their production, sale and distribution.
REPORT OF REPRESENTATIVES TO THE ANNUAL MEETING OF ASSOCIATION OF STATE DEPARTMENTS OF AGRICULTURE


Mr. President, members and guests of the Association: The meeting of Secretaries, Directors and Commissioners of Agriculture was held at the Edgewater Hotel, Madison, Wisconsin, on September 28 through October 3. In attendance were two official delegates from this Association, namely the speaker and Dr. Ralph West. In addition, Dr. J. R. Hay was present in his capacity of Commissioner and Dr. J. Milligan was present as a representative of the Commissioner from Alabama. The meeting was well attended, the members worked very hard, and I believe accomplished a great deal. The following resolutions pertaining to Animal Health were adopted by the Association:

RESOLUTION NO. XII—INTERNATIONAL BOUNDARY FENCE

WHEREAS, the livestock industry of the southwestern areas of the United States continues to suffer exposure of its domestic animals to diseases borne by drifting animals from foreign sources because of the disrepair or complete destruction of stretches of the International Boundary Fence, which was intended to, in fact, exist as a protection against straying animals across the border strip between the Rio Grande River and the Pacific Ocean; and

WHEREAS, such lack of restraint upon the indiscriminate movement of infected animals from foreign sources through such a neglected fence in large measure nullifies the value of careful quarantine inspections and holding at established border entry points to prevent introduction of injurious animal diseases; and

WHEREAS, during the past two years there have been numerous instances of hazardous animals having drifted across said unprotected border strip, resulting in additional expense in carrying on necessary protective measures to insure against disease outbreaks;

NOW THEREFORE, BE IT RESOLVED, by the 40th Annual Convention of the National Association of State Departments of Agriculture assembled at Madison, Wisconsin, September 29 to October 3, 1958, that its Executive Committee work toward the reintroduction of legislation comparable to S. 76 of the 84th Session, to make provision for the construction and/or repair of an adequate boundary fence, and toward granting authority and finances to the Secretary of Agriculture for the adequate maintenance of such fence so that it will, in fact, serve as a necessary protection against indiscriminate entrance of diseased livestock.

24
RESOLUTION NO. XIII—HOG CHOLERA VIRUS

WHEREAS, there have been developed methods for immunizing swine against hog cholera which do not require the use of fully virulent virus which is a means of perpetuating the disease; and
WHEREAS, hog cholera cannot be eradicated while virulent hog cholera virus is in general use; and
WHEREAS, many states have passed laws prohibiting the sale of virulent hog cholera virus, except for experimental purposes and then only by permit issued by the responsible state agency;

NOW THEREFORE, BE IT RESOLVED, by the 40th Annual Convention of the National Association of State Departments of Agriculture assembled at Madison, Wisconsin, September 29-October 3, 1958, that they urge those states which have not passed such legislation to enact laws which will prohibit the sale of virulent hog cholera virus as soon as possible.

RESOLUTION NO. XIV—MASTITIS CONTROL

WHEREAS, a condition in dairy cattle known as Mastitis is causing a heavy financial loss to dairy owners throughout the nation annually, and there is a marked division of opinion as to whether Mastitis is a distinct disease or the result of several types of infection and contributing factors;

THEREFORE BE IT RESOLVED, that the 40th Annual Convention of the National Association of State Departments of Agriculture assembled at Madison, Wisconsin, September 29-October 3, 1958, urge the states and the Federal Government to carry on an extensive coordinated research on Mastitis.

RESOLUTION NO. XV—INTERSTATE BRUCELLOSIS REGULATION

RESOLVED, that the 40th Annual Convention of the National Association of State Departments of Agriculture assembled at Madison, Wisconsin, September 29-October 3, 1958, request the Executive Committee to review with the Agricultural Research Service the interstate brucellosis regulation with a view to considering any problems arising from its operation.

RESOLUTION NO. XVI—SCRAPIE DISEASE QUARANTINE AND RESEARCH

WHEREAS, scrapie is a serious disease of sheep for which there is no known remedy except that of condemning the infected sheep for slaughter; and
WHEREAS, there have been instances in which this disease has been introduced into the United States by the importation of sheep from flocks known to be infected with scrapie; and
WHEREAS, the importation of such sheep has resulted in the United States Department of Agriculture and the states being required to expand large sums of money for indemnities;

NOW THEREFORE, BE IT RESOLVED, by the 40th Annual Convention of the National Association of State Departments of Agriculture meeting at Madison, Wisconsin, September 29-October 3, 1958, that its Executive Committee urge...
REPORT

the United States Department of Agriculture to establish regulations which will prohibit the entry of sheep into the United States from flocks where scrapie is known to have ever existed, or from premises whereon the disease has existed in past years; and

Be It Further Resolved, that intensive research be undertaken by the Agricultural Research Service on this disease.

RESOLUTION NO. XVII—BOVINE BRUCELLOSIS

WHEREAS, the Congress of the United States has recognized the importance of Brucellosis and has appropriated considerable amounts of money for this program;

Therefore Be It Resolved, by the 40th Annual Convention of the National Association of State Departments of Agriculture meeting at Madison, Wisconsin, September 29-October 3, 1958, that this organization express its appreciation to the Congress for its fine cooperation in the Brucellosis program and it is hoped this fine cooperation will continue in the future; and

Be It Further Resolved, that a copy of this resolution be sent to the chairmen of the sub-committee on agriculture of the Appropriations Committee of each house, with copies to the members thereof.

RESOLUTION NO. XVIII—BOVINE TUBERCULOSIS

WHEREAS, the United States Department of Agriculture and the State Departments of Agriculture have been engaged in a bovine tuberculosis testing program for many years; and

WHEREAS, $350,000,000 of county, state and Federal moneys have been expended in conducting the said program; and

WHEREAS, through the said testing program the incidence of the disease has been materially reduced but has not been eradicated from the herds of this country; and

WHEREAS, there is evidence of lack of uniformity in testing procedures and that tests are not being conducted at pre-determined and regular intervals; and

WHEREAS, there seems to be some evidence of an increase in the incidence of the disease in many states;

Now Therefore, Be It Resolved, that the 40th Annual Convention of the National Association of State Departments of Agriculture meeting at Madison, Wisconsin, September 29-October 3, 1958, request the Executive Committee to urge the United States Department of Agriculture to re-evaluate the bovine tuberculosis testing program, including reaccreditation of counties, and place the entire program on a basis which will eradicate bovine tuberculosis from the cattle in this country in the shortest possible time;

Be It Further Resolved, that the United States Department of Agriculture be urged to immediately request sufficient funds of the Congress to implement this program and to completely eradicate the disease.
REPORT OF COMMITTEE ON LAWS AND REGULATIONS


Efforts have previously been extended by this Association looking toward the unification of health requirements governing the entrance of animals into individual states. Owing to differing conditions affecting the maintenance, handling as to sale and traffic of animals, in the several states, uniformity of entrance regulations has never seemed possible. However, during the past few years increasing interest has become manifested by the organized livestock industry of the United States and others in measures which might tend to simplify and unify state entrance requirements. Your Committee realizing that complete unification, or, the acceptance of one set of standard requirements regulating entrance of animals, acceptable to all states seems as yet impossible. It is, therefore, considered as a definite step in advance, that regulations with slight modifications, may be drawn meeting the requirements of states by groups. As a definite service to that segment of the livestock industry which necessarily transports livestock from one state to another, your Committee recommends that further study be given by a committee of this Association to an analysis of individual state requirements governing the admission of animals to the end that a designation of states by groups may become possible; not necessarily, however, by geographical location. The findings of this Committee would be calculated to permit of the formulation of one master entrance regulation for each group, subject possibly to slight modification thus making the entrance regulations or requirements acceptable to states in each designated group.

Your Committee being fully cognizant of the great havoc which might be wrought to the sheep industry should scrapie become indigenous to one or more states in this country, urges the adoption by each state of the following regulation covering the admittance of sheep and goats: "Sheep originating in a state in which scrapie is known to exist or has existed within 42 months prior to shipment, in addition to being accompanied by usual health certificate, must bear especial notation thereon, or, a separate statement properly attested or certified to by the livestock sanitary authorities of the state of origin, to the effect that such sheep are not the progeny of scrapie exposed or scrapie infected sheep, or have been exposed to scrapie exposed or infected sheep."

Your Committee urges the several states to comply with the use of the "Federal Shield" for permanent identification of official vaccinated animals as recommended by the Committee on Brucellosis.
Your Committee further recommends that hereafter in the publication of Circular No. 1, "State Requirements Governing the Admission of Livestock" that the portion or section setting forth Standard Regulations be deleted from the booklet and that instead, details of existing import regulations or of each state governing the admission of animals be included and that the booklet be published in loose leaf form so that the regulations of each state may be supplemented as required from time to time so that it may thus remain current between publications. In its present form the booklet soon becomes obsolete. Although the cost of the publication of the booklet in loose leaf form initially would be somewhat greater, the longer up-to-date usefulness and more infrequent need for new editions would more than compensate for the additional original cost. It is further recommended that Circular No. 1 be published by this Association, including later supplements as may become necessary and that an appropriate cost charge for the booklet including supplements be set up by the Secretary, the cost to be borne by state or individuals desiring to obtain and use this publication.
UNITED STATES LIVESTOCK SANITARY ASSOCIATION,
COMMITTEE ON LEGISLATION


The principal activity of the Committee on Legislation of the United States Livestock Sanitary Association for the current year 1958 involved representation on behalf of our association to the 85th Congress, 2nd Session, dealing with the appropriation bills for the Agricultural Research Service, and with an amendment to the law involving the importation of certain animals into the United States.

The Advisory Committee to the Agricultural Research Service on program and budget of the United States Livestock Sanitary Association directed the Committee on Legislation to make representation to the proper committee of congress in support of the Department of Agriculture’s attempt to get a considerably increased appropriation for the Department’s meat inspection activity. Such representation was made, both in written and verbal testimony, to the congress and a supplemental appropriation in approximately the amount requested was provided. A copy of this testimony, along with factual data presented to justify this increase, is available and will be supplied to any interested member at the Association who might like to have it.

The Advisory Committee to the Agricultural Research Service on program and budget of this Association has in the past recommended increased financing and activity by the Department of Agriculture in the licensing and inspection of animal biological products. The Virus, Serum Toxin Act of 1913 places responsibility on the United States Department of Agriculture for the prevention of the interstate shipment of worthless, dangerous, harmful or contaminated animal biologics. The sum provided annually for this service is entirely inadequate, and does not allow the Department to anything like fulfill its obligations. No real effort has been made by the Department of Agriculture to increase these operations, although it is understood that when the facilities currently under construction at Ames, Iowa, are available an attempt will be made to instigate additional activity in this field. The Advisory Committee directed the Committee on Legislation to make direct representation to Congress for increased funds for fiscal 1959 in an amount sufficient to allow the Department to make full use of existing facilities, and to permit some contract testing. Such representation was made to the appropriate committees of the congress, and while considerable interest was expressed in this subject no additional funds were provided. Copies of this testimony are also available to any member of this Association who cares to request them.

29
As a result of a ruling by the United States Court of Appeals for the District of Columbia the Department of Agriculture found itself powerless to prohibit, control or regulate the importation of wild animals into the United States from countries where such importation would be considered dangerous. Existing legislation prohibited the importation of domestic ruminants and swine into the United States, but made no mention of wild animals. (Section 306-A, Act June 17, 1930, 19 United States Code 1306.) On April 23, 1958, H.R. 12126 was introduced into the House of Representatives by Mr. Mathews, which extended the prohibition on domestic ruminants and swine contained in existing law to wild ruminants and swine. This bill was actively supported by the Department of Agriculture and at the Department's request by the United States Livestock Sanitary Association. This bill passed the House of Representatives and was referred to the Senate. Opposition developed to the bill lead by the American Association of Zoological Parks and Aquariums. An amendment was offered to the bill by that group which would deny the Department the right to prohibit the importation of wild animals but would grant specific powers to regulate such importation and the consequent distribution, maintenance and exhibition of such animals in the United States. At the Senate Committee Hearing the committee chairman, Mr. Humphrey, suggested a conference between the interested parties on both sides to explore the possibility of reaching an acceptable compromise. The conference was held between representatives of the Department of Agriculture, the Smithsonian Institute, the American Association of Zoological Parks and Aquariums, the American Veterinary Medical Association, the National Association of State Departments of Agriculture, the United States Livestock Sanitary Association and the Senate Committee on Agriculture and Forestry. A compromise was not reached, but the proposed amendment prevailed, and the bill was finally enacted into law as amended.

The Committee on Legislation of this Association has the responsibility, principally through its chairman, of speaking for the United States Livestock Sanitary Association to the congress on matters of interest to or effecting livestock health. Bills dealing with these subjects before the congress that are carried over from one session to the next offer opportunity for debate by this Association and permit a stand for or against. This, of course, serves as a guide and a directive to the Committee on Legislation. Frequently, however, matters arise and a position must be taken with no opportunity afforded the Committee on Legislation, or its chairman, to know what the Association's position would be. This presents a serious dilemma that should be solved at this meeting. It would be a formidable task for the Committee on Legislation to notify each member of the Executive Committee of the United States Livestock Sanitary Association each time a matter of interest and concern presents itself and to ask for guidance. In such an operation too, the answers probably would leave the Committee as much in the dark as ever. The United States Livestock Sanitary Association has at present standing committees dealing with the specific areas of interest or concern to this Association. These committees from time to time are increased as the need arises, or discontinued or consolidated again on the basis of need.
REPORT OF THE COMMITTEE ON PUBLIC RELATIONS

C. L. CAMPBELL, Chairman, Tallahassee, Florida; T. C. Green, Charleston, West Virginia; W. D. KNOX, Fort Atkinson, Wisconsin; R. L. KNUDSON, Columbus, Ohio; J. E. STUART, Sacramento, California.

The Committee on Public Relations wishes to reaffirm the comments and recommendations made by this Committee in its 1957 report at St. Louis. The early submission of papers for abstraction and distribution through publicity channels is of paramount importance if annual meetings of this organization are to receive recognition through the nation's agricultural press. Aside from Committee reports, there were 24 papers scheduled for presentation at this meeting. Of these, eight were submitted to your Public Relations Committee chairman for pre-convention publicity purposes, two of the papers being received in the second week of October, and the remaining papers within the past two weeks. As a result, the Committee has been able to obtain very little coverage from the standpoint of papers presented here.

Fortunately, we have had some local projects with which we tied in the United States Livestock Sanitary Association that have had regional news appeal, namely: the hog cholera eradication pilot test area, the Southeastern screwworm eradication program, and the inspectional tour made by officials of this organization immediately preceding this meeting. Through the able assistance of Mr. A. U. Spear, a professional public relations specialist employed in one of Florida's disease eradication projects, your Committee has been able to focus state-wide attention upon this meeting at semi-weekly intervals over the past three weeks. Additional television, radio, and newspaper coverage has been obtained during this week that we have been in session. Individual reports covering these activities will be filed with the secretary following the close of this meeting through a press clipping service to which we subscribed.

Public relations may be defined as the activities of an organization in building and maintaining sound and productive relations with special groups and with the public at large, so as to interpret itself to those groups. This Committee recognizes that while publicity concerning the Association's annual meetings is of great value in acquainting the public with our activities, there is a vast difference between this and a sound long-range public relations program. It is felt that the adoption of such a program is not only desirable but essential if this organization is to assume its proper role of leadership in the formulation of sound livestock disease control and eradication programs throughout the nation.

To this end your Committee recommends that the Executive Committee and the newly elected officers devote serious consideration to the establishment of a strong public relations program in the promotion of this organization.
REPORT OF THE COMMITTEE ON REGULATORY EDUCATION


The past few years have witnessed a growing concern for and emphasis on regulatory veterinary medicine, especially in educational circles. Adequate control of animal diseases requires a good background in many disciplines, such as animal ecology, microbiology, epizootiology, pathology, and nutrition; in fact, in all of the biological components that are put together as medicine—in this case, veterinary medicine. To this must be added competent administration, development, and utilization of laboratory techniques and facilities and the development of regulations based upon the latest scientific medical information.

Those responsible for education in our schools and colleges of veterinary medicine are placing more emphasis on this entire area of the veterinary profession. Attempts are being made to better equip the present-day veterinary medical graduate with the fundamental information he needs to carry out satisfactorily the applied aspects of meat hygiene, as well as that necessary to function as a veterinary practitioner in the control of infectious diseases.

The emphasis on research in the biological and medical sciences during the past decade is providing us with factual information at a rate unsurpassed in our history. The application of these facts by our disease control officials in the most effective way will require in the future a continuing educational process. The United States Department of Agriculture in establishing regional conferences, either in conjunction with State Veterinary Medical Society meetings or separately for discussions on problems concerned with animal disease control, is certainly progressing in the right direction. They have also provided for the undergraduate in veterinary medicine special lectures which relate to the state and Federal disease control programs. Both processes are important, since in some areas practitioners receive up to 50 percent of their annual income from participation part-time in the state and Federal disease control programs. The Committee agrees that this is fine for the present, but what about the future?

As indicated earlier in this report, tremendous advances in medical science are increasing the available information. If we take the position that veterinary medicine is, after all, medicine applied to animals, then the whole area of medical science is brought to bear upon animal disease regulatory work. Not only should we consider training in the regulatory phases, we must consider also all areas of the curriculum which provide the knowledge for effective animal disease control. This field of veterinary medicine is
rapidly coming to require more than just a superficial knowledge of forms and regulations. Although these are necessary, it is obvious scientifically that advanced training beyond the D.V.M. Degree would certainly be advantageous for future officials responsible for regulatory veterinary medicine. Whether this be in the form of an M.S. Degree or an M.P.H. Degree, can be debated pro and con. However, since regulatory veterinary medicine involves all areas of medical science, some of the methods used in Public Health could be applied to corresponding areas in veterinary medicine. It is gratifying to note that the Department of Agriculture is permitting a number of employees to take graduate work while carrying out certain duties in cooperative programs with the Schools and Colleges of Veterinary Medicine. This must be encouraged and developed further, if we are to keep pace with the other medical professions. If this is not done, regulatory veterinary medicine may become perfunctory and vocational and may soon lose its place as a distinct area of the veterinary profession which will attract capable individuals with the proper training.

Through this report, the Committee has tried to stimulate some thinking on our present operations in animal disease control work or regulatory veterinary medicine. Three main points can be made:

1. The schools and colleges are being encouraged, and the faculties have taken steps, to graduate veterinarians more proficient in animal disease control.

2. Federal and state agencies are taking steps to disseminate information pertinent to regulatory veterinary medicine to present and future members of the profession.

3. Steps, although limited in scope at this time, are being taken to provide additional training that will improve the competence and prestige of those in key positions in this area of veterinary medicine.
REPORT OF COMMITTEE ON REVISION OF CONSTITUTION AND BY-LAWS, UNITED STATES LIVESTOCK SANITARY ASSOCIATION

R. L. West, Chairman, St. Paul, Minnesota; C. L. Campbell, Tallahassee, Florida; K. J. Peterson, Salem, Oregon; R. W. Smith, Concord, New Hampshire; K. F. Wells, Ottawa, Canada.

At the 1957 meeting of this Association in St. Louis, a resolution consisting of a proposal to amend the Constitution and By-Laws, was submitted to the Committee on Resolutions by Dr. Ab Quin. This resolution, however, was submitted at such a late hour the Committee had no time to give it consideration, and it was not included in their report; however, it was presented to the Executive Committee at the final meeting in St. Louis. At that time, by motion duly made and carried, the resolution was tabled for further consideration, and the President was directed to select a committee to bring in a report at this meeting after studying the resolution.

Pursuant to that action, President Milligan selected the Committee on Nominations to make this study and report. This selection was made, at least partly because this Committee consists of five members so distributed that one member represents each of the recognized districts in this country and Canada. The resolution reads as follows:

"Whereas, livestock and poultry diseases are not only continental but international in character, and
"Whereas, the original and long-established name of this Association designates the United States only, and
"Whereas, meetings no longer draw the attendance of the livestock and poultry leaders of America;
"Therefore: We suggest that the name 'American Livestock Regulatory Officials Association', replace the present name of this organization; and further,
"That active membership and majority representation on the Executive Committee shall be restricted to those directly engaged in official livestock and poultry regulatory activities; and, further,
"That associate membership shall be granted to all reputable citizens of this nation, Canada, Mexico and respective territories who are engaged in or allied to the production and marketing of livestock and poultry; and, further,
"That such associate members shall be given strictly minority representation on the Executive Committee of this Association."

The members of your Committee have corresponded throughout the year and have discussed the proposals among themselves and with other members of the Executive Committee. They have been in session during this meeting and wish to present the following report. The question of a change of name
REVISION OF CONSTITUTION AND BY-LAWS

has been previously discussed by the Executive Committee, which at that time voted no change be made. Your Committee agrees with this action and again recommends no change.

The question of increasing the membership of the Executive Committee to include representation by the industry, resolves itself in two main questions: (1) Is such a change desirable for the welfare of the Association, and would it increase their influence and the area of their activities, and (2) If it is decided that such an increase is desirable, how shall such additional membership be chosen, and who and what shall they represent?

It seems to your Committee that interest in the activities of this Association by livestock groups, can and should be engendered by means other than by representation on the Executive Committee. The Executive Committee as presently constituted, represents the organized disease control agencies of all the states and territories as well as our neighboring countries of Canada, Mexico, and Cuba. It is your Committee's considered opinion, that the organization as now constituted, is performing its functions competently and harmoniously, and we recommend rejection of the Quin Resolution as submitted in 1957. Your Committee does further recommend, more representation on the several committees of the Association by persons representing the industry, and particularly those committees having to do with control matters which directly affect the production, marketing, and transportation of livestock. We also recommend that more speakers be chosen from the industry to present papers on practical phases of livestock disease control.

PROPOSED AMENDMENT TO CONSTITUTION AND BY-LAWS

Mr. President, we the undersigned members of the United States Livestock Sanitary Association in good standing submit the following amendments to the Constitution of this Association:

I. Amend Article V, line 44 by deleting the last 10 words in said line 44 and adding after the word "territory" "the Director of Livestock Regulatory Programs of the United States Department of Agriculture."

II. Further amend by deleting the sixth word in line 47, and adding in its place "",

III. Further amend by adding after the word "Association" in line 48 "and eight delegates at large representing the livestock industry including poultry. No more than two delegates from each of the four districts of the United States shall be elected. Said districts shall be known as the Northeast; consisting of the States of Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island and Vermont; The Northcentral; consisting of the States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Montana, Nebraska, North Dakota, Ohio, South Dakota and Wisconsin; The Southern; comprising the following states, Alabama, Arkansas, Georgia, Florida, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina,
Tennessee, Texas, Virginia, West Virginia, Puerto Rico and The Virgin Islands; and the Western district consisting of the States of Alaska, Arizona, California, Colorado, Hawaii, Idaho, Nevada, New Mexico, Oregon, Utah, Washington and Wyoming. It shall be the duty of the Committee on Nominations to canvass the membership of this Association and select eight (8) nominees for delegates at large. Said nominees must be selected from and represent the livestock industry, including poultry. No more than two (2) delegates at large shall be elected from each of the four designated areas or districts, nominations from the floor of the convention may be made for additional nominees by districts and shall be bona fide residents of the respective district for which they are nominated. Such delegates shall be elected at the time and place as are the elected officers of this association, so that Article V, as amended, shall read as follows:

ARTICLE V—OFFICERS

The officers of this Association shall be: President, First Vice-President, Second Vice-President, Third Vice-President, Secretary-Treasurer, and an Executive Committee.

EXECUTIVE COMMITTEE

The Executive Committee shall be composed of the executive officer representing the livestock sanitary departments of the various states and territories, the Director of Livestock Regulatory Programs of the United States Department of Agriculture, the Veterinary Director General of Canada, The executive regulatory officer of Cuba and Mexico, The Territories, Puerto Rico, the Virgin Islands, Los Angeles County, California, the elective officers of this Association and eight delegates at large representing the livestock industry including poultry. No more than two delegates from each of the four districts of the United States shall be elected. Said districts shall be known as the Northeast; consisting of the States of Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island and Vermont; the Northcentral; consisting of the States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Montana, Nebraska, North Dakota, Ohio, South Dakota and Wisconsin; the Southern; comprising the following states, Alabama, Arkansas, Georgia, Florida, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia, Puerto Rico and the Virgin Islands; and the Western district consisting of the States of Alaska, Arizona, California, Colorado, Hawaii, Idaho, Nevada, New Mexico, Oregon, Utah, Washington and Wyoming. It shall be the duty of the Committee on Nominations to canvass the membership of this Association and select eight (8) nominees for delegates at large. Said nominees must be selected from and represent the livestock industry including poultry. No more than two (2) delegates at large shall be elected from each of the four
designated areas or districts, nominations from the floor of the convention may be made for additional nominees by districts and shall be bona fide residents of the respective district for which they are nominated. Such delegates shall be elected at the time and place as are the elected officers of this Association.

The Executive Committee shall constitute the administrative body of this Association and shall determine its activities and policies.

All recommendations and reports of officers and committees shall be referred for consideration to the Executive Committee.

The First Vice-President shall be ex-officio chairman of the Executive Committee.

The Executive Committee shall elect yearly a Secretary-Treasurer for the Association. The Secretary-Treasurer shall receive such salary and allowance as may be fixed by the Executive Committee.

The Executive Committee shall cause to be audited annually or oftener if deemed necessary, the receipts and disbursements of the Secretary-Treasurer, and shall have authority to hear and determine all complaints filed before it in writing relative to the conduct of any member; and shall have authority to accept or reject applications for individual membership properly placed before them. Three negative votes shall disqualify for such membership.

Mr. President, I wish to make the following amendment to the By-Laws of the United States Livestock Sanitary Association.

Amend ARTICLE IV—Quorum of the By-Laws by striking out the first word “five” in line 161 and substituting therefor the word “twenty”, so that Article IV as amended will read as follows:

ARTICLE IV—QUORUM

Twenty-five members of the Association shall constitute a quorum. Twenty members of the Executive Committee shall constitute a quorum.

Respectfully submitted:

ROBINSON W. SMITH, Concord,
New Hampshire
Ten years ago the prospect of a trip to the Soviet Union appeared as remote
to me as a trip to the moon. I’m sure the other members of our delegation
felt the same way until a few months ago. Then on July 24 our Russian jet
plane came down at the Moscow airport and the trip to Russia became a
reality. Who knows . . . in another 10 years we may be having a report from
some young scientist of a trip to the moon!

This delegation of veterinarians was one of six agricultural delegations
which the Department of Agriculture sent to the Soviet Union this year.
The trip was arranged under an agreement between the two countries for a
general program of exchanges in cultural, technical, and educational fields.
Similar teams have come to our country from Russia. This report reflects
the combined note-taking and observations of our whole group.

Veterinary medical activities in the Soviet Union are guided and controlled
by the central government in Moscow through the Veterinary Collegium of
the Ministry of Agriculture. It might be called the policy-making body. Each
republic also has its Veterinary Collegium and Ministry of Agriculture, respon-
sible to Moscow.

Second in power to the Collegium is the Academy of Agricultural Sciences.
It has six departments. One of these, Animal Husbandry and Veterinary
Science, has supervisory jurisdiction over three of the largest veterinary
research institutes in the Soviet Union. They are the All-Union Institute of
Experimental Veterinary Medicine (known as VIEV) the All-Union Research
Institute of Veterinary Sanitation and Ectoparasitology, and the All-Union
Institute of Helminthology. We visited these in Moscow. Each of the 15
republics has one or more republic research institutes, the number determined
by the size of the republic and the livestock population.

We visited a republic research institute at Leningrad—the Veterinary Re-
search Institute, which was established in 1898. It is the immediate ancestor
of VIEV and for many years was the center of veterinary research in Russia.
After the Revolution, it was divided. One part was moved to Moscow as
VIEV, and the other part—reduced to a regional research basis which also
produced a few special biological preparations—remained in Leningrad.

Generally, the activities of these institutes are confined to research, although
a few make a limited number of biological preparations. Some also give
refresher courses of six weeks’ or three months’ duration to veterinarians.
There are 158 agricultural scientific institutions, and 650 experiment stations
and field laboratories.

Animal husbandry research is under supervision of the Academy of Agri-
cultural sciences. Progress in breeding better quality livestock probably has
been retarded by the Lysenko theory that environment’s influence is stronger than that of heredity. The emphasis in cattle improvement is on what the Ukrainians call the universal-type animal—equally good for milk and meat production.

It is common practice to import purebred bulls, such as Symmenthal cattle from Switzerland, Holstein-Friesians from Holland, or Jersey bulls, and cross them on the native cattle. Apparently, the breeders soon report the establishment of a new breed as a result of this crossing, to which they give a name, such as the Red Steppe, or the Kastroma, or the Russian Black-and-White.

Artificial insemination is being used widely, not only in cattle but also in sheep, horses, and to some extent, experimentally in swine. It is required in herds where trichomoniasis and brucellosis exist.

Research in animal diseases is conducted in veterinary schools and research institutes. There are also major research institutions in the Ukraine, Byelorussia, Kazakhstan, Uzbek, Turkmen, Georgia, and Azerbaijan.

Many diseases which we know and deal with are reported to occur in Russia, such as tuberculosis, brucellosis, anthrax, hog cholera, fowl pox, and anaplasmosis. Foot-and-mouth disease is one of their most serious problems. Other diseases which exist there and not here include sheep pox, Brucella melitensis in sheep, contagious agalactia in sheep and goats, glanders, and some parasitic diseases.

Most of the schools and laboratories have little more than a minimum of equipment. Improvement is being made as modern equipment becomes available. Some laboratories are equipped with electron microscopes and some are experimenting with isotopes. Some are also beginning to work with tissue culture.

A major deficiency in animal disease research is the apparent lack of facilities for experimental animals, at least near the research centers. Experimental studies involving livestock and poultry were not observed at any of the institutions visited. Repeated requests to see experimental animals and facilities were denied because they were some distance away, and time would not permit.

This suggests that many biological investigations, chemotherapeutic trials, and other experimental control measures are probably pursued largely on the collective and state farms when specific problems arise.

Veterinary parasitology in the USSR seemed to compare more favorably with work in the United States than was evident with any of the other veterinary sciences we observed. Their strong point is definitely taxonomy and systematics, as exemplified by the classical works of the renowned Skriabin and his many students and followers. Their parasite collections, particularly at the All-Union Institute of Helminthology in Moscow, were impressive.

Control activities, including meat and dairy inspection and processing, and production of biological preparations, are also directed from Moscow. The State Scientific Control Institute for Veterinary Preparations is headed by Professor Siurin, who traveled with us and is one of the best informed veterinarians we met in the USSR.
In animal disease control, the principal effort for most infectious diseases is directed toward immunization. This is apparent from the variety and volume of preparations available, and the high priority which seems to be attached to biological production and control activities.

Production of biologicals is handled through bio-combines located in the various republics. The Control Institute provides cultures to the bio-combines and has its representatives in each bio-combine for inspection and testing. Distribution of biologicals to the republics and regional veterinary installations is carried on by the Biological Trust.

Apparently most of the preparations known in the United States and Europe are also known in Russia, and improvements are adopted promptly.

Chemical controls for parasitic infections seem to be those largely developed outside the Soviet Union. Much of their experimentation seems to be an adaptation of known antiparasitic agents and measures to the specific conditions found in Russia.

The central government's control of meat slaughtering and processing and of dairy products includes jurisdiction over some 3,000 municipal and district control stations. Carcasses moving to market must be accompanied by a certificate from the veterinarian on the farm where the animals were slaughtered. At the market, carcasses are checked mainly for condition. Milk inspection procedures included testing for butterfat, sediment, and bacterial culture.

We visited only one slaughter plant... at Alma Ata, in southern Russia. It was reported to be one of the oldest, dating from 1939, with additions in 1945. Procedures seem to be similar to those followed in the United States and Europe. The plant is capable of handling 5,000 sheep and approximately 800 cattle a day on an average, using two eight-hour shifts.

Fifteen veterinarians were assigned to this plant for inspection, both ante-mortem and post-mortem. Animals found to be diseased on ante-mortem inspection are not allowed in the main plant, but are sent to a separate slaughtering house for this purpose.

We were assured that all milk for human consumption is pasteurized. We saw many milk trucks, similar to our bulk tanks, parked on the street, dispensing milk to people with buckets to carry it home. Whether or not this milk had been pasteurized was questionable.

The USSR depends on its strong educational program for an adequate supply of veterinarians for employment at State and collective farms, diagnostic laboratories, food inspection stations, and research and teaching institutions. The Government operates 99 agricultural high schools and 34 veterinary schools, widely distributed geographically. We visited four—all located in major cities and in centers of large livestock populations. Some schools of veterinary medicine are combined with zootechnical institutes for the training of veterinarians and animal husbandmen. Three of the four we saw were such.

A total of 30,000 teachers instruct in veterinary and animal husbandry schools, some 14,000 in veterinary medicine. Their primary purpose is the
teaching of students, although research projects are conducted, especially during summers.

The ratio of teachers to pupils is highly favorable . . . ranging from one to 10 at the Moscow Veterinary Academy to the highly individualized teaching of the Leningrad Veterinary Institute, where the ratio is one to four.

The curriculum of the veterinary medical schools is uniform throughout the USSR, with five years in professional education. Students of high scholastic ability may go directly to the professional schools on completion of high school. Students of average ability are expected to spend three years in practical training on a farm before admission to a school of veterinary medicine. Increasing emphasis is being given to this practical experience—presumably to discourage development of a bourgeois class.

The schools are highly selective, with admission based on previous scholarship and examinations. At the Alma Ata Zoo-veterinary Institute, for instance, a class of 225 new students is selected from approximately 1,300 applicants. No desirable student is excluded because of personal inability to finance his education. Instruction and lodging are provided without cost and needy students receive a stipend sufficient for food and clothing needs. Students whose parents are financially able to pay their way do not receive government help.

One graduate degree is conferred. The Candidate of Science requires approximately three years of study and work. The Doctor of Science degree requires five to 10 years of experience. In graduate study emphasis is on research. The Doctor of Science conveys honor and usually qualifies the recipient to full professorship on a teaching or research faculty.

Although their libraries are strong in publications in the Russian language, German, French, British, and American periodicals are available. The American Journal of Veterinary Research appeared to be popular and well used. The veterinarians with whom we had contact were familiar with our literature. Many have a reading knowledge of English because they had chosen it for their foreign language requirement during their first 10 years of schooling.

In some respects, Soviet veterinary science may lag behind ours, but the Russians are closing the gap. It is essential for us to face the fact that they are determined to surpass the United States on all fronts. Their competitive urge, their singleness of purpose, and their emphasis on education are driving them forward industrially, agriculturally, and scientifically. In the specific field of veterinary medicine, insofar as we could see, they had little from which we could learn. But, we could and did learn from their determination to succeed. We dare not minimize their ability and their dedication to a cause—to outstrip the acknowledged leader of the world in this field.
from industry representation in the past. We look with chagrin on them. We also recognize the reason for them, those of us in the industry.

I comment to this extent: that usually in my own state, when I appear, or in other states, I have to prelude my remarks with this: No matter what I say or do not say, I probably will be misunderstood.

I do not intend to more than just take a moment, but I do want to express this feeling. We have a dissension rising in the western states that I feel you gentlemen should be alerted to. I happen to be the President of the Stockmen's Disease Control Association, an organization that got its impetus and structure from a local situation, which, as it rose and developed, encompassed other states, particularly the western states. And instead of being what we thought was just a little, small group trying to express their sincere belief in their understanding of their own personal problems, the thing expanded until now it has become an interstate organization.

We only want to come to you people with the thought that you are our professional advisors. We want to hold you on the same high plane, on the technical plane, that has always typified the articles that I have read in your publications over the many years that I have been a silent member of your organization.

We want to have that confidence that you want us to have in you. We want you to merit it, and we are sure that in most cases you do.

The things that I say are very difficult to say, because it is so easy to be misunderstood.

We have confidence in you. We want you to have confidence in us. We do not want under any circumstances to have a relationship that will embarrass those persons who are so close to us, that you referred to, Mr. President.

Those are the people that we go to first. We do not want to be embarrassed in misunderstanding them, because they are not being understood on a national level.

I would like to talk to any of you who are interested in our portion of the western area, in regard to problems of brucellosis at this time. I will see you in the lobby.

And I thank you for this opportunity. [Applause.]
SIMPLIFIED STANDARDS FOR LIVE VIRUS VACCINES

DOUGLAS S. ROBSON, PH.D. and JAMES A. BAKER, D.V.M., PH.D.

From the Biometrics Unit and the Veterinary Virus Research Institute, Cornell University, Ithaca, New York.

Within the last decade, new techniques for cultivation of viruses have yielded a considerable number as the cause of various illnesses in animals. In proving etiology under conditions of isolation, there has been an increased awareness that viruses probably are an important factor in animal production and, consequently, that their control could add greatly to efficiency in production. Obviously, live virus vaccines offer the greatest possibilities for control at present, for it is the method proven to be effective, especially by performance in the instances of smallpox in man and hog cholera. Final proof of the contribution made to animal production by vaccines, however, must be determined by incidence studies and then a cost analysis to determine economic efficiency as indicated by the formula:

\[
\frac{\text{total loss number of diseased animals} \times \text{average loss per diseased animal}}{\text{total cost number of animals} \times \text{cost per vaccination}} = \frac{\text{incidence of the disease} \times \text{average loss per diseased animal}}{\text{cost per vaccination}}
\]

As more live virus vaccines are being developed singly and in combination, standards and potency control become correspondingly more complex. When consideration needed to be given to only a few vaccines, supervision to ensure a satisfactory product was provided by an inspection service plus certain animal tests. This type of standard now needs improvement. In an effort to resolve the complexity posed by numerous vaccines, simplified but exacting standards which are based on the newer techniques that made these vaccines possible are offered for consideration herewith.

Standards for Vaccine Virus. Any vaccine for a virus disease now considered to be acceptable should contain virus modified in virulence. This is brought about by initiating cultivation of virulent virus in some alien host cell with continuous transfer thereafter until passage levels are reached in which the virus is considered suitably altered. Unfortunately, modification in virulence does not reach a certain point and then stabilize, but seems to be a continuous process. Not only does prolonged cultivation affect virulence but, paralleling modification, there is an increase in amount of virus required to infect and therefore immunize. It becomes imperative that the safety and immunizing capacity of vaccine virus be defined in relation to specific passage levels. Safety should be determined on the lowest passage level of virus to be used in vaccine and immunizing capacity determined on highest passage level of virus to be used in vaccine.
DOUGLAS S. ROBSON AND JAMES A. BAKER

Naturally, safety requirements of vaccine virus are to be fulfilled by its effect on inoculated animals and whether it spreads by contact exposure and, if so, whether it readily reverts to virulence. Reversion to virulence should be determined by a specified number of continuous passages in animals like those to be vaccinated.

Immunizing capacity must be assured by simplified but realistic tests. The following procedure is suggested:

1. A minimal immunizing dose for vaccinated animals, of the modified virus at the highest passage level it is to be used in vaccine, should be calculated by the Reed-Muench formula. This dose is to be related to a titrated number of laboratory tested units. A laboratory test may be defined as making use of laboratory animals, embryonated eggs, tissue-cultured cells or other acceptable methods.

2. This minimal dosage then must be multiplied by a factor sufficiently large to assure an amount of virus considered ample to be a commercial dose, which then is to be tested for efficacy in animals similar to those for which the vaccine is being produced, according to the statistical design described below.

3. After acceptance as a suitable vaccine, each and every lot of commercial vaccine need be tested by producer and governmental control agency only for content of virus by laboratory means in order to be released for sale.

4. In order to assure that commercial lots continue to immunize, (1) the defined passage level must remain unchanged, (2) a standardized acceptable virus content must always be present, and (3) acceptable efficacy must be confirmed by annual application of the statistical acceptability test on field vaccinated animals. If immunity is based on serological tests, serum samples collected before and after vaccination should be tested.

A Statistical Definition of Efficacy Standards. The efficacy of a vaccine is understood to mean the percentage of the existing population of susceptible animals which would develop immunity if all were vaccinated. Conceptually, then, efficacy is a well defined number, but it can be determined with certainty only by testing the entire susceptible population. Commercial vaccines are, of necessity, released to the market without this certain knowledge of their effectiveness, and the consumer is thereby obliged to accept some small chance that the efficacy of the product he purchases is slightly overrated. The minimal level of effectiveness considered acceptable in a commercial vaccine and the degree of certainty that a vaccine does satisfy this minimal requirement together define the efficacy standards for commercial vaccines.

Theoretically, there is no reason to believe that a live virus vaccine when handled with sufficient care and administered in adequate doses should not be 100 percent effective. In practice, however, such perfection is rarely attained and, as with other type vaccines, efficacies of less than 100 percent may be expected to arise. The lowest level of effectiveness considered accept-
able should, ideally, vary with the economic importance of the disease and the urgency of the need; in the absence of such pertinent information as disease incidence and dollar loss per diseased animal, however, the only available alternative is to establish arbitrary and universal standards. For the purposes of the present discussion an efficacy of about 90 percent will be regarded as a reasonable dividing line between acceptable and unacceptable vaccines.

A degree of uncertainty will always attach to the acceptability of a vaccine because the acceptance test will always be carried out on a sample representing only a very small fraction of the population of susceptible animals for which the vaccine is intended. The degree of uncertainty is measurable in a statistical sense, however, and may be reduced to practically nil by making the acceptance test sufficiently severe. In the statistical sense, absolute certainty is equivalent to a probability equal to one, while "practical certainty" has, conventionally, come to mean any probability greater than or equal to .95 (chances of 19 in 20 or odds of 19 to one). Thus, if an acceptance test were devised to ensure that vaccines of less than 90 percent efficacy would be practically certain to be rejected for commercial use then the consumer could feel practically certain that any vaccine he buys will be at least 90 percent effective. A test with this property is easily derived; if, for example, acceptability were to be determined on the basis of a 30-animal test with the rule that a vaccine be rejected if any immunization failures occur among the 30 susceptible test animals then a vaccine of 90 percent efficacy would stand a one in 20 chance of passing the test, and vaccines of less than 90 percent efficacy would, of course, stand an even smaller chance of being accepted. This acceptance test is a very poor one, however, from both the producer's and consumer's point of view, for it tends also to reject vaccines of greater than 90 percent efficacy. In fact, a vaccine which would immunize 97 percent of the entire population would stand only a 50-50 chance of immunizing all 30 animals in the acceptance test, and would thus stand a 50-50 chance of being rejected for commercial use. Producer and consumer, alike, would therefore suffer under such a test procedure.

Ideally, the rules for acceptance testing should be such as to guarantee with practical certainty that a vaccine which is truly more than 90 percent effective will pass the test and that one which is truly less than 90 percent effective will not pass. This ideal is not attainable but may be approached arbitrarily closely by a rule which will reject with practical certainty any vaccine which is slightly less than 90 percent effective and will accept with practical certainty any vaccine which is slightly more than 90 percent effective, while one which is exactly 90 percent effective stands a 50-50 chance of acceptance. The 90 percent dividing line was, of course, arbitrarily chosen in the first place. A vaccine which will immunize only about 90 percent of the susceptible population may be considered as a borderline case in the sense that its rejection for commercial use would be no great loss to the consumer, nor would its acceptance be particularly undesirable from the consumer viewpoint. This vagueness and arbitrariness associated with the dividing line between acceptability and nonacceptability is actually consistent
with the less than ideal, but attainable, test properties mentioned above. An
efficacy slightly below 90 percent, say two or three percentage points below
90, will be considered unacceptable and should be rejected with practical
certainty, while an efficacy of 92 percent or 93 percent will be considered
acceptable and should be accepted with practical certainty. Between these two
boundaries is the zone of indifference where neither acceptance nor rejection
is regarded as a serious error; even in this zone of indifference, however,
any reasonable test will have the property that vaccines of greater effectiveness
will stand a greater chance of acceptance.

Thus, while efficacy standards in their simplest terms are defined by a
minimal acceptable level of effectiveness and the degree of certainty that a
vaccine does satisfy this minimal requirement, a complete description of the
standards actually specifies the probability of acceptance for every level of
efficacy between no percent and 100 percent. The pioneers in acceptance
sampling, such as the Army Quartermaster Corps, the Army Ordnance De-
partment, and Bell Telephone Laboratories, have found that for their purposes
an adequate description of acceptance standards is generally given by the
"tolerance level," which is the quality level standing only a one in 20 chance
of acceptance, and the "acceptable-quality level," which is the quality level
standing a 19 in 20 chance of acceptance. The "indifference quality level,
which stands a 50-50 chance of acceptance, usually falls about midway
between the tolerance level and the acceptable-quality level. Perfect quality,

![Diagram](image)

Fig. 1. A sketch of the operating characteristic of the proposed efficacy standards
showing the probability that a vaccine of any given efficacy will be accepted for release to
the market.
in this case 100 percent effectiveness, is certain to pass the acceptance test and, at the other extreme, zero quality (no percent effectiveness) is certain to be rejected by the test. A complete description of the efficacy standards suggested here is illustrated schematically in Figure 1, and an experimental procedure which satisfies these standards is described in the next section.

Experimental Procedures for Testing the Acceptability of Vaccines. The first problem in designing an experiment to test the efficacy of a vaccine is the selection of susceptible test animals. Since efficacy is, by definition, measurable only in animals which are susceptible at the time of vaccination, the exclusion of immune animals from the test is essential. When serological techniques are available this problem is relatively simple, and may be expedited by the construction of nomographic devices in the manner of the distemper nomograph derived by Baker (1) et al. In the distemper case, for example, the economically most desirable test subject is a puppy at the age when it loses the distemper immunity which it received from its mother in utero and through colostral transfer. A serological test of the mother alone may be used with the aid of the nomograph to predict the age at which the litter will come available as susceptible test subjects. Such a procedure would virtually eliminate the waste due to the discarding of test animals which were immune at the time of vaccination. When serological techniques are not available the efficacy of a vaccine is considerably more expensive to evaluate, in that direct tests of immunity must substitute for serological ones. In this case, litter mates of animals which test susceptible are commonly used as test subjects for evaluating efficacy.

When means of ensuring susceptibility of the test animals are established, the problem of designing an experiment to test whether a vaccine is of acceptable effectiveness reduces simply to the problem of determining the minimum number of animals which must be tested in order to satisfy the probability requirements of the efficacy standards. This is a purely mathematical problem which was solved by Abraham Wald (2) in 1943 and has since found widespread application in the form of the so-called “sequential acceptance sampling plans.” The optimum experimental procedure is, in this case, a sequential procedure in which animals are tested singly or in groups until the rules of the experiment dictate either the acceptance or the rejection of the vaccine.

The rules for carrying out a sequential acceptance sampling plan are extremely simple, as will be illustrated here by the sampling plan which satisfies the numerical efficacy standards used earlier—namely, that vaccines which are only 87 percent effective stand only a one in 20 chance of acceptance, vaccines which are 90 percent effective stand a 50-50 chance of acceptance, and vaccines which are 93 percent effective stand a 19 in 20 chance of acceptance. The best rule of conduct which satisfies these probability requirements is to vaccinate and test animals in sequence until the number of vaccination failures plotted against number of animals tested falls in
either the acceptance region or the rejection region of the graph shown in Figure 2. The acceptance and rejection lines appearing in the graph are determined from the general formulas given by Wald (3).

![Graph showing the rules of conduct for a sequential acceptance test](image)

**FIG. 2.** A chart giving the rules of conduct for a sequential acceptance test which has the operating characteristic shown in Figure 1.

\[
\text{acceptance number for n tests} = \frac{B}{1 - a} + n \frac{\log p_0}{\log p_1}
\]

\[
\text{rejection number for n tests} = \frac{1 - B}{a} + n \frac{\log p_0}{\log p_1}
\]

where

- \(100p_0\) percent = the "acceptable-quality level"
- \(100p_1\) percent = the "tolerance level"
- \(a\) = the probability of rejecting a vaccine which is 100 \(p_0\) percent effective
- \(B\) = the probability of accepting a vaccine which is 100 \(p_1\) percent effective
Thus, in the present example,

\[
p_0 = .93 \\
p_1 = .87 \\
\alpha = .05 \\
B = .05
\]

so that

\[
\frac{.05}{.95} + n \frac{.93}{.87} \\
\frac{.13}{.07} - \frac{.87}{.93}
\]

acceptance number for \( n \) tests =

\[
= -4.294 + .097 n
\]

\[
\frac{.95}{.05} + n \frac{.93}{.87} \\
\frac{.13}{.07} - \frac{.87}{.93}
\]

rejection number for \( n \) tests =

\[
= 4.294 + .097 n
\]

Examination of the graph reveals that the experiment may terminate after only five vaccinations if all are failures, and that the minimum number of tests required for acceptance of the vaccine is 45. If one failure occurs then 55 tests are required before the vaccine can be accepted; if two failures occur then 65 tests are required for acceptance, and so on. The average number of tests required to terminate the experiment depends upon the true efficacy of the vaccine; a vaccine which is 87 percent effective will require, on the average, about 118 tests, while one which is 93 percent effective will require an average of 142 tests; the average number of tests will be greatest for efficacy levels in the neighborhood of 90 percent, and the maximum average sample size will be about 210 test animals.

The most efficient sampling plan from the point of view of minimizing the expected number of animals tested involves, as described above, the vaccination and test for immunity of individual animals singly in sequence. Practical considerations may, in some circumstances, lead to a modification of this plan involving the vaccination of animals in lots of, say, 25 or 50 at a time. The same graph may be used in this case as when animals are tested singly in sequence; the probability requirements are still satisfied, however the average number of animals tested will be greater when they are tested in groups. Another modification of the sequential plan which is sometimes used in inspection sampling is the so-called "curtailed," or "truncated" sequential plan in which an upper bound is placed on the number of animals to be tested. If this upper limit to sample size is placed as high as three times the maximum average sample size (as high as 600 in this example) then the effect of curtailment upon the probability properties of the plan is negligible since the probability is nearly one that the truly sequential procedure would terminate before that time.
A vaccine which passes the acceptance test is "practically certain" to be at least 93 percent effective. The vaccine may, in fact, be 99 percent or even 100 percent effective, but on the basis of the acceptance test alone the only statistical statement which can be made with practical certainty (95 percent confidence) is that the efficacy is at least 93 percent. The producer may feel that his vaccine is nearly 100 percent effective, and wish to validate this belief statistically. This may be accomplished by testing additional animals, beyond what were required in the acceptance test. A "practically certain" lower bound on efficacy may then be determined from binomial probability tables or, if the number of animals tested is greater than 50, from the formula

\[
\frac{n - x + 1.3448 - \sqrt{1.8085 + 2.6896 \frac{x(n-x)}{n}}}{n + 2.6896}
\]

where \( n \) is the number of susceptible animals tested and \( x \) is the number of failures. Figure 3 is given to illustrate the type of results which may be expected from the application of this formula. For example, if 300 susceptible animals are tested and no vaccination failures occur then the producer may claim an efficacy of at least 99 percent for his vaccine.

Once a vaccine has passed the initial acceptance sampling test and goes into commercial production the efficacy should be maintained at a high level through periodic testing. The quality control of an accepted vaccine presents essentially the same problems as the initial acceptance test, for the question of whether the quality has been maintained after a period of production is essentially the question of whether the vaccine is still acceptably effective. Consequently, the periodic testing should consist of repeating the acceptance sampling test at yearly intervals.
Discussion. The standards proposed here are designed to take advantage of several convenient properties of live virus vaccine as they are now understood. It is now known, for example, that safety and effectiveness can be maintained for relatively long periods of time simply by confining passage level to the predetermined range between the lowest passage level which is safe and the highest passage level which is acceptably effective. The routine test of individual lots of an accepted vaccine therefore need involve nothing more than a laboratory assay of the amount of virus present in the vaccine. Annual field tests of efficacy are added as a precautionary measure to detect any unforeseen changes in the immunizing capacity of the vaccine virus over a period of one year.

The innate effectiveness of live virus vaccines is exploited here by the use of a sequential acceptance test. A sequential acceptance sampling plan will show the greatest gains over the conventional, fixed sample size plan when the quality of the product is, a priori, likely to be high. The efficacy of a live virus vaccine is likely to be very near 100 percent, so that the sequential acceptance test suggested here is, a priori, likely to terminate with 45 successful vaccinations. In order to satisfy these same efficacy standards by a fixed sample size acceptance test, the producer would be required to test 260 animals and reject the vaccine if the number of failures exceeded 25. Thus, if most live virus vaccines are, in fact, highly effective then the sequential acceptance test will usually require about one-sixth as many test animals as the corresponding fixed sample size test. At worst, if most vaccines tested for acceptance were only about 90 percent effective, the sequential plan would still require, on the average, 210 test animals as opposed to 260.

The efficacy standards employed in this discussion are, of course, offered only as one possible set of standards which appear adequate and reasonable to the authors. The objective is to devise standards which provide reasonable assurance not only that accepted vaccines will be effective but also that effective vaccines will be accepted. In the standards laid down here, “reasonable assurance” is interpreted to mean odds of at least 19 to one, and an “effective vaccine” is interpreted to mean any vaccine which will immunize at least 90 percent of the susceptible animals in the population. Since these interpretations are more or less arbitrary they will not find universal acceptance. The principle of the objective itself, however, represents the foundation of a large part of modern statistical theory and practice, so, unless the foundations of statistics are to be questioned, the only points of contention are the above interpretations. Once an interpretation is agreed upon, a sequential sampling plan, or the less efficient fixed sample size plan, may be easily constructed to fulfill the objective.

The reasoning underlying the proposed efficacy standards for live virus vaccines applies almost equally well to all biological and pharmaceutical products which elicit an all or none response comparable to the “immune” or “non-immune” response to a vaccine. This general applicability would permit control agencies to use a common procedure for a large class of products, and therefore simplify and increase the efficiency of their operations.
DOUGLAS S. ROBSON AND JAMES A. BAKER

Summary. Standards suggested for the quality control of live virus vaccines consist of

1. an initial determination of the lowest passage level for safety and the highest passage level for effectiveness,
2. a statistical demonstration that the efficacy of commercial doses of vaccine at the highest passage level exceeds 90 percent,
3. continued control of quality by
   a) maintaining passage levels between the predetermined lowest safe level and highest effective level
   b) testing every lot of vaccine for virus content by laboratory assay methods
   c) annually repeating the statistical demonstration of efficacy using field test animals.

The efficacy standards, in particular, are discussed in considerable detail and a statistical design for testing efficacy is described. These suggestions promise improvement over present standards and in application should ensure that commercial products will give reliable immunity in the field.

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REPORT OF COMMITTEE ON BIOLOGICS AND PHARMACEUTICALS 1958

A. A. CREAMER, Chairman, West Point, Pennsylvania; J. A. BAKER, Ithaca, New York; E. A. CAHILL, Jr., Kansas City, Missouri; J. COLLINS, Latham, Maryland; H. G. GEYER, Columbus, Ohio; J. M. HEJL, Washington, D. C.; A. L. KLECKNER, Athens, Georgia; A. G. PICKETT, Topeka, Kansas; M. WELSH, Secretary, Maryland.

REPORT OF COMMITTEE ON BIOLOGICALS AND PHARMACEUTICALS 1958

Historically the Committee on Biologicals and Pharmaceuticals has presented a report covering new or significant agents which have become available as implements in the hands of those individuals or groups concerned with the health and production of the livestock of our nation. This approach becomes a progress report having a dual message. First, it mentions and describes products at hand which, if used properly, have a direct bearing on the health and productive capacity of livestock. Secondly, it measures in a truly related sense the intensity of research that is carried on by the medical sciences. The investment in concentrated and prolonged research with its yearly dividends in the form of effective biologicals and pharmaceuticals is by no mere assumption a major pillar in the success of livestock sanitation in its broadest sense. The following material is presented with comments upon some new agents for their effectiveness and appropriateness in controlling disease or otherwise influencing the health of livestock.

BIOLOGICALS

The current activity in the research and preparation of biological products if described as having a trend would be in the direction of polyvalent products. Such products, combined for the multiple immunizing effect, are not new in veterinary or human medicine. Much consideration was given to combined vaccines by Baker (1) at the last meeting of this Association. Continued efforts prepare and prove these vaccines have yielded several polyvalent products which have been licensed since the last report of this Committee. The products in mind provide new agents directed at canine distemper, hepatitis and leptospirosis complexes. The appearance of these products indicates success in the necessary techniques involved and insures the advent of others as they become appropriate. Serious consideration must be given the various combinations of antigens chosen. A statistical design for disease incidence to serve as a basis for combined vaccines and for epidemiological evaluation as presented by Robson and Baker (2) at the 61st annual meeting offers one practical approach. Emphasis is placed on critical need, supported by actual economic justification.
Serum products containing single or multiple antibody components continue to provide assistance to biological problems. One new polyvalent product combines specific antibody components against the pathogens of canine distemper, hepatitis, leptospirosis and bronchisepticus, streptococcus, typhimurium infections. It is timely to comment here on the so-called hyperimmune serums that we have known and used extensively for many years with varying results. Standards for the antibody value of such serums, not formerly determined, are now suggested. Practical egg tests can be used to standardize serums to appropriate antibody levels for a protective dose.

Johnin, previously supplied by the Federal Government, is now being produced as a licensed biological. It continues to be important as a diagnostic agent to facilitate the Animal Disease Eradication program.

Autogenous vaccine-Mink has been developed and accepted as a licensed biological for use on specific mink ranches in the northern states as an aid in the control of mink enteritis. Mink is an economic problem requiring specific study for the isolation and identification of the etiological agent and the development of an effective prophylactic.

Clostridium Botulinum Toxoid, types A, B and C has been released as a newly licensed biological since the last report.

Modified live virus vaccines presently offer economical and effective means of controlling animal diseases. Virulent agents whose effect upon the cell is tempered by the use of some influencing substance such as hyperimmune serum continue to produce valuable immunity. Less complex substances performing the same function as the hyperimmune serum are being investigated. The use of iron in conjunction with Hog Cholera live virus is an example.

The Committee recommends that, in view of the isolation and identity of more and more virus agents, efforts be made to create a national system of disease incidence and disease prevalence reporting on a statistical design basis thereby measuring the accuracy and efficiency of prophylactic or eradication programs and setting an accurate course for the pursuits of research.

CONTROL OF BIOLOGICALS

The regulating and control of all immunizing agents falling under the authority of the Animal Inspection and Quarantine Division of the Agriculture Research Service continues with emphasis on uniform inspection of all veterinary biologicals. Accomplishment of this inspection is performed by on-the-spot inspection of all phases of biological production and testing at individual licensed laboratories. The Federal Government Biologics Control Laboratory at the National Animal Disease Laboratory, Ames, Iowa, will be of paramount importance to this program.
Specific approaches to the control of mastitis have been made with the appearance of new combinations of antibiotics with efforts to encompass all etiological possibilities of the disease. More effective therapy is being accomplished by use of special diffusible bases and by steroid influence.

ANTHELMINTICS

Anthelmintics have contributed a major offensive against the ravages of parasitic infestation. Considerable advances are now recorded against cattle grubs with the use of organic phosphorus insecticides administered orally and by external spray. Screwworns in cattle, sheep and goats may be effectively eliminated with these compounds by either systemic or direct contact treatment. Correlation of data on the effect on parasites, host toxicity and tissue residues indicates a great advance in control of these insect pests. Brief reports indicate success in the treatment of canine demodectic mange using ET-57 (3).

Cyanacethydrazide, previously reported as a promising treatment for lungworms of cattle, sheep and swine is available in injectable form for control of the parasite. Treatment results in the expulsion of the adult parasite from unobstructed air passages. Still another organophosphate, dimethoate (CL-12880) gives 97 percent efficiency in controlling nasal bot flies. High kills are performed in the first, second and third instars (4).

Greater range of activity against nemotodes of sheep is reported using fine-particle purified phenothiazine. Determination of the optimum effective particle size range has not been accomplished; however, sizes ranging from one (1) micron to fifty (50) micron have been studied. Using fine-particle (fifty (50) micron average) purified phenothiazine, Fritts et al., demonstrated effective results in ninety-one (91) to one hundred (100) percent in removing mixed nemotodes from sheep (5).

In the successful use of these chemicals for the control of parasites the physiochemical balance within the host must be maintained. To be able to preserve this equilibrium the user must be aware of the pros and cons of the chemical compounds in question. In the case of the organic phosphate compounds, which are allied to the nerve gases developed for chemical warfare, primary activity is upon the nervous system. The result of these compounds together is a potentiation action inhibiting cholinesterase—the essential enzyme which prevents the accumulation of excessive amounts of acetylcholine in the body. Atropine sulfate is a good antidote but must be administered initially by the intravenous route and maintained by large doses in the animal system. Parathion and other organophosphate pesticides were reported to be rapidly destroyed when 100 p.p.m. were added to bovine rumen fluid (6). In this species there is an apparent lack of toxicity. Of practical significance, however, are the simple and rapid colorimetric tests designed to detect organic phosphate insecticide poisoning in cattle and swine (7, 8).
For the control of coccidiosis in animals and poultry, new chemical compounds providing broader or safer therapy have been made available. Some of the newer compounds appearing since the last report are 3,5 dinitrobenzamide; arsenical and sulfonamide combination; glycarbylamide, and bithional-methiotriazamine. These compounds are made effective for the control of coccidiosis by addition to feeds. Also administered through feed is a new treatment for blackhead in turkeys, nithiazide.

**MEDICATED FEEDS**

Medicated feeds have become the spring board for a multiple approach to animal growth stimulation. Effects are established by a variety of influences on the metabolism of the host, both directly and indirectly. Hormones, antimicrobial agents, antifungal agents, antibiotic residues, vitamins, anthelmintics and coccidiostats, to name a few, provide means of influencing growth as additives to feed. At the present time a current listing of compounds incorporated into feed numbers over fifty (50). New to the list since the last Association Meeting are the several coccidiostats named previously in this report and nithiazide an antihistomonad. Mystatin, an antifungal compound and oleandomycin having fresh antibiotic activity are listed as recent additions to feed. Hydroxyzine hydrochloride, an ataractic tranquilizing compound is also found among the newer feed additives.

**TRANQUILIZERS**

Nearly all species of livestock have received attention where the use of tranquilizing drugs may offer a desirable effect. Accumulating data on successful application establishes these compounds as a logical tool in the livestock and veterinary fields. The new addition to animal sedation is ethyl isobutrazine, another phenothiazine derivative. This psychosedative is suggested for use in cattle to minimize “in transit” problems.

**ANTIBIOTICS**

Many known antibiotic compounds have become incorporated into therapeutic agents in various combinations designed to encompass major susceptible disease problems. This does not eliminate the development of antibiotic-resistant pathogens. Several drugs (9) receiving preliminary attention in allied medical fields may find application to the treatment of livestock diseases. Kanamycin, a broad spectrum antibiotic displays less toxicity than neomycin and when used where pathogens have developed resistance to other antibiotics has achieved successful results. Resistance to this antibiotic is known to develop. Ristocetin, which has to be administered intravenously, has been used as a last resort, with success especially in resistant staphylococcal infections. The antibiotic, triacetyloleandomycin, is longer lasting and attains higher levels than the antibiotic erythromycin but is not so active. The new propionate dosage form of erythromycin is now preferred, however,
BIOLOGICS AND PHARMACEUTICALS

attaining higher blood levels than triacetyloleandomycin. Of academic interest is a new broad-spectrum antibiotic, actinobolin, having anti-bacterial, anti-protozoan, and anti-tumor activity.

Among the antibiotics customarily used in veterinary medicine broader applications are being made such as their effect on shock and stress mechanisms. In the anticipation of surgery, antibiotics have merit prophylactically.

The prophylactic use of antibiotics in bovine leptospirosis has been studied and effective prophylaxis demonstrated by chlortetracycline (10). Through feed medication disease was suppressed but significant antibody titers were obtained. The feeding of antibiotics as a chemotherapeutic agent for the elimination or suppression of the carrier state of porcine leptospirosis has been described using chlortetracycline and oxytetracycline (11, 12, 13).

DiethylaminoethylpenicillinGhydroiodide may be mentioned as a new antibiotic with affinities for mammary and respiratory tissues. The advantage of this predilection for specific tissues, with concentrations several times greater than with procaine penicillin G in these tissues is obvious.

OTHER CHEMOTHERAPEUTIC AGENTS

Antibiolyphins, or lymphotrophic antibiotics, are antibiotics in combination with higher molecular compounds. These compounds demonstrate affinity for tissues, namely lymphoid, not usually demonstrating concentrations of antibiotics. Toxicity for these antibiolyphins is reported to be less than for the antibiotics.

Polyvinylpyrrolidone, an organic chemical derived from high pressure synthesis of acetylene and known as a plasma expander, has been used in conjunction with antibiotics in mastitis therapy. Possessing anti-inflammatory and detoxifying properties one (1) Gm. of the material, infused with penicillin and dihydrostreptomycin, produced results superior to those achieved by using the two antibiotics alone (14).

New sulfonamides, sulfadimethoxine, sulfamethoxypridazine, and sulfaphenylpyrazol, receiving attention in therapy of humans are long-acting, well absorbed, highly soluble and diffusible. Administered orally at infrequent intervals, they are bacteriostatically superior to other sulfonamides.

AIR SHIPMENT OF BIOLOGICAL MATERIAL

A proposed regulation of the Postal Department carried in the Federal Register for August 23, 1958, page 6557, indicated the attitude of air carriers towards shipments of biological materials. These regulations if made effective would have seriously encumbered many phases of medical research, diagnostic procedures as well as drug production and distribution. A critical service would become curtailed in the event of disaster or epidemics. The influence of such regulations in respect to livestock health programs is obvious. Acting upon the opportunity to comment on the proposed regulations the Chairman of your Committee on Biologicals and Pharmaceuticals directed correspond-
ence to the Postal Services Division urging that the regulations not be adopted. Word has been received that the regulations as proposed will not be made effective and with the exception of several commercial carriers, biological materials under question will be carried if conforming to packaging requirements.

A.V.M.A. COUNCIL ON BIOLOGICAL AND PHARMACEUTICAL AGENTS

During the 1958 Session of the House of Representatives, American Veterinary Medical Association, a Council on Biologicals and Pharmaceutical Agents was created. This Council, subject to the direction of the Executive Board, shall study the merits of various biologicals and pharmaceuticals, cooperate with regulatory and other medical groups and advise upon the acceptability of advertising literature. It is noteworthy that the consideration of this Council will parallel some of the activities of the Committee on Biologicals and Pharmaceuticals of this Association and indicates collective thinking on the value of appraisal of these products.

REFERENCES

THE INCIDENCE OF LATENT *ANAPLASMA MARGINALE* INFECTION IN WILD DEER IN AN AREA WHERE ANAPLASMOSIS IS ENZOOTIC IN CATTLE*

JOHN F. CHRISTENSEN, PH.D., D.V.M.,1 JOHN W. OSEBOLD, D.V.M., PH.D.,1 AND MERTON N. ROSEN, B.S.2

Any program for the control of anaplasmosis of cattle in California and other areas of the western United States must take into consideration the fact that deer are carriers of the infection. The role of the deer as a reservoir of *Anaplasma marginale* infection was first demonstrated in this country by Boynton and Woods (1) in 1940 when they produced typical acute anaplasmosis in one cow by inoculation of blood pooled from seven wild deer. Little attention was paid to the possible significance of this observation until recently, when Osebold et al. (2) reported the transmission of the disease to 17 out of 22 susceptible calves by injection of pooled or individual blood specimens collected from 64 wild deer in two widely-separated areas of the coastal hill and mountain country of California.

One of the deer collection areas (Mendocino County) described by Osebold et al. (2) was a 5,000 acre University of California experimental area where cattle have been excluded for several years. The fact that blood from these deer, which had little or no contact with cattle, produced anaplasmosis in 10 of the 14 calves inoculated, and certain other considerations, led the authors to the conclusion that *A. marginale* may be better adapted to the deer host than to the bovine host. This suggested that the host-parasite relationship in deer may have begun at an early period, with cattle appearing as a more recent animal in the infection chain. The second area for deer blood collection (northern San Benito County) was typical of vast portions of California where cattle and deer occupy the same range lands, and where anaplasmosis is enzootic in the cattle population. Seven out of eight inoculations of deer blood collected from this area produced the disease in calves.

Many of the transmissions reported by Osebold et al. (2) were effected by means of inoculations of pooled deer blood, since the primary purpose was to transmit the infection if it existed in the deer. However, transmission of infection occurred in seven of 12 calves inoculated with blood from individual deer, thus giving some information on the incidence of infection in deer. Following the study cited, deer blood from a third geographic area of California has been inoculated individually into splenectomized calves. It is the purpose of this paper to report the incidence of *Anaplasma* infection in deer

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* This study was in part a contribution of Federal Aid in Wildlife Restoration, California Project W-52R.
from this particular area and to provide additional information on the status of deer as reservoirs of *A. marginale* infection.

**MATERIALS AND METHODS**

Blood specimens were obtained by arrangement with four landowners in February, 1958, from Columbian black-tailed deer (*Odocoileus hemionus columbianus*) on cattle ranches in southern San Benito County, California. This area was located approximately 50 miles south of the northern San Benito County deer blood collection area described in the paper by Osebold et al. (2), and lies in the coastal hill and mountain country where anaplasmosis is enzootic in the cattle population. The deer were shot in the head or neck and bled immediately by cardiac puncture into vacuum serum bottles containing Alsever's solution, and into tubes without anticoagulant for serum separation. Merthiolate* was added to the serum. The whole blood was kept chilled until inoculations were made into splenectomized calves, which was a maximum of three days from the time of collection. The deer varied in age from approximately eight months to six years and were predominantly females.

The experimental calves were of both sexes and mixed breeding. They were splenectomized at two to four months of age and held for 40 days or longer before inoculation. Eighteen calves were inoculated on February 6, 1958, with blood from individual deer. Each calf received 100 ml. of deer blood with the exception of two calves which were inoculated with 80 and 90 ml., respectively. Part of the inoculum was administered intravenously and part subcutaneously. All calves were negative for microscopically demonstrable blood parasites and *Anaplasma* complement-fixing antibodies at the time of inoculation. The animals were kept in fly-proof enclosures. Starting at the time of inoculation, they were bled at weekly intervals for the first three weeks, at two to five day intervals during the next four weeks when infection was most likely to be detected, then weekly until the 83rd day post inoculation in calves in which no infection developed. Calf 77, in which infection was detected on the 83rd day, was kept under observation until the 118th day.

The complement-fixation (CF) test was performed each time blood was collected as described previously by Christensen et al. (3) and Osebold et al. (2), using antigen supplied by the Animal Disease and Parasite Research Division of the Agricultural Research Service. The hematological methods used were also described in the earlier work (2, 3).

**RESULTS**

The sera from all of the deer whose blood was used for inoculation were either negative at the 1:5 dilution in the CF test or gave only a ± reaction. Careful examination of blood films failed to reveal the presence of bodies in the red blood cells that could be identified positively as *A. marginale*.

* Sodium ethyl mercurithiosalicylate.
TABLE I
Results of Inoculations of Splenectomized Calves With Blood From Individual Deer Collected in Southern San Benito County, California

<table>
<thead>
<tr>
<th>Calf Number</th>
<th>Donor Deer</th>
<th>Age of Inoculum</th>
<th>Transmission Results</th>
<th>Complement-fixation Data</th>
<th>Anaplasma Body Data</th>
<th>Packed Cell Volume of Red Blood Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(No.)</td>
<td>(Days)</td>
<td>(Age)</td>
<td>Antigens Detected</td>
<td>Bodiede</td>
<td>(Percent Parasitized RBC) (Percent)</td>
</tr>
<tr>
<td>220</td>
<td>25</td>
<td>3 years</td>
<td>3</td>
<td>Pos. 26</td>
<td>1:1280</td>
<td>26 41 25.0 33.0 48 22.0</td>
</tr>
<tr>
<td>250</td>
<td>40</td>
<td>8-12 mos.</td>
<td>1</td>
<td>Pos. 21</td>
<td>1:640</td>
<td>21 26 4.0 24.0 36 28.0</td>
</tr>
<tr>
<td>257</td>
<td>33</td>
<td>12-18 mos.</td>
<td>2</td>
<td>Pos. 29</td>
<td>1:1280</td>
<td>29 41 18.0 31.0 48 22.5</td>
</tr>
<tr>
<td>258</td>
<td>28</td>
<td>6 years</td>
<td>3</td>
<td>Pos. 26</td>
<td>1:640</td>
<td>26 41 10.0 35.0 48 19.0</td>
</tr>
<tr>
<td>261</td>
<td>34</td>
<td>8-12 mos.</td>
<td>2</td>
<td>Pos. 21</td>
<td>1:320</td>
<td>34 41 2.0 34.0 41 28.0</td>
</tr>
<tr>
<td>265</td>
<td>32</td>
<td>4 years</td>
<td>3</td>
<td>Pos. 34</td>
<td>1:160</td>
<td>34 48 45.0 40.0 48 19.0</td>
</tr>
<tr>
<td>266</td>
<td>24</td>
<td>3 years</td>
<td>3</td>
<td>Pos. 34</td>
<td>1:1280</td>
<td>34 83 10.0 37.0 83 23.0</td>
</tr>
<tr>
<td>268</td>
<td>30</td>
<td>5 years</td>
<td>3</td>
<td>Pos. 39</td>
<td>1:640</td>
<td>43 55 6.0 28.0 62 18.5</td>
</tr>
<tr>
<td>272</td>
<td>42</td>
<td>3 years</td>
<td>1</td>
<td>Pos. 21</td>
<td>1:320</td>
<td>21 41 30.0 38.0 41 18.0</td>
</tr>
<tr>
<td>273</td>
<td>38</td>
<td>2 years</td>
<td>2</td>
<td>Pos. 26</td>
<td>1:320</td>
<td>29 41 15.0 37.5 55 21.5</td>
</tr>
<tr>
<td>276</td>
<td>36</td>
<td>2 years</td>
<td>2</td>
<td>Pos. 21</td>
<td>1:1280</td>
<td>34 34 25.0 37.5 36 18.0</td>
</tr>
<tr>
<td>77</td>
<td>35</td>
<td>3 years</td>
<td>2</td>
<td>Pos. 83</td>
<td>1:640</td>
<td>83 95 12.0 31.0 102 19.0</td>
</tr>
<tr>
<td>275</td>
<td>46</td>
<td>8-12 mos.</td>
<td>1</td>
<td>Neg.</td>
<td>&lt;1:5</td>
<td>— — &lt;1.0? 38.5 — —</td>
</tr>
<tr>
<td>253</td>
<td>26</td>
<td>8-12 mos.</td>
<td>3</td>
<td>Neg.</td>
<td>&lt;1:5</td>
<td>— — — 33.5 — —</td>
</tr>
<tr>
<td>264</td>
<td>29</td>
<td>4 years</td>
<td>3</td>
<td>Neg.</td>
<td>&lt;1:5</td>
<td>— — — 35.0 — —</td>
</tr>
<tr>
<td>270</td>
<td>43</td>
<td>2 years</td>
<td>1</td>
<td>Neg.</td>
<td>&lt;1:5</td>
<td>— — — 32.0 — —</td>
</tr>
<tr>
<td>271</td>
<td>37</td>
<td>3 years</td>
<td>2</td>
<td>Neg.</td>
<td>&lt;1:5</td>
<td>— — — 39.0 — —</td>
</tr>
<tr>
<td>277</td>
<td>31</td>
<td>8-12 mos.</td>
<td>3</td>
<td>Neg.</td>
<td>&lt;1:5</td>
<td>— — — 32.0 — —</td>
</tr>
</tbody>
</table>
The results of inoculations of calves with deer blood are shown in Table I. Twelve of the 18 splenectomized calves inoculated with blood from individual deer developed *Anaplasma* infections that were demonstrated by blood smear examination for anaplasma bodies, by increase in CF titers, and by variable clinical manifestations.

The period from inoculation of deer blood to first detection of anaplasma bodies in red blood cells of the infected calves varied from 14 to 43 days (average 28 days), with the exception of calf 77 in which bodies were not detected until the 83rd day. These periods may have been a few days shorter in some animals, considering the time-intervals of several days between collections of blood. Percentages of parasitized erythrocytes at the observed peaks of infection varied from as few as two percent to as many as 45 percent of the cells. The degree of anemia, as indicated by the packed cell volume of erythrocytes also varied considerably, readings ranging from 18.0 percent in the most severely affected calves to 28.0 percent in calves with the mildest infections.

None of the affected calves died of the infections, which were on the average only mildly to moderately severe. The most severely affected calves revealed body temperature readings up to 104.1° F., increased pulse and respiratory rates, and varying degrees of depression and inappetence. Only two calves showed increase in the icteric index, but this icterus was not observable clinically. The least affected calves showed little or no clinical disturbance; however, the infections were easily detected by the presence of anaplasma bodies in the erythrocytes and by increase in CF titers.

Usually antibodies were detected on the same day or a few days before anaplasma bodies were first observed. In two calves the organism was seen before the serological test became positive. The peaks of antibody response occurred between the 34th and 62nd days following inoculation (excluding calf 77). The geometric mean for these peak titers was 1:602 and this had fallen to 1:85 by the 83rd day as the titers were declining to carrier levels.

Observations on calf 275 require some clarification. Examination of blood smears from this animal revealed the presence of extremely few marginal bodies in the red blood cells on every observation made between the 14th and 34th days after inoculation. However, the CF antibodies were not detected. Considering the inconclusive nature of the microscopic observations and the absence of increase in CF titer, the animal was classified as negative for *Anaplasma* infection.

*Eperythrozoon* infections were detected in seven of the 18 calves following inoculation with deer blood. However, only one calf revealed what seemed to correlate with the interference phenomenon between eperythrozoan and *Anaplasma* infections described by Foote et al. (4). Calf 77 had a heavy eperythrozoan infection between the 14th and 43rd days post inoculation, while *Anaplasma* infection was first detected on the 83rd day. This could relate to the theory of an interference phenomenon as an instance where an eperythrozoan infection prolonged the incubation period of *A. marginale*. 

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However, this did not appear to be a factor in four other infections in which calves began to show anaplasma bodies after 26 to 34 days, even though they had previously been shown to have eperythrozoan infections. Two calves classified as *Anaplasma* negative after 33 days showed light eperythrozoan infections on single examinations. None of the sera from calves infected with eperythrozoa showed increase in *Anaplasma* CF titer associated exclusively with the presence of eperythroza.

### TABLE II

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Number of Deer in Group</th>
<th>Transmission of Infection to Calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fawns (8-12 mos.)</td>
<td>5</td>
<td>2 Positive 3 Negative</td>
</tr>
<tr>
<td>Yearlings (12-18 mos.)</td>
<td>1</td>
<td>1 Positive</td>
</tr>
<tr>
<td>2 years</td>
<td>3</td>
<td>2 Positive 1 Negative</td>
</tr>
<tr>
<td>3 years</td>
<td>5</td>
<td>4 Positive 1 Negative</td>
</tr>
<tr>
<td>4 years</td>
<td>2</td>
<td>1 Positive 1 Negative</td>
</tr>
<tr>
<td>5 years</td>
<td>1</td>
<td>1 Positive</td>
</tr>
<tr>
<td>6 years</td>
<td>1</td>
<td>1 Positive</td>
</tr>
<tr>
<td>Totals</td>
<td>18</td>
<td>12 Positive 6 Negative</td>
</tr>
</tbody>
</table>

The *Anaplasma* carrier status of the deer in relation to age is shown in Table II. Transmission of anaplasmosis to splenectomized calves was effected by inoculation of blood from two of five deer classified as fawns (actually eight to 12 months old) and from 10 of 13 deer past the age of one year.

### DISCUSSION

The results reported in this paper reveal that 12 of 18 wild deer collected from four cattle ranches in southern San Benito County, California harbored latent *A. marginale* infection. This represents the fourth distinct area in the state where carrier infection in deer has been demonstrated by inoculation of blood into susceptible cattle. The source of the seven deer blood specimens used as a pooled inoculum in the one transmission reported by Boynton and Woods (1) was eastern Santa Clara County. Osebold et al. (2) obtained deer blood from Mendocino County and northern San Benito County in their study, in which inoculations of pooled or individual blood samples from deer produced anaplasmosis in 17 of the 22 calves inoculated. The closest of these areas were approximately 50 miles apart, while the four areas were scattered along nearly 250 miles of the coastal hill and mountain country of California, where anaplasmosis is enzootic in the cattle population.

In the earlier demonstrations of deer as carriers of *Anaplasma* infection by Osebold et al. (2) a suggestion of the high incidence of infection in deer was obtained by the fact that all 10 calves receiving pooled deer blood inoculations of two to 18 samples per inoculum developed the disease, while seven of 12
calves receiving blood from individual deer became infected. The transmission of infection to 12 of 18 calves inoculated with blood from individual deer from southern San Benito County in the present study offers additional evidence that the incidence of carrier infection in deer may be high in areas where the disease is enzootic.

California has a deer population estimated at approximately 1,500,000. These deer occupy public and private range lands which are used for the grazing of cattle, particularly beef cattle. Both of these species of animals are parasitized by ticks known to be capable of transmitting Anaplasma infection. This particular association of deer, cattle and ticks characterizes the areas of California and other parts of the west where the disease is considered to be enzootic in the cattle population.

It has now been demonstrated convincingly that a high percentage of deer in enzootic areas for anaplasmosis harbor latent A. marginale infection, and that infection is readily transferred from deer to cattle and from cattle to deer by blood inoculation. It is logical to assume that transfer of infection between the two species may also occur in nature through the bites of ticks, and possibly other vectors. However, additional work is necessary to establish the transmissibility of infection from deer to cattle by means of ticks taken from carrier deer.

It is expected that the demonstration of the role of deer as carriers of Anaplasma infection may place serious obstacles in the way of eradication of anaplasmosis in these areas. CF testing of cattle and elimination of carriers could not be expected to eradicate the disease where reservoir deer are present, provided ticks and other vectors are capable of transmitting infection from deer to cattle. Control of ticks on cattle that have been turned out on range under western conditions is impractical considering the difficulties of rounding up the cattle frequently enough for effective treatment. Elimination of vector ticks would also have to be accomplished on deer and other mammals on the range in order to break the infection chain completely.

This study has explored more fully the extent of the anaplasmosis carrier problem and the results indicate that the disease agent will likely not be removed from the environment by eliminating carrier cattle. This new fact must be considered in plans for control of the disease. Under existing circumstances investigators must search at an accelerated pace for means of raising the resistance of cattle by immunization procedures that will be universally acceptable.

SUMMARY

Twelve of 18 splenectomized calves inoculated with blood from individual Columbian blacktailed deer (Odocoileus hemionus columbianus) collected in southern San Benito County, California, developed anaplasmosis, indicating an incidence of 67 percent. The incubation periods in the infected calves varied from 14 to 43 days with the exception of one calf in which the period was 83 days; percentages of erythrocytes containing anaplasma bodies at the observed peaks of infection ranged from two to 45 percent; and the packed
cell volumes of erythrocytes varied from 18.0 to 28.0 percent. All infections in the calves were associated with marked rises in CF antibodies in a test using *Anaplasma marginale* antigen. Complement-fixation at the antibody peaks occurred at serum dilutions of 1:160 to 1:1280, as compared with preinfection levels of no reaction at the 1:5 dilution.

The results of this study offer additional evidence to support the earlier observations that wild deer in areas of California where anaplasmosis is enzootic in cattle are reservoirs of the infection. It is believed that the carrier role of deer may prove to be an important complicating factor in efforts to eradicate the disease in areas where carrier deer and cattle occupy the same range lands.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of the following: J. Boyd Harrold, Doris Devine and Jean Abare, University of California, Davis, for technical assistance in the hematological and serological work; personnel of the Wildlife Investigations Laboratory, Region III, California Department of Fish and Game, for aid in obtaining blood from deer; and the California State Fish and Game Commission, for granting a permit to collect deer in San Benito County.

REFERENCES


ANAPLASMOSIS CONTROL BY TEST AND SUBSEQUENT TREATMENT WITH CHLORTHETRACYCLINE


Stillwater, Oklahoma

College of Veterinary Medicine, Oklahoma State University in cooperation with the Oklahoma Agricultural Experiment Station.

Chlortetracycline and partial financial support of this study were provided by American Cyanamid Company.

The complement-fixation test, an accurate method of diagnosing anaplasmosis carrier cattle, has provided a tool for the control of this disease (1, 2). Concurrent with the development of the complement-fixation test, treatments using the tetracycline antibiotics have been devised to eliminate the carrier infection (3, 4). A combination of these two recent developments offers a method for the control of anaplasmosis which can be used when it is desirable to retain valuable blood lines in a herd.

The control of infectious diseases such as tuberculosis and brucellosis necessitates slaughter of all reactors. In anaplasmosis control slaughter may be avoided if carrier infection can be successfully treated. In order to be successful, the treatment not only requires accurate identification of carrier animals but must also eliminate the infection at a cost commensurate with the value of the treated cattle.

At present the tetracyline antibiotics are the only drugs known to cure anaplasmosis carrier infection. They have been administered parenterally for the destruction of carrier infection and orally mixed in the feed for prophylaxis (5). Parenteral administration of these antibiotics is impractical when used on more than one or two exceptionally valuable animals. Oral administration of the drugs in sufficient dosage to eliminate carrier infection would permit treatment on a herd basis and the use of more economical antibiotic preparations.

The object of this experiment was to determine if chlortetracycline could be given orally in sufficient dosage to eliminate carrier infection and if such treatment would be both practical and successful under the range conditions.

PART I. TREATMENT UNDER CONTROLLED CONDITIONS

Methods:

Fourteen two-year-old Hereford cattle were used. Each of these cattle had survived an acute attack of experimentally induced anaplasmosis. At the time of treatment each animal had $4+$ or $3+$ reaction to the complement-fixation test for anaplasmosis. These animals were therefore assumed to be carriers of the infection.
The cattle were divided randomly and placed in three adjoining dry lots—three cattle in lot one, seven cattle in lot two and four cattle in lot three. The cattle in lot one averaged 952 lb. with a range of 895 lb. to 1,035 lb.; lot two averaged 964 lb. with a range of 920 lb. to 1,095 lb.; lot three averaged 937 lb. with a range of 770 lb. to 1,035 lb. The dosage of chlortetracycline for each lot was determined by the average weight of the animals in that lot. The cattle in lot one received an average of five mg. of chlortetracycline per pound of body weight daily for 60 days; those in lot two averaged 2.5 mg. of the drug per pound of body weight daily for 60 days; those in lot three averaged 1.5 mg. of the drug per pound of body weight daily for 60 days. Chlortetracycline in the form of Aureofac-10* was mixed with cottonseed meal at a commercial mill to provide the proper dosage of the drug for each lot when three lbs. of feed per animal was fed daily.

The complement-fixation test was run on each animal at approximately two week intervals during the 60 day treatment period and at monthly intervals for the following three months.

Subinoculation into splenectomized calves of 500 ml. samples of whole blood from each treated animal was made at the end of the 60 day treatment period and also after an additional 60 day period following the end of treatment. The splenectomized calves were examined for infection by both the complement-fixation test and hematologic methods weekly for 60 days following inoculation.

Results:

All of the cattle in the three lots gave a negative reaction to the complement-fixation test for anaplasmosis sometime before the end of the 60 day treatment (table 1.). Two cattle in lot one (5.0 mg. of chlortetracycline per pound of body weight daily) showed a negative reaction after the forty-seventh day and the other animal in this lot was negative to the test after the fifty-fourth day.

* American Cyanamid Company.
One animal in lot two (2.5 mg. of chlortetracycline per pound of body weight) gave a negative reaction to the test after the forty-seventh day, three cattle were negative to the test after the fifty-fourth day and three animals were negative after the fifty-eighth day.

Of the cattle in lot three (1.5 mg. of chlortetracycline per pound of body weight daily) two were negative to the test after the fifty-fourth day and two showed negative reactions after the fifty-eighth day.

After developing a negative reaction to the complement-fixation test the cattle in the three lots remained negative through the one-hundred-fiftieth day following the start of the treatment period.

All of the cattle in the experiment showed varying degrees of reaction to the treatment for five days to one week. The reaction consisted of diarrhea, anorexia and loss of weight. The diarrhea and anorexia disappeared after the first week of treatment and the weight was soon regained.

The results of the subinoculation tests of blood from the treated cattle into susceptible splenectomized calves were negative at both 60 and 120 days after treatment began.

### TABLE 1

**Complement-Fixation Test Reactions of Cattle Treated Orally With Chlortetracycline**

<table>
<thead>
<tr>
<th>Lot</th>
<th>Animal</th>
<th>Wgt. (lbs)</th>
<th>Days From Start of Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>1,035</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>925</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>895</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>935</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1,095</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>646</td>
<td>920</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>648</td>
<td>980</td>
<td>4</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
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</tr>
<tr>
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<td>699</td>
<td>975</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>643</td>
<td>770</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>645</td>
<td>1,000</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>647</td>
<td>945</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>653</td>
<td>1,035</td>
<td>3</td>
</tr>
</tbody>
</table>

**PART II. TREATMENT UNDER FIELD CONDITIONS**

**Methods:**

A herd of approximately 475 purebred Angus cattle were tested for anaplasmosis carrier infection by the complement-fixation test. Nineteen of the cattle were positive to the test.

One of the positive cattle was sold for economic reasons. The other 18 reactor cattle were placed on five mg. of chlortetracycline per pound of body weight for 60 days. The dosage of chlortetracycline was determined by the
average weight of the treated cattle. The drug was mixed with the feed in a mechanical mixer at the ranch under the supervision of the ranch foreman.

The 18 cattle were tested by the complement-fixation test approximately four months after the start of treatment.

This part of the experiment was conducted during the winter months when there was the least chance of natural spread of anaplasmosis.

Results:

Fifteen of the 18 cattle treated under field conditions showed a negative reaction to the complement-fixation test after four months. The other three cattle remained positive to the test.

DISCUSSION

It was possible to eliminate anaplasmosis carrier infection by oral administration of 1.5 mg., 2.5 mg., or 5.0 mg. of chlortetracycline per pound of body weight daily for 60 days under controlled dry lot conditions. When this method of treatment (five mg. dosage) was applied to cattle under field conditions, including lay supervision of the treatment, three out of 18 cattle or almost 17 percent of the animals apparently remained anaplasmosis carriers.

In spite of the difference in results between the two parts of this experiment identification of carrier animals combined with this method of herd treatment appears to be a satisfactory means of control to save breeding and show stock.

The cost of the treatment described is estimated to be $60.00 per animal when the five mg. dosage is used. Although its use seems limited to valuable cattle, treated either individually or on a herd basis, this procedure should receive consideration in any anaplasmosis control program. Under a voluntary testing plan the opportunity to save certain infected cattle could mean the difference between acceptance and rejection of the program.

SUMMARY

Anaplasmosis carrier infection was eliminated by feeding chlortetracycline mixed with feed at dosages of 1.5 mg., 2.5 mg., and 5.0 mg. per pound of body weight daily for 60 days. The complement-fixation test for anaplasmosis gave a negative reaction on all treated cattle by the end of the 60 day treatment period. The treated cattle did not transmit anaplasmosis on subinoculation into splenectomized calves.

Of 18 cattle treated with five mg. of chlortetracycline per pound of body weight daily for 60 days mixed with the feed under normal ranch operation 15 gave negative and three gave positive reactions to the complement-fixation test two months after completion of treatment.
BIBLIOGRAPHY


DISCUSSION

Doctor Brock: I would like to ask Doctor Christensen: In your opinion, do you think that the infections in the calves inoculated from the deer generally had a lighter infection than you would expect in splenectomized calves?

Doctor Christensen: In the first infections occurring in calves by inoculation from the wild deer, generally we felt that these were milder than the typical one that you expect in your usual experimental work.

However, we did make subinoculations; then from the calves into further splenectomized calves and also into splenectomized fawns, and we got fatal cases, with incubation periods more like the normal 14 to 20 days.

This is perhaps just a stage, then, in the adjustment of the parasite as it is coming from deer into cattle, and when established in cattle probably assumes the typical anaplasma pathogenicity and incubation period.

Dr. L. E. Foote, Baton Rouge, Louisiana.

Bill, I would like to ask you if you challenged any of these cattle after you eliminated the chlortetracycline?

Doctor Brock: We did not challenge any of these. Previously, however, I might say, we have challenged some of the animals that we have cured, with not exactly this technique, but other techniques—that we have cured with chlortetracycline. And those animals were equally susceptible, apparently, to anaplasma.

Doctor Foote: But not after the feeding technique?

Doctor Brock: No, not after the feeding technique. It was after the intravenous type of treatment.
REPORT OF THE COMMITTEE ON ANAPLASMOSIS

K. J. Peterson, Chairman, Salem, Oregon; V. D. Chadwick, Jackson, Mississippi; L. R. Noyes, Fort Worth, Texas; W. T. Oglesby, Baton Rouge, Louisiana; L. J. Poelma, College Park, Maryland; T. O. Roby, Silver Spring, Maryland; J. W. Safford, Helena, Montana; E. E. Saulmon, Washington, D. C.; E. H. Willers, Honolulu, Hawaii; D. Isben, Little Rock, Arkansas; M. N. Riemenschneider, Oklahoma City, Oklahoma.

Losses from anaplasmosis have been particularly great during the past two years in the Mississippi River Delta and Gulf Coast area. It is believed that the previous several years of drought, with decreased insect vector populations, allowed for a build-up in numbers of susceptible cattle. 1957 and 1958 have brought, in these areas, an increased rainfall, luxuriant pastures and heavy insect populations. Such conditions are optimum for severe outbreaks of anaplasmosis when carrier cases of the disease are in the same herds with adult susceptible cattle.

Last year your Committee made three specific recommendations concerning future research and development studies on the control of this disease. These were (1) that the standardized complement-fixation test as recommended by the subcommittee appointed at the third National Research Conference on Anaplasmosis be adopted for use in field diagnosis, survey and control programs, for import and export shipments, and for further research studies; (2) that funds be made available to the Animal Disease Eradication Division of Agriculture Research Service for production of anaplasmosis complement-fixing antigen and complement, and (3) that the Agriculture Research Service in cooperation with one of the Western States conduct an experimental field trial control program on a heavily infected herd in the Rocky Mountain region where the disease is believed to be transmitted primarily by the Rocky Mountain Wood Tick, Dermacentor Andersoni Stiles.

At this time your Committee is pleased to report that definite progress has been made in carrying out these recommendations. A kit containing a supply of antigen, standard reference positive and negative control sera, and complement, with a Manual of Instructions for Conducting the Complement-Fixation Test for Anaplasmosis has been supplied to those laboratories which are engaged in conducting the test. In order to assure a continuing supply of antigen the Animal Disease Eradication Division of Agriculture Research Service has negotiated for a supply of anaplasmosis antigen with the Texas A & M Experiment Station. Also the Agriculture Research Service, in cooperation with regulatory officials in the State of Wyoming, is developing an experimental field trial program in a large herd located in southwestern Wyoming. The incidence of the disease in this herd, based on serological findings, has been determined to be slightly over 50 percent, with a much higher percentage incidence in the adults, when calves are excluded. The
objectives of this field trial are (1) to study the duration and transmission of anaplasmosis by the Rocky Mountain Wood Tick, other vectors and wildlife, indigenous to the pastures where the disease is a serious problem, and (2) to study the test and segregation plan for anaplasmosis control in this Western area which is known to be infested with a tick capable of transovarian passage of the etiological agent to the next generation of seed ticks.

In connection with the anaplasmosis eradication program in the Territory of Hawaii, the following statement from Dr. Ernest H. Willers, Territorial Veterinarian, is included in this Committee report as it is an excellent presentation of the current status of that program:

"Progress of the anaplasmosis program in Hawaii during the past year indicates that all carrier animals have probably been eliminated from island herds. No clinical disease was observed and no native carrier animals were uncovered by testing. One valuable Hereford bull that had been imported prior to the inception of the cooperative eradication program in November 1955 was found as a reactor and proved by calf inoculation to be a carrier. However, all cattle that had been in contact with this bull proved to be test negative.

"Sixteen reactors were found among 3,342 animals imported during the year. Of these, five were detected on entry test and eleven were found on a 60-day retest following a negative entry test. The Hawaiian workers want to emphasize this point—in case any State or area is contemplating adopting regulations to require that imported cattle be test negative—there must be a 60-90 day retest on all cattle entering a clean area, unless they originate in a test negative herd.

"The cooperative program will be continued in Hawaii on a surveillance basis for two more years. Blood samples from all cattle slaughtered in the Territory will be tested. Cattle tested for inter-island movement and all cattle receiving an initial test for tuberculosis or brucellosis will be tested for anaplasmosis. These screening tests will be discontinued at the end of the two-year period providing the results are similar to those of the past fiscal year. Testing of imported cattle will continue indefinitely."

Research on anaplasmosis is being conducted at 12 state universities and experiment stations, as well as at Beltsville, Maryland, under the Agriculture Research Service. You have heard on this program a paper relating to recent developments in Oklahoma on the control of anaplasmosis by testing and treatment with antibiotics, also a paper on the incidence of latent anaplasmosis in wild deer in an enzootic area in California. During the past year a report was made by Louisiana workers on the nature of the anaplasma body as revealed by the electron microscope. Also, ovine anaplasmosis was recognized in Wyoming in a flock of sheep which had been debilitated by copper poisoning. It should be pointed out, however, that ovine anaplasmosis is not believed to be transmissible to cattle.

Additional information on anaplasmosis is gradually becoming known. It is more and more apparent that the disease problem varies in terms of incidence, methods of transmission, and reservoirs in different regions of the country. For this reason, the Committee recommends that emphasis should continue on fundamental research, as well as on field trial control programs to broaden the understanding of these regional variations of the disease.
STATUS OF STATE-FEDERAL COOPERATIVE BRUCELLOSIS ERADICATION

C. K. MINGLE, D.V.M., M.S.C.*
Washington, D. C.

Over the past few years our annual reports to this Association have reflected cautious optimism about the progress being made in the cooperative brucellosis eradication campaign. In view of the continuing favorable trend shown during fiscal year 1958, our enthusiasm is bound to be somewhat less constrained than usual.

Certainly, prospects for complete control and eventual eradication of bovine brucellosis are brighter now than at any time since 1934 when the nationwide program was initiated. This does not mean, of course, that all of our problems are solved. As the project advances, new problems are being revealed. However, from experience we have learned to approach these situations in a manner that usually results in solutions being developed before the program is seriously retarded. In our report this year we will endeavor to present a clear picture of the progress being made in the nationwide brucellosis eradication campaign, together with observations on certain factors that are influencing the program.

The brucellosis eradication campaign has now reached the stage where progress is determined largely by the level of service that can be provided. In other words, service requirements are greater than can be met with available finances and manpower. Industry support is at an all-time high and as a consequence each dollar expended on the project is buying more brucellosis control and eradication than ever before. By taking full advantage of this favorable situation, increasingly rapid progress can be made toward the ultimate goal of complete eradication.

LEVEL OF PROGRAM ACTIVITIES REMAINS STEADY

With minor fluctuations, field activities have been maintained at a fairly constant level over the past three years. Such being the case, it would appear that available funds and manpower will not support significant expansion beyond the present volume of work.

* Chief, Brucellosis Eradication Section, Agricultural Research Service, United States Department of Agriculture.
## NATIONAL STATUS—COMPARATIVE BRUCELLOSIS DATA

<table>
<thead>
<tr>
<th>ACTIVITIES</th>
<th>FISCAL YEARS</th>
<th></th>
<th></th>
<th></th>
<th>TOTALS</th>
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<th>TOTALS</th>
<th>Percent Change (4 Years)</th>
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<td><strong>Blood Tests</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>*Herd Tested</td>
<td>565</td>
<td>671</td>
<td>660</td>
<td>696</td>
<td>2,592</td>
<td>985</td>
<td>1,155</td>
<td>1,171</td>
<td>1,177</td>
<td>4,488</td>
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<td>*Reactor Herd</td>
<td>72</td>
<td>116</td>
<td>108</td>
<td>96</td>
<td>392</td>
<td>144</td>
<td>156</td>
<td>124</td>
<td>108</td>
<td>532</td>
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<td>Percent</td>
<td>12.8</td>
<td>17.3</td>
<td>16.4</td>
<td>13.8</td>
<td>15.1</td>
<td>14.6</td>
<td>13.5</td>
<td>10.6</td>
<td>9.2</td>
<td>11.9</td>
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<tr>
<td>*Cattle Tested</td>
<td>5,641</td>
<td>7,491</td>
<td>7,861</td>
<td>9,002</td>
<td>29,995</td>
<td>14,186</td>
<td>16,754</td>
<td>15,913</td>
<td>16,251</td>
<td>63,104</td>
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<td>*Reactor Cattle</td>
<td>172</td>
<td>314</td>
<td>268</td>
<td>236</td>
<td>990</td>
<td>365</td>
<td>367</td>
<td>280</td>
<td>260</td>
<td>1,272</td>
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<td>4.2</td>
<td>3.4</td>
<td>2.6</td>
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<td>Percent of Reactors</td>
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<tr>
<td>Slaughtered</td>
<td>40.1</td>
<td>30.6</td>
<td>32.0</td>
<td>51.3</td>
<td>37.6</td>
<td>71.1</td>
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<td>97.7</td>
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<tr>
<td>*Herd Tested</td>
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<td>671</td>
<td>932</td>
<td>2,058</td>
<td>1,201</td>
<td>1,728</td>
<td>1,866</td>
<td>1,751</td>
<td>6,546</td>
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<td>*Susp. Herd</td>
<td>136</td>
<td>176</td>
<td>243</td>
<td>555</td>
<td>279</td>
<td>256</td>
<td>213</td>
<td>164</td>
<td>912</td>
<td>+</td>
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<tr>
<td>Percent</td>
<td>29.9</td>
<td>26.2</td>
<td>26.1</td>
<td>27.0</td>
<td>23.2</td>
<td>14.8</td>
<td>11.4</td>
<td>9.4</td>
<td>13.9</td>
<td>+ 14.1</td>
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<tr>
<td>*Vaccinations</td>
<td>2,542</td>
<td>3,179</td>
<td>3,688</td>
<td>3,999</td>
<td>13,408</td>
<td>4,381</td>
<td>4,773</td>
<td>5,501</td>
<td>6,277</td>
<td>20,932</td>
<td>+ 56.1</td>
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<td>*Certifications</td>
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<tr>
<td>New Counties</td>
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<td>18</td>
<td>46</td>
<td>33</td>
<td>46</td>
<td>169</td>
<td>241</td>
<td>490</td>
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<tr>
<td>County Removed</td>
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<td>93</td>
<td>79</td>
<td>11</td>
<td>1</td>
<td>48</td>
<td>6</td>
<td>8</td>
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<tr>
<td>Total Cert. Counties</td>
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<td>345</td>
<td>312</td>
<td>334</td>
<td>379</td>
<td>500</td>
<td>735</td>
<td>1,217</td>
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* (Thousands.)
† Three-year total for comparison purposes (1956-57-58).
‡ Actual difference.
## TABULATED NATIONWIDE REPORT ON BRUCELLOSIS ERADICATION ACTIVITIES

<table>
<thead>
<tr>
<th>ACTIVITIES</th>
<th>July 1, 1957 Through September 30, 1957</th>
<th>July 1, 1958 Through September 30, 1958</th>
<th>Percent Change</th>
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<tr>
<td><strong>Blood Tests</strong></td>
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<tr>
<td>Herds Tested</td>
<td>313,056</td>
<td>261,427</td>
<td>-16.5</td>
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<tr>
<td>Reactor Herds</td>
<td>32,141</td>
<td>24,685</td>
<td>-23.2</td>
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<tr>
<td>Percent (Blood Test Only)</td>
<td>10.3</td>
<td>9.4</td>
<td>(-0.9)</td>
</tr>
<tr>
<td>Percent (Blood and B. R. T. Negative Herds)</td>
<td>4.36</td>
<td>3.78</td>
<td>(-0.58)</td>
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<tr>
<td>Cattle Tested</td>
<td>3,534,489</td>
<td>3,146,396</td>
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<tr>
<td>Reactor Cattle</td>
<td>68,200</td>
<td>64,881</td>
<td>-4.9</td>
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<tr>
<td>Percent (Blood Test Only)</td>
<td>1.93</td>
<td>2.06</td>
<td>(+0.13)</td>
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<tr>
<td>Percent (Blood and B. R. T. Negative Cattle)</td>
<td>0.67</td>
<td>0.69</td>
<td>(+0.02)</td>
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<tr>
<td><strong>Percent Reactors Slaughtered</strong></td>
<td>102.9</td>
<td>84.3</td>
<td>18.6</td>
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<tr>
<td><strong>Ring Tests</strong></td>
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<td></td>
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<tr>
<td>Herd Tests</td>
<td>467,771</td>
<td>426,156</td>
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<tr>
<td>Suspicious Herds</td>
<td>84,269</td>
<td>35,730</td>
<td>-57.6</td>
</tr>
<tr>
<td>Percent</td>
<td>18.01</td>
<td>8.38</td>
<td>(-9.63)</td>
</tr>
<tr>
<td><strong>Vaccinations</strong></td>
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<td>1,016,359</td>
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<td><strong>Certification</strong></td>
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<td>New Counties</td>
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<td>Counties Removed</td>
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<td>0.0</td>
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<tr>
<td>Total Certified Counties</td>
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<tr>
<td>(9/30/57)</td>
<td>(9/30/58)</td>
<td></td>
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</tbody>
</table>

( ) Actual difference.

Tables 1 and 2 show, on a comparative basis, the activities carried out during the last four years and a direct comparison of operations conducted in fiscal years 1957 and 1958.

**Blood Testing:** For the four-year period of July 1, 1954 through June 30, 1958, a total of 63.1 million blood serum agglutination tests for brucellosis were conducted on cattle from 4.4 million herds. As a result of these tests, evidence of infection was disclosed in 1.2 million cattle (2.0 percent) and 532 thousand herds (11.9 percent). These figures represent slightly more than a 100 percent increase in the number of blood tests run and a 73 percent increase in herds tested over the cumulative totals shown in Table 1 for the preceding four years. It is significant to note also that even with greatly expanded testing, cattle infection rates declined 1.3 percent and herd infection rates were reduced by 3.2 percent. For the past three years approximately 16 million official blood tests have been recorded annually.

**Brucellosis Ring Testing:** The milk and cream ring test continues to provide an efficient and economical means of detecting *Brucella* infected dairy
herds. This is evidenced by increased utilization of the test and the progress being made in those areas where it is employed as a complement to the blood test. During fiscal years 1955 through 1958 a total of 6.5 million herds were ring tested, or approximately 150 percent more than were tested over the preceding four-year period. In spite of this greatly expanded use, the percentage of ring-test suspicious herds has steadily declined from 23.2 percent in 1955 to 9.4 percent in 1958.

Vaccination: As the area certification program moves into new sections of the country, increasing evidence is seen of the benefits associated with calf vaccination. It is not unusual to find infection rates markedly reduced by vaccination in areas where the disease was known to have been extensive before. Official vaccinations have increased steadily each year since Strain 19 vaccine was adopted as part of the program in 1941. Within recent years, the real value and limitations of vaccine have been more fully recognized than during early stages of the vaccination program. As a consequence, the benefits derived from this procedure have been enhanced.

A total of 20.9 million official vaccinations were reported over the period of fiscal years 1955 through 1958. This is an all-time high for any similar reporting period and in comparison with the previous four years represents an increase of 56 percent.

Area Certifications: Continued emphasis is being placed on the development of Modified Certified Areas. By operating in this manner, advancements made in combating bovine brucellosis are given increasingly effective protection.

During the past four years, encouraging progress has been made in the establishment and maintenance of Modified Certified Areas. In fact, more has been accomplished along these lines than had been expected. As of June 30, 1954, there were only 334 counties listed as being currently qualified in this category. This number had increased to 1,217 by the end of June 1958. In addition there were 594 other counties operating on a complete area basis leading directly to early certification. At that time, therefore, 57 percent of all domestic and territorial counties were either certified or nearing this goal. Over the last four years, 946 new county certifications were recorded, 490 of which qualified during fiscal year 1958.

NEW BRUCELLOSIS FILM DISTRIBUTED

During the past fiscal year a new brucellosis color movie entitled, “Back the Attack on Brucellosis” was distributed in all of the States. This film has as its themes the importance of conducting brucellosis eradication activities on a complete area basis and the need for exercising continued vigilance in the Modified Certified Areas. Audience reactions from initial showings are quite favorable and indicate that the picture should be useful in States where the program is just getting underway as well as in those areas which are already certified.
FIELD STUDIES FURTHER CONFIRM THE VALUE OF BRUCELLOSIS RING TESTING

In cooperation with the State of Wisconsin, a comprehensive field investigation was carried out during fiscal year 1958 to determine the adequacy of area certifications established and maintained, through full use of the brucellosis ring test, as recommended in the Uniform Methods and Rules. This study was conducted in two selected counties and incorporated parallel blood and ring testing of all eligible cattle. Based upon blood test results alone, the combined infection rates for the two counties was 0.11 percent for animals and 1.4 percent for herds. These figures are well below the minimum one percent animal and five percent herd infection rates required for the certification of areas. On the initial round of ring testing, blood test results on ring suspicious herds revealed 4.2 percent animal and 30.2 percent herd infection rates in one county and 5.7 percent animal and 41.0 percent herd infection rates in the other county. Obviously, brucellosis was a serious problem in both counties at the time intensive efforts to combat the disease were undertaken. Nevertheless, full use of the ring test along lines recommended by this Association and approved by the Agricultural Research Service, resulted in suppression of the disease in these counties to an amazingly low level. Moreover, it was determined in this survey that the cost of locating a single Brucella-infected herd was approximately 80 percent less with the screen ring test than with blood testing alone.

RECOGNITION OF MODIFIED CERTIFIED AREAS

As already pointed out, the current brucellosis eradication program is directed towards the qualification of entire counties as Modified Certified Areas. While good progress has been made in this connection during the past few years, there are many areas still to be certified. In order to assure early certification of the remaining counties, it is essential that worthwhile incentives be provided. One, that has strong appeal to the livestock industry, is freer movement of cattle. With this in mind, the Federal Interstate Regulations includes provisions for shipping cattle from Modified Certified Areas under less restrictions than apply to unqualified areas. It was hoped that this regulation would have the effect of modifying individual State regulations along similar lines. Unfortunately, there are still many States that do not accept cattle from non-quarantined herds in Modified Certified Areas without additional requirements being imposed. If this situation continues, it may become increasingly difficult to obtain support for area certification work in some of the exporting States.

It is important that the benefits of nationwide certification be carefully weighed against the calculated risk associated with recognition of cattle originating in areas that are currently qualified according to approved standards. It seems reasonable to believe that by developing more Modified Certified Areas, additional protection against Brucella exposure is being provided the cattle population in general. If the brucellosis status of animals cannot be considered higher in these areas than for animals in noncertified
areas, then we should take a new look at the Uniform Methods and Rules and strengthen them accordingly. Many of the States not yet certified need the incentive of freer movements in order to keep working toward this goal.

**BRUCELLOSIS IN SWINE AND GOATS**

In our 1953 report to this Association, we presented data collected over an 18 months' period on blood agglutination tests conducted on a limited number of swine and goats throughout the country. Since that time, considerable more information has been assembled relative to the brucellosis problem in these species.

For the seven-year testing period involved, cumulative totals represent 633,965 swine in 74,506 herds, and 96,760 goats in 15,593 herds. Of the swine tested during this time, 5.01 percent were classed as reactors and/or suspects. In the case of goats, 1.18 percent were similarly identified. These rates have remained fairly constant each year since the survey was initiated. During fiscal year 1958, 4.09 percent of the 137,805 swine tested and 1.53 percent of 12,185 goats tested could be considered as reactors and/or suspects.

Inasmuch as these results are based largely on voluntary testing, it is reasonable to assume that a significant number of the herds involved were tested because there was some reason to suspect that brucellosis might be present. Consequently, the indicated infection rates are probably somewhat higher than would be reflected by random sample testing. Nevertheless, a brucellosis problem does exist in our swine and goat populations that cannot be ignored if final eradication of this disease is to be achieved.

**PROSPECTS FOR THE FUTURE**

For the current fiscal year, prospects are reasonably good for continued progress in the bovine brucellosis eradication campaign at about the same level as recorded for the previous 12 months' period. During the first quarter of fiscal year 1958, 112 new counties were added to the list of Modified Certified Areas, making a total of 1,327 counties, including 15 complete States and Puerto Rico, qualified for certification as of September 30, 1958. Figure 1 presents the distribution of certified counties on this date.
These call for the certification of 616 additional counties, including five more complete States by June 30, 1959. This would make a total of 1,833 certified counties, or 58 percent of all counties in the United States, Puerto Rico, Alaska and the Virgin Islands. On a State-wide basis, we should have a total of 21 States and Puerto Rico, and the Virgin Islands completely certified by the end of the current fiscal year.

While Federal funds available for brucellosis eradication activities are approximately 10 percent less this year than for 1958, there has been a slight increase in support provided by the States. Consequently, the over-all financial situation for fiscal year 1958 should provide for a level of program operations only slightly lower than last year.

The problem of meeting manpower requirements of the program has never been fully solved. There is no question but that the program would be further advanced today had it been possible to satisfy all service needs. For the most part, veterinary practitioners have cooperated reasonably well in helping meet these requirements. As of June 30, 1958, there were 7,149 accredited veterinary practitioners signed up for work on the brucellosis program. However, with only about 60 percent of these veterinarians ever participating at the same time, the productive work level still fails to meet existing demands. It is essential that every effort be made to encourage the widest assistance possible on the part of the veterinary profession in order to assure success of the program.

Although our immediate goal is qualifying the entire country as a Modified Certified Area, we must be looking ahead and planning for the final phase leading to complete eradication of brucellosis. Some of the Certified States already are taking steps in this direction. It is hoped that from these pilot investigations sufficient information can be developed for establishing the standards necessary to designate and maintain brucellosis-free areas. With our present knowledge and tools, there is every reason to believe that procedures can be designed which will qualify areas as completely free of this disease.

SUMMARY AND COMMENTS

Over the past four years, 4.4 million herds containing 63.1 million cattle were blood tested for brucellosis. Of these, 11.9 percent of the herds and two percent of the cattle were classed as reactors. For the preceding four years—1951 through 1954—out of 2.5 million herds and 29.9 million cattle blood tested, respective infection rates of 15.1 percent and 3.3 percent were disclosed.

During the past year, the bovine brucellosis eradication campaign has continued to show reasonably good progress. As presented in Table 2, the results of 16.2 million blood agglutination tests conducted during fiscal year 1958 show that indicated infection rates have been reduced to 1.6 percent for cattle and 9.2 percent for herds. These figures compare with 1.76 percent animal infection and 10.6 percent herd infection rates found in the preceding fiscal year. With program activities extended into new areas and the volume
of testing increased during 1958, it is significant to note that both herd and cattle infection levels continued to decline. These results tend to confirm the value of procedures being employed and the effectiveness of approved diagnostic tests and Strain 19 vaccine when intelligently applied.

Each year since it was approved in 1952, increased use of the brucellosis ring test has demonstrated the value of this procedure in detecting dairy herds in which Brucella infection probably exists. This is evidenced by the fact that over the past seven years the volume of ring testing has increased approximately 150 percent and the percent of ring suspicious herds has decreased from 29.9 to 9.4 percent. The results of additional field investigations completed during the year fully support the establishment and maintenance of Modified Certified Areas, based on full use of the ring test where applicable.

The qualification of counties as Modified Certified Areas is proceeding at an encouraging rate. During fiscal year 1958 the number of such areas was increased by 490, the most ever recorded for any 12 months' period. With 42 percent of all counties in the United States, Alaska, Puerto Rico and the Virgin Islands currently certified or working on programs leading directly to certification, there is every reason to believe this status may be attained nationwide within the next five years. Modified Certification is, of course, only a long step toward eradication. We cannot afford to relax our efforts until all Brucella infected livestock, including cattle, swine, goats, and sheep are detected and eliminated. Indecision at this point could have the effect of unnecessarily delaying final solution of the brucellosis problem—control alone is not enough.

Although the use of Strain 19 vaccine has increased steadily each year to the point where about six million calves are being vaccinated annually, there is still a need for wider coverage. These six million vaccinations represent only about 41 percent of the eligible calves. With the usefulness and limitations of vaccine now clearly defined, we cannot expect this procedure to accomplish more than effective control of brucellosis. When employed as an adjunct to test and elimination of reactors, the real value of vaccination is being realized. Even in certified areas, there will be a continued need for vaccination until eradication is complete.

The benefits to be derived from eventual elimination of the economic and public health threats associated with brucellosis in animals will more than repay the concerted efforts of all groups concerned.
Knowledge of swine brucellosis is extensive.† The proposals for swine brucellosis control and eradication are efficient, practicable, and adequately field tested (Proceedings of the Fifty-Third Annual Meeting of the United States Livestock Sanitary Association pp. 68-72; Proceedings of the Fifty-Eighth Annual Meeting of the United States Livestock Sanitary Association pp. 204-206; Proceedings of the Fifty-Ninth Annual Meeting of the United States Livestock Sanitary Association, pp. 146-147). This is a happy situation. Nevertheless, there is urgent need to consider the disease. This need arises from misunderstandings and misinterpretations relative to the facts on swine brucellosis which in turn have resulted in widely divergent state brucellosis regulations. Widely divergent regulations in the face of well established facts make for confusion among producers who cannot be expected to know the background or basis for such regulations. Just as serious is the fact that such divergency is impossible to explain on the basis of sound disease control. For example, there is a great difference of opinion on what constitutes a brucellosis-free swine herd or, indeed, how to define a brucellosis-free hog in law or regulation.

On these points (as well as on others) there is complete agreement in only one way—everyone agrees to disagree with the other fellow. This cannot be said to be sound disease control or can it be expected to win the confidence of producers.

Going into the matter a step further on what constitutes brucellosis-free stock, it is said in some regulations that a herd is brucellosis-free when none of the hogs in the herd reacts to the “agglutination test” in “any dilution.” In addition, it is stated that swine herds are certified brucellosis-free on the basis of two or more such tests and yearly tests, thereafter. Those who have worked with the disease or those who are informed know that these kinds of regulations make for an impossible situation. They know this “thinking” is not in conformity with the recommendations of the United States Livestock Sanitary Association—see the 1949 Proceedings of the United States Livestock Sanitary Association—see the 1949 Proceedings of the United States Livestock Sanitary Association.

* Prepared with the assistance of A. B. Hoerlein & L. M. Hutchings.

† It has been pointed out that such knowledge is not employed to a reasonable extent. Some have said that it has done little good to develop such knowledge. At the same time these individuals admit that unemployed scientific knowledge is a most valued asset, ranking in value with such things as Billing’s “good set of bowels.” Never was there a time when scientific knowledge was so quickly put to use—never was there a time when there was such a small backlog of unused scientific knowledge. Even though the information on swine brucellosis is largely unused, all admit research and development of additional knowledge on the disease is most desirable.
Livestock Sanitary Association, page 69, the last paragraph. One just does not find herds of any size where all animals are without titers to the agglutination test on repeated testing.*

Kernkamp and Roepke (Amer. Jour. Vet. Res. 9, Jan. 1948, 46-49) in a study of five brucellosis-free herds found that a "significant percentage" of the breeding animals showed agglutination titers to Brucella antigen in the 1:25 to 1:50 range with several having incomplete agglutinations at 1:100. They state, "It is believed a herd can be diagnosed as brucellosis-free if two successive agglutination tests, sixty days apart on all breeding stock do not disclose titers of 1:100 or higher, and no new animals have been introduced into the herd within 30 days preceding the first test. Subsequently, single annual tests of the breeding stock which do not disclose titers in the 1:100 range or higher, may be interpreted as representing a brucellosis-free herd if no new animals have been introduced into the herd within 60 days preceding the tests. Titers of 1:25 or, less often, 1:50 were found in the breeding animals in these herds in 14 to 38 percent of the hogs. In a single clean herd, Hoerlein and Leith (Vet. Med. 47, 1952, 448-450) found only 2.8 percent titers of the 1:25 or 1:50 among 5,726 samples over a three-year period. Still later in the same herd, Hoerlein (Cornell Vet. 43, 1953, 28-37) after a careful search failed to find Brucella infection in nine reacting animals—four of these nine were positive 1:25, two were positive 1:100, two positive 1:400, and one partial in the 1:800 dilution. When Hoerlein subjected these and like samples from clean herds to the agglutination technique of Feinberg and Wright (Jour. Immunol., 67, 1951, 115-122) where the incubation temperature is 56° C for 16 hours he uncovered a main cause for titers among uninfected swine. In this work by Hoerlein "eight swine droves, free of brucellosis, were represented by 136 individual serum samples. The initial titers at 37 C were distributed as follows: 106 at 1:25, 25 at 1:50, three at 1:100, one at 1:400, and one at 1:800. Incubation of the tests at 56 C for 16 hours gave negative titers with three exceptions; One titer of 1:100 did not change, one of 1:400 was decreased to 1:50 and one of the 1:800 was depressed to 1:25." Detailed bacteriological examinations after slaughter of the hogs failed to reveal any infection. In contrast to this, when the serums of hogs known to be infected with Brucella were subjected to similar test procedures, their titers remained essentially the same at the 56 C incubation. Because of these findings and the work of others which tends to confirm them, it is common practice to conduct additional tests at 56 C where there is question as to whether or not titers of the conventional tube (or plate test) actually indicate infection. It is generally concluded that incubation of tests at 56 C inactivates the non-specific agglutinins that result in false reactions.

The Animal Husbandry herd of hogs at the University of Wisconsin is brucellosis-free on the basis of negative evidence of the disease including negative cultures. During the past five years 1312 blood samples from this

* The plate agglutination test of swine blood is less apt to show non-specific titers than the tube test.
herd have been examined by the agglutination test for brucellosis. Of these 150 have reacted at 1:25, 13 at 1:50 and one at 1:100. This is at the rate of one reactor at 1:25 or above for each 8 animals tested. When 230 blood samples selected at random from this herd were tested using the incubation temperature of 56 C for 16 hours only one animal reacted at 1:25 or higher—at the rate of one reactor for each 230 animals tested. Except for one animal, the recommendations of the United States Livestock Sanitary Association would have been satisfactory in this herd, while according to some state regulations 164 of these brucellosis-free animals would have been declared “infected.” Incubation of the tests at 56 C would have been satisfactory except for a very few animals.

In the face of all this and like evidence and in the face of recommendations by the United States Livestock Sanitary Association, it seems a bit silly to propose that a herd is free of brucellosis only if all the swine are entirely free of titers on repeated tests. Many who have worked with swine brucellosis feel that they could not meet such requirements in justice to swine herds without falsification of the test records. Where the regulations are so arbitrary that they allow no titer whatever by any test, one wonders if the 1:25 dilution is employed and indeed if the 1:50 dilution is interpreted strictly. It has been suggested that the lowest dilution be 1:100 in the testing of swine blood.

"Regulatory officials are urged to study pertinent literature so they can recognize the necessity of making flexible interpretations on the agglutination test rather than using the specific yardsticks employed in the control of bovine brucellosis." (Proceedings of the Fifty-Ninth Annual Meeting of the United States Livestock Sanitary Association, page 147.)

To continue with the agglutination test, it has been said that it is “necessary to use the agglutination test as a herd diagnostic procedure and base any attempts to control of entire herds or units rather than on individual swine.” (Proceedings Fifty-Third Annual Meeting, United States Livestock Sanitary Association, page 69.) To many, this is a slight overstatement of fact. These would rather say that “the diagnostic value of the agglutination test is greatest when applied to all the swine in a herd, and least valuable on individual animals.” It has been well established that certain hogs fail to react even though infected, and that certain other hogs may cease to react although still infected. This situation is common enough so that it often adversely influences the results of any test and slaughter plan.

It has long been known that it was unsafe and unwise to move an animal from one cattle herd to another on a single negative tuberculin test unless the herd of origin was accredited. It is also well known that it is unsafe and unwise to move a cow from one herd to another on a single negative brucellosis test unless the herd of origin is certified free of brucellosis. To move a hog into a clean herd on the basis of a single negative agglutination test is downright foolish unless the herd of origin is certified. It has been proposed that where it is “necessary” (!?) to obtain a boar from an unknown herd; such a boar should be isolated for 60 to 90 days and subjected to
one or more additional tests. Still, the safe plan is to purchase from a certified herd subject to test, isolation and retest.

Contaminated ground is a potent source of infection for swine. All ground that infected swine have run on is unsafe. Such ground must be allowed time for self-sterilization. Two months may be suggested as a time for ground to become self-sterilized in the warmer periods of the year. Ground cannot become self-sterilized when frozen. On page 204 of the Proceedings, Fifty-Eighth Annual Meeting, United States Livestock Sanitary Association is the recommendation that "used hog lots be rested if possible" before clean hogs are put on such lots. This is a mistake. There is no such "milk toast" words as "if possible" if one is to eradicate swine brucellosis. The contaminated lots must be rested. In the same report is the following: "3. Replace the stock from certified brucellosis-free herds, preferably placing them on clean ground for as long as possible." Here again, there is no choice—clean hogs must be placed on clean ground and not for "as long as possible" but for as long as necessary. On the next page of the same report, it is said that "the disease has a greater tendency to spread as swine approach sexual maturity." Hutchings and his group and McNutt were unable to show that the disease spread appreciably faster in older animals but they did demonstrate that there was a great deal of difference in the disease manifestation depending on sexual maturity. The immature were much less apt to show evidence of brucellosis and were much more apt to recover and to recover much more quickly than the mature. Such recovered animals could be readily reinfected when they reached maturity, but they were resistant or immune to the extent that they usually recovered from such reinfection in a month or less usually without evident signs of the disease; whereas, the sexual mature, that had no previous experience with the disease, did not readily recover and in these animals all the expected manifestations of brucellosis developed. These findings largely account for lack of evident infection in many diseased herds.

Much enabling legislation, both state and federal, was necessary before Texas fever was eradicated. The same was true for bovine tuberculosis. Still greater amounts of legislation have been passed to enable work on bovine brucellosis eradication. All of these control and eradication procedures have required huge organizations, again both state and federal. Through habit many have taken it for granted that swine brucellosis would require much legislation and organization. Those who have attempted to think the matter through on the basis of the evidence have concluded that better swine brucellosis control and eventual eradication could be effected with essentially no enabling legislation and only minor changes in present organization. This conclusion has been reached because of the nature of the disease which makes it possible for the producers to do the job without appreciable cost to themselves.

If things are in readiness for better swine brucellosis control or eradication, why are not appreciable strides made toward this end? Very largely the producers see no reason for taking better control measures inasmuch as they
can move breeding stock intrastate without determining whether or not it is brucellosis-free (see definition of "brucellosis-free" in United States Livestock Sanitary Association reports). The same applies to the movement of swine interstate to considerable degree (hogs can be moved on a single negative test or no test at all). In addition, producers do little to better the swine brucellosis situation because of fear. That fear is mostly misplaced. They might well fear if their herds are infected because of the danger to themselves and their families but they fear because they think swine brucellosis is very prevalent and that their own herds are probably infested. Actually the situation is not nearly as complicated or so serious as many believe. If the various states would each provide for certification of individual herds as brucellosis-free, and the producers, or at least a part of the producers, would apply for certification through their local people, everyone would be amazed to find how many herds are already entirely free of brucellosis—how many could be certified brucellosis free on the initial tests. Many states provide for certification and machinery for blood testing but expect the producers to supply the blood samples. This procedure is most simple and direct, and with understanding by all would be the most effective.

In addition, producers remember how they have been penalized in the past when they have tried to do something about animal disease through animal health regulatory officials. They remember the situation with bovine brucellosis—the individual who tried to do something about bovine brucellosis in his own herd (not too many years ago), immediately had his herd quarantined and was placed under all kinds of so-called regulations. Whereas the producer across the road did nothing with the result his herd was not quarantined; although, it was full of brucellosis and he could ship his animals any place. Producers are not apt to walk into that trap again. Some swine producers in the midwest have found that once their herds were officially certified, their boar sales were so good they could no longer supply the demand.

Presently, it is believed that swine brucellosis will not greatly influence bovine brucellosis eradication; but, if it does, swine producers can expect drastic rules and regulations, which we all hate, directed toward eradication of swine brucellosis. Janney (Master’s thesis, University of Wisconsin, 1958) while investigating reasons for infections in certified counties (and also better means of eradication) encountered a cattle herd that was infected with Br. suis. On continued investigation of conditions on the farm involved, it was found that hogs had been kept on the farm but they had just been sold for slaughter and could not be examined. It is likely that the hogs were responsible for the outbreak of brucellosis in this herd that had been clean.

If this sort of thing is recognized often, pressure will be brought on the swine producers and in this case demands will be arbitrary. Swine producers themselves can do the best job on control and eradication without outside pressures. In addition, the whole process of better swine brucellosis control
and eradication appears to be simple as compared to bovine brucellosis. Three main factors make for simplification in swine brucellosis. They are:

1. There is less herd infection in swine.
2. The disease is self-limiting especially in herds of reasonable or small size—much more so than in the case of cattle herds.
3. Infected boars are a most significant source of spread.

The first of these factors has already been mentioned. Surveys show no increase in infection and there is reason to believe producers generally are more careful of their breeding stock so there has been a decrease in swine brucellosis. The incidence of the disease has never been estimated at more than two percent.

Most infected hogs recover if given time. It follows that infected herds become brucellosis-free—there are not enough susceptible hogs in herds, especially small herds, to keep the infection going; and it must be admitted that following recovery, most swine have a degree of resistance regardless of age (McNutt and Leith, M.S. Vet. 4, Fall, 1943, pp. 28-35 and Proceedings of the Iowa Academy of Science 53, 1946, 307-311; Hutchings, Delez and Donham, Amer. Jour. Vet. Res. 7, 1946, 11-20 and Vol. 5, 1944; 195-208 Hoerlein et al., Swine Brucellosis Bulletin of the Veterinary Medical Research Institute, Iowa State College).

In regard to the spread of swine brucellosis, the infected boar has long been recognized as a main factor. Producers generally are putting forth increased effort to select brucellosis-free herd sires. Many investigators are of the opinion that if infected boars were controlled, swine brucellosis would be greatly reduced—almost to the vanishing point (Hoerlein-Hutchings). At the same time, all are aware that the infected hog, either male or female, can spread the disease either directly or indirectly. In Iowa, Br. melitensis was shown to spread from cattle to swine thence to man (Jordan, Borts and McNutt, Jour. Amer. Med. Assoc. 131, Jan. 12, 1946, p. 966), and it has been demonstrated that swine pick up Br. abortus infection (McCullough and others).

State regulatory officials can do much to further the eradication of swine brucellosis on a sound animal disease control program. It must be admitted that the policies are often outside the jurisdiction of such officials. For example, state legislatures sometimes pass regulatory legislation that is unsound and when the courts take over the functions of the legislative bodies as is being done more and more from the highest to the lowest courts, the situation is worsened. In such instances, regulatory officials can do little except to exert a moral influence. For example the state of Illinois once made it unlawful to prohibit the sale of raw tuberculous milk anywhere in the state. It must be admitted that regulatory officials can make mistakes the same as all others. But if no one is willing to listen to reason so that we add the mistakes of the regulatory officials to the mistakes of the legislatures to the mistakes of the courts, we are apt to come out with a jumble of regu-
lations and laws that mean little but trouble for producers and result in little
disease control. It is the desire to avoid this in swine brucellosis.

The following is proposed on swine brucellosis control:

1. That the recommendations of the United States Livestock Sanitary
   Association for control and eradication of swine brucellosis be followed.

2. That the term “agglutination test” be defined by each state that has
   strict regulations and laws on swine brucellosis.

3. That each state arrange for certification of brucellosis-free swine herds—
   and that the term “brucellosis-free” be as defined already by the United
   States Livestock Sanitary Association. It is suggested that producers
   arrange for meeting the requirements of a “brucellosis-free herd”
   without expense to the state except for conducting tests and book-
   keeping.

4. That each state consider prohibiting the movement of breeding stock
   unless such stock comes from brucellosis-free herds as defined by the
   United States Livestock Sanitary Association.
REPORT OF COMMITTEE ON BRUCELLOSIS


Mr. President, members of the Association, and invited guests: The last official report of the Agricultural Research Service of the United States Department of Agriculture dated September 30, 1958, indicates that we now have 15 states plus Puerto Rico in the modified certified free area list. In addition to these states there are 537 counties, the entire state of Alaska, and the Virgin Islands listed as having completed area testing. All states are now participating in the eradication program. However, no program of disease eradication can be carried on successfully year after year unless every advantage of modern science and research is considered and incorporated in the program.

Each year as we meet in convention, new plans backed up by research and controlled experiments are presented for consideration of your Committee. Many resolutions and suggestions for improvement of the program have been received during the past 12 months from different sections of our country. All of these have been considered in public hearings which started Tuesday morning at 9:15 in this hotel. We believe that everyone has had an opportunity to present his case and every proposal has been given careful consideration.

The coveted goal of attaining a modified-certified brucellosis-free status is the desire of every state. Upon achieving it, however, a state may be in its most dangerous period. There is the real prospect of a let-down in the vigorous effort to attain final and complete eradication. While state and federal expenditures can be reduced considerably following attainment of the modified certified brucellosis-free status, industry and professional pressure must be continued, not only to attain final eradication but to prevent losing hard-fought ground.

Therefore, your Committee wishes to emphasize the fact that there is no short road to success in disease eradication, and that increasing warfare against all contagious and dangerous economic livestock diseases demands of us constant watchfulness and service. It has long been true in the affairs of man that only time can confirm the gift of greatness. Artists, statesmen, writers, craftsmen, each must await the years for the maturity of his talent,
and for the verdict of his judges. In every human effort, time is the essence. No single act, no brief moment in the sun has ever overshadowed the life work of a master, and as it is with the affairs of man, so it is in the world of disease control. Nothing great and lasting was ever created or accomplished suddenly, whether the product is an individual or an organization, and when the last chapter of brucellosis eradication has been written, the accomplishment will be of lasting value to our livestock industry and in the end will meet the critical verdict of its judges.

With these few introductory remarks, we are now ready to report to you what, in our best judgment, should be incorporated into the uniform methods and rules governing the program of brucellosis eradication in the United States, and to review with you some of the problems under discussion during the Committee hearings.

1. The question of extending the age for official calf vaccination was presented for discussion and was given a great deal of thought. While an extension of the age limit on calves to be vaccinated might be desired in a few states; as a national wide policy, your Committee believes the age limit should remain as it is today, four to eight months.

2. The resolution presented by the Western States Livestock Sanitary Association and the American National Cattlemen’s Association asking that provisions be adopted whereby “A herd or ranch would be eligible to maintain its modified certified status toward area or state recertification when the female offspring or female herd replacements have been officially calfhood vaccinated or spayed and no evidence of infection is demonstrated in such herd.” This proposal was carefully considered by the Committee and after hearing all the evidence presented feel that vaccination alone is not a safe procedure to follow in the re-certification of herds or areas.

3. Your Brucellosis Committee welcomed the opportunity to review with Dr. R. J. Helvig the proposed changes in the United States Public Health Service, Standard Milk Ordinance and Code. The proposals dealt with Item 1R, Cows’ Health and in particular the brucellosis phase of this item. In general the proposal will provide that all herds producing Grade A milk for pasteurization shall be from Plan A herds. Provision was also made for classifying herds that have passed three satisfactory milk ring tests as having met the Plan A requirement.

4. This Committee urges the Agricultural Research Service to explore a further use of the Milk Ring Test. There is the possibility of utilizing the composite milk samples that are assembled for butterfat determinations.

5. As you know parts of the 1956 Report as pertaining to Uniform Methods and Rules, have never been approved by the Agricultural Research Service Animal Disease Eradication Division. All items in question have been thoroughly thrashed out by your Committee and we have been assured the corrective proposals that are part of this report will enable adoption of the Uniform Methods and Rules proposed in 1956, amended in 1957, and the 1958 corrective proposals.
REPORT OF COMMITTEE

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ANIMAL DISEASE ERADICATION DIVISION

UNIFORM METHODS AND RULES FOR THE ESTABLISHMENT AND MAINTENANCE OF CERTIFIED BRUCELLOSIS-FREE HERDS OF CATTLE AND MODIFIED CERTIFIED AREAS, AND FOR THE ESTABLISHMENT OF CERTIFIED BRUCELLOSIS-FREE AREAS

PART I: DEFINITIONS

"Positive" or "Reactor"

1. An official vaccinate more than thirty (30) months of age that discloses a complete agglutination reaction in the blood titer dilution of 1/200 or higher.

2. A non-vaccinated animal more than six (6) months of age that discloses a complete agglutination reaction in the blood titer dilution of 1/100 or higher.

3. An adult vaccinate that discloses a complete agglutination reaction in the blood titer dilution of 1/100 or higher.

"Suspect"

1. An official vaccinate more than thirty (30) months of age that discloses agglutination in the 1/100 dilution and less than complete in the 1/200 dilution.

2. A non-vaccinated animal more than six (6) months of age that discloses agglutination in the 1/50 dilution and less than complete agglutination in the 1/100 dilution.

3. An adult vaccinate that discloses agglutination in the 1/50 dilution and less than complete agglutination in the 1/100 dilution.

"Negative"

1. An official vaccinate more than thirty (30) months of age that discloses a reaction of not more than complete agglutination in the 1/50 dilution.

2. A non-vaccinated animal more than six (6) months of age that discloses a reaction of less than incomplete agglutination in the 1/50 dilution.

3. An adult vaccinate that discloses a reaction of less than incomplete agglutination in the 1/50 dilution.

The "Herd" Test

Shall include all cattle over six (6) months of age except steers, spayed heifers and official vaccinates not more than thirty (30) months of age.
“Approved brucella biologic”
A product that is approved by and produced under license of the United States Department of Agriculture to be used in the control of brucellosis through its injection into cattle.

“Official vaccinate”
A bovine animal that was administered an approved brucella biologic when not less than four (4) months or 120 days of age nor more than eight (8) months or 240 days of age under the supervision of a licensed, accredited veterinarian and such record of vaccination has been filed with the State Livestock Sanitary Authority on forms provided.

(a) Range and semi-range official vaccinate means a bovine animal of the recognized beef breeds that was administered an approved brucella biologic when not less than four (4) months or 120 days of age, nor more than twelve (12) months or 365 days of age under the supervision of a licensed, accredited veterinarian and such record of vaccination has been filed with the State Livestock Sanitary Authority on forms provided except as heretofore provided for range and semi-range cattle of the recognized beef breeds.

“Adult vaccinate”
A bovine animal that was administered an approved brucella biologic when more than eight (8) months or 240 days of age under the supervision of a licensed, accredited veterinarian and such certificate of vaccination has been filed with the State Livestock Sanitary Authority on forms provided except as heretofore provided for range and semi-range cattle of the recognized beef breeds.

Identification of Vaccinated Animals
(a) Adult animals tattooed “AV” in the right ear or branded “AV” on right jaw.
(b) Calves tattooed “V” or “shield and V” in right ear or branded “V” on right jaw.
(c) If the tattoo is used, then the “V,” “shield and V,” or “AV” shall be preceded by a numeral indicating the quarter of the year in which the vaccination was done. The “V,” “shield and V,” or “AV” shall be followed by the last digit of the year in which the vaccination was done.
(d) If the brand is used, then the “V” shall be applied in four different positions—one each year over a four-year period to indicate in which year the vaccination was done. The fifth year will repeat the first year, and so on indefinitely. In 1959 the “V” shall be placed with the open end facing forward and so on clockwise indefinitely.

“Range Area”
An area in which all cattle are maintained on natural forage and/or browse during the entire calendar year.
"Semi-Range Area"

An area in which all cattle are maintained on natural forage and/or browse during the entire calendar year with occasional supplemental feeding.

Exception: When in the judgment of the State and Federal Livestock Sanitary Authority local factors prevail which are not conducive to the eradication of brucellosis, such areas need not be classed as Range or Semi-Range.

"Immediate slaughter"

The delivery of animals to the slaughtering establishment within a ten (10) day period from the date the animals were either consigned for slaughter or permit issued for their consignment to slaughter. Such animals, upon delivery to the slaughtering establishment, shall be slaughtered as soon as practicable.

PART II: RECOMMENDED PROCEDURES

Section I. Individual Herd Plans

Plan A. Testing of cattle, permanent identification, and prompt disposal of positives, for slaughter only, with or without vaccination of calves. Herds that have passed three successive satisfactory milk ring tests at intervals of not less than four (4) nor more than six (6) months may be considered as having met the brucellosis requirements of Plan A for Grade A milk production.

Plan B. Testing of cattle, permanent identification, and temporary retention of positives pending their disposal for slaughter, with vaccination of calves. Positives may be retained in a quarantined herd for a period not to exceed three years from the date retention of positives was started. All plan B herds should be retested at least every six months.

Plan C. Calf vaccination without test of any part of the herd. This plan is to be confined to those herds in which the movement of animals is restricted by special permits issued by the State Livestock Sanitary Authority.

Plan D. Testing of entire herds with vaccination of negative cattle only within ten (10) days after completion of test and permanent identification of positives. This plan to be used only in emergencies in herds where there is evidence of a rapid spread of brucellosis, and then only with the written permission of the State-Federal cooperating agencies. Whenever the plan provided under this subsection is used, written notice that vaccination may not prevent the spread of such disease shall be given by the state and Federal agencies to the owner of the livestock.

Section II. Participation on Area Basis

A. Voluntary—When 65 percent of the cattle owners representing at least 51 percent of the cattle in an area have placed their cattle under any one or a combination of the four plans.

B. Compulsory—When 75 percent or more of the cattle owners in an area have placed their cattle under any one or a combination of the four plans.
C. Compulsory—When 75 percent or more of the counties representing a majority of the cattle in the state have placed their cattle under any one or a combination of the four plans.

Section III. Supervision

The official brucellosis eradication programs shall be supervised by full-time employed state and/or Federal veterinarians.

Section IV. Entering Premises

Persons engaged in the brucellosis project should be authorized to enter premises to carry out eradication procedures.

Section V. Services to Owner

Services to owner should be made available without expense to him so long as funds for such purposes are available (owner to provide for handling of his cattle). Provision should be made if possible to pay practicing veterinarians for brucellosis eradication services on a per head or on a per head and per farm basis.

Section VI. Classification of Animals

The following tables shall be used in classifying the blood titers of tested animals:

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<td>+</td>
<td>+</td>
<td>I</td>
<td>Suspect</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Positive</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Section VII. Branding Positives

A permanent brand with the letter “B” (at least 2x2 inches) must be placed on the left jaw of all cattle classed positive. Positives must remain on premises where disclosed until a state or Federal permit has been obtained for movement to immediate slaughter where state approved and/or Federal inspection is maintained.

Section VIII. Vaccine

Only vaccine approved and manufactured under license of United States Department of Agriculture, Agricultural Research Service, shall be used in any brucellosis control program.
Section IX. Movement of Cattle

No female cattle or breeding bulls over six months of age shall be sold and/or moved interstate except:

a. Animals consigned for immediate slaughter.
b. Those tested and found negative for brucellosis within 30 days prior to date of movement.
c. Cattle under 30 months of age which are official vaccinates.
d. When official records establish that the animal is a part of a certified brucellosis-free herd, a Modified Certified Brucellosis Area, or a Certified Brucellosis-Free Area at the time of sale or movement.

Section X. Reports

All activities, conducted either privately or as part of the official program, such as results of agglutination tests and vaccination, must be reported promptly to state and Federal cooperating agencies.

PART III: INDIVIDUAL CERTIFIED HERD PLAN

A herd may be placed under supervision for certification as brucellosis-free upon complying with provisions governing the testing requirements of the respective State-Federal Cooperative Program.

Section I. Herd Certification

A. Herd Blood Tests shall be made at intervals of not more than sixty (60) days until all evidence of infection has been eliminated. A herd may be certified as brucellosis-free when it has passed at least two consecutive negative blood tests, with the first clean test and the certifying test not less than twelve (12) months nor more than eighteen (18) months apart. Additional herd tests may be conducted on herds if the owner so desires, or if the certifying agency deems it advisable.

B. If there is no evidence of infection on the first blood test, a herd may be certified as brucellosis-free when it has passed one additional negative blood test conducted not earlier than six (6) months nor more than eighteen (18) months from the date of the first test.

C. Where the Milk Ring Test is employed, herds may be initially certified as brucellosis-free with a minimum of three satisfactory milk tests conducted at not less than ninety (90) day intervals and followed by a negative herd blood test conducted within ninety (90) days after the last negative milk ring test. Herds in which infection has been disclosed shall be tested in accordance with provisions of paragraph A of this section.

Section II. Herd Recertification

A. Upon evidence of a negative herd blood test at the end of one year, certification of that herd may be extended for one year.

B. If the retest of a certified herd or of animals from such a herd reveals one reactor, the herd may be recertified on the result of a negative herd
retest conducted not earlier than sixty (60) days following removal of the reactor. Intervening blood tests, if any, must be negative.

C. If the retest of a certified herd or of animals from such a herd reveals more than one reactor, the herd must requalify for certification as under Section I, A.

D. If the retest of a certified herd discloses suspects but no positives, only the suspicious animals need be retested. If all suspects are available for retest and are negative, the herd test may then be considered negative. If the retest does not include all suspicious animals, or if one or more positives result, the herd shall be retested as provided in Section II, B or C. If only one suspect was not available for retesting and if that suspect had been an official vaccinate, the herd test will be considered negative and the herd recertified.

Section III. General Provision

A. Vaccination titers. Official vaccinates under thirty (30) months of age are not required to be tested, or if tested, are not required to be negative. Official vaccinates over thirty (30) months of age, classed suspect, may be retained in certified herds for retesting until their final determination is made.

B. Additions to certified herds or herds with negative tests shall be limited to the following:

1. To certified herds:
   a. Natural herd increase.
   b. From herds with equal status.
   c. From herds that have passed a negative blood test within the past twelve (12) months.
      (1) Official vaccinates under thirty (30) months of age on certificate of vaccination—over thirty (30) months of age if negative within thirty (30) days prior to addition.
      (2) Non-vaccinated animals on evidence of negative retest not less than sixty (60) days from date of negative herd test.

2. To herds that have passed a negative blood test within the past twelve (12) months:
   a. Natural herd increase.
   b. From herds with equal or superior status.
   c. From other herds:
      (1) Official vaccinates under thirty (30) months of age on certificate of vaccination—over thirty (30) months of age if negative within thirty (30) days prior to addition.
      (2) Non-vaccinated animals if tested negative within thirty (30) days prior to addition, then segregated and retested negative in not less than sixty (60) days from date of first test.

C. Additions from herds without equal status. Under qualifying conditions of Subsections B. 1, c. and B. 2, c., such animals shall not receive new herd
status for sale purposes until they have been members at least thirty (30) days and included in a complete herd retest.

D. Certificates. Certified Brucellosis-Free Herd certificates which shall be valid for one year unless revoked, may be issued by cooperating State or Federal officials.

E. Quarantine. The entire herd must be confined to the premises if reactors are disclosed by the blood test. Movement of all cattle shall be prohibited until the herd has passed a negative herd retest at least thirty (30) days following removal of reactors, except cattle consigned for immediate slaughter under permit.

F. Cleaning and disinfecting. Premises shall be cleaned and disinfected under regulatory supervision within fifteen (15) days following removal of reactors, unless an extension of time is granted.

PART IV: MODIFIED CERTIFIED AREA PLAN

The provisions of the individual certified herd plan that relate to testing, cleaning, quarantining and disinfecting shall apply to the Modified Certified Brucellosis Area plan. The extent of the area shall be determined by the cooperating State and Federal agencies. All tests for area certification shall be performed within an eighteen (18) month period. When an area has been designated and the required percentage of herds and cattle included under any of the plans, the area should be placed under quarantine and the following rules apply:

Section I. Area Certification

A. If as the result of a blood test of all cattle within an area the number of positives does not exceed one percent and the herd infection does not exceed five percent, the area may be declared a Modified Certified Brucellosis Area for a period of three (3) years. Infected herds shall be quarantined until they have passed one negative blood test at least thirty (30) days following removal of the cattle classed positive, except cattle consigned for immediate slaughter under permit.

B. An area may be declared a Modified Certified Brucellosis Area by the application of two milk ring tests not less than six months apart, together with a blood test of all milk reacting herds, such other herds as are not included in the milk test, and herds in which the BRT does not represent a majority of the cattle in the herd. The number of positives must not exceed one percent of the cattle and the herd infection rate must not exceed five percent. Infected herds shall be quarantined until they have passed one negative blood test at least thirty (30) days following removal of the cattle classed positive, except cattle consigned for immediate slaughter under permit.

C. (1) Range and semi-range areas may qualify as Modified Certified Brucellosis Areas for a period of three years if as the result of a blood test of all dairy cattle, all purebred cattle, and not less than 20 percent of the range and semi-range cows over three years of age in each herd, the number
of positives does not exceed one percent of the area cattle population over six (6) months of age (excluding steers and spayed heifers) and five percent of the herds.

(2) Should evidence of infection be disclosed in any of the animals required to be tested in the range or semi-range herds under provisions of Part IV, Section I, C (1), such herds shall be quarantined until the entire herd has passed one negative blood test at least thirty (30) days following removal of the cattle classed positive, except cattle consigned for immediate slaughter under permit.

D. If testing as outlined under Part IV, Section I, Paragraphs A, B, or C reveals an animal infection rate of more than one percent, but not over two percent, and an initial retest of the infected herds applied not less than thirty (30) nor more than one hundred twenty (120) days following removal of the cattle classed positive, discloses not more than one percent animal infection and not over five percent herd infection, the area may then be certified. Percentages shall be computed on the totals of the last test.

E. If the test of an area as outlined under Part IV, Section I, Paragraphs A, B, or C results in more than two percent positives, or if an initial retest of infected herds as under Section I, Paragraph D does not qualify the area for certification, it shall be necessary to make a complete area retest.

F. Bulls and female cattle being held under quarantine for feeding purposes separate and apart from dairy or breeding cattle, may be exempted from test provided such cattle are sold for immediate slaughter under permit from the appropriate Livestock Sanitary official at the end of the feeding period.

Section II. Area Recertification

A. At the expiration of the three-year period areas certified under the provision of Part IV, Section I, Paragraph A or B may be recertified for another three-year period. To do so, the results of a test of all herds in which infection was reported at the time of the previous certifying test or since, together with the results of a test of at least 20 percent of other representative herds, must reflect a rate of infection which does not exceed one percent of the cattle and five percent of the herds. The number of herds required for retest shall be computed from the last area test and shall not include the same herds previously tested for this same purpose.

B. Areas certified under the provisions of Part IV, Section I, Paragraphs A or B may be continued as certified with the application of semi-annual milk ring tests, follow-up blood tests of milk reacting herds, and blood tests at three-year intervals on at least 20 percent of all herds not included in the milk test and herds in which the BRT does not represent a majority of the cattle in the herds, if the incidence of infection does not exceed one percent of the cattle, and five percent of the herds. The herds tested to meet the 20 percent requirement shall not include the same herds previously tested for this purpose.

C. (1) At the expiration of the three-year period, range and semi-range areas may be certified for another three-year period when at least 20 percent
of the herds, including animals as outlined under Part IV, Section I, Paragraph C (1) have been retested and the animal infection rate does not exceed one percent in not more than five percent of the herds. The number of herds required for retest shall be computed from the last area test and shall not include the same group previously tested for this same purpose.

(2) Modified Certified Brucellosis Range or Semi-Range Areas may be maintained in a certified status for additional periods of three years, provided:

(a) That at least 80 percent of the heifer calves retained in the area annually are officially vaccinated; providing that vaccination is not mandatory in strictly range areas where winter feeding is not practiced.

(b) That during each year at least five percent of the breeding cows in the area, as determined by the statistics of the Agricultural Marketing Service, or a total of 15 percent during a three-year period, are subjected to the agglutination test for brucellosis; the blood samples to be taken from cull and slaughter cows at ranches, sale yards, or slaughtering establishments.

(c) That herds of origin of cattle reacting at a titer of complete at 1-100 are subjected to an official blood test, and are handled according to the provisions of Part IV, Section I, Paragraph C (2).

(d) That herds found infected during the preceding certification period are blood tested at least one year following release from quarantine.

(e) That dairy herds in the area are screened semi-annually by the milk ring test, with blood test of herds reacting to the milk ring test, or are blood tested annually.

(f) That the percentage of infection disclosed as a result of such tests as conducted under the provisions of the above four paragraphs does not exceed five percent of the herds, and one percent of the area cattle population over six months of age (excluding steers and spayed heifers); the number of reactors used in computing the percentage to be the number accumulated over the three-year period.

D. If testing as outlined under Part IV, Section II, Paragraphs A, B, C (1), or C (2) reveals an animal infection rate of more than one percent, but not over two percent, and an initial retest of the infected herds applied not less than thirty (30) nor more than one hundred twenty (120) days following removal of the cattle classed positive, discloses not more than one percent animal infection and not over five percent herd infection, the area may then be recertified. Percentages shall be computed on the totals of the last test.

E. Any area not qualifying for recertification under the provisions of Part IV, Section II shall be required to re-establish its certified status through testing procedures as outlined under Part IV, Section I.

F. Bulls and female cattle being held under quarantine for feeding purposes separate and apart from dairy or breeding cattle, may be exempted
Section III. Additions to Modified Certified Brucellosis Areas

A. Cattle from officially Certified Brucellosis-Free Herds or Areas and cattle from negative herds in Modified Certified Brucellosis Areas may enter Modified Certified Brucellosis Areas without being retested for brucellosis. All such cattle shall be individually identified and shall be accompanied by an approved certificate of health indicating herd and animal status.

B. Cattle from herds under Federal-State supervision for the control of brucellosis may enter a Modified Certified Brucellosis Area or an area in the process of such certification when all animals in the herd were negative to the official blood agglutination test for brucellosis within 90 days of the date of entry. Individual animals to be moved must be negative to an official retest at least 30 days from the date of the previous herd test and within 30 days of entry or be official vaccinates under 30 months of age.

C. Cattle under 30 months of age officially vaccinated and coming from (a) negative herds in Modified Certified Brucellosis Areas, (b) Certified Brucellosis-Free Herds or Areas, or (c) herds under Federal-State supervision which have passed a test as under paragraph B may enter a Modified Certified Brucellosis Area or an area in the process of certification without further test when individually identified by mark, brand, tattoo or other acceptable identification, and accompanied by a certificate of health approved by the proper sanitary official of the state of origin.

D. Breeding cattle not over 30 months of age, officially vaccinated, which do not qualify under paragraph C may enter a Modified Certified Brucellosis Area providing they do not show blood agglutination reactions higher than incomplete in dilution of 1-200 and the animals are maintained in quarantine until they have passed a negative blood retest.

E. All other male or female cattle over six months of age, including official vaccinates over thirty (30) months of age, except steers, spayed heifers, and cattle intended for immediate slaughter, shall be required to pass a negative officially recognized blood agglutination test for brucellosis within 30 days prior to the date of entry. They shall be maintained in quarantine separate and apart from all other cattle and be retested in not less than 30 nor more than 90 days after date of entry. If retested and classed negative, they shall be released from quarantine.

PART V: CERTIFIED BRUCELLOSIS-FREE AREAS

Whenever an area, by utilizing the Uniform Methods and Rules, discloses no animal and no herd infection, the area may be declared "Certified Brucellosis-Free."
PLANS FOR CONTROL OF SWINE BRUCELLOSIS

A. Certification of Swine Herds as Brucellosis-free

Certification is made on the basis of two consecutive negative tests on the entire herd 90 days apart. This includes all animals six months of age and over. This certification is valid for 12 months. Recertification is made annually by the passing of a single negative test on the entire herd.

B. Plans of Control for Infected Herds

Plan 1. This plan is recommended for commercial herds.
1. Market the entire herd of swine for slaughter.
2. Clean and disinfect houses and equipment.
3. Replace with stock from certified brucellosis-free herds, placing them on clean ground.
4. Following two consecutive negative tests 90 days apart, the herd is eligible for certification.

Plan 2. This plan is recommended for use in purebred herds where it is desirable to retain valuable blood lines.
1. Separate pigs from sows at 42 days of age or younger and isolate.
2. Market infected herd as soon as practicable. If sows are held for later litters, complete isolation is essential.
3. Test the gilts to be used for the following breeding season about 30 days before breeding. Save only those gilts which are negative. Breed only to negative boars.
4. Retest the gilts after farrowing and before removing them from individual farrowing pens. Should reactors be found, they should be segregated from the remainder of the herd. Select only pigs from negative sows for breeding gilts.
5. If herd is not negative at this time, the process is repeated. When the entire herd passes two consecutive negative tests 90 days apart, it becomes eligible for certification.

Plan 3. This plan is not recommended in general but has been found useful in herds where only a few reactors are found and where no clinical symptoms of brucellosis have been noted.
1. Remove reactors from farm.
2. Retest herd at 30-day intervals, removing reactors, until entire herd is negative.
3. Two negative tests, 90 days apart, qualify the herd for certification.
4. If the herd is not readily freed of infection, abandon this plan in favor of Plan 1 or Plan 2.
C. *Accessory Regulations*

1. Blood samples are to be taken by approved accredited veterinarian.

2. Reactors must be sold for immediate slaughter.

3. Replacement swine may be added without test if procured directly from a certified brucellosis-free herd.

4. All other replacement breeding animals shall have passed a negative agglutination test and be held in isolation until passing a second negative agglutination test. The second test shall be at least 30 days after the first, in the case of boars and open gilts, or after farrowing in the case of breed sows and gilts.

5. All swine on the farm kept for feeding purposes shall be segregated from the breeding herd until moved for slaughter.

6. (A) Negative animal from a herd of unknown or infected status means an animal that discloses no agglutination in test dilutions of 1/25 or higher.

(B) Negative animal from a certified herd is one showing a reaction no greater than incomplete in 1/100 dilution.

(C) Negative herd means a herd that discloses no animal reacting more than incomplete in dilutions of 1/100.

(D) Reactor animal means an animal that discloses a reaction of complete in the dilution of 1/100 or higher.

(E) Infected herd means a herd that discloses one or more animals reacting complete in the dilution of 1/100 or higher, then any animal in the herd showing a reaction of complete in dilution of 1/25 or higher shall be considered a reactor.
A LEPTOSPIRA WILDLIFE SURVEY IN OHIO

HARRY E. GOLDSTEIN, D.V.M., HENRY A. PECK, B.Sc. AND EUGENE KNODER, Forest Game Research Specialists

Reynoldsburg, Ohio

In any discussion of leptospirosis it is usually agreed that the disease is classical. An accurate test for evaluation and diagnosis is available. A means of vaccination for prevention is available, and a treatment to eliminate a high percentage of carrier animals can be utilized. With these points in mind one of the important factors that needs further exploration is the animal reservoirs of leptospirosis other than the domestic animal carrier.

Leptospirosis has become an economic problem confronting the Ohio livestock industry. The disease occurs in widespread geographic distribution throughout Ohio, and manifests itself primarily in cattle and swine. Five flocks of sheep have demonstrated clinical manifestations of leptospirosis and have been confirmed by agglutination lysis tests. Many individual equine leptospirosis cases have been observed. The seasonal incidence is highest in the spring and fall with little variation in incidence rates.

The first recognized case of leptospirosis in farm animals in Ohio occurred in 1952. Since 1952, either from increased incidence or awareness a higher percentage of samples have disclosed significant reactions.

In July of 1957, a localized zoonosis of leptospirosis occurred in Logan County, Ohio. Twenty-eight farms were evidencing severe economic losses in cattle. Abortions and atypical mastitis with mild hemoglobinuria were common clinical symptoms in the adult cattle. In young calves and yearlings acute leptospirosis was observed with rapid onset and high mortality. Each case was confirmed by agglutination lysis tests.

Exhaustive field investigations were conducted in this area. Each premise was visited and attempts were made to correlate a common etiological exposure. The rodent population was explored with no conclusive findings. At this season an extremely high number of horse flies was observed on every farm. The possible role of the Hematophagus flies has been studied by Rieter and Ramme, who showed that Haemotopota pluvialis may transmit leptospires to guinea pigs mechanically (1). The same possibility was demonstrated by Uhlenhuth and Kuhn for Stomoxys calcitrans (2). It has been demonstrated that the horsefly may transmit the disease as long as six days after having bitten an animal in the leptospiremia stage (3).

Therefore, a number of horseflies were obtained from several of the infected farms and submitted to the laboratory. These studies were inconclusive but are considered of sufficient importance to warrant further consideration.
It is of interest to note that Burgdorfer (4) succeeded in transmitting leptospirosis by permitting infected ticks to feed upon susceptible guinea pigs and also found a naturally infected L. ballum tick (5).

These studies are reported because it is believed that there is a need for more research conducted on the possibility of biting insects transmitting leptospirosis, when the host animals are in a leptospiremia state.

At the time of the initial investigations in Logan County the question arose on many farms as to the possibility of deer acting as carrier animals. These questions could not accurately be evaluated and it was quite apparent that some evaluation was necessary. It was determined that in this area the deer population had increased to the point that more deer had been observed than in previous years. Therefore, an attempt was made to evaluate the status of the deer population.

For the past three and one-half years the Division of Wildlife, Ohio Department of Natural Resources and the Division of Animal Industry, Reynoldsburg Pathological Service Laboratory have cooperated on disease problems of Ohio wildlife. In their continuing program of cooperation the two agencies conducted a survey to determine the incidence of leptospirosis in wild mammals.

This survey was instituted with a two-fold purpose. One, to evaluate blood titers in wild mammals to demonstrate the possible role of the carrier animal and two, to evaluate the effects on the wild mammal population either from abortion or mortality.

The initial phase of the survey utilized only blood samples obtained from deer. The samples were obtained from deer shot during the period of December 11, through December 14, 1957.

A total of 226 blood samples were tested by means of the agglutination lysis test for *Leptospira pomona*. These 226 samples were obtained from 38 counties. A total of 43 deer samples were positive at a titer of 1:100 or greater, which represents 20 percent of the total sampling.

A titer breakdown of the positive samples was as follows:

<table>
<thead>
<tr>
<th>Titer</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:3200</td>
<td>4 samples positive</td>
</tr>
<tr>
<td>1:1600</td>
<td>3 samples positive</td>
</tr>
<tr>
<td>1:800</td>
<td>4 samples positive</td>
</tr>
<tr>
<td>1:400</td>
<td>8 samples positive</td>
</tr>
<tr>
<td>1:200</td>
<td>5 samples positive</td>
</tr>
<tr>
<td>1:100</td>
<td>19 samples positive</td>
</tr>
</tbody>
</table>

When this data had been compiled an immediate interest was aroused among the wildlife people to obtain a more complete study on other species of wildlife. This additional study was performed to gain additional information regarding other species and to take the pressure off the deer population.

In the short period of January 23, to January 30, 1958, an additional 708 wildlife blood samples were obtained and tested.
The 708 samples were broken down by species as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Counties Sampled</th>
<th>Number of Blood Samples Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit (Sylvilagus floridanus)</td>
<td>38</td>
<td>150</td>
</tr>
<tr>
<td>Rats (Rattus norvegicus)</td>
<td>33</td>
<td>165</td>
</tr>
<tr>
<td>Mice (Microtus, Peromyscus) (Mus musculus)</td>
<td>24</td>
<td>156</td>
</tr>
<tr>
<td>Raccoon (Procyon lotor)</td>
<td>24</td>
<td>70</td>
</tr>
<tr>
<td>Fox (Vulpes fulva) (Urocyon cinereoargenteus)</td>
<td>18</td>
<td>55</td>
</tr>
<tr>
<td>Opossum (Didelphis virginiana)</td>
<td>26</td>
<td>43</td>
</tr>
<tr>
<td>Muskrat (Ondatra zibethica)</td>
<td>12</td>
<td>37</td>
</tr>
<tr>
<td>Skunk (Mephitis mephitis)</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>House Cats (Felis domesticus)</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Squirrel (Sciurus, Tamiasciurus)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Shrew (Blarina brevicauda)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Mole (Scalopus aquaticus)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mink (Mustela vison)</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

A breakdown of these species revealed:

- Raccoon: 16 positive or 23% of sampling
- Fox: 14 positive or 25% of sampling
- Skunk: 6 positive or 54% of sampling
- Vole Mouse: 1 positive or .7% of sampling
- Rat: 1 positive or .8% of sampling
- Opossum: 1 positive or .2% of sampling

A titer breakdown of these species were as follows:

**Raccoon**

- 1 sample positive: 1:1600 L. pomona
- 3 samples positive: 1:800 L. pomona
- 5 samples positive: 1:400 L. pomona
- 5 samples positive: 1:200 L. pomona
- 3 samples positive: 1:100 L. pomona

16 Positive Samples

**Fox**

- 3 samples positive: 1:3200
- 1 sample positive: 1:1600
- 1 sample positive: 1:400
- 4 samples positive: 1:200
- 5 samples positive: 1:100

14 Positive Samples
The titers demonstrated in the fox became significant when compared to the studies performed on silver foxes in the Union of Soviet Socialist Republic.

**Skunk**
- 1 sample positive: 1:3200
- 2 samples positive: 1:400
- 1 sample positive: 1:200
- 2 samples positive: 1:100
- 6 Positive Samples

It is also of interest to note that this survey indicated one positive deer and four positive fox from Logan County.

After the 932 blood samples were tested with the *leptospira pomona* antigen, the positive samples were then tested with a battery of 10 different serotypes. In so doing we observed concurrent or para-specific reaction in only five samples.

One fox sample had a titer of 1:1600 for *L. pomona* and a titer of 1:6400 for *L. autumnalis*.

A second fox sample had a titer of 1:200 for *L. pomona* and a titer of 1:1600 for *L. ictericemorrhagiae*.

A third fox sample had a titer of 1:3200 for *L. pomona* and a titer of 1:12,800 for *L. autumnalis*.

A skunk sample which had a 1:100 titer for *L. pomona* exhibited a titer of 1:400 for *L. autumnalis*.

A rat sample which exhibited a 1:100 for *L. pomona* titer afforded a 1:800 titer for *L. ictericemorrhagiae*.

The 165 rat samples were all cross tested with *L. ictericemorrhagiae* and only one exhibited the titer.

In summarizing the initial survey, 932 wildlife mammal blood samples were tested with *leptospira pomona* antigen by means of the agglutination lysis test. Of the 932 samples 82 were positive at 1:100 or greater. It is realized that these numbers are not of great statistical significance but warrant additional studies in deer, raccoon, fox and skunk and are reported as interesting data with no conclusive fact.

In Ohio we are now in the process of enlarging the initial survey. This survey is projected to sample all 88 counties in Ohio and will afford 130 blood samples from each county. The 130 blood samples will be composed of:

- 6 deer
- 32 rabbits
- 10 raccoon
- 6 fox
- 6 skunk
- 6 opossum
- 32 squirrel
- 6 muskrat
- 10 Norway rats
- 6 groundhogs
- 10 chipmunks or spermophile

In so doing a total sampling of 11,440 wildlife bloods will be surveyed by the end of this year. This should afford statistical significant data.

Thus far in Survey No. 2, 1,436 squirrel blood samples have been tested. Only one sample has afforded a positive titer. This titer was 1:204,800. The second survey is being conducted in the following manner. All wildlife...
serum samples are first screened against *L. pomona* by the rapid plate test. All positives detected in this manner are then retested by the agglutination lysis method and titered out to an end point. The positives are also tested against a battery of 10 different serotypes to detect a possible reaction. In cases where a para-specific reaction is noted the reacting serotypes are reported along with their concurrent titers. There is much controversy at the present time as to whether the highest titer denotes the infective serotype.

The leptospirosis survey reported in this paper is based primarily upon blood samples obtained at random from the wildlife mammals population in Ohio.

No intent is made in this survey to falsely incriminate animals with blood titers for leptospirosis as shedders or carriers of the disease. Babudieri states that we must distinguish between the temporary shedder and the true carrier of leptospirosis. The shedder classification is a normal process of any infected animal and may only exist for a relative short period of time. The temporary shedder may be a factor in the epidemiological importance as a source of infection, but not nearly as important as the carrier animal which has the potential of shedding organisms over a long period of time.

To date we have many conflicting evaluations of blood titers in establishing what titer indicates infection, carrier, or exposure and recovery. If this data were available many of the questions regarding titers would be answered.

After the statewide survey has been completed we plan to perform a much more intensive wildlife study on a smaller area. A farm experiencing leptospirosis in domestic animals will be surveyed from the wildlife standpoint with emphasis based on the findings of the statewide survey. At that time attempts will be made to carry out isolation studies as well as histopathological examinations.

In conclusion it must be restated that the data presented affords no definite findings but is presented to precipitate further research. The titer findings of the wildlife mammals sampled are only a means of evaluating agglutins and therefore the actual role of the wildlife animal as a carrier is yet to be determined. It is however conclusive that there was a definite difference in agglutin characteristics of the positive and negative sampling and therefore further studies are needed.

REFERENCES

CURRENT DIAGNOSTIC PROBLEMS IN LEPTOSPIROSIS

ERSKINE V. MORSE*

The diagnosis of leptospirosis presents a major problem to the laboratory worker and the veterinary practitioner. *Leptospira pomona* is the etiological agent in approximately 98 percent of the outbreaks in swine and cattle in the United States. However, as in the case of salmonellae, many species or serotypes of leptospiroa are known and some have cellular antigens in common. Serological cross reactions may present a frustrating and complex situation to those engaged in routine laboratory diagnosis.

This discussion of leptospirosis diagnosis can best be delineated by the question-answer method of presentation. Unfortunately, many questions cannot be answered completely and some will require revision as newer knowledge becomes available. The major questions are:

I. What is the prevalence of active *L. pomona* infection in cattle and swine in the United States today?

*L. pomona* infection, according to serological surveys, is or has been present in five to 50 percent of our swine (2, 3). The incidence of active infections can be closely correlated with general husbandry practices, traffic in livestock, population susceptibility, population density, host(s) involved, virulence of the agent, pH of the soil, amount of rainfall, access to surface water, season and presence of reservoir hosts in nature as well as vectors. Since leptospiral serum antibodies will persist in cattle for six years (4, 5), it can be assumed that most of the higher figures for prevalence include past infections. Considering all the conditions influencing the presence of leptospirosis, it is estimated that the probable prevalence of active porcine and bovine infection is two to four percent.

II. What procedure(s) will give an unequivocal diagnosis of leptospirosis?

Isolation of the etiological agent with subsequent antigenic characterization is the only means by which a positive diagnosis can be made. Such laboratory procedures are often adaptable and successful in the diagnosis of human leptospirosis. However, the techniques and number of trained personnel involved, as well as laboratory animals and media required, will not be routinely available in many animal disease diagnostic laboratories.

The recognition of ascending serological titers for a specific leptospira in an acutely ill individual over a period of several weeks will enable a quite accurate diagnosis. Even by this method technical difficulties are encountered. Such diagnosis is not uncommonly accomplished in medical laboratories. In the case of livestock, immediate vaccination and control measures should

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be initiated. The veterinarian and his client will be reluctant to wait several weeks for a definite diagnostic answer. However, it is recommended that the application of the ascending titer procedure be utilized in order to recognize the true incidence of active bovine and porcine leptospirosis.

III. What is the meaning of the *L. pomona* serum titer?

A. Past or present infection with *L. pomona*.

B. Past or present infection with an antigenically related leptospira causing the so-called serological cross reactions. These are dependent upon the phase of infection, the strain of the infecting serotype, and the species of host infected (6). In this respect *Leptospira icterohemorrhagiae* and *Leptospira canicola* appear to present a diagnostic problem in *L. pomona* infections (6, 7, 8). In the vast majority of instances the homologous titer is in a significant range and exceeds that of the heterologous titers.

C. Antigenically related microorganisms other than leptospirae. Rothstein (9) has found significant serological cross reactivity between *L. pomona* and *Shigella dysenteriae*. For the present such reactions should probably be of minimal concern. However, the importance of sharing common antigens with pathogenic and saprophytic microorganisms by leptospiral species may gain in ascendancy.

D. Passive transfer of antibody *in utero*, by colostrum, or administration of antisera. Commercial hog cholera antisera (10, 11), as well as apparently normal bovine and porcine *gamma* globulin preparations (12), have been found to react with *L. pomona* antigens. Under normal dosage levels the biologics would not appear to produce significant levels of titer when properly administered. Placental leptospiral antibody transfer was not observed in cattle (13), swine (14) or sheep (15). *L. pomona* serum titers appear in lambs (16) and pigs (14) nursing infected or recovered dams. Such reactions may persist for as long as four months, but remain at a significant diagnostic level for only approximately one month. It would appear that passive antibody transfer is not confusing the issue in the over-all serodiagnosis of leptospirosis.

E. Vaccination with commercially prepared bacterins. The use of killed vaccines or bacterins produces serum titers which are transitory and of relatively low level. Most workers agree that, for the present, such reactions are not a major source of confusion. However, the use of living, attenuated, vaccinal strains in the future may result in persistent titers which will be diagnostically problematic.

F. Nonspecific reactions. This category includes the positive reactions due to the following: pH of the test reagents, temperature of test incubation and individual differences in sera. The significance or frequency of these reactions is unknown.
IV. What serological titer indicates active infection?

This is not known. A 1:10,000 titer, using a modified agglutination-lysis test, has been suggested to indicate active infection in swine (17). Experience indicates the same condition may exist for cattle. By Stoenner's method, the 1:160 titer is considered to have significance since it indicates probable past or present infection with a specific serotype. This interpretation is applicable for both swine and cattle. Obviously, more concrete information is needed in order for diagnostic facilities to provide the most helpful service. It is felt that such will be forthcoming from the various researchers working on this very problem.

V. What effect does antibiotic therapy have upon the development of titers?

Information is meager. Cholvin (7) observed that dogs naturally infected with *L. canicola* and treated in the acute phase of infection with antibiotics developed titers for the infecting agent, *L. icterohemorrhagiae* and *L. pomona*. The antibody levels were all in the significant range and considerable fluctuation occurred. Definitive serotype diagnosis was not possible unless the history of the hosts was known. Antibody levels, which were insignificant and disappeared within a few weeks, were observed for experimentally infected swine fed low levels of antibiotic at the time of exposure (14). More investigative effort should be directed toward clarification of the situation.

VI. How should serological test reports be rendered to the practicing veterinarian?

The terms "reactor" and "suspect" or "positive" and "negative" are confusing to all concerned. They do not have the same connotation in any two laboratories. The veterinary practitioner and livestock producer consider these terms in the same light as the bovine brucellosis "reactor," e. g. positive at 1:100 or 1:200. Obviously, the situation is not comparable and is leading to consternation and some animosity. This is most unfortunate and only complicates matters. It is suggested that the term "positive" be applied to a specified titer and test, such as "positive at 1:160 (Stoenner's method," or "positive at 1:100 (agglutination-lysis)." Perhaps "negative" should be used only for sera which do not react at any dilution. An explanatory note should accompany the report and delineate what various titers mean in the light of our present knowledge as applied to the test employed. Many state agricultural extension or diagnostic service groups have prepared bulletins explaining the etiology, transmissibility, symptoms, laboratory diagnosis, treatment and control of leptospirosis. Such information is sent to individuals inquiring about possible infection in their herds. It is felt that these communications have improved public relations and given both veterinarians and livestockmen insight into the magnitude of the problem. It is recommended that all states prepare and send out such a pamphlet.
VII. How many serum samples should be submitted?

Few laboratories are equipped to do unlimited testing for leptospirosis. At the Iowa Veterinary Diagnostic Laboratory, during the first eight months of 1958, 21,839 porcine and 32,811 bovine sera were submitted, and during one day 1,200 samples were received (8). The status and magnitude of this problem is shown in Table 1. Many samples are received for testing to rule out leptospirosis as a cause of abortion or because a brucellosis test is also being conducted and the leptospirosis status would be informative. A herd which does not contain animals which react to the leptospirosis test(s) is more easily sold. The leptospirosis negative status is also an asset from the standpoint of advertising. This information is valuable and should be used to promote the interests of livestockmen. The aim of diagnostic laboratories has always been to provide accurate and useful data to the livestock owner and his veterinarian. Unfortunately, if titers are found, the presence of active infection cannot be established without retesting or submitting a rather detailed clinical history. Simple economics, i.e., available personnel, laboratory space, available time and financial support will preclude unlimited testing by most laboratories for the present at least.

Leptospirosis is a herd problem. The diagnosis of active infection can best be made on a herd basis. It is an all or none principle, for if one actively infected animal is present, the entire herd is potentially infected. In Wisconsin during 1952-55, it was suggested that serum samples from large herds along with a complete clinical history be submitted somewhat as follows: a few sera from cattle which aborted, some from those currently sick, and a few sera from the recovered cases. Titers of 1:10,000 or higher, using the modified agglutination-lysis test (17), were indicative of active leptospirosis if substantiated by clinical history typical of leptospirosis. The results of most testing procedures can be put into more concrete and meaningful terms if the clinical status of a herd is known.

### TABLE 1

*Approximate Number of Serological Tests for Leptospirosis Conducted Monthly in the United States*<sup>*</sup>

<table>
<thead>
<tr>
<th>Animal Species</th>
<th>Total Reported</th>
<th>Median Number For Laboratories</th>
<th>Average Number For Laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>29,973</td>
<td>200</td>
<td>575</td>
</tr>
<tr>
<td>Porcine</td>
<td>8,576</td>
<td>25</td>
<td>197</td>
</tr>
<tr>
<td>Equine</td>
<td>40</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Canine</td>
<td>390</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Human</td>
<td>1,922</td>
<td>10</td>
<td>119</td>
</tr>
</tbody>
</table>

VIII. What serological test should be used?

Unfortunately, the most accurate tests are expensive with respect to personnel, time, space and technique; in addition, there may be an element of human health hazard. These tests are excellent research tools. A plate-type test would seem to offer diagnostic laboratories greater adaptability and versatility. The Stoenner plate test method is widely used by laboratories testing animal sera. It is agreed that a standard test, technique and method of reporting results of the test are needed. Careful evaluation of the situations in both large and small diagnostic laboratories is required before final standardization of the procedures can be made.

Various groups, e. g., diagnosticians, Federal agencies, universities and commercial biological firms are devoting considerable thought, time, effort and money to the many perplexing facets of leptospirosis serodiagnosis. Such investigations will help accomplish what is desired by the practicing veterinarian and the livestock industry. Progress has been made and will continue to be made through concerted research efforts.

REFERENCES


DISCUSSION LEPTOSPIROSIS

PRESIDENT MILLIGAN: Has anyone any questions for Doctor Morse on this subject?

DOCTOR BAKER: I could add a little bit on this finding of Doctor Goldstein's.

About three years ago, in New York State, Doctor Marshall made a survey of about 400 deer and about 100 foxes. There were 400 deer with an incidence of leptospira of about 25 percent. In foxes the findings were about 25 percent.

A survey of the fox sera for the presence of antibodies yielded that more than half of them had distemper antibodies. On infectious hepatitis, 10 percent had antibodies. In 500 dogs in New York State the incidence of leptospira ran from 10 to 25 percent.

I think if you were to try to draw a conclusion from this data, it is an indication that foxes are truly a reservoir of disease.

Now a question for Doctor Morse. Has any use been made of urine antibodies as an indication of the carrier's state?

DOCTOR MORSE: Doctor Baker, I believe a gentleman in Texas, E. L. Nowicki, has made somewhat of a survey on that. I believe, too, that perhaps VanderHoden has looked into these things.

Now, these are just things I am recalling from memory, so I may be mistaken on them.

With regard to the carrier's state, it appears that at least in our experience the high serological titer in experimental animals with L. pomona occurs in about three weeks. The urine antibody titer peak occurs in about three months, and at about that time, at least in our experience, the incidence of carriers was pretty well down to its lowest level. In other words, we would be getting pretty close to the zero point in our experimental work.

I do not know whether that is any help to you or not.

DOCTOR BAKER: I just thought that this round cell infiltration we get probably represents lymphocytes which migrate into the kidney in response to the presence of the leptospira, since all of the evidence indicates that these round cells are the antibody producers. I still think it might be a useful means of indicating the carrier's state.
DOCTOR MORSE: These reactions which one does see on the histopathological side—one does get a lot of plasma cells, as you mentioned, and they remain there for periods of time after the infection, the active infection, has ceased, in our experience. When there are no longer leptospira there, we had many evidences of histopathologic change and evidence of activity.

DOCTOR BAKER: This return to normal seems to occur about two to three weeks or a month after the disappearance of the leptospira.

So I still think it might be explored as a useful means of indicating a carrier condition.

DOCTOR MORSE: It may have possibilities.

DOCTOR SCHEIDY: Scheidy is the name, of Pennsylvania. I would like to ask Doctor Goldstein a question. The mice to which he referred—I am wondering whether they were field mice that he examined.

DOCTOR GOLDSTEIN: For the most part they were field mice, Doctor. I can give you the breakdown on the species. Microtis paramiscus and Mus musculus are the species of mice.

DOCTOR ROTHE: Roth of Louisiana.

We had the occasion to examine 100 deer samples by agglutination lysis and we found approximately 25 percent of the deer in northern Louisiana positive.

FROM THE FLOOR: Considering the short life a wild deer must have, I wonder what a one to 300 agglutination lysis titer really means.

DOCTOR GOLDSTEIN: In my opinion, I do not think you can place any significance on a one to 300 titer. That is just my personal opinion. In my opinion, the titers that go from 800 to 3,200 are far more significant there than the one to 300. We did not know whether these animals did have a high titer at some time and were just showing a residual titer of one to 300. But I agree with you wholeheartedly that a one to 300 titer, particularly in a wild deer, is of little or no significance when you are trying to evaluate a disease problem.
As members of the United States Livestock Sanitary Association are probably aware, this is only the second year that the Committee on leptospirosis has existed. The first report consisted primarily of a review of work on the disease up to that time without going into details regarding any one phase of the subject. The Committee recognizes that there are still a number of controversial areas regarding leptospirosis, some of which fairly specific remarks may be made, while others must frankly await further research.

Part of the confusion that exists regarding leptospirosis stems from the fact that all too frequently veterinarians and livestock officials compare leptospirosis in cattle too closely to brucellosis. While similarities do exist, there are so many differences, including the fact that there are a number of different leptospira serotypes that may create confusion and a wide variety of possible carrier hosts, including both domestic and wild animals, so that specific control measures developed for brucellosis cannot readily apply to this disease.

One of the problems that has created considerable controversy is the matter of serological diagnosis of leptospira infection. Too many men in veterinary medicine look on laboratory serological tests with an over generous amount of wishful thinking, not realizing the limitations of a test for antibodies for any disease. All the laboratory can do is provide information, on whether the animals tested have or have not experienced a leptospiral infection. The diagnosis of the disease must depend on the clinical signs of illness and the history of the herd with the serology from the laboratory used as an aid in arriving at a decision. Ideally for laboratory use acute and convalescent samples should be collected and accurately speaking this is the only sure way of confirming by serological means that the disease observed was leptospirosis. But it is recognized that, in the control of a herd outbreak of this disease, it is difficult to wait 10 days or so for a second set of samples. Accordingly we have tried here to suggest a procedure for control of the disease in a bovine herd with history and clinical signs of illness suggestive of this type infection. Note we have used the word suggest rather than recommend because there are many facets of this disease that varies in different situations that cannot be anticipated in a brief report such as this. Thus the following points covered are intended as a guide for the men who are actually faced with the specific problem in the field.
LEPTOSPIROSIS

1. Ideally serum samples should be obtained from at least five and preferably 10 animals from the herd. Of these samples some should be from cases that history shows have recently recovered from infection (say 7-10 days), some from acutely ill animals being observed on the day of visit, and some from cattle with no evidence of having had the disease. If serums from animal recently recovered have a high titer and the proper history and clinical signs are present, then a diagnosis of leptospirosis is indicated.

2. Under usual circumstances all animals not having had the disease clinically are vaccinated as soon as the diagnosis is made.

3. All cattle introduced into this herd for the next six months should be vaccinated two weeks prior to admission.

4. Revaccination on an annual basis should be considered if the possibilities of re-exposure exists.

5. In situations where abortions are the major problem, efforts should be made to eliminate both by medical examination and laboratory tests other factors that may produce abortion such as brucellosis, vibrioses, virus diarrhea, and infectious bovine rhinotrocheitis. It should be emphasized at this point that leptospirosis produces abortions 10-28 days following infection, thus cows will usually have a positive serology at the time of abortion. Furthermore, one can expect some abortions for at least four weeks following vaccination, since it is likely that some animals will be incubating the disease at the time of vaccine injection.

In herds that have not had leptospirosis, the following suggestions can be made:

1. In all newly assembled herds vaccination should take place the day of introduction of the animals.

2. If an established herd is to be kept free of infection without vaccination, all newly purchased cattle should be serologically tested and the introduction of positive animals be considered on the height of antibody titer and the history of the animal in respect to possible previous vaccination experience.

3. If replacements are not tested then introductions should be only made into vaccinated herds.

4. One situation that frequently is responsible for the spread of leptospirosis is the show circuit. It is felt that it would be more feasible for show and fairs, rather than require a blood test before showing, to develop a program whereby only animals vaccinated for leptospirosis just prior to the show season would be allowed to be exhibited.

5. It should be re-emphasized in maintaining a bovine herd free of infection that the movement of cattle is not the only way leptosporal infection can be introduced. Swine offer a serious problem to the cattle raiser. Deer, opposums, skunks, dogs and very likely other animals can carry L. pomona organisms. Furthermore, in the spread of infection, it should also be emphasized that vaccination itself has absolutely no effect on the renal carrier condition of cattle infected at the time of vaccination.
In consideration of the problem in swine, suggestions for control are similar to those made for cattle. It should be noted that there are usually no signs of illness in swine other than abortion or the birth of weak and dying pigs, which usually occurs in the last two weeks of the gestation period. The following points can be made:

1. Diagnosis should be made as described for cattle.
2. All swine on the farm should be vaccinated as soon as a diagnosis is made.
3. Ideally, where infection is likely to occur, all sows should be vaccinated just prior to breeding, since it is not known for sure when, during the gestation period, infection injures the fetus.
4. Evidence indicates that considerable leptospiral infection is brought about by the introduction of infected boars. Only serologically negative boars should be introduced into a herd, or if necessary, serologically positive animals must be used, they should be isolated and given an adequate course of a suitable antibiotic, to attempt to eliminate the renal carrier state.

One reason for not making stronger recommendations for a specific control program, is the lack of a uniform serological test for use throughout the country. Differences in type of test, methods of using the same test, source of antigen and strains of organisms, exist from laboratory to laboratory. It is recognized that research may well devise a test for use in the future that will replace all present methods. In this regard however, this Committee in cooperation with interested agencies, associations and diagnostic laboratories, is exploring the possibilities of setting up a cooperative study in an effort to obtain information that may lead to standard serological procedures.

It has been urged by a number of individuals that a state wide control program be outlined by this Committee. Until uniform serological procedures are adopted it is far premature to outline any such program. The question regarding antibodies in cattle that react to *L. serjoe* is another reason for our hesitation in outlining a specific control program. This situation lacks explanation and the answer must await further research. For the time being we must consider that the primary pathogenic leptospira for swine and cattle is *L. pomona*.

Further research is needed to develop vaccines that will provide a more rapid and longer lasting immunity; the development of methods of controlling leptospirosis by combinations of immunization and treatment procedures that are within the limits of economic feasibility; as well as the development of improved laboratory methodology. Considerable amount of research is being conducted at this time. Other investigations will be initiated this coming year. We recommend that the work of this Committee be continued and by next year we hope to be able to bring to your attention some answers to currently unexplained problems.
THE RELATIONSHIP OF INFECTIOUS PUSTULAR VULVOVAGINITIS
VIRUS TO INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS

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Infectious pustular vulvovaginitis (IPV) is a disease of cattle characterized,
as the name suggests, by the formation of pustules on the mucosal surface of
the vulva and vagina. The veterinary literature of both Europe and America
contains numerous reports (1, 3, 8, 9, 13, 15, 17, 18) about this disease since
it was first reported from Germany in 1886 (20). A filterable virus was
shown to be the cause in 1928 (14) but it was not until 1958 that application
of tissue culture methods successfully yielded a cytopathogenic virus (7, 9)
which permitted further study, especially serological tests (9).

In a brief survey of incidence for IPV among 53 herds in New York State,
serums of five cows from each herd were pooled and each pool tested for
neutralizing antibodies. About 15 percent of these herds were positive and
this indicated that one or more of the five animals had been infected pre-
nviously (4). This was puzzling, since IPV, as such, is sporadic and clinical
observations alone would not support this high incidence.

Infectious bovine rhinotracheitis (IBR) is a disease of cattle characterized,
as the name suggests, by inflammation of the upper respiratory tract. It
was first recognized in 1954 as a clinical entity of cattle in the western
United States (11, 12, 16). By use of tissue culture methods, a cytopathogenic
virus was isolated and proven to be the etiological agent (10). When sero-
logical tests were applied to 43 herds of cattle in New York State, about
12 percent were positive, indicating that this virus also infected cattle in
the east. A serum sample that had been procured from a calf in New Jersey
in 1941 also proved positive, a further indication that IBR virus had actually
been in eastern cattle for a long time (5). Yet no clinical syndrome which
compared with the one described in western cattle was recognized at the
time in the east. This finding also was puzzling.

Both IBR and IPV viruses produce a similar type of generalized illness
in that each invokes a febrile reaction. Local effects are so different, it
seemed inconceivable that the same virus was responsible for both diseases.
It was surprising, therefore, when a test showed that serum from an ani-
mal which had recovered from IBR neutralized the IPV virus. This was
encouraging, if true, for at last the failure to correlate clinical disease with
incidence of neutralizing antibodies might have an explanation. Accordingly,

* Collaboration from the Department of Pathology and Bacteriology, New York State
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the following comparisons were made in an attempt to clarify the relationship between IBR and IPV viruses.

- Tissue culture features
- Pathogenicity for calves
- Pathological findings
- Cross-immunity tests in calves
- Reciprocal serological tests

**COMPARISON OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS WITH INFECTIOUS PUSTULAR VULVOVAGINITIS VIRUS**

1) *Tissue Culture Features.* All isolations of IPV virus which had been made from affected dairy cattle in New York State produced complete degeneration in tissue-cultured bovine kidney cells within 48 hours after inoculation with 0.2 ml of undiluted virus (9). Undiluted IBR virus also produced complete degeneration within 48 hours (10). Greig et al. also had isolated a virus by tissue culture from the vaginal exudate of a Canadian cow which showed a pustular vullovaginitis (7). It, too, behaved like the other viruses.

Titrations of fluids from tissue cultures infected with IBR virus and with IPV virus showed similar titers (6). Furthermore, the character of degeneration was indistinguishable for each virus. Small, clear areas in the cell sheet appeared which were surrounded by enlarged, rounded cells with granular cytoplasm. These cells soon separated from neighboring ones and clustered together, resembling grapes on a vine. The process gradually diffused throughout the culture. Eventually the cells became shrunken and then detached from the glass. Cheatham and Crandall (2) observed that IBR virus produced intranuclear inclusion bodies in tissue-cultured bovine kidney cells. Similar intranuclear inclusion bodies were observed in tissue-cultured bovine kidney cells after inoculation with IPV virus (9). (Figs. 1 and 2).
THE RELATIONSHIP OF INFECTIONOUS VIRUS

Comparison of infectious pustular vaginitis (IPV) with infectious bovine rhinotracheitis (IBR). Fig. 2 courtesy R. A. Crandall, Armed Forces Inst. of Pathology; Figs. 1, 3 and 5 Cornell Vet.
(2) **Pathogenicity for Calves.** In comparing the effects of these viruses in cattle (6), the plan of study consisted of placing IBR virus vulvovaginally in five heifers and IPV virus in the nasal passages of five bull calves. Observations for signs of illness were made daily. Material for virus isolation and titration was obtained from the nasal passages on the third, fourth and fifth day after inoculation. Exudate for virus titration was collected from the vagina on the third day after vulvovaginal inoculation with IBR virus. After three weeks, each animal was tested for immunity against the same virus originally inoculated and, finally after a further period of three weeks, each calf was given the other virus for comparison, i.e., if given IBR first, then the test virus for comparison was IPV and vice versa. Serums were procured before the initial inoculation and again three weeks later for reciprocal serological studies. In addition, biopsies were taken from the vulva of two heifers inoculated with IBR virus for comparison of histological changes with those produced by IPV virus.

Each of the five calves inoculated vulvovaginaIly with IBR virus showed signs of illness and lesions that were indistinguishable from those produced by IPV virus. Two to three days after inoculation, numerous pustules and a mucoid exudate were observed on the mucosa of the vulva and vagina. One day later the mucoid exudate became purulent. A rise in temperature accompanied these changes which lasted for two to five days. There was little difference in the lesions until the seventh day, when healing began. As a rule, uneventful healing occurred. Virus was isolated from vaginal exudate obtained three days after inoculation, and virus titers ranged from TCID$_{50}$ 10$^{4.8}$ to 10$^{6.5}$. Except for one animal, virus was not isolated from nasal passages. In this instance, virus was isolated only from swabs taken on the fifth day after inoculation. This animal had a titer of TCID$_{50}$ 10$^{2.4}$ in contrast to titers above TCID$_{50}$ 10$^{6}$ which were found after nasal inoculation of IBR virus (5).

Most calves inoculated intranasally with IPV virus showed a febrile reaction two to three days after inoculation that lasted one to four days. The animals were depressed during the course of fever. When IBR was given calves, a nasal discharge usually occurred; in contrast, no nasal discharge was observed in those calves given IPV intranasally. Nasal swabs taken on the third day after inoculation and titrated for virus content had titers of approximately 10$^{4.5}$ TCID$_{50}$ or less. This was at least 100-fold less virus than usually was found at this time in calves inoculated with IBR virus.

(3) **Pathological Findings.** Biopsies that were taken from the vulva of the two heifers given IBR virus showed histological changes (6) indistinguishable from those produced by IPV virus (9). Pustules appeared over lymphatic follicles with a predominance of neutrophiles occupying the area of epithelial necrosis. There was a diffuse infiltration of lymphocytes in the connective tissue (Figs. 5 and 6). Intranuclear inclusion bodies were produced by IBR virus in epithelial cells which resembled those seen in calves inoculated with IPV virus (Figs. 3 and 4).
THE RELATIONSHIP OF INFECTIOUS VIRUS

(4) Cross-Immunity Tests in Calves. (6) Results of the cross-immunity tests are presented in Table I.

TABLE I
Cross-Immunity Tests Between Infectious Bovine Rhinotracheitis (IBR) and Infectious Pustular Vulvovaginitis (IPV) Viruses in Cattle

<table>
<thead>
<tr>
<th>Immunizing Virus</th>
<th>Route of Inoculation</th>
<th>IPV - Test Virus*</th>
<th>IBR</th>
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</thead>
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<tr>
<td>IPV</td>
<td>Nasal</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>IBR</td>
<td>Vulvovaginal</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

* IPV was given vulvovaginally and IBR intranasally in tests for cross-immunity. Denominator indicates number of animals tested and numerator those showing signs of illness.

It can be seen that IPV virus given intranasally immunized against itself and also against IBR virus when it was inoculated as test virus. In a reciprocal manner, IBR immunized against itself when inoculated vulvovaginally and also against IPV test virus.

(5) Reciprocal Neutralization Tests. (6) Serums on all calves were taken prior to the first inoculation and again three weeks later. In a similar manner, serum samples were taken from five heifers that had been inoculated vulvovaginally with IPV virus and from five calves that had been inoculated intranasally with IBR virus. All serums were tested for neutralizing antibodies against both IBR virus and against IPV virus in complete reciprocal tests.

As shown in Table II, neutralizing antibodies for both IBR and IPV viruses developed in the serums of all calves whatever virus had been inoculated.

TABLE II
Reciprocal Neutralization Tests Between Infectious Pustular Vulvovaginitis (IPV) Virus and Infectious Bovine Rhinotracheitis (IBR) Virus*

<table>
<thead>
<tr>
<th>Immunizing Virus</th>
<th>Route of Inoculation</th>
<th>Before Inoc. IPV</th>
<th>After Inoc. IPV</th>
<th>Before Inoc. IBR</th>
<th>After Inoc. IBR</th>
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<td>IBR</td>
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<td>5/5</td>
<td>0/5</td>
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</tr>
<tr>
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<td>5/5</td>
<td>0/5</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>Vulvovaginal</td>
<td>0/5</td>
<td>5/5</td>
<td>0/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

† Denominator indicates number of serums tested; numerator indicates number of serums having neutralizing antibodies.
DISCUSSION

Certain properties of IBR and IPV were studied comparatively. The findings are summarized in Table III.

**TABLE III**

*Comparison of Infectious Bovine Rhinotracheitis Virus (IBR) With Infectious Pustular Vulvovaginitis Virus (IPV)*

<table>
<thead>
<tr>
<th>Features Studied</th>
<th>IBR</th>
<th>Findings</th>
<th>IPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Tissue culture</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Effect on cells</td>
<td>Cytopathogenic</td>
<td>Cytopathogenic</td>
<td></td>
</tr>
<tr>
<td>Inclusion bodies</td>
<td>Intranuclear</td>
<td>Intranuclear</td>
<td></td>
</tr>
<tr>
<td>2. Clinical features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Pathological features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vulvovaginal inoc.</td>
<td>Similar to IPV.</td>
<td>Similar to IBR.</td>
<td></td>
</tr>
<tr>
<td>Inclusion bodies</td>
<td>Intranuclear.</td>
<td>Intranuclear.</td>
<td></td>
</tr>
<tr>
<td>4. Cross-immunity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal inoc.</td>
<td>Immunizes against itself.</td>
<td>Immunizes against IBR.</td>
<td></td>
</tr>
<tr>
<td>Vulvovaginal inoc.</td>
<td>Immunizes against IPV.</td>
<td>Immunizes against itself.</td>
<td></td>
</tr>
<tr>
<td>5. Reciprocal serology</td>
<td></td>
<td>Neutralizes itself and IPV.</td>
<td>Neutralizes itself and IBR.</td>
</tr>
</tbody>
</table>

The ability of IBR virus and IPV virus to produce similar lesions, immunize against themselves and each other, and similarity by reciprocal neutralization tests indicates these two viruses are indeed the same. The only differences found were lack of nasal discharges in calves inoculated with IPV virus and the correlated result of less virus developing in the nasal passages. These findings suggest that the IPV-IBR virus modifies in virulence for nasal tissues after residence in the vulvovaginal tract. While only one test has been made, it was noted in this instance that IPV virus inoculated vulvovaginally transmitted by contact exposure to another heifer. An increased temperature was the only sign of illness noted. Neither nasal discharge nor reproductive tract lesions could be seen. It may be that virus maintains itself in New York State cattle by transfers in this heretofore unrecognized fashion, thus accounting for failure to correlate the incidence of 15 percent infected herds with either respiratory disease or vaginitis.

Obviously, further studies must be made in a full exploration of the pathogenic potential of this versatile virus. The suggestions that this virus causes infertility (19) and abortion must be investigated and certainly its effect on newborn calves must be determined.

At this time, no attempt will be made to propose any revision of nomenclature or to discuss such details as prior claims. Properly this should wait confirmation of the findings reported here and further studies on the clinical entities associated with this virus.
THE RELATIONSHIP OF INFECTIOUS VIRUS

SUMMARY

Comparison of infectious pustular vulvovaginitis virus with infectious bovine rhinotracheitis virus showed similar effects in tissue culture. Both viruses were cytopathogenic, attained the same virus titers and produced intranuclear inclusion bodies in tissue-cultured bovine kidney cells. When inoculated into cattle, each virus produced similar clinical and pathological features. Each virus immunized cattle against itself and against the other and, in reciprocal neutralization tests, sera from cattle recovered from either virus neutralized both viruses. These features indicate that IBR virus and IPV virus are identical. Since the clinical entities are quite different, the versatility of this IPV-IBR virus is emphasized. In view of this, it is suggested that nomenclature revision be postponed until the full pathogenic potential is explored further.

REFERENCES

SPORADIC BOVINE ENCEPHALOMYELITIS
IN CALIFORNIA

J. B. ENRIGHT, PH.D., W. W. SADLER, D.V.M., M.P.H.
AND E. A. ROBINSON, B.ED.*

The disease referred to today as Sporadic Bovine Encephalomyelitis (SBE) was first reported by McNutt (1) in 1940. In the study of an outbreak of disease occurring in eastern Iowa, he recognized that the syndrome was previously unreported and called it Buss Disease. Later, McNutt (3, 4, 6, 7) and Stearns and McNutt (5) reported on further studies characterizing this disease. In the meantime Boughton (2) in 1941 reported his encounter with the disease in Texas and his descriptions of the symptoms and pathology were similar to those reported by McNutt.

During the period from 1942 to 1951, the disease remained unreported in the literature. In 1951, Harshfield (8) described an outbreak of sporadic bovine encephalomyelitis recognized for the first time in South Dakota. In the next two years extensive investigations into the epizootiology, etiology, pathogenesis and natural history of the disease were reported by a group including Wenner, Harshfield, Menges, Chang and Carter. (9, 10, 11, 12, 13, 14).

Excellent descriptions of the symptomatology, pathology and epizootiology are to be found in the studies referred to above. It is the purpose of this communication to present our experiences with this disease in California and to describe some ancillary observations of interest.

ETIOLOGY

Sporadic bovine encephalomyelitis is caused by a virus antigenically related to the psittacosis-lymphogranuloma group. Comparative studies using strains of the virus isolated in California and a strain isolated by McNutt in Iowa did not reveal differences in antigenicity.

Complement-fixing antigen was made using McNutt’s strain of the virus according to the method of Topping and Shepard (18). This antigen fixed complement equally well in the presence of sera from cases of the disease as it occurs in California, as well as in the presence of the sera of animals immunized against strains of the virus isolated elsewhere. Complement was also fixed in the presence of sera containing antibodies against the virus of sheep pneumonitis. Complement fixation was not always obtained in the presence of antisera to the 6BC strain of the psittacosis virus nor in the presence of the serum from a recovered human case of ornithosis of turkey origin. However antigens made from the psittacosis virus fixed complement in the presence of antibodies to the virus of sporadic bovine encephalomyelitis contained in bovine sera.

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127
It may be assumed that the virus of SBE contains common antigens with other members of the psittacosis-lymphogranuloma group of viruses and will cross react with them. It therefore becomes necessary to interpret serological findings with this fact in mind.

No evidence was obtained indicating an antigenic relationship with the viruses of lymphocytic choriomeningitis, western equine encephalomyelitis, St. Louis encephalitis or Japanese B encephalitis. In addition, the antigen made from the McNutt strain of the virus of sporadic bovine encephalomyelitis would not fix complement in the presence of antibodies to the rickettsiae of Q fever, to *Listeria monocytogenes* or to the brucella and pleuropneumonia groups of organisms.

**EPIZOOTIOLOGY**

In our experience the disease in the bovine usually affects calves, but is occasionally seen in older animals. It is usually mild with a low case-fatality rate. In only one instance have we observed the acute disease with convulsions and opisthotony. This occurred in a feed-lot operation and involved four animals all of which died. In the milder form of SBE the animal becomes noticeably ill rather suddenly. The duration of illness is quite variable. Recovery may be quite rapid or the animal may remain in poor condition for an extended period.

On a herd basis the epizootic may develop rapidly and completely subside in one or two months or sporadic cases of the disease may occur for several months following the initial outbreak. Serologic evidence indicates that many more inapparent than apparent infections occur. In one large dairy herd an outbreak involving approximately 100 calves developed during a period of three weeks and abruptly subsided. During the next two years examination of 734 calves born on the premises revealed 48 (6.4 percent) had developed antibodies to this group of viruses by the time they were weaned, although no frank case of the disease was identified.

Outbreaks of SBE have generally been confined to the ranch upon which they occur and seldom spread to neighboring ranches. The transfer of normal, recovered, and serologically positive calves after weaning from the dairy herd referred to above to another ranch, did not result in the introduction of the disease. In addition, of 461 animals returning to the home ranch as springing heifers, only five had complement-fixing antibody titers of 1:16. Included in the 461 returning animals were 164 animals born and weaned on the home ranch during or immediately after the outbreak of disease.

In another outbreak of SBE involving a large beef operation, frank cases of the disease occurred in the younger animals. The outbreak developed more slowly than the one on the dairy farm, but involved more animals. It is estimated that this epizootic reached its peak in two to three months and then subsided only to reappear in the young animals born the following year. The disease then seemed to become enzootic with sporadic cases being identified on a clinical and serological basis over the next few years.
Isolations of the virus or a combination of clinical and serological evidence has indicated the presence of the virus of SBE in 12 counties in California. These counties are located in widely separated areas and include mountain, coastal and valley environments with wide varieties of temperature and other climatic and topographical features. Outbreaks have occurred in both beef and dairy breeds and under many different conditions encompassing many different kinds of husbandry and nutrition. The disease has been seen in susceptibles of both sexes. No particular seasonal predominance has been evident.

Little is known concerning the mode of transmission from an intermediate host to the bovine except that contact or close association with an infected animal seems to be necessary. McNutt (3) reported transmission of the infectious agent to calves by feeding milk containing the organism. We infused the udder by way of the teat canal of two lactating cows. Each cow was nursing a calf at the time. Isolations of the virus could not be made from the milk of the first cow on the third day, but could be made on the seventh day after infusion. Her milk remained "positive" for at least 18 days at which time she developed an acute but transitory mastitis. The next milk sample taken 30 days after infusion was negative. Other than the mastitis this animal did not show a temperature rise nor exhibit any other signs of illness although complement-fixing antibodies appeared in her serum and reached their maximum titer in six weeks. The calf she was nursing exhibited no temperature rise nor any other signs of illness, but developed complement-fixing antibodies four weeks after exposure. The other cow developed a significant rise in temperature 11 days after infusion which was sustained for three days and then abruptly returned to normal. Otherwise this animal exhibited no signs of illness. Antibodies first appeared in her serum 14 days after exposure, reached their maximum on the twenty-eighth day, and at the end of three months had returned to an insignificant level. The virus was isolated from her milk on the seventeenth and thirtieth day, but was negative on the sixty-first day after infusion. The calf she was nursing developed a significant rise in temperature 14 days after exposure and exhibited other symptoms of the disease on the fifteenth day post-exposure. This calf developed antibodies to the virus which reached their maximum titer by the thirty-fifth day after exposure. This antibody level was maintained for eight weeks and returned to an insignificant level by the end of the third month.

These experiments indicate that the bovine can be infected by way of the teat canal under experimental conditions and that the suckling calf may be infected by the oral route. However, we have not been successful in demonstrating this mode of transmission under field conditions in which natural infection of calves has occurred.
SYMPTOMS

After an incubation period of approximately two weeks the onset of symptoms in the young susceptible animal is sudden without a noticeable prodrome. A high temperature (105° F. to 107° F.) anorexia and depression are usually the first signs noticed. Along with this may be a moderately increased flow of a clear watery saliva and sometimes diarrhea. We have observed that the animal from the beginning exhibits a reluctance to stand and when forced to run tends to avoid bending the legs as much as possible. This results in a stiff, unsteady gait and a tendency to trip over small objects. The animal will often knuckle over which seems to result from difficulty in quickly and correctly placing the feet to recover from tripping and stumbling rather than a loss of balance due to central nervous system origin. Examination of the stifle and hock joints at this time may reveal swelling and when squeezed the animal may exhibit discomfort. The signs of tenderness in these joints disappear within a week, but the swelling and stiffness remain for longer periods of time. The illness may progress and depression, apathy and stupor increase until fatal termination, but usually the animal recovers. Recovery may be prompt and complete especially following the milder forms of the disease, but in other instances animals have been observed in which stiffness of joints, failure to attain normal weight and a general poor condition has persisted for months, resulting in appreciable economic loss. Nervousness and excitability have not been observed except in the four cases occurring in a feed lot. These four animals were first noticed because they were thin and refused to eat. On the next day they exhibited irritability and would charge at moving objects and soon developed a tendency to circle in either direction with a tendency to twist the head to one side or the other. Later, weakness in the hind quarters developed and convulsions occurred in which opisthotony was pronounced, after which the animal usually died. The brain of one of these animals was examined and the virus of SBE isolated. There were no negri bodies and attempted isolation of the rabies virus or bacteria was negative.

PATHOLOGY*

Lesions in the central nervous system have not been observed except for a suggestion of hyperemia in some cases. In acute cases terminating fatally, little evidence of disturbed physiology is noted in the peritoneal and pleural cavities except for the presence of variable amounts of a yellowish fluid having a tendency to clot on standing. In cases of longer duration a serofibrinous peritonitis and pleuritis may be observed. In some instances, the pericardial sac may contain excessive fluid with fibrin deposited on the serous surfaces. The spleen may be somewhat enlarged due to engorgement with blood, and the liver may be yellowish-brown in color. The kidneys have

* We are indebted to Dr. Donald R. Cordy, Chairman, Department Pathology, School Veterinary Medicine for this descriptive account of the pathology of SBE.
applied normal. Examination of the carpal and tarsal joints have revealed a moderate excess of synovial fluid and on occasion this fluid has contained extensive deposits of fibrin.

Histologically, evidence of hyperemia and edema is usually observed in the brain together with perivascular cuffing and parenchymal foci of infiltrating cells. The cuffs are made up of polymorphonuclear and monocellular cells. The number of these latter cells is greater than the number of polymorphonuclear cells. The foci are composed of necrotic debris, polymorphonuclear cells and large mononuclear cells (actually many are gitterzellen filled with debris). While the cuffs resemble those seen in listeriosis, the foci tend to show more gitterzellen and fewer polymorphonuclear cells than in that disease. Elsewhere in the body, except for evidence of hyperemia and a more or less extensive serofibrinous exudation, the histopathologic findings are not remarkable.

**DIAGNOSIS**

It is the combination of symptoms referrable to the central nervous system together with the pathological findings, expressed mainly by a serofibrinous peritonitis, that constitute the elements of differential diagnosis. Either alone is not definitive. It has become generally accepted that this disease may be characterized by these findings.

**ANTIBIOTICS**

Experiments designed to determine the effect of antibiotics on the virus were conducted. These experiments were designed to determine the proper use of antibiotics in attempts to isolate the virus and to indicate a possible rationale for therapy in the naturally-occurring disease.

The antibiotics under test in different concentrations was mixed with the virus and after 30 minutes at room temperature the mixture was inoculated into the yolk sac of embryonating chicken eggs. The survival of these embryos was compared to that of embryos receiving only the virus. It was found that auremycin, terramycin, a chromycin, chloromycetin and penicillin were equally effective, enabling 85 to 90 percent of the embryos to survive. Erythromycin enabled 100 percent while dihydrostreptomycin enabled only 10 to 20 percent of the embryos to survive. In each trial all the control embryos died not longer than five days after inoculation with the virus. The results of this experiment agree in general with those of Wenner et al. (12) and with those of Price and Hardy (16) except that in our experiments erythromycin was relatively more effective than the other antibiotics tested. These findings permit the use of dihydrostreptomycin in isolation procedures and suggest antibiotic therapy to be of value in the naturally-occurring disease.
SOME ANCILLARY OBSERVATIONS

In addition to the clinical symptoms referrable to the central nervous system, information suggesting that this virus may be involved in other diseases of the bovine has been collected, although the evidence is not sufficient to be conclusive.

In 1954, a veterinarian was called by the owner of a dairy herd in central California. The owner complained of a reduction of milk flow in many of his animals and a cessation of flow in some. The veterinarian was puzzled by the outbreak and collected blood and milk samples from six animals showing early signs of recovery. The serum of five of the six animals was positive by complement fixation in titers of 1:64 to 1:256. Virus was not isolated from the milk samples. No evidence of involvement of the central nervous system was exhibited by any animal in the herd. This type of episode has not occurred on this ranch since.

The serum from four cows in a beef herd in which 300 abortions had occurred in two months was obtained. These abortions were characterized by their occurrence during the later stages of pregnancy. A seasonal incidence is seen in that these abortions occurred during the summer months, but whether this connotes a breeding, nutritional, husbandry or environmental factor is not known. The cows under study were bled two and three days before they aborted and one and three months later. All pre-abortion bloods were negative by complement fixation for antibodies to the virus of SBE. The serum from three of the four animals collected one month after abortion were positive, while serum samples collected at the end of three months were negative. The disappearance of antibodies after three months, or their reduction to insignificant levels, was seen in our experimentally infected dairy cows and calves and has been repeatedly observed in guinea pigs. Attempts to isolate the virus from the aborted fetus and placental membranes from one of these cows were unsuccessful.

One of our experimental calves infected by the oral route, while feeding on her mother whose mammary gland was infected through infusion by way of the teat canal, was observed for three years following infection. During this time this animal aborted two calves at almost full term. Attempts to isolate the virus of SBE from the tissues and membranes of one of these aborted calves were unsuccessful.

In further studies of this problem in an attempt to identify this agent or a member of the group to which it belongs with abortions in the bovine, a survey of 18 herds in which this problem had occurred for several years was made. These were all beef herds located in the foothills surrounding the great central valley of California. The survey consisted of confirmation of the existence of the problem and collection of blood samples by several veterinarians connected either with the University of California or with the California State Department of Agriculture. The blood samples were collected at various times throughout the year without regard to the seasonal incidence of abortion or to the history of pregnancy of the individual cow.
that was bled. For the most part these bloods were collected at the convenience of the veterinarian who was engaged in other duties. While a survey of this type can offer very little possibility of definitive results, it was hoped that enough information might be obtained to justify more intensive study. Two hundred and thirty-nine animals from six different herds were bled and 39 of the 239 had complement-fixing titers of 1:16 or better while 116 animals from 11 other herds contained no demonstrable antibody in their serum. In the herds in which positive serological findings were made an average of about 18 percent of the samples were positive.

In 1955, Schoop and Kauker (17) described an outbreak in a State-owned dairy herd in Germany characterized by abortions and cessation of milk flow. This outbreak followed the introduction of two cows imported from Holland into the herd. Serological findings and studies of several viral isolates indicated that the causative agent was a virus belonging to the psittacosis-lymphogranuloma group of viruses.

The definite findings reported by Schoop and Kauker in Germany, together with the implications of four observations on the abortion problem in the bovine, suggest that a more intensive study of the relationship of the psittacosis-lymphogranuloma group of viruses to bovine abortion should be undertaken.

INFECTION OF HUMANS

In 1953, during a multiphasic health survey conducted jointly by the Department of Public Health, School of Veterinary Medicine, University of California, and the California State Department of Public Health assisted by the United States Public Health Service, sera were collected from 481 individuals with repeated exposure to animals. Fifty-one (51) of 481 sera had titers of 1:32 by complement fixation using an antigen made from the McNutt strain of the SBE virus (15).

Many of these 51 individuals were questioned in an effort to recall any clinical episode in their recent history. A surprising number recalled headaches in the occipital region and stiffness in the neck which lasted about a week. Most of these complained of dull joint pain. Admittedly, this information was without specific value, but it did stimulate interest. Arrangements were made with the Student Health Service to procure samples of blood from any case of encephalitis or meningitis not readily attributed to another cause.

C. P. a student on the University of California campus at Davis, became ill in March of 1957. His chief complaints were headache, sore throat and nausea of 48 hours duration. There was no abdominal pain, diarrhea or other symptoms. He had not to his knowledge been exposed to any communicable disease nor had he been working around animals.

The positive findings on physical examination were; temperature of 101.2° F., throat moderately inflamed and neck slightly stiff to flexion. Flexion caused pain to radiate down along the spine. Straight leg raising likewise caused pain in the region of the spine. No abdominal reflexes were elicited and the cranial nerves were intact.
Laboratory work revealed a urinalysis to be negative, a white blood count of 4600 with a normal differential, a hemoglobin of 14.8 grams and a red cell count 5.19M. A lumbar puncture revealed a spinal fluid containing 10 mononuclear cells per cubic millimeter and a protein of 18 milligrams per 100 ml. Serology for mumps, St. Louis encephalitis and western equine encephalomyelitis were consistently negative. Complement-fixation studies were done on the blood serum by the Department of Veterinary Public Health, University of California at Davis, which showed the first sample to be less than 1:8 for sporadic bovine encephalomyelitis while 10 days later (the twelfth day of illness) the second sample revealed a positive titer of 1:128.

The clinical course was that of a mild encephalitis. The original fever of 101.2°F. was the highest recorded and the patient was afebrile after three days. Headache disappeared on the third day as did the soreness in the throat. At no time were there any abnormal neurological findings indicated. His treatment consisted of hospitalization for eight days and aureomycin therapy for five days. Recovery was complete. Follow-up examinations to date have revealed no sequelae.

In an effort to evaluate the role of the virus of SBE in human encephalitis, sera from humans whose illness indicated a viral encephalitis, the etiology of which was undetermined, were obtained through the courtesy of Dr. Edwin Lennette. Serial samples collected in the summer of 1956 from 88 individuals have been tested so far. None revealed antibodies that would fix complement in the presence of the SBE antigen. More will be tested.

**SUMMARY**

Our observations of sporadic bovine encephalomyelitis are similar to the experiences of others in the United States.

The virus is readily communicable to susceptibles within the same herd, but remains confined to a premise, suggesting contact transmission or possibly a host-specific arthropod.

The disease has been recognized in many widely separated areas of the United States and will probably be recognized in others as more information on the essentials of differential diagnosis are disseminated.

Sex, breed of bovine, nutrition, husbandry and environmental factors have not been observed to be related to prevalence of the disease. The disease occurs mostly in young animals. Because of the apparently continued presence of the parasite on a premise and its ready communicability to susceptibles, the immunity following recovery engenders the enzootic situation.

Certain features of symptomatology and pathology have been emphasized as cardinal points in differential diagnosis.

Limited experiments suggest that certain antibiotics may be useful in treating the disease, while others may be used in laboratory isolation procedures.

The virus of sporadic bovine encephalomyelitis or another member of the psittacosis-lymphogranuloma group may have a relationship to the bovine abortion problem.
A human case of encephalitis has been described together with the presentation of certain evidence suggesting that the etiologic agent is a member of the psittacosis-lymphogranuloma group and possibly the virus responsible for SBE in the bovine.

REFERENCES

CURRENT STATUS OF THE NEWER VIRUS DISEASES OF CATTLE


Davis, California

With the exception of several circumscribed outbreaks of foot-and-mouth disease which were quickly stamped out, the United States has in the past been singularly free of virus diseases of any consequence in cattle. Within recent years, however, the situation has been undergoing a definite change. While the menace of exotic disease is none the less real—in fact, with rapid transport on a global scale it is probably even greater than at any time in the past—several diseases of cattle have appeared of late in various sections of the country. All are of proven or suspected viral etiology, and most appear to have originated in the United States within the past several years. In order of their appearance or recognition these new diseases are, namely; virus diarrhea (New York), mucosal disease, virus diarrhea (Indiana), infectious bovine rhinotracheitis (IBR), catarrhal vaginitis, and coital vesicular exanthema.

Virus diarrhea (VD), which was the first of the new diseases to be recognized, was observed by Olafson et al. (1) in 1946 in New York State. These investigators demonstrated that it was caused by a virus, and assigned the name by which the condition is now known. In view of the close clinical similarity of this new disease to rinderpest, reciprocal cross-protection tests were conducted in cattle which demonstrated, however, that VD is immunologically distinct from rinderpest (2). Later, Baker, et al. (3), using an isolate from a typical clinical case of VD, adapted the virus to rabbits. More recently, Lee and Gillespie (4) succeeded in propagating the virus in cultures of bovine tissue cells in vitro. The virus used in these studies is assumed to be the same as the one isolated by Olafson and his co-workers but which, in the meantime, was unfortunately lost.

Mucosal disease was reported by Ramsey and Chivers (5) in 1953 in Iowa, and later, by Prichard (6) in Indiana. Ramsey (7) has given an excellent description of the pathological features of the disease as it occurs in Iowa although he failed to demonstrate convincingly that he had transmitted the infection by experimental inoculation. Prichard (6), however, reports having transmitted a viral agent to cattle from early cases of mucosal disease in Indiana.

Pritchard et al. (8) observed a clinical entity in Indiana that differed in minor respects only from VD as described in New York State and, accordingly, named it virus diarrhea (Indiana). However, preliminary cross-protection tests indicated that the virus of this disease was immunologically distinct from the New York strain of VD virus.
Catarrhal vaginitis and coital vesicular exanthema are infections of the reproductive tract of cattle. The former infection was observed in California in 1955 in herds experiencing reproductive disorders. A virus was isolated (9) from the vaginal discharges of affected cattle which caused a mild form of the disease on inoculation into virgin heifers. At approximately the same time the same, or a similar condition or conditions, was reported from South Africa (10), England (11), and New Zealand (12). In each case a virus was isolated which produced the clinical syndrome that was observed under field conditions. Thus far, comparative studies have been conducted only between the virus isolated in South Africa and the American isolate. While the clinical entity with which the virus is associated in the United States and South Africa is identical in each case, reciprocal cross-neutralization tests indicated that the American and South African isolates are antigenically distinct (13). Although circumstantial evidence suggests that the condition is accompanied in each case by lowered fertility, this has not as yet been confirmed by critical experimentation.

Coital vesicular exanthema, which has existed in Europe for many years, has been suspected in the past of occurring in the United States (14) (15). A virus, which reproduces the clinical syndrome in cattle, has recently been isolated from outbreaks of the disease—which has been renamed infectious pustular vulvovaginitis (16)—in the United States (16) and in Canada (17). A determination of the extent and economic importance of the condition in the United States awaits further study.

While a variety of respiratory infections, including shipping fever, of cattle are currently recognized, the only one that has been studied successfully is IBR. This condition was first observed in Colorado in 1950 (18) and was reported in California in 1953 (19) (20). It has since spread to most of the western states and to some of the midwestern and eastern states. The causative virus was isolated in 1955 (21), and a vaccine developed shortly thereafter (22) which has proven very effective in preventing and controlling the disease. Most of the unresolved aspects of the condition concern its epizootiology.

It is apparent from this brief resume that virus diseases of cattle are on the increase in the United States and in certain other areas of the world also. The importance of these conditions is difficult to assess at the present time, due partially to deficient knowledge and, in some cases, because of conflicting data as to their nature, extent, and distribution. While the economic importance of these conditions might be considered by some to be more apparent than real, it would be ill-advised to assume an attitude of unconcern with respect to the situation. Should, in reality, these diseases be of great potential danger to the livestock industry of this country, it would become increasingly difficult to bring them under control as they became more widespread in the country. Every effort should, therefore, be made to study these conditions in order to determine their true nature so that preventive and control measures, developed on a rational basis, can be instituted at the earliest possible moment.
Much of the work currently underway on IBR at the School of Veterinary Medicine at Davis deals with the epizootiology of the disease. The studies include, specifically, attempts to determine how long cattle shed the virus following infection, and whether the virus subsequently reappears in the nasal secretions under normal conditions and under conditions of stress; the duration of immunity as determined by experimental challenge; the correlation between immunity and antibody titer; and the host range of the virus among the domestic species of animals. Studies of other respiratory syndromes in cattle are being conducted concurrently, the main one being shipping fever.

The studies referred to above were carried out in a series of experiments, some of which are described in this report.

**Materials and Methods**

**Experimental Animals:** The great majority of cattle used in the experimental work described herein were of the Hereford breed. Both heifers and steers were employed. Occasionally dairy breed animals were included but mainly as control subjects. The beef cattle were obtained directly from the range in groups of varying sizes. The ages of the cattle varied from six to 12 months. In some cases the animals were examined before purchase for the presence of cytopathogenic (CP) respiratory viruses, and the blood serum tested for neutralizing antibodies to the IBR virus. Pending experimental use they were held separately from other cattle in a well isolated pasture. The ration consisted mainly of dry hay in addition to pasture forage. The length of time the animals were held before being used varied from several days to months. Dairy cattle breed animals that were used from time to time were raised on the premises of the School of Veterinary Medicine at Davis. The goats were purchased locally and were used for experimental purposes when from five to eight months of age. Pregnant animals were not employed in any of the experimental studies.

**Virus:** A strain of IBR virus, referred to as LA, was employed for all studies reported in this publication. This strain of virus was obtained from an outbreak of IBR in dairy cattle in Los Angeles County during the summer of 1955. Although the outbreak from which it was isolated was mild, the virus regularly produced a severe infection by experimental inoculation. It was selected for use in experimental work because of its pathogenicity.

Cultures of the virus at low passage levels in tissue culture were each pooled, and small amounts stored in sealed glass ampoules under dry ice refrigeration. These pools of virus served as the source of inoculum for preparing large amounts of virus for animal inoculations.

**Tissue Culture:** For the most part bovine embryo kidney (BEK) cell cultures were used in the studies described herein. However, goat and sheep cells, and lymphoid tissue cells of cattle were also employed but to a limited extent. Occasionally primary cultures of these tissues were used but for the major part of the work established cell lines, ranging from the 10th to the
45th passage level, were employed. Both the primary and the established cell lines were prepared according to the method of Madin et al. (23) (24), with slight modifications. The cell suspensions, containing from 80,000 to 100,000 cells per ml, were dispensed in one ml amounts in roller tubes and incubated at 37.0° C in a forced draught egg incubator. After three days' incubation, one ml of fresh medium, containing six percent by volume of lamb serum, was added to each tube. At intervals of five days thereafter until the cultures were used, the medium was withdrawn from each tube and replaced with one ml of fresh medium, containing three percent lamb serum.

_isolation Procedures:_ Essentially the same method for obtaining materials for viral isolations was employed in the case of cattle, sheep, and goats. Nasal secretions were obtained by flushing the nasal passages several times with 30-50 ml of tissue culture medium without serum but containing penicillin (500 units per ml), streptomycin (200 mcg per ml), and mycostatin (100 units per ml). The fluid was injected through the external nares by means of a 50 ml syringe, the tip of which was fitted with a piece of rubber tubing three to four inches in length, and collected in a pan as it ran from the nostrils. It was then transferred to tubes. During the operation the animal was restrained with the head held vertically in order to prevent the fluid from gravitating into the pharynx and being swallowed. The passages of each animal were flushed several times with the same fluid. Swabs were also used to obtain nasal secretions but a lower virus recovery rate was obtained by this method than by the one described above.

The nasal washings were centrifuged at low speed to sediment debris and the supernatant fluid withdrawn. If not cultured immediately it was dispensed in small amounts in ampoules, shell frozen, and stored under dry ice refrigeration. Blood was obtained by venapuncture and allowed to clot at room temperature. The serum was then held in a mechanical freezer until required for use.

Penicillin 5000 units, streptomycin 2000 mcg, and mycostatin 300 units, were added to each ml of nasal specimen. The mixtures were held either at room temperature for several hours or overnight in the refrigerator. Serial decimal dilutions of each specimen were prepared in tissue culture medium containing three percent by volume of lamb serum, and inoculated into BEK cultures in roller tubes from which the medium had just been withdrawn, in a volume of one ml per tube. In some cases two-tenth ml amounts of undiluted, 10-fold, and 100-fold dilutions of treated nasal washings were inoculated to cell cultures containing one ml amounts of fresh medium. In most instances the inoculated tubes were incubated on a roller drum but occasionally in stationary racks. The cultures were examined daily for evidence of CP changes. When no CP effects were detected in the original passage, two additional blind passages were made before the specimen was considered to be negative for virus. All isolates causing CP effects were inoculated to PPLO medium to exclude the possibility that pleuropneumonia-like organisms might be mistaken for viruses.
Inoculation of Virus: Cattle and goats were inoculated intranasally by means of an ordinary hand sprayer. Instillation of virus into the nasal passages by means of a syringe has also proven to be a satisfactory method of inoculation. However, since the spray method was employed in the initial studies undertaken by the authors of this report, this procedure was used throughout for the sake of uniformity.

DURATION OF IMMUNITY IN EXPERIMENTALLY PRODUCED IBR IN CATTLE AS DETERMINED BY (I) ANTIBODY TITER RESPONSE AND (II) RESISTANCE TO EXPERIMENTAL CHALLENGE

Antibody Titer Response

This experiment was carried out on a group of animals consisting of 20 Hereford steers, each of which was approximately eight months of age when the experiment was initiated. In addition, studies of the persistence of virus, which will be published subsequently as a separate report, were also carried out concurrently on these cattle. For facility in handling, the group was subdivided into two groups of 10 head each. These are referred to subsequently as group I and group II.

The animals of each group were placed in experimental pens several days prior to the beginning of the actual experimental work, and the temperature of each animal was recorded once daily during this time to determine the normal range. Nasal washings and blood were obtained from each animal at the time of inoculation. The nasal washings were tested for viral agents as already described, and the blood for the presence of IBR antibodies as described under "Test Procedure." Each animal was exposed intranasally by inoculating 10 ml of a freshly harvested culture of IBR virus at the third to fifth passage level in BEK. During the course of the studies reported herein, the inoculum was, on some occasions, titrated to insure that each animal received approximately the same amount of virus. One control animal was included each time challenge inoculations were made. Temperatures of the inoculated animals were recorded twice daily for 10 to 12 days following inoculation, or until such time as they returned to the normal range. When virus could no longer be recovered from the nasal washings, the cattle were removed to a small corral where they were held for a short time. They were again checked for virus before being transferred to a large pasture removed from other areas containing cattle. The same procedure was followed for the inoculation and subsequent handling of both groups of animals.

Following inoculation, each animal was bled and nasal washings were collected at predetermined intervals. In the case of group I, blood for serological studies was obtained from each animal on the 42nd day after inoculation and at each two-month interval, beginning four months from the time of experimental infection. Blood was collected from the members of group II on the 12th day following inoculation and again at each two-month interval, beginning at the second month. Thus, blood from cattle prior to, and at 12, 42, and 60 days after inoculation, and at each subsequent in-
interval of two months beginning four months after inoculation, was made available for serological study.

**Test Procedure:** In the preparation of stock virus for use in the serum-neutralization (SN) test, cultures of BEK in roller tubes from which the fluid had been withdrawn, were inoculated with one ml of a $10^{-5}$ dilution of virus at the tenth passage level in tissue culture. The virus was harvested on the fourth day, by which time CP effects were almost complete, and the content of each tube was pooled. The pooled material was diluted with an equal volume of fresh tissue culture medium containing six percent by volume of lamb serum, and the culture was then centrifuged at 2000 RPM for 30 minutes. The supernatant fluid was dispensed in four ml amounts in ampoules, fast frozen, and stored under dry ice refrigeration. The virus was titrated after 24 hours' storage and at each time it was used for test purposes thereafter. A fresh ampoule of virus was used for each test run.

The reference sera used in the test consisted of paired samples from the same animal prior to, and 30 days following intranasal exposure to IBR virus at the fifth passage level in cultures of BEK. The sera were stored in a mechanical freezer and used as required.

BEK cultures were used exclusively for SN tests from seven to 12 days after preparation. Only tubes which were shown by microscopic examination to contain a uniform diffuse sheet of typical cells were used. At the time of inoculation the fluid was withdrawn from each tube and replaced by the test mixtures. The latter were prepared in the following manner: Equal volumes of stock virus, diluted in tissue culture medium containing two percent by volume of lamb serum so that each ml contained 100 TCID$_{50}$ of virus (i.e. 50 TCID$_{50}$ of virus per ml of diluted serum), and serial two-fold dilutions of serum were mixed, and incubated at room temperature for 90 minutes. The sera were not inactivated. The serum-virus mixtures were then inoculated in a volume of one ml per tube, using five tubes for each dilution of test mixture. Each test run contained the following control series: A serum control consisting of each serum sample in the lowest dilution tested, the paired reference sera in the presence of virus, the virus titration series in the absence of serum, and the virus control containing the amount of virus used in the test. A diluent control was also included to ensure that the tissue culture medium itself, which was used throughout as the diluent, was not capable of producing cell changes of a CP nature. The inoculated tubes were incubated on stationary racks in a forced draught egg incubator at a temperature of 37.0° C. Readings were made under low power magnification beginning 48 hours after inoculation, and the final reading was made on the fifth day. Any degree of CP activity was recorded as a positive reaction. Serum titers were expressed in terms of 50 percent neutralizing endpoint according to the method of Reed and Muench (25).

**Results:** The results cover a period of 14 months with the number of animals tested at each interval varying from two to 16. The variation in numbers is due to the fact that blood was obtained from each group at different
periods during the first four months following inoculation, while the number of cattle on experiment decreased progressively since two animals were removed at each four months interval for challenge.

In the case of each animal there was a rapid rise in titer, the average, after 12 days, being close to the highest average figure which was recorded 10 months after inoculation. A striking feature was the wide variation in the titers of some of the cattle, the extremes being recorded in Graph 1. In some cattle the titers fluctuated widely as shown by one or more high peaks, in others they remained fairly constant, while in other animals they were still rising 10 months after initial exposure to virus. On the other hand, the titer in some cases decreased gradually over the period tested, following the initial rise. Inasmuch as the titer of the reference serum remained within the limits of experimental error (1–89 to 1–115), and the amount of virus used varied but slightly from one test run to the other (46–56 TCID$_{50}$, average 51.5), the results obtained are regarded by the authors as indicative of the antibody response in IBR infection.

**Duration of Immunity Based on Resistance to Experimental Challenge**

Two animals were selected for challenge at the following intervals after experimental inoculation, namely; 12 days, six weeks, four months, and at each subsequent interval of four months thereafter until the conclusion of the experiment.
Cattle scheduled for challenge were removed to experimental pens several days, if possible, before inoculation in order to allow them to become adjusted to their surroundings. Blood and nasal washings were obtained, following which each animal was given 10 ml of a freshly harvested culture of IBR virus at the third to fifth passage level in cultures of BEK. The challenge inoculations were carried out as described for the initial exposure to virus. Temperatures of the challenged and the control animals were recorded twice daily, and nasal washings were collected once or more from each animal for virus isolation. A close check was maintained for any clinical signs of infection in the inoculated animals. Following completion of the challenge studies, the cattle were either disposed of or utilized for studies other than IBR.

Results

All 20 animals responded with a febrile reaction on initial exposure to virus accompanied, in most cases, by clinical signs of illness, and virus was isolated from each animal. As shown by the individual temperature readings, which are recorded in Graph 2 (morning readings exclusively), there was a fairly wide variation in the degree of febrile response although the curve depicting the average for the group is typical of that of the individual animal. Challenge of two animals at intervals of 12 days, six weeks, and four, eight, and 12 months after initial exposure to virus revealed that in each case the animals were solidly immune in that they did not manifest clinical signs of illness, and the temperatures remained within the normal range as shown in Graph 2. However, the control animals in each instance responded to

Graph 2. The Febrile Response of Cattle to Intranasal Exposure with IBR Virus and to Challenge at Variable Intervals Thereafter.
inoculation with a marked febrile reaction and with clinical signs of infec-
tion. On the basis of the degree of febrile and clinical response to challenge,
there was no indication that the immunity was waning over the period of 12
months during which a total of 10 animals were challenged at the intervals
specified above.

In most cases IBR virus was recovered daily from the nasal secretions until
the eighth or ninth day, and occasionally as late as the twelfth day follow-
ing initial exposure. It then disappeared abruptly. In the case of the immune
animals, however, the virus persisted for a short time only in the nasal
passages following challenge. This might suggest that the virus recovered
following challenge was residual challenge virus which had not undergone
multiplication in the animal. The fact that virus could not be recovered 10
to 12 days after initial exposure, by which time serum antibodies had ap-
peared, might suggest that the apparent failure of the virus to multiply in the
nasal passages of immune animals is due to the presence of antibodies in the
nasal secretions.

EPIZOOTIOLOGICAL STUDIES

One of the most puzzling features of IBR is the manner in which the virus
is perpetuated. The outbreak pattern suggested two possibilities: (i) that
other of the domestic species become carriers of the virus as the aftermath of
either clinical or subclinical infection and, (ii) that cattle themselves serve
in some manner as the chief medium of spread, despite the fact that it could
not be demonstrated that they harbor the virus for any length of time fol-
lowing infection. Accordingly, studies were undertaken to explore these
possibilities.

Host Range Studies

In a preliminary experiment, sheep, goats, swine, and horses were inocu-
lated with tissue culture propagated IBR virus by either the intranasal or in-
travenous routes, after ascertaining that they did not harbor CP agents in
the nasal secretions, or contain serum antibodies to the virus. Of the four
species tested, only a young goat that was inoculated by the nasal route dis-
played signs of infection, and virus was recovered from the nasal secretions
of this animal on the twelfth day after inoculation. It was later demonstrated
that an antibody titer of 1-35 developed in this goat and in another one that
had been injected intravenously. The titers of the pooled sera of the other
three species were somewhat lower being, in the case of the swine 1-8, the
horses 1-10, and the sheep 1-11. In view of these findings a more detailed
experiment was undertaken, using goats between five and six months of age.

Procedure: Five goats, housed in the same experimental pen, were each
inoculated by the nasal route with five ml of the strain of IBR virus used for
the studies conducted in cattle. The temperature of each animal was recorded
twice daily and observations made for clinical indications of illness. Nasal
washings were cultured for the presence of virus prior to and at various in-
tervals following inoculation, and blood was obtained for complete blood
counts and for serological studies at appropriate intervals.
Results: A fever developed in all five animals, the peak occurring between the fifth and tenth day after inoculation. The reaction was more marked in the case of one of the younger goats, and this particular animal underwent a relatively severe clinical episode characterized by depression, nasal discharge, and some respiratory distress (Graph 3). The nasal mucosa of all was hyperemic and some of the goats became inappetent. Complete blood counts indicate that the blood picture remained essentially normal. Virus was isolated from each animal on the fifth day after inoculation, from two on the 10th day, and on the 20th day from the one that underwent the severe course of infection. However, this goat was negative for virus on the 25th day after exposure. Attempts to recover virus from the blood during the febrile peak in this animal were unsuccessful. However, blood drawn at this time, when inoculated into other goats, provoked a febrile reaction although virus could not be recovered from the nasal or ocular secretions of these animals.

The sera of the five test goats, drawn prior to inoculation, were negative for IBR antibody. Twenty days after inoculation the titers ranged from 1-7 to 1-26 while after an additional 20 days they were found to have increased slightly in the majority of the animals. No antibody could be detected in the serum of the goats 20 and 40 days after the inoculation of febrile blood.

Subclinical Infection With the IBR Virus

The possibility that cattle themselves could harbor the virus was supported by experimental evidence in which it was shown that contact cases of the infection were frequently quite mild, being manifested in some instances by a
slight fever only. However virus could be readily isolated from the nasal secretions of these cattle which, for all intents and purposes, appeared clinically normal. Under field conditions such mild cases would obviously escape detection.

During early field studies of IBR, cattle manifesting conjunctivitis were observed in affected herds although, at the time, it was believed that this occurrence was coincidental with the infection rather than being one of its manifestations (26). Later, when the same observation was made in experimentally infected cattle, the thought occurred that it might be part of the disease picture. Preliminary supporting evidence was obtained by demonstrating that the lacrimal secretions of these cattle contained IBR virus. The implication of this finding was then investigated in more detail.

Procedure: Cattle, whose ocular secretions were shown to be free of CP agents, were selected for studies of the intraocular route of exposure to IBR. Five ml of tissue culture propagated IBR virus at the fifth passage level were instilled into the lacrimal sac by means of a serological pipette. Sterile tissue culture medium, which served as the control inoculum, was instilled into the other eye. Temperatures were taken twice daily for several days prior to inoculation and for 15 days following exposure to virus. The animals were observed closely for clinical evidence of infection following inoculation and nasal and ocular secretions were obtained for isolation attempts.

Results: A typical reaction is shown in Graph 4 in which a mild febrile response, very similar to that resulting from contact exposure, occurs following

![Graph 4. The Febrile Response of Cattle Following Exposure to IBR Virus by the Ocular Route and the Persistence of Virus in the Ocular and Nasal Discharges.](image)

ocular instillation of IBR virus. Apart from lacrimation, shown in Figure 1, and a slight nasal discharge, the animals remained clinically normal, yet virus was present in the nasal secretions by at least the fifth day after ex-
posure. Virus was recovered also from the secretions of the right eye, the uninoculated eye remaining virus free. In the particular case shown, the virus persisted for a longer period in the ocular secretions than in the nasal discharges. This might suggest that despite the presence of lysozyme in the ocular secretions, cattle are capable of harboring the virus for variable periods of time in this site in the body, and thus remain potential carriers.

**STUDIES ON SHIPPING FEVER OF CATTLE**

The successful application of tissue culture techniques to studies of IBR indicated that tissue culture is the most useful method available for studies of respiratory infections in general of cattle. Because of the prevalence and economic importance of shipping fever, it was obvious that this condition would be among the first of the unresolved respiratory diseases of the bovine to be attacked by this new research tool.

Shipping fever does not occur as extensively in California as in many other areas of the country. However, during the late summer and fall months outbreaks are relatively common, particularly in cattle that have been shipped in from out of state. It occurs also to some extent in feedlot cattle shortly after being brought from the range pastures.

An outbreak of shipping fever that occurred in the vicinity of Davis in dairy type cattle that had been recently imported from Idaho and Montana occurred in November of 1957. The studies that were carried out on this particular group of cattle are reported herein.
Etiological Studies

Procedure: Nasal washings and blood samples were obtained from several of the cattle during various stages of the infection. The washings were treated as described for the isolation of the IBR virus, and each specimen inoculated to cultures of BEK in roller tubes.

Results: Since no evidence of cell changes were present on the first passage, a routine subpassage was made on the sixth day. Three days later cellular changes, unlike those produced by the IBR virus, were observed in the tubes inoculated with subpassage material of one of the early cases of the disease. The other tubes did not show changes following a second subpassage and were, consequently, considered to be negative. After several serial passages, the recovered agent produced complete CP effects after 72 hours' incubation. These changes were preceded by the appearance of large, clear cells in cultures of both primary and established cultures, scattered throughout the monolayer. With the disappearance of these cells, large, spindle-shaped vacuoles developed in the cell sheet which appeared to result from a coalescence of the cells, forming long strand-like formations. These strands gradually disintegrated leaving cell clumps and individual cells adhering to the wall of the tube. These finally disintegrated completely and separated from the glass surface. The sequential changes in the culture are shown in Figure 2.

Pathogenicity Studies

Two calves, each approximately six months of age, were each given 12 ml of the viral isolate at the third passage level in tissue culture by the nasal route, after obtaining samples of blood and nasal secretions from each animal. Blood for complete blood counts was also obtained from each. Temperatures were recorded for several days prior to, and twice daily following inoculation, while observations were maintained for clinical signs of illness. Nasal washings were obtained at intervals after exposure and blood was drawn for complete blood counts and, later, for serological studies.
Results: Within 48 hours the temperature of one of the calves rose to a peak of 104.1°C, which was approximately two degrees higher than any of the previous readings. It remained elevated for 24 hours and then dropped rapidly to the preinoculation level where it remained fairly constant for the duration of the study (Graph 5). The blood picture was essentially normal at the time of inoculation and at the febrile peak. A slight serous nasal discharge appeared on the third day after inoculation but otherwise the animal remained clinically normal. Virus was isolated on the second, third, and fourth days after exposure but 11 attempts to recover virus over the next 18-day period yielded negative results. The second animal remained essentially normal in all respects although virus was recovered daily for 12 days following inoculation.

Subsequent attempts to infect older cattle with this virus, both by itself and in combination with cultures of Pasteurella multocida and P. hemolytica, gave inconclusive or negative results. In addition, attempts to enhance the effects of the virus by inoculating it into splenectomized cattle and by lowering the resistance of other cattle, following inoculation, by cortisone injections and by holding at low environmental temperatures, failed to result in a clinical infection.

Serological Studies

Paired serum samples from each animal, obtained preinoculation and 24 days postinoculation, were tested for neutralizing antibody to the isolate according to the procedure described earlier.

Results: The preinoculation samples were negative in a dilution of 1-10 which was the lowest dilution tested. The postinoculation samples in the
case of one animal had a titer of 1-40, while in the animal that responded to inoculation the endpoint was not obtained in a dilution of 1-160, which was the highest dilution of serum tested. Six weeks following isolation of the virus the serum titer of the animal from which the recovery was made, and that of a second affected animal from the same herd exceeded 1-128. Unfortunately, preinoculation sera were not obtained from these cattle.

Low titers to the virus, ranging from 1-8 to 1-16, were obtained in the sera of normal animals and also in the sera of cattle prior to and following a series of parenteral injections with IBR virus. The fact that there was no rise in titer in the postinoculation serum specimens of the latter animals indicated that the virus under study was unrelated to the IBR agent. Confirmation of this was provided later when it was found that animals which had been previously exposed to the virus isolate from shipping fever were fully susceptible to subsequent infection with the IBR virus.

**DISCUSSION**

Over the past number of years virus diseases of cattle have increased in number and economic importance in the United States. VD, mucosal disease, IBR, and catarrhal vaginitis are conditions that were unknown, or at least unrecognized, prior to 1946 but are today, in some cases, widely distributed in this country, and some are believed to occur in other countries as well. Coital vesicular exanthema, which has only recently been positively identified in the United States and Canada, has occurred for many years in Europe. It is, therefore, not a new disease in the true sense of the word. Nevertheless, its recognition in this country adds one more virus infection to the list of those that must be dealt with in cattle.

It is interesting, although possibly futile because of the voids in our present knowledge, to speculate as to where each of these new diseases originated. It would appear, nevertheless, that VD, mucosal disease, and IBR originated in the United States. However, reports indicate that some of these diseases, or similar diseases, exist in Canada (27) and possibly in some European countries (28) (29). The rapid sequence in which the vaginitis syndrome was reported from England, South Africa, the United States, and New Zealand suggests that the condition originated in each of the countries mentioned, rather than having spread from one country to the other. It must be borne in mind, however, that because of the problems inherent to the conduct of comparative disease studies, particularly at the international level, the identity of some of the conditions with each other has been assumed on the basis of clinical descriptions rather than having been established by etiological studies. It is, therefore, possible that a clinical syndrome occurring in different countries, or in different areas within the same country, might not be the same disease in each case.

Of the new diseases that are believed to have widespread distribution, the vaginitis syndrome, or syndromes, that occurs in South Africa and the United States is the only one that has been studied on a comparative basis. Although clinically similar and characterized by much the same outbreak
pattern in each case, serological studies have indicated that the South African strain of virus is antigenically distinct to the American strain. It is quite possible that in this case two different antigenic types of the same virus are implicated, the disease being similar in this respect to bluetongue. On the other hand, it might indicate that two distinct diseases are involved. This example serves merely to illustrate the somewhat tenuous basis on which the identity of clinically similar syndromes has been established. Thus, the relationship of the various clinical entities that have been encountered during the recent past must be ascertained before any logical attempts can be made to determine their origin. While comparative studies have been conducted to a large extent with the newly recognized diseases that occur within the United States, the relationship of certain of these diseases to clinically similar entities that occur elsewhere is, thus far, not established.

While many aspects of the recent developments in virus diseases of cattle are not yet clear, there is little or no doubt that these diseases are new, or at least diseases that were hitherto unrecognized. One theory is that they existed in the past but were either unnoticed or mistaken for other conditions. With the development of more precise virus techniques, it became possible to isolate the causative agents in some cases with the result that these diseases were recognized thereafter on the basis of etiological identity.

In keeping with this postulation, it is granted that IBR might have been mistaken in the past for shipping fever although IBR was recognized at a distinct clinical entity some time prior to the isolation of the virus. This would tend to discount the possibility that it is not a new disease. Likewise, while it is possible that catarrhal vaginitis might have, until recently, been mistaken for a non-specific infection, the fact that investigators, working independently in widely separated areas of the world, reported this syndrome as a new condition would tend to suggest otherwise. It is, likewise, difficult to visualize that diseases characterized by such distinctive features as are VD and mucosal disease, could have gone unrecognized or been mistaken for other conditions in the past.

A second possibility is that these conditions are new manifestations of disease caused by mutants of viruses that existed in the past or exist at the present time. This would presuppose the existence, either past or present, of diseases bearing at least some clinical resemblance to the mutant-produced infections. Since diseases of the VD-mucosal disease complex share certain features in common, it is possible that they are caused by a different antigenic type of the same virus, or by different viruses that share a common origin. If it may be assumed that this is the case, the rinderpest virus would then appear to be the most logical one from which these viruses arose because of the clinical similarities between rinderpest, VD, and mucosal disease. While no immunological relationship has, thus far, been demonstrated between rinderpest and VD, much the same situation exists in bluetongue in that cross protection between different antigenic types of the virus is, at most, only partial. Thus, the lack of a demonstrable immunological relationship between VD and rinderpest would not necessarily exclude the possibility that
the VD virus arose, through the process of mutation, from the rinderpest virus.

A third possibility is that these viruses always existed in cattle as members of the normal microbial flora, but only recently emerged as pathogens as a result of certain innovations that exerted a profound influence on the normal microbial populations of the body. In considering recently introduced practices that would, logically, be held accountable, the most obvious is the widespread use of antibiotics as therapeutic agents and feed additives. The effect of altering certain components of the bacterial flora of the gut on the co-resident bacterial populations by antibiotic therapy has already been well established in the case of several species of animals and birds. By means of tissue culture and other techniques it has been demonstrated that many orphan viruses reside in the respiratory and intestinal passages of cattle (and undoubtedly in the reproductive tract also), while at least one large antibiotic sensitive elementary body producing virus is known to inhabit the bovine gut. Therefore, viruses must now be included among the biological flora of cattle, and hence, in the interrelationships that exist between the various populations that make up this flora. It is entirely possible that the qualitative and quantitative effects of antibiotics on the bacterial flora, which result in altered characteristics in certain component members of this group of organisms, may likewise alter the characteristics of other co-resident populations, and possibly the susceptibility of the body cells to invasion by microorganisms not normally capable of invasion. It is also possible that, as in the case of the bacterial flora, similar manipulations of the antibiotic sensitive members of the viral flora induce altered characteristics in other component members of the virus group. In the case of either eventuality or both the normally harmless orphan viruses might, in the process, assume invasive properties with disease production as the end result. Having once assumed a dominant position in the flora of the body of one animal, it is conceivable that these agents would then be in a position to invade and establish themselves in other members of the same species of animal, with the consequent production and spread of disease.

Studies on IBR that are reported herein confirm certain impressions that have been gained over a considerable period of time in observations of the naturally-occurring disease. While it has been known for some time that antibodies develop in response to the infection, it was rather surprising to find that they attain such a high level in so short a time following exposure, and maintain this level over such a protracted period. Another interesting disclosure was that the antibody curve is extremely erratic, varying widely from one animal to another, and sometimes from one bleeding to another in the same animal. While the exaggerated peaks which appear occasionally in the antibody curve of some individuals might appear to represent an anamnestic reaction, no evidence could be obtained that these increased titers were related in any way to virus exposure.

The observation made under field conditions that immunity to IBR is of relatively long duration was amply confirmed in these studies in which it
was shown that a resistance to reinfection is operative as early as the 12th day after exposure, and persists for at least 12 months, which was the longest interval at which the immunity was tested at the time of writing. Thus far, there has been a close correlation between the presence of antibody and immunity, with no indication that either is declining to any appreciable extent.

The studies reported herein have shed little light on the epizootiology of IBR, particularly with respect to the manner in which the virus is spread and maintained. However, preliminary studies indicate that swine, sheep, and horses are not involved. Although goats can be infected and have been demonstrated to harbor the virus—sometimes for a longer period than do cattle—the circumstances surrounding most outbreaks would exclude the implication of goats in any way. Nevertheless, this finding demonstrates that IBR is not strictly a host-specific disease.

The opinion is held by some that cattle, either as subclinically infected animals or as true carriers, constitute the chief medium of spread of IBR. Evidence for the first possibility is provided by the finding that cases of the disease induced by contact, and by intraocular exposure to the virus under experimental conditions, were so mild in some cases as to be clinically undetectable. In addition, studies currently in progress provide strong evidence that virus reappears in the nasal secretions of a low percentage of cattle months after clinical recovery has taken place. It is possible that the virus might be harbored in the ocular system, and periodically make its way through the lacrimal duct to the nasal passages. With this possibility in mind, further studies are currently in progress.

Limited comments only can be made with respect to the findings reported herein on shipping fever. While the agent that was isolated might be one of the harmless orphan viruses that inhabit the upper respiratory tract of cattle, it would then appear unusual that it was not isolated previously when the nasal secretions of a large number of normal and diseased cattle were cultured by the authors of this report for the presence of viruses. Moreover, it is difficult to reconcile the possibility that this isolate is a harmless agent with the fact that it produced clinical disease, although in one animal only, followed by the development of a high antibody titer. Furthermore, the animal from which the isolate was recovered, as well as several others that underwent the infection in the same herd, were found also to possess a high titer to this agent.

If a virus is implicated in shipping fever, as has been generally postulated, it might be widespread in cattle existing, under normal conditions, at a minimal survival level only. It would be difficult, if not impossible, to isolate at this time yet it undoubtedly could stimulate a low level antibody response. Under certain predisposing conditions, which are believed to involve factors of a stress nature, the virus increases rapidly and initiates a series of reactions—in which certain bacteria undoubtedly play a major role—that is manifested by the characteristic clinical syndrome. The virus might be present for a short time only in sufficient numbers to be readily isolated before being submerged or masked by the bacteria directly involved in the disease
process, and by the secondary invaders. Animals free of the virus would thus be susceptible to infection under both natural and experimental conditions although the response in the absence of the necessary predisposing factors would undoubtedly be mild. Cattle which already harbor the agent would be expected to be refractory to such exposure.

The finding that only one of 10 animals that were inoculated with the isolate described herein displayed evidence of infection, and the fact that low antibody titers to this agent were found to exist in cattle which, insofar as is known, had not previously undergone an attack of shipping fever, would not be in conflict with the possibility that the agent is involved in the manner postulated above. While no claim is made to have isolated the causative agent, it might well be that the major problem yet to be resolved in connection with shipping fever is not so much a matter of etiology as an understanding of the predisposing factors involved. It is possible that the virus is present under ordinary circumstances in many animals, and that it has been isolated by a number of investigators. The real problem is, then, to determine the factors that cause it to become active, and thus initiate the series of reactions which result in the clinical syndrome known as shipping fever.

SUMMARY

Recent studies have revealed that infectious bovine rhinotracheitis (IBR) can be transmitted to goats and that these animals harbor the virus for a longer period of time in some cases than do cattle. Cattle exposed to the virus by ocular inoculation undergo a mild form of the disease, which would escape detection under field conditions, but harbor the virus in the ocular secretions. The possible implications of these findings in the perpetuation and spread of the disease are considered.

Cattle were found also to develop neutralizing antibodies in the blood to the IBR virus, and to be solidly immune to reinfection for at least one year after clinical recovery.

A virus was isolated from a case of shipping fever which, on inoculation into calves, produced a mild reaction. Antibodies which developed in response to inoculation with this agent were demonstrated also in cattle that had recovered from shipping fever. The authors postulate the possible role that a virus might play in the disease.

The authors also review the current status of the virus diseases of cattle that have been encountered over the past 10 to 12 years and offer several possibilities in speculating as to their origin.

ACKNOWLEDGMENTS

The authors are indebted to Dr. H. E. Adler of the School of Veterinary Medicine, University of California, Davis, for demonstrating by cultural means that the agent isolated from the outbreak of shipping fever was not a pleuropneumonia-like organism; and to Dr. E. A. Rhode, also of the School
of Veterinary Medicine at Davis, for assistance in the clinical aspects of the study on shipping fever reported herein.

Financial assistance for this study was furnished, in part, by the Animal Disease and Parasite Research Branch, Agricultural Research Service, United States Department of Agriculture.

REFERENCES

McKercher, Saito, Wada and Straub


President Milligan: You have heard these three papers on infections of cattle. Are there any questions for these men?

Doctor Baker, of New York: I would like to make several comments and raise several questions, if I may.

Doctor McKercher's talk was exceedingly interesting.

The point is that I am greatly disturbed about the fact that we are raising a great deal of complications in our etiology of cattle agents. I think the time has come to simplify the situation rather than complicate it.

Through the courtesy of Doctor York, a strain of Indiana virus diarrhea was sent to Cornell. Doctor Gillespie, in certain preliminary tests, has shown this strain of Indiana virus diarrhea to be similar to that reported as New York virus diarrhea.

A little comment made at this point is that this agent does not produce diarrhea anyway. It seems to be more septicemic. I do not think that we need to dignify the situation by creating types at this point. Types must be proven, not postulated.

I would like to comment on Doctor Enright's paper just a moment. Doctor York, working at Cornell, isolated what was probably one of the first orphan viruses. He did a beautiful piece of work. Subsequently, as he presented to the New York Academy of Sciences, this agent could produce disease.
The relationship of this agent to the one previously described by Doctor McNutt is not proven. They seem similar and, if so, Doctor McNutt was the first man to report a member of the psittacosis lymphogranuloma group as producing an infection in mammals.

Now, the Japanese have studied this situation very, very thoroughly, and I recommend to all of you to read the Japanese work, in which they have shown that this member of the psittacosis lymphogranuloma group can produce abortion and encephalomyelitis.

The need for a clarification of this picture in cattle was clearly indicated by Doctor Enright, and I hope that more will join in the study of this agent. You must study this agent not only in relation to concentrations of population, but colostral protection as conferred by the mother; as against dairy herds and beef cattle herds. Doctor York worked in dairy cattle herds; he saw no disease. Doctor Enright works in a diluted cattle population; he sees disease.

The thing that I think is most important is that we should clarify the cattle situation such as the effort made by Doctor Gillespie this afternoon. We should begin relations rather than differentiations. Our attitude should be reoriented so that we can then devise a program to increase animal production, rather than to call for more research by our splitting programs.

PRESIDENT MILLIGAN: Thank you, Doctor Baker.

Is there any further discussion?

DOCTOR MANTHEI: I would like to say a few words.

I discussed with Doctor McKercher last night the desirability of saying a few words on shipping fever. I do not want to confuse us any more, Doctor Baker; I will try to identify what I am talking about.

We started working about a year ago on shipping fever, and we isolated a virus in a hemo absorption group, and it has been classified as Type 1 of the myxo viruses. This Type 1 is closely related to the one found in respiratory diseases of children.

Serologically they cannot be differentiated, or we have not been able to differentiate them. But the one that we have isolated from herds where the symptoms of shipping fever existed will produce disease in calves. It is of short duration, and the paper will be coming out soon, so I am not going into detail on it.

The one isolated from children was used to expose calves. They did not produce disease, and those calves were susceptible to the virus that we had isolated.

I do not know for sure whether this virus is related to the one that Doctor McKercher spoke about; it may or may not be. I have given you the identification of our virus as far as I know up to date.

In examining a number of herds in Maryland, we had what we consider shipping fever. We isolated two different viruses, but not from the same herds. We isolated IBR from some herds, and we isolated this Type 1 myxo virus from other herds. Clinically, we could not tell the diseases apart. In all cases we had a high percentage of isolation of Pasteurella. I do not think
that we have typed enough of those cultures at this time to say what they are, or to say what significance they have.

**Doctor Baker:** Three years ago Doctor Gillespie and I did some work on shipping fever. This was from an outbreak in Georgia. From this, a cytopathogenic agent was isolated in tissue-cultured bovine kidney cells. We maintain a herd of cattle at Cornell which so far as we know is free of any known virus infections. When Doctor Gillespie inoculated these cattle, the agent produced a slight febrile reaction, but it certainly did not simulate shipping fever.

Doctor Gillespie has been exceedingly reluctant to report on this agent.

The role of the Pasteurella that Doctor Manthei mentioned and the types of Pasteurella are important to this problem.

**Mr. Dutton:** Howard Dutton, Pennsylvania State.

I would like to ask Doctor Baker if he would clarify just one thing. We all recognize that New York virus diarrhea is similar to the Indiana, or Indiana's is similar to New York virus diarrhea. He said that Doctor Gillespie had done some preliminary work in which he has shown that the Indiana virus was similar to the New York virus diarrhea.

Now, I would like to ask Doctor Baker: Is he saying that it is a similar virus, or that it is the same virus? If it is a similar virus, then this has not particularly changed things.

**Doctor Baker:** Through the courtesy of Dr. Charles York, one strain was received from Indiana. It was inoculated into Rockefeller calves. It elucidated a reaction, and the calves were immune to this agent.
REPORT OF COMMITTEE ON INFECTIOUS DISEASES OF CATTLE


Mr. Chairman, members of the Association and guests: Your Committee is not including in its report today further reference to anaplasmosis, brucellosis, leptospirosis, parasitic diseases, rabies, tuberculosis and the vesicular diseases, as we feel that these diseases, as they pertain to cattle, have been ably covered by the special committees assigned to their study and the many fine papers arranged for by those committees.

For our report we have endeavored to have certain authorities present situation reports on other important diseases of concern to many. Some of these are newer entities with which we wish to keep abreast, and others are older diseases that need dusting off, but all are of concern.

The chairman has had most helpful suggestions from the Committee and others. It has not been possible to report on all of the conditions brought to our attention. The Committee, therefore, recommends certain diseases, and practices not covered today, as being worthy of consideration for special study and reporting next year. These diseases, in the order in which the Committee feels they need attention, are: Genital diseases (with particular attention to nonspecific abortion and interstate movement of semen as a means of spreading some diseases), anthrax, mastitis, salmonellosis, and virus diarrhea or mucosal disease.

Your Committee has received the best of cooperation from those authorities who consented to prepare situation reports on a few diseases of current interest. These reports are as follows:

LISTERIOSIS

(A Situation Report)

Losses from listeriosis continue to occur among domestic ruminants and other classes of livestock throughout the United States. Epizootics attract most of the attention but widely dispersed sporadic cases may have a greater total effect on economic loss. The infection manifests itself primarily as a fatal encephalitis or as a genital infection causing fetal death and abortion.

Veterinary and public health laboratories should be encouraged to search for this organism. This means that they must be on guard against discarding it as a "contaminating diphtheroid" and that they should employ the reculture procedures that have been described in the literature.

Studies on immunization using sheep have indicated that the ruminant can develop active resistance when immunized with live organisms. However,
practical immunization procedures with vaccines that are safe from the public health standpoint are still of uncertain value.

*Listeria monocytogenes* has been classified into five serotypes (1, 2, 3, 4a, and 4b) and it will undoubtedly be broken down into further subgroups as antigenic analysis is extended. The identification of serotypes can serve a valuable function in epidemiological studies. The most common serotype occurring in the United States is 4b although others have been encountered.

The accumulating case records of human listeriosis and the interest they generate in public health agencies again point to a disease problem which will take the combined efforts of the medical and veterinary professions to offer means for prevention and control. (Submitted by John W. Osebold, D.V.M., Ph.D., School of Veterinary Medicine, University of California, Davis.)

**MUCOSAL DISEASE COMPLEX**

*(A Situation Report)*

During the past 12 months mucosal disease has been reported in all months of the year. The number of cases by months is not large. If fact, only during the month of March of this year were there more than 100 cases reported. The mortality rate in mucosal disease continues to run high but with usually few animals in any one herd. There have been isolated cases where a considerable number of animals died.

Virus diarrheas were reported by Ohio, Indiana, Nebraska, and North Carolina. There were not enough cases reported to determine any seasonal influence.

The number of cases of rhinothlacheitis reported in the various states has been on the increase. In Colorado and adjacent states the use of the vaccine has been reported to be effective. Even though more than 16 states reported the condition, the figures are not considered to be too factual since most diagnoses were clinical.

There has been a slight increase in the number of cases of malignant catarrh reported. However, the total number of cases was very low.

In the matter of reporting progress, it appears that we now have a satisfactory test for rhinotracheitis. We still must rely on immune animals for testing for virus diarrheas, and we do not have a test for mucosal disease.

The research authorities on the mucosal disease complex have made considerable progress in solving some of the problems presented by these disease conditions.

It is hoped that in the not too distant future satisfactory tests can be made available so that a more accurate account of the true extent of these diseases can be determined. (Report submitted by Frank J. Mulhern, D.V.M., Animal Disease Eradication Division, United States Department of Agriculture.)
Prior to 1957 infections of the bovine mammary gland by *Nocardia asteroides* were unknown in this country. Canada had reported a single quarter infection of one cow as had Australia. Early in 1957 the organism was isolated from 28 animals in a single herd of California dairy cattle. Shortly thereafter the infection was recognized in four additional herds in California. During this same period of time the organism was isolated from two severely involved herds in Hawaii where an estimated 250 head were involved on the two dairies. The disease has since been reported as single cow infections from Texas and Alabama.

The extent of infected animals in a single herd has varied from one or two animals up to 20 percent of the entire herd. (Estimates of total losses in severely infected herds exceed this figure.)

We have never isolated the organism from a cow's udder in the absence of detectable signs of mastitis and only rarely did infected animals escape the notice of the herd manager. The infection is usually accompanied by febrile reaction of from 104 to 108°F.; marked enlargement and unusual hardening of the affected mammary glands were prominent. Such glands produced a pasty exudate which often contained grossly visible white flecks or mycelial masses. Infected animals undergo considerable emaciation and die or are destroyed. Metastatic lung foci have been found in a few cases of long duration.

*Nocardia asteroides* has been shown to have antigenic components closely associated with the tubercle bacillus. Skin testing of a number of cows known to have nocardial mastitis has failed to show cross reactions with commercial tuberculin. Preliminary studies of the organism's high heat tolerance have indicated that some strains may survive exposures to 145°F. for 30 minutes and 161°F. for 15 seconds. Thermal death curves are currently being conducted to determine definite end points in thermal resistance. In that the organism is a known pathogen of man, such studies are of primary practical import.

Studies on epidemiology indicate that one of the chief vectors of infection is faulty aseptic technique used in lay treatment of cows by udder infusion for the more common types of mastitis.

Current investigation is under progress to determine the extent of infected herds in California.
Where surveys have been made, *Vibrio fetus* was found to be the most important single cause of bovine infertility. According to reports, vibriosis is a countrywide problem. Vibriosis is estimated to be responsible for approximately 40 percent of bovine infertility which costs the cattle producer more than $100,000,000 annually. This loss is the result of decreased reproductivity, abortions, decreased milk production, and replacements.

Research results indicate that the greatest loss is caused by decreased reproductivity. There was a significant difference in the interval from first service to conception between Vibrio-free females bred to Vibrio-free bulls and those bred to Vibrio-infected bulls. The average number of days for conception in noninfected females bred to Vibrio-free bulls was 17, as compared to 128 in similar females bred to bulls with low virulent infection, or to 215 days in those bred to bulls with high virulent infection.

*Vibrio fetus* was found widespread among herds using natural service, with repeat-breeding in females being the most constant clinical symptom. All females are susceptible to infection; whereas, approximately 50 percent of experimental bulls have become infected after the first exposure, with a smaller percentage becoming infected after repeated exposure.

Cattle owners who have Vibrio-free herds should be advised concerning methods of preventing the introduction of vibriosis. They also should be advised that it is a venereal disease and to avoid addition of either bulls or cows of unknown status that have been used for breeding. Vibriosis is spread rarely, if ever, from female to female, but there is evidence that it may be spread from bull to bull. Another precaution to practice is add only sexually immature animals from *Vibrio fetus*-free herds. Negative results obtained with some diagnostic tests should not be accepted as evidence of freedom from infection in individual animals.

Artificial insemination has been used successfully both to prevent introduction and spread of *V. fetus* and to eradicate the disease from infected herds. Semen from *V. fetus*-free bulls is preferred; however, properly treated unfrozen semen from infected bulls has been equally good for restoring the conception rate to a satisfactory level.

It has been reported that *V. fetus* was spread by the use of treated frozen semen. Therefore, with our present knowledge concerning vibriosis, it seems that an effort should be made to at least consider ways of reducing the disease in studs with the purpose of eventual eradication. This has been accomplished with trichomoniasis in most studs. (Report submitted by A. H. Frank, D.V.M., Animal Disease and Parasite Research Division, United States Department of Agriculture.)
A COMPARISON OF VARIOUS DIAGNOSTIC TESTS WITH MICROSCOPIC POST-MORTEM FINDINGS IN CATTLE INFECTED WITH JOHNE'S DISEASE*

AUBREY B. LARSEN, D.V.M., M.S., and THOMAS H. VARDAMAN, D.V.M., M.S.

Auburn, Alabama

A purebred herd of Guernsey cattle infected with Johne's disease was made available for study through a memorandum of understanding between the owner and the United States Department of Agriculture. The herd, which consists of approximately 190 animals, has been under observation for two years. During this period it was tested six times at regular intervals with intradermic johnin, and blood was drawn each time for serological tests. The owner sells animals for slaughter only, and intestinal specimens are obtained from each animal slaughtered. Through this procedure the accuracy of the diagnostic tests is studied and compared with post-mortem microscopic findings. In addition, the owner keeps production records of each animal which make possible an accurate study of economic losses experienced in the herd. Methods of controlling the disease without slaughtering all reactors are also being studied.

The premises leave much to be desired in the way of management practices. As an example, baby calves are permitted to suck old nurse cows and are kept in sheds where the sun never shines. Two nurse cows that were scouring were each nursing two calves in a shed that was seldom cleaned. The cows were slaughtered two days after being observed and acid-fast bacilli were found in the intestines of both. Some of the largest intradermic reactions were on calves sucking cows in the calf shed. On one occasion five of six suckling calves reacted to the test. Calves as young as 30 days of age have reacted.

To date, six intradermic tests, six hemagglutination tests, six hemagglutination tests modified by the addition of complement, and three complement-fixation tests have been conducted on this herd. The complement-fixation tests were conducted on sera from the first, fifth, and sixth tests. Complement-fixation studies were started 15 months after the initial test, but sufficient stored sera from the first test were available for study.

Detailed procedures for conducting the serological tests have been published. Essentially the hemagglutination tests consist of adding sensitized sheep erythrocytes to various dilutions of sera (1). The hemolytic modification of the hemagglutination test consists of adding complement to the mixture of serum and sensitized erythrocytes and reading the test on the basis of hemolysis (2). A titer of one to 32 or above is considered positive for both

* Regional Animal Disease Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Auburn, Ala.
The complement-fixation test is conducted according to standard procedures (3) with an antigen prepared at our laboratory. A titer of one to eight or above is considered positive. Approximately 15 percent of the cattle show intradermic johnin reactions large enough to be considered positive; 50 percent react to the hemagglutination test, 75 percent to the hemolytic modification of the hemagglutination test, and 50 percent to the complement-fixation test. These percentages remain fairly constant from one test to another, since in the normal course of events some reactors are culled and new ones disclosed.

A study of the intradermic reactors has disclosed the following information: (1) Of 21 animals that reacted before they reached one year of age, 18 (85 percent) lost their sensitivity to johnin within 15 months after the first test. (2) Of 31 animals that reacted as adults, 16 (52 percent) also lost their sensitivity within 15 months. (Several reacting animals that were slaughtered in less than 15 months are not included.) (3) During the two-year period, 40 calves were tested at less than nine months of age and 29 (72 percent) reacted to the intradermic test. (4) Of 72 animals from one to 15 months of age, 52 (70 percent) reacted to the skin test one or more times. Of the 52 reactors above, 24 had two or more tests after becoming positive and of these, 18 (75 percent) did not react on the last two tests. To date, three of the remaining six continue to react. (5) Of 10 animals showing clinical symptoms, that have been observed since the study started, seven were reactors to the intradermic test as adults; none of the animals that reacted at less than one year of age have shown clinical symptoms to date. (6) The milk production of the reactors that showed no clinical symptoms of the disease was equal to the production of the non-reactors.

A total of 34 animals was examined post-mortem. Nineteen were reactors, and 15 were negative to the intradermic test. Small acid-fast bacilli were demonstrated in the intestinal tract of 10 (56 percent) of the reactors and five (33 percent) of the non-reactors.

Twenty of the 34 culled animals had positive or suspicious serum titers to the hemagglutination test. Small acid-fast bacilli were found in nine (45 percent) of these and in six (43 percent) of the 14 showing no hemagglutination titer.

Thirty-two of the 34 animals showed positive or suspicious serum titers to the hemolytic modification of the hemagglutination test. Small acid-fast bacilli were found in 14 (44 percent) of those and in one (50 percent) of the two showing no titer.

Twenty-seven of the 34 had positive or suspicious titers to the complement-fixation test. Small acid-fast bacilli were found in 13 (48 percent) of those and in one of the five (20 percent) showing no titer. Two had not been tested with a complement-fixation test.

From the results it appears that the intradermic test is the best single test for Johne's disease since bacilli were found post-mortem in the intestines of 56 percent of the reacting animals, and at the same time the removal of all intradermic reactors would have eliminated only about one-sixth of the herd.
FINDINGS...IN CATTLE INFECTED

However, the test leaves much to be desired since bacilli were also found in the intestines of five non-reactors, three of which were showing clinical symptoms of the disease. Therefore, if the owner had eliminated all reactors as well as those that showed clinical symptoms at least two infected animals would have remained in the herd, and since only about one-fifth of the herd was examined post-mortem there were probably others.

It is unusual to obtain hemagglutination titers higher than one to 16 in sera from normal bovines. It has not been determined why so many cattle in this herd showed titers well above that level but have not developed clinical symptoms. Since infective bacilli are probably present on the premises, many of the animals may become sensitized by them. Since most of these cattle spend their normal productive life in the herd without developing clinical evidence of the disease they appear to develop an immunity.

It has been observed that many animals in an infected herd will react to johnin at less than one year of age, and frequently develop large reactions as a result of exposure to infection. To prevent such needless exposure, it is recommended that calves should be removed from their dams and raised separately on clean premises (4, 5). However, the observation that a large percentage of the reactions of these young animals will change from positive to negative within 15 months is a finding of considerable interest. It indicates that some of these young animals may be capable of recovering from the infection. It is hoped that these animals can be kept under observation for several lactations and then examined post-mortem. The reactions of adult reactors are more persistent than those of young animals, and about one-half of these adults continue to react until they are eliminated from the herd for one reason or another.

At the time this herd was brought to our attention the owner was losing about 15 animals a year from Johne's disease. At the completion of each test, he receives a list of the reactors and he makes a special effort to cull the adult reactors. In addition, he quickly culls animals that become unthrifty. As a result, after only two years, he has reduced his losses from this disease to three cows during the past 12 months and stated that the dairy is now showing a profit for the first time in several years. It is believed that he could reduce his losses still further if he initiated a strict program of sanitation and management along with a better system of raising his calves. In fact, the results he has achieved thus far by close culling alone indicate that with real effort he could possibly eliminate the disease from the herd.
SUMMARY

1. A herd of 190 cattle, many of which were infected with Johne's disease, has been tested for two years at intervals of three to four months by both intradermal tests and serological tests.

2. Approximately 15 percent of the cattle showed intradermic johnin reactions large enough to be considered positive; 50 percent react to the hemagglutination test, 75 percent to the hemolytic modification of the hemagglutination test, and 50 percent to the complement-fixation test. The intradermic test is the method of choice based on post-mortem results. However, it still leaves much to be desired as a diagnostic test since several animals negative to the intradermic test were found to be infected when examined post-mortem.

3. Eighty-five percent of the animals reacting to the intradermic test before they reached one year of age lost their sensitivity within 15 months, while only 48 percent of adult reactors lost their sensitivity during the same period.

4. During the two-year study seven of 10 animals showing clinical symptoms were reactors to the intradermic johnin test. Typical small acid-fast bacilli were found in intestinal specimens from all 10 animals.

REFERENCES


The November elections are just passed and the campaign year, as always, has supplied us with a vivid display of democracy in action. This year, however, the campaign has also supplied me with the theme of my report, because I plan to borrow from the tried-and-true technique of the politician who "points with pride," while he "views with alarm."

Actually, if I were to cast my vote for the one area of most progress in tuberculosis eradication, I believe it would have to be for the upsurge of program interest and activity—for the revitalizing of our efforts to eradicate tuberculosis—that has taken place in recent months. All of us can surely point with pride to the beginnings of the positive change we have worked so hard together to achieve.

This renewed enthusiasm has been seen at the college short courses and State Veterinary Medical Association conferences in recent years, as many of them have staged demonstrations of approved tuberculin testing techniques using artificially sensitized animals. Such demonstrations are proving invaluable for training the undergraduate and reorienting the practicing veterinarian.

The highpoint of the year was the Tuberculosis Eradication Conference held at Michigan State University in June, which was followed by specialized field training. During the week-long meeting, the participants were divided into groups each headed by an experienced and well-qualified leader. The groups worked together on the tuberculin testing of previously sensitized cattle, the daily observation of test results, and the 72-hour classification of each tested animal. Swine and poultry were also tested and reactions observed. Complex epizootiological tracing problems were studied by the groups which then presented their solutions at a formal conference session.

Following this, a week of specially supervised work under field conditions was provided for each participant. Standard program procedures and testing techniques were reviewed and applied. The reports received from the participants tell of the high value they attached to this practical phase of the conference.

During formal conference sessions the speakers emphasized the five points of major importance which were drawn to your attention a year ago. Progress has been made in each of these areas, but each also has its "view-with-alarm" aspects. Let me review these five points briefly:

* Dr. A. F. Ranney, Chief, Tuberculosis Eradication Section, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture.
First, the tuberculin test. As one of the most reliable diagnostic aids known to medical science, the proper application and interpretation of the tuberculin test was the subject of many lectures and of special clinical sessions held daily throughout the conference week. While no biological test is entirely free of inaccuracies it was repeatedly emphasized that fullest confidence can be placed in the tuberculin test when it is properly used. This was dwelt on at some length because the post-mortem report appears to have replaced the tuberculin test in the thinking and practice of some. This, we view with alarm. For, while the finding of tuberculous lesions confirms the diagnosis, a negative report is not conclusive. Reliance upon the test and confidence in our professional techniques are justified by experience and they are basic to the success of this program. Experience and reason dictate that test results ought not to be rejected on the basis of a subsequent negative post-mortem report.

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**TUBERCULIN TESTING**

- **Area Testing and Herd Accreditation**
  - 77.9% of cattle tested
  - 57.5% of reactors found

- **Quarantine**
  - 29.4%

- **Other**
  - 17.3%
  - 13.1%

**Fiscal Year 1958**

*Fig. 1*
A second major point of the Conference dealt with quarantine requirements for infected herds. If tuberculosis is to be eradicated, it is vital that all cattle which may have been exposed to the disease be closely restricted in movement until danger of infection has been eliminated.

Table 1 shows the tuberculin testing results for fiscal year 1958. It will be noted that nearly 30 percent of all reactors were found on retests applied to infected herds which involved less than five percent of the total animals tested. The test results may be broken down into three major categories as shown in the table and graphically illustrated in Figure 1.

We may expect to find a high percentage of our reactors when we are retesting infected herds. For that very reason, it is obvious that we can do this whole job much faster and much more efficiently if the reasons for quarantines are fully explained and we apply and enforce proper measures to help confine the disease.

We go on now to the third point, cleaning and disinfection, which has from the beginning been considered an integral part of a successful program. During the year, pick-up trucks have been purchased from Federal funds for use in 27 States and arrangements are being made to supply modern powerspraying equipment for use in each of these States. More thorough cleaning and disinfection of infected premises will help bring us more quickly to our eradication goal. Attention is being given to the possibility of wider use of sodium orthophenylphenate, and the development of other effective disinfectants for use on those premises where cresylic disinfectants create a major problem due to distasteful odors.

The fourth major point deals with epizootiological investigations and, as last year’s report emphasized, we must determine the origin of animals which react to the tuberculin test and we must trace exposed animals removed from infected herds. In each such case we must apply the appropriate tests to herds that contain, or may have harbored, tuberculous or exposed animals.

The regular kill lesion case, of course, presents special problems which field regulatory personnel cannot solve alone. We need the help and cooperation of many others in this “trade and tracing” cycle—from slaughter through marketing channels, to the herd of origin. While it is true that our first knowledge of a non-reactor lesion case comes to us from meat inspection personnel the story does not begin at slaughter nor, indeed, does it end there. This all-important cycle begins, in fact, at the herd of origin and responsibility first rests upon each one of us who may hold responsible field positions. We have the responsibility to devise and effectively apply a system whereby all cattle, when leaving the herd of origin may be identified by tag, brand, tattoo, or other satisfactory method. Such identification is vital to successful tracing.

The cycle next brings us, in most cases, to the stockyards markets, large and small, which provide another vital link in this tracing chain. Credit is paid to interested personnel at market centers for what they have done to promote sound tracing procedures. Much remains to be accomplished so that each animal may constantly be identified with the premises of origin.
and expeditiously traced thereto. Too many of our trace-backs are not successful due to inadequate records of livestock moving into and through stockyards markets.

Another step in this cycle, and a most important one, is the slaughtering establishment. This report would not be complete without a sincere word of thanks to Meat Inspection Division personnel. In the midst of their other responsibilities they have undertaken to provide us with the basic post-mortem information and animal description required to conduct sound tracing operations. Much credit goes to them and to cooperating State and Municipal Meat Inspectors for their contribution to the program.

Finally, and in addition to providing sound animal identification so essential to all stages of the cycle, it is a field responsibility, to trace all regular-kill lesion cases from slaughter back to herd of origin. Thus, the cycle is successfully completed when each person involved appreciates and discharges his full responsibility.

A striking illustration of successful tracing, and by no means an isolated one, is provided by an investigation conducted in the State of Idaho as the result of a report of two tuberculosis lesion cases found on regular kill by a California state meat inspector. These animals were traced to the premises of origin where the owners maintained two separate units. In the face of some initial owner objection, arrangements were finally made to test all cattle in the herd. Following the tests on 420 grade and 322 purebred cattle, a total of 124 reactors were disclosed. Eight other herds which had received animals from the infected premises were tested and 43 additional reactors were found. This investigation and one other resulted in the disclosure of 177 reactors on 10 premises in a state which had a record of but six reactors 'reported in the previous five years.

All cases are not so effectively dealt with. During fiscal year 1958, meat inspectors reported extensive lesions of tuberculosis in 27 animals that were not traced to their herds of origin. Nineteen of those animals had been purchased at 10 different stockyards. Some of the factors contributing to these loses were:

1. No eartags, brands, or other specific identification reported to the field in some of these cases.
2. Adequate records of previous owners were not available at some stockyards, especially when dealers and speculators were involved.
3. Some packing plants apparently did not make available adequate records of purchase and other essential information so that the names of previous owners and sufficient animal description could be obtained.
4. Lack of prompt and thorough field investigation for some cases.

Effective identification of cattle and sound tracing procedures go hand-in-hand. Such a dual system, properly developed, can bring great returns to a State and its livestock industry. It gives much promise and may lead to new and more efficient procedures, not only for tuberculosis eradication but for other diseases as well. It is a tool complementary to those in use for
years and one which, when perfected, will give new impetus to eradication programs.

The fifth, and last, major point deals with funds. During the past 17 years the amount of funds available to the cooperative program has increased from $5,500,000 in 1941 to $6,751,886 in fiscal 1958. The 1.25 million dollar increase comes mainly from individual states and counties and is indicative of their interest in eradicating tuberculosis.

Ways and means must be found to increase program efficiency so that an even greater return can be realized from the available funds. Adequate identification and tracing will do more toward conservation of those funds than, perhaps, anything else we can undertake. The amount of costly tuberculin testing required to eradicate the disease will, without doubt, be directly proportional to the efficiency of field identification and tracing methods.

These five areas—tuberculin testing, quarantine, cleaning and disinfection, epizootiological investigations, and funds—were stressed in last year’s meeting and at the Michigan Conference.

Now, looking to our program platform for the campaign of the coming year, we should add to these five points certain other important matters requiring attention:

First, we should be cognizant of the fact that the number of counties overdue for reaccreditation continues to be of considerable concern. In November, 1955, there were 277 delinquent counties and on September 30, 1958, 243 counties in 32 states and Puerto Rico were still in that category. Of that number, 62 counties in 12 states had been overdue for periods in excess of 12 months. (The maps attached as Figures 2 and 3 illustrate this problem.)
Some states have specific plans for bringing their counties up to date and, in some instances, have materially decreased the number of overdue counties since September 30, when these figures were prepared.

Second, the standards for area reaccreditation require attention. The program need in this regard is a change in philosophy from "how little can we do to remain in a modified accredited status" to "how much must be done to eradicate tuberculosis?" Many states have intensified their area testing programs but as can be seen from these figures approximately two-thirds of the areas reaccredited during this period have been qualified at minimal or near-minimal requirements. The number of counties reaccredited during the period January 1, 1955, to June 30, 1958, and the percentage of cattle tested to qualify these counties is illustrated in Table II.

### TABLE II

<table>
<thead>
<tr>
<th>Percent Cattle Tested for Area Reaccreditation</th>
<th>Number of Areas (Counties)</th>
<th>Percent of Total Counties Reaccredited During Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 25%</td>
<td>1,450</td>
<td>67</td>
</tr>
<tr>
<td>25 to 74%</td>
<td>484</td>
<td>21</td>
</tr>
<tr>
<td>75 to 99%</td>
<td>94</td>
<td>4</td>
</tr>
<tr>
<td>100%</td>
<td>337</td>
<td>14</td>
</tr>
<tr>
<td>1/1/55 to 6/30/58—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Areas Reaccredited</td>
<td>2,365</td>
<td>100</td>
</tr>
</tbody>
</table>
Third, tuberculosis in swine and poultry, and paratuberculosis, are closely related to the bovine program and, therefore, call for special consideration. In this connection the following is reported:

Avian Tuberculosis: Statistical Tables for Fiscal Year 1958, available on request, show that very little attention has been given to testing for avian tuberculosis. Consideration has been given to the tracing of tuberculous poultry found on slaughter to farms of origin. Due to the manner in which live poultry are marketed, and handled prior to slaughter, tracing is extremely difficult and results have not, thus far, been too encouraging. Efforts will be continued and intensified to solve this problem.

Tuberculosis in Swine: As shown by Federal Meat Inspection Records, tuberculosis in swine has steadily declined. In 1924, over 15 percent of all swine were retained at slaughter because of tuberculosis. Although that high incidence has been reduced to 2.8 percent it still represents the retention of 1.64 million carcasses in fiscal year 1958. Of that total, nearly 10,000 carcasses were condemned, or passed subject to cooking. We may expect that tuberculosis in swine will continue to decline as we further reduce the infection rate in cattle and poultry. The tracing of infected swine to herds of origin is effective and of great assistance in locating additional infection in swine and other species.

Paratuberculosis: Tests were conducted for paratuberculosis on 213 premises in 22 states during the past fiscal year. Out of a total of 8,406 cattle tested 395 or 4.7 percent were classified as reactors. These reactors were found in 85 different herds in 14 states. State and Federal indemnity was paid in nine of the states involved.

Finally, it has been proposed that another National Tuberculosis Eradication Conference be held this coming year. It is anticipated that a large number of state and Federal regulatory veterinarians will participate in this conference.

As I mentioned at the beginning of this report, the problems associated with tuberculosis eradication are presently receiving more widespread and careful attention than has been the case for several years. And rightly so. For the fourth consecutive year, the percentage of reactors has slowly but steadily increased from a low of 0.11 in 1954 and the two preceding years to 0.17 in 1958. This increase should be viewed with alarm by each one of us. It was predicted a few years ago that such increases might be anticipated when increased attention was given to the program; and, although these increases were to be expected, they should serve to remind us that tuberculosis has not been eradicated and that it will not be unless still more progressive and positive action is directed toward that goal. There is no single field of veterinary effort where the need for a very clear appreciation and recognition of current problems is more necessary than in the field of tuberculosis eradication.
So, while on the national scene we see a campaign year end at the polls, those of us concerned with tuberculosis eradication must continue to take the stump—to plug for the old techniques and procedures that have stood the test of time, while we work diligently for new procedures which may be developed and perfected to meet the changed conditions of the day.
REPORT OF COMMITTEE ON TUBERCULOSIS—1958


The Annual Reports of the Committee on Tuberculosis of this Association since 1945, have repeatedly called attention to the fact that while since 1940 all states have been maintained in a modified accredited status, tuberculosis still exists in most of the 48 states. These Committee reports have warned time and again against a growing sense of complacency. Apparently little heed has been given to these warnings.

Prior to 1940, when all states attained the modified accredited status, tremendous progress was made in the eradication of tuberculosis from the cattle of the United States. Since that time, however, there has been a feeling on the part of livestock owners, as well as many veterinarians, that tuberculosis is no longer a problem. Progress towards complete eradication, the goal originally established and for which we are still striving, is not being made as rapidly as we should expect.

It seems imperative that some means be devised to impress on the livestock industry and the veterinary profession, the menace we face as long as centers of infection remain; which if not disclosed and eradicated, can well undo the progress made at tremendous expense and effort during the first 20 years after the program was initiated. Many suggestions have been made for the accomplishment of this end; some of them have been put into effect with encouraging results. Among these measures are—(1) increased testing in some states which were delinquent for a period of years. (2) Improved methods for and more diligent tracing of animals showing tuberculous lesions on post-mortem at abattoirs, and (3) more attention to the subject of tuberculosis in veterinary colleges, including demonstrations regarding the technique and interpretation of the tuberculin test, emphasis on the epidemiology of tuberculosis, and in some states, participation by students in field activities connected with the tuberculosis eradication program.

The Agricultural Research Service is to be congratulated on the excellent material submitted to Federally employed veterinarians at the Tuberculosis Conference held in June, 1958 at Michigan State University, and also for the plans now in progress for conducting a similar program for selected state and Federal personnel.

Progress to date, however, in overcoming the complacent attitude is meager indeed in many areas. It seems to your Committee that some method should be devised to offer more incentive to the cattle industry to induce renewed interest in tuberculosis eradication. It seems imperative that our present
program be reviewed and many of the loopholes now existing be filled. It seems necessary that we reaffirm our faith in the tuberculin test, that we step up the amount of testing for reaccreditation to a point where we can reasonably determine the incidence of tuberculosis in any area before reaccreditation. We must also establish and more carefully enforce quarantines on infected herds until we can be reasonably assured they are free from disease, and insist on more careful and thorough cleaning and disinfection of premises and vehicles where tuberculous animals have been maintained or transported.

The “no gross lesion” reactors continue to present a serious problem, and further study and research pertaining thereto, is badly needed. There is much to be learned regarding the effect of paratuberculosis and exposure of cattle to tuberculous animals of other species in sensitizing cattle to the tuberculin test. We need to know particularly to what extent sensitization by exposure to avian and human tuberculosis may exist without actual infection. Some progress has been made in the development of a serological test which it is hoped may complement or supplement the tuberculin test.

It has been repeatedly demonstrated that a significant percentage of reactors passing routine Federal inspection and reported as “no gross lesion cases,” actually are infected with bovine tuberculosis and may have been dangerous to other livestock if they had been allowed to remain in a herd. Almost all of the success so far attained in eliminating tuberculosis from the cattle of the United States was accomplished through the tuberculin test, and there is no evidence at this time to discount its value. We are recommending in this report that the Uniform Methods and Rules be amended to delete reference to gross lesions for determination of the tuberculosis status of any herd or area.

The present Uniform Methods and Rules make provision for reaccreditation in range and semi-range areas if properly identified post-mortem reports are produced showing that a percentage of the breeding herd have been slaughtered and that such post-mortem examination failed to disclose lesions of tuberculosis. Doubtless in these areas where tuberculosis is and always has been infrequently diagnosed, this procedure is sound with regard to range cattle. Your Committee believes, however, that in such areas it is vitally necessary that the testing of dairy, farm and purebred herds be materially increased, preferably to the point where all such cattle in the area are tested prior to each reaccreditation. We also believe that the privilege of reaccreditation based on a test of a percentage of cattle as now permitted under range conditions be not allowed in areas in which the incidence of tuberculosis was high at the time the program started, and in which there still exist many centers of infection. We recommend that testing on a systematic basis as now required by the Uniform Methods and Rules, be not only continued but significantly increased, and in addition thereto, every effort be made to trace all non-reactors disclosing lesions at slaughtering establishments to the herds of origin, and to promptly test all cattle directly or remotely exposed to such animals.
Your Committee recommends the following changes in the Uniform Methods and Rules for the Establishment and Maintenance of Tuberculosis-Free Accredited Herds of Cattle and Modified Accredited Areas:

(1) Amend Part I, Section 1 (a) to read as follows:

A tuberculosis-free accredited herd is one in which no reactors have been found on at least two consecutive annual tuberculin tests and physical examinations. Herds in which reactors occur shall be quarantined and must successfully pass a tuberculin test in not less than 60 days before the herd may be released from quarantine. If there is indication of well established infection in one or more reactors, a second test in not less than 60 days from the prior negative test (unless the Cervical Test is used), shall be applied before releasing the herd from quarantine. All herds in which reactors occurred shall be retested in not less than 12 nor more than 15 months following the first negative test following disclosure of reactors, at which time the herd may be accredited or reaccredited if it otherwise qualifies. The physical examinations and tuberculin tests shall be applied by a veterinarian employed in a full-time capacity by the state or the Federal Animal Disease Eradication Division, or by an accredited veterinarian.

(2) Part I. Delete Section 1 (b) and reletter Section 1 (c) to 1 (b).

(3) Add a new paragraph Section 1 (c) to read as follows:

When suspects to the tuberculin test are disclosed in herds not containing reactors, such suspects shall be quarantined to the premises where disclosed, and the accredited herd status suspended until the status of the herd is determined, by a retest of the suspect 60 to 90 days subsequent to the original test. A complete herd test shall be conducted along with the suspect or within a period of 12 months.

(4) Amend Part I, Section 10 to read as follows:

Herd additions must originate directly from tuberculosis-free accredited herds or herds in a modified accredited area that were tested and found negative within a 12-month period immediately prior to being added to the herd. Any other cattle to be added to an accredited herd shall be segregated from the remainder of the herd until retested and found negative at least 60 days after entering the premises where the accredited herd is maintained.

(5) Amend Part II, Section 14 to read:

A modified accredited area, excepting as otherwise provided in Section 15 applying to range and semi-range areas, may be reaccredited for the period designated below if prior to date of expiration of accreditation one of the following procedures is followed:

(a) If all cattle in the area are tested disclosing not to exceed 0.2 percent infection, the area may be reaccredited for a period of six years.

(b) If all cattle in the area are tested, disclosing more than 0.2 percent but less than 0.5 percent infection, the area may be reaccredited for a period of three years.
(c) If all cattle in the area are tested disclosing more than 0.5 percent but less than one percent, the area may be reaccredited for a period of three years provided a retest of all infected and suspicious herds in not less than 60 nor more than 120 days, discloses less than 0.5 percent of infection among the cattle in the area.

(d) If at least 20 percent of all cattle in the area, including all previously infected herds are tested, disclosing a percentage of infection in the cattle tested of less than 0.2 percent, the county may be reaccredited for a period of three years. The herds selected for testing shall be distributed throughout the area and excepting previously infected herds, shall not be the same herds tested on a previous partial test of the same area.

(e) If, following a retest of all cattle in an area the degree of infection exceeds 1 percent but the percentage of herds infected does not exceed 2 percent, the area may be reaccredited for a period of three years provided a retest of the infected herds within a period of 60 to 120 days discloses that the total number of reactors as a result of this retest is less than 0.5 percent of the entire cattle population of the area.

(6) Delete Sections 15, 16, 17 and 18, and renumber present Sections 19, 20 to 15 and 16 respectively.

(7) Amend Part II by adding a new Section 17 to read as follows:

If an area does not requalify for modified accredited status on the due date, it shall automatically be removed from the modified accredited list, provided that the due date may be extended not to exceed one year, provided the period of extension be mutually agreed upon by the state and Federal authorities, and further provided such action shall not be taken excepting in extenuating circumstances, and for good and sufficient reason. Any area so removed and not qualifying for an accredited status under paragraph 14 and 15, within a six-month period immediately following such removal, may reestablish its modified accredited status under the provisions of 14 (a), (b), (c), or (e), under (5) above. Testing to requalify suspended counties in the range and semi-range regions shall include all dairy, farm and pure-bred herds.

(8) Delete Section 21 in its entirety.

Your Committee has considered the proposal of October 1, 1958, by the United States Public Health Service for amending the Milk Ordinance and Code. This proposed amendment calls for a retest of herds at least once in three years. We recommend that milk from herds located in areas wherein all cattle have been tested within six years and the area maintains modified accredited status be included in the proposed amendment.
UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ANIMAL DISEASE ERADICATION DIVISION

UNIFORM METHODS AND RULES FOR THE ESTABLISHMENT AND MAINTENANCE OF
TUBERCULOSIS-FREE ACCREDITED HERDS OF CATTLE AND MODIFIED
ACCREDITED AREAS

PART I

Individual Accredited Herd Plan

1. (a) A tuberculosis-free accredited herd is one in which no reactors have been found on at least two consecutive annual tuberculin tests and physical examinations. Herds in which reactors occur shall be quarantined and must successfully pass a tuberculin test in not less than 60 days before the herd may be released from quarantine. If there is indication of well established infection in one or more reactors, a second test in not less than 60 days from the prior negative test (unless the cervical test is used), shall be applied before releasing the herd from quarantine. All herds in which reactors occurred shall be retested in not less than 12 nor more than 15 months following the first negative test following disclosure of reactors, at which time the herd may be accredited or reaccredited if it otherwise qualifies. The physical examinations and tuberculin tests shall be applied by a veterinarian employed in a full-time capacity by the State or the Federal Animal Disease Eradication Division, or by an accredited veterinarian.

(b) When an accredited herd or a herd in the process of accreditation is to be tested by an accredited veterinarian the following regulations are to be observed:

(1) The accredited veterinarian shall not conduct such tests until he has received written authorization from the proper cooperating state or Division officials.

(2) The accredited veterinarian shall submit a report of such tests in accordance with the regulations of the cooperating state and Federal authorities. These officials reserve the right to supervise any tests conducted by an accredited veterinarian.

(c) When suspects to the tuberculin test are disclosed in herds not containing reactors, such suspects shall be quarantined to the premises where disclosed, and the accredited herd status suspended until the status of the herd is determined, by a retest of the suspect 60 to 90 days subsequent to the original test. A complete herd test shall be conducted along with the suspect or within a period of 12 months.

2. (a) The official tuberculin test shall be the intradermic or the subcutaneous test. The intradermic injection shall be a measured amount of tuberculin, not less than 0.1 cc. for routine testing—nor less than 0.2 cc. for
testing known infected herds, when intradermic injections are made in the caudual or cervical areas. The intradermic injection of tuberculin in the cervical area shall be made only in infected herds, and then only upon approval by state and Federal cooperating officials.

(b) State and Federal authorities may require that any herd in which infection has been found shall not become accredited unless the final or accrediting test has been made by a combination of tests listed under Paragraph (a) above.

(c) The veterinarian who applies the tuberculin test shall inform all cattle owners concerning tuberculosis of other domestic animals, including poultry and swine. Owners or caretakers should also be informed of the possibility of cattle becoming sensitized as a result of exposure to people affected with tuberculosis.

3. The entire herd, or any cattle in the herd, shall be tuberculin tested or retested at such times as are deemed advisable by the cooperating state and Federal authorities.

4. No animal that has been designated as a reactor at any time shall be presented for retest.

5. Reactors to the tuberculin test shall be promptly removed from the farm, and after their removal the infected premises shall be thoroughly cleaned and disinfected with a disinfectant approved by the United States Animal Disease Eradication Division, and in a manner satisfactory to the cooperating state and Federal authorities. Full information is desired with respect to every factor that might have a bearing on the appearance of infection in the herd, such as past history of herd; water supply; light; ventilation; sanitation; management; manner of making additions to the herd (source, isolation pending retest, and retests); disposal of waste products; human infection; avian infection; Johne's disease; etc.

6. Herd owners are required to house, feed, and care for their cattle under such sanitary conditions as will tend to promote good health, and to follow such recommendations as are made by the cooperating state or Federal authorities.

7. Calves in accredited herds shall not be fed milk or other dairy products from other herds not fully accredited, or from unknown sources, unless such materials have been properly pasteurized.

8. (a) The herd owner is required to establish satisfactory evidence of the identity of each registered or grade animal, the grade animal to be marked by a tag or other means satisfactory to the cooperating state and Federal authorities.

(b) Each herd owner is required to keep a record of all additions.

9. All vehicles shall be cleaned and disinfected before they are used for transporting cattle to herds maintained under this plan.

10. Herd additions must originate directly from tuberculosis-free accredited herds or herds in a modified accredited area that were tested and found negative within a 12-month period immediately prior to being added to the herd. Any other cattle to be added to an accredited herd, shall be segregated
from the remainder of the herd until retested and found negative at least 60 days after entering the premises where the accredited herd is maintained.

11. Accredited herd certificates may be issued by the cooperating state and Federal authorities and shall be valid for one year unless revoked.

12. Failure on the part of an owner to comply with these methods and rules shall constitute sufficient cause for the revocation of the accredited herd certificate.

PART II

Modified Accredited Area Plan

13. The provisions of the individual accredited herd plan that relate to testing, removal of reactors, cleaning, disinfecting and sanitation shall apply to the modified accredited area plan. All infected herds shall be quarantined and tested as provided in paragraph 1.

14. A modified accredited area, excepting otherwise provided in Section 15 applying to range and semi-range areas, may be reaccredited for the period designated below if prior to date of expiration of accreditation, one of the following procedures is followed:

(a) If all cattle in the area are tested disclosing not to exceed 0.2 percent infection, the area may be reaccredited for a period of six years.

(b) If all cattle in the area are tested, disclosing more than 0.2 percent but less than 0.5 percent infection, the area may be reaccredited for a period of three years.

(c) If all cattle in the area are tested disclosing more than 0.5 percent but less than 1 percent, the area may be reaccredited for a period of three years provided a retest of all infected and suspicious herds in not less than 60 nor more than 120 days, discloses less than 0.5 percent of infection among the cattle in the area.

(d) If at least 20 percent of all cattle in the area, including all previously infected herds are tested, disclosing a percentage of infection in the cattle tested, of less than 0.2 percent, the county may be reaccredited for a period of three years. The herds selected for testing shall be distributed throughout the area, and excepting previously infected herds, shall not be the same herds tested on a previous partial test of the same area.

(e) If, following a retest of all cattle in an area the degree of infection exceeds 1 percent but the percentage of herds infected does not exceed 2 percent, the area may be reaccredited for a period of three years provided a retest of the infected herds within a period of 60 to 120 days discloses that the total number of reactors as a result of this retest is less than 0.5 percent of the entire cattle population of the area.

[Former 15, 16, 17, 18 deleted.]

15. [Formerly 19] A county or area may be reaccredited in the range or semi-range region upon compliance with paragraph (a) or (b) and other provisions of this section.
(a) When not less than 10 percent of the bulls, purebred breeding cattle, milk cows, and semi-range breeding females, with such other cattle as may be considered necessary by the state and Federal cooperating officials are tuberculin tested.

(b) When not less than 10 percent of the bulls, purebred breeding cattle, milk cows, barnyard cows, and home fed cattle are tuberculin tested, or properly identified post-mortem reports are produced showing that at least 10 percent, and not less than 25 animals, of the breeding herd have been slaughtered within a year, and that such post-mortem examination failed to disclose lesions of tuberculosis.

If under paragraph (a) or (b) of this section a reactor or any other evidence of infection is revealed in any herd by post-mortem reports, etc., including post-mortem inspection at packing plants of those branded cattle that are sold direct from the range for immediate slaughter, all the cattle in that herd or associated with the diseased animal shall be immediately tuberculin tested in accordance with the provisions of the modified accredited area plan. The area may then be reaccredited for a period of six years, if the total number of reactors and cattle found tuberculous upon post-mortem examination from the area is not more than 0.2 percent of all cattle tested in the area.

16. [Formerly 20] The movement of cattle interstate under any and all conditions shall be subject to the approval of the proper livestock sanitary official of the state of destination.

17. If an area does not requalify for modified accredited status on the due date, it shall automatically be removed from the modified accredited list, provided that the due date may be extended not to exceed one year, provided the period of extension be mutually agreed upon by the state and Federal authorities, and further provided such action shall not be taken excepting in extenuating circumstances, and for good and sufficient reason. Any area so removed and not qualifying for an accredited status under paragraphs 14 and 15, within a six-month period immediately following such removal, may re-establish its modified accredited status under the provisions of 14 (a), (b), (c), or (e), under 5 above. Testing to requalify suspended counties in the range and semi-range regions shall include all dairy, farm and purebred herds.

[Former 21 deleted.]
SCREWWORM ERADICATION PROGRAM IN THE
SOUTHEASTERN UNITED STATES

R. S. SHARMAN, D.V.M.*

The story of screwworm eradication in southeastern United States was sparked 20 years ago when an alert entomologist suggested that the female screwworm fly probably mates only once. Confirmed later, that observation opened the door to the present eradication program.

Superficially, that seems a flimsy principle upon which to base a $9 to $10 million eradication program; but it has proved to be the weakest link in the habits of the pest—a weakness which we have been able to attack successfully. How that weakness was exploited is a lesson in applied science.

The development of the eradication program is so complex that it required the combined talents of scientists in many fields—entomology, veterinary medicine, engineering, nuclear energy and many others. This is an imposing force brought to bear on this pest which costs livestock producers of the Southeast up to $20 million each year just to live with it; $10 million by the State of Florida, alone.

Here is a fly, relatively few in numbers, but capable of causing great damage every season. The maggots or larvae of this fly feed only on the living flesh of warm-blooded, wild or domestic animals. The larvae feed for five to seven days, drop to the ground and pupate—to emerge as flies in about a week in warm weather. One wound, continuously reinfested, is capable of killing a full-grown steer in 10 days. Any break in the skin is inviting to the female for depositing eggs. Navels of calves are particularly attractive and the result—crippling or death—follows quickly unless prompt treatment is given.

First reported in Texas about 1825, the fly migrates northward as the summer advances and “freezes back” each winter to subtropical areas. Conditions were not favorable for the fly to migrate eastward much further than the Mississippi River each season, but as so often happens, man was the instrument in spreading the pest beyond natural borders. In 1933, it was transported to Boston, Georgia by means of infested animals. The infestations quickly spread over the Southeast and particularly into southern Florida where it is able to survive the winter—providing the source for infestations to move north each summer.

It would be tedious to cover in detail all the patient study and work expended in the development of this program—monotonous trial after trial and test after test—endured only by the seasoned research worker—entomologists in this instance; the difficulties in finding an artificial medium in which to rear this pest that feeds only in living flesh; the progress in adapting electric elements to heat the rearing vats instead of depending on the surrounding air, which can now be kept cool for the comfort of workers.

* Dr. Robert S. Sharman, Assistant to the Director, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture.
Once the rearing technique was developed, the entomologists selected highly prolific strains of the fly which could produce large numbers of healthy specimens under the laboratory conditions and yet survive when released in nature. In selection of the strain, attention was given to retaining a strain of flies producing young that completed each life stage uniformly. But how to attack the weak link, provided by the mating habits of the fly, still hadn’t been found.

Somehow, the one-time mating of the female fly had to be brought about without causing production of fertile eggs. A logical line of search was to find some way of rendering the male sterile without impairing his ability to mate one to 15 times. Chemicals proved to be unsatisfactory, so entomologists turned to the geneticists who had been working with X-rays since 1927 to effect genetic changes, including sterility, of the fruit fly in their studies of heredity. Here they found the answer. Native female screwworm flies mated with irradiated males produced only infertile eggs.

But after all their work, entomologists found that X-rays were too expensive to use on a large-scale, screwworm eradication program. Working with nuclear energy scientists, the entomologists adapted the use of radioactive cobalt to cause essentially the same effect but with a much cheaper and more convenient source of gamma rays. This technique was built into the present program which calls for the production and sterilization of 50 million flies weekly, half of them males.

The facilities for rearing these flies were prepared by converting an airplane hangar at Sebring, Florida. The hangar provides 100,000 square feet of floor space on two levels. Inside, 66 cages confine three million flies that produce the millions of eggs needed to maintain output. The larvae are reared in a thousand four by five foot, shallow, aluminum vats suspended in units of three from overhead monorails on the upper floor. Each vat has a thermostatically-controlled heating unit. The mature larvae crawl off these vats, after feeding on the rearing medium for about five days, and drop through grates into huge funnels that lead to the lower floor. The larvae are caught in trays of sand aligned on conveyor belts.

The larvae pupate in about eight hours and are sifted out of the sand and stored until they reach the proper age to be irradiated. A powered monorail, moving almost imperceptibly through the pupal storage room, delivers the pupae at the end of 5½ days to a location where they are prepared for sterilization.

About 18,000 pupae are poured into a canister which is lowered into one of the six irradiation units. The radioactive cobalt-60 is fastened in stainless steel casks mounted in five-ton cylinders of lead and steel. The pupae are exposed to the gamma rays for around 10 minutes and receive a dosage of 8,000 roentgens. Both males and females are sterilized at this dosage.

Following irradiation the pupae are conveyed to a packaging room where they are loaded automatically into small cardboard boxes—200 to 500 per box. The flies emerge from the pupae inside the boxes which are trucked to distribution points where they are loaded on airplanes. The boxes are opened
SCREWWORM ERADICATION PROGRAM

and released automatically as the airplanes fly a systematic course at about 1,500 feet over the infested area. Normally, the course is east and west, two miles apart.

To produce 50 million sterile flies per week requires an immense amount of fresh media: 40 tons of ground meat; 4,500 gallons of beef blood; 9,600 gallons of water; 350 pounds of formaldehyde.

Since the small larvae are more sensitive to diet, they are started on a mixture of ground horsemeat, blood serum, formaldehyde, and water. For older larvae, ground whale meat and whole blood are substituted for the horsemeat and serum. New supplies of the mixture are added and the expended media removed as necessary.

Turning out 50 million flies per week is a 24-hour, seven-day week process. Full security measures must be followed to prevent escape of fertile flies. All personnel entering the rearing facilities must change into clothing provided and street clothing donned when leaving the plant. Many other security measures are employed inside the completely sealed hangar. Since there has been no precedent for such large-scale rearing of insects, many of these processes had to be worked out literally from "scratch."

In spite of many difficulties, the rearing facilities were put into operation on schedule on July 11, 1958. As rapidly as possible, the production of sterile flies was brought up to a level sufficient to meet the program needs.

While the facilities were under construction at Sebring, however, a series of events occurred which gave us an advantage in the eradication program. Unusually cold weather eliminated screwworms in the Southeast in all areas north of the Florida Peninsula and even there, populations were greatly reduced. Taking advantage of the situation, even before the impact of the cold weather on screwworm populations could be appraised, small-scale laboratory rearing facilities at Orlando, Florida, were expanded to capacity to produce sterile flies. It was anticipated that release of sterile flies over a belt across the peninsula might prevent the northern migration of the fly during the spring and summer, until the Sebring plant was in production. At the same time, an opportunity was provided for proving equipment and training personnel. Accordingly, release of sterile flies was begun January 15, 1958.

The results have been most gratifying. By September 1, only two cases of screwworms had been reported north of the Florida-Georgia line—in southern Georgia. In addition, the number of cases of screwworms in Florida has been greatly reduced over previous records for the state. The results confirm those obtained in three pilot test areas performed since 1950.

From 1951 to 1953, tests were conducted on Sanibel and Captiva Islands off the Coast of Florida. The operation was considered successful, although reinfestation occurred from the mainland. In 1954, with cooperation of the Netherlands Antilles government, United States Department of Agriculture's Entomology Research Division eradicated the screwworm from the Island of Curacao off the Coast of Venezuela. At the end of four months the fly population had declined to the point that it was impossible to find egg masses or infested animals. However, release of sterile flies was continued two more
months to insure eradication. Since then, there have been no reports of screwworms on the island.

In 1957 a 2,000-square-mile area near Orlando, Florida, was used as a pilot test to develop the technique further and to train personnel and to test equipment. Eradication of the fly was not expected in this area since infestations could infiltrate from surrounding infested territory. However, screwworm populations were reduced greatly and confirmed results of successful tests conducted previously.

Progress with the present program is much as anticipated. We are approaching a period when the flies are usually eliminated by frost in the northern states as far south as peninsular Florida and the over-wintering habitats. Cool weather in the peninsula also prolongs the pupal and adult stages and reduces populations. Thus, incidence is difficult to determine.

Once eradication is achieved in the Southeast, however, our work is not finished. Infested animals will again be the source of reinfestation in the area after eradication since the fly does not migrate from the western states or other countries into that area. Concurrently with the planning for eradication, a system of inspection of animals and regulation of movements of cattle was put into effect and maintained.

Inspection facilities have been established on the eastern state lines of Arkansas and Louisiana to screen cattle as they move into the Southeast. Here any infested animals are freed of screwworms before they enter the eradication area which includes Mississippi, Alabama, Georgia, Florida, and South Carolina. A state quarantine line has been established in Florida east and west through Ocala to prevent the transportation of infested animals from south of the line. Thus, the eradication area is isolated from reinfestations of other territories.

It is essential at all times to keep close watch on the screwworm situation in the eradication area. We have about 80 livestock inspectors throughout the five-state area who are assigned specifically to the program. Livestockmen have been encouraged to report screwworm cases and to locate and treat all suspected infestations to keep down the screwworm populations.

Traps have been distributed systematically in Florida to capture flies whether native or sterile. By treating the sterile pupae with a dye we are able to distinguish between the laboratory-reared flies and the native ones. From this data, the vigor, relative numbers, and longevity of the sterile flies can be determined.

The estimated time to achieve eradication is two years, but we will not be certain until surveys over a period of time fail to turn up any infestations or flies.
The relationships of parasites and their animal hosts are of considerable complexity. Parasites are injurious to livestock in many different ways. They injure and annoy them by biting, drawing blood, crawling, scratching, and by the injection of irritants or other substances. Some live within the animal’s body causing irritation and destruction of tissues and interfering with the normal processes.

There are no areas in which livestock are free from their depredations. Certain parasites are relatively host specific whereas others attack many or all species. Some are local or regional in distribution—because of climatic and other factors—while others are generally distributed, although less numerous in some areas than others. Certain of them are troublesome only during particular seasons while others plague livestock the year around.

Parasites are vectors of a number of diseases. They may be merely mechanical carriers but in other instances play an essential role in disease transmission. Their role may be such that a specific disease may be controlled or eradicated by eliminating the parasite vector. The parasites may be attacked by destroying their breeding places, through sanitation, poison baits, by the application of parasiticides either on or off the animal hosts, in animal feeds, by injection, etc., and by the use of repellents. Research is continuing to find better materials to control internal parasites and substances that can be used internally in animals to destroy lice, mites, ticks, flies, grubs, etc. A few materials have been found which have this systemic action when given orally or injected subcutaneously. Bayer 21/199 acts as a “dermal” systemic when applied as a spray.

The importance of the livestock industry in the United States is reflected in the fact that it provides a cash income of approximately $18 to $20 billion annually. Livestock parasites extract a heavy toll from both producers and consumers. It is difficult for us to perceive the actual losses caused by parasites as such a high proportion of the livestock population suffer in some degree. Even a small monetary loss per animal results in total losses that are virtually incomprehensible.

Your Committee feels that more intensive research should be done to explore basic problems of livestock parasite control and eradication including improved chemical materials and methods of administration, problems of residues, parasite resistance to insecticides, and on the use of systemics. It feels that intensive studies should be done on exotic parasites also to
strengthen our efforts to keep them from entering this country and to aid us in fighting them should they be introduced.

Your Committee had planned to have Dr. H. O. Peterson, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Albuquerque, New Mexico, prepare and present a paper on the control of *Oestrus ovis*. We regret to say that Doctor Peterson has been ill and unable to prepare and deliver the paper. We are pleased to report that at this time Doctor Peterson's health has improved considerably.

Dr. H. E. Kemper was kind enough to discuss the matter with Doctor Peterson and has prepared the material included in our report. Doctor Kemper was present when some of the treated sheep were autopsied and, therefore, has personal knowledge of the project. I am sure the majority of you will remember him. Before his retirement he was veterinarian-in-charge at the Animal Disease and Parasite Research Division Laboratory at Albuquerque.

Doctor Kemper wrote your Committee as follows:

[Doctor Peterson related to me the substance of his proposed paper on the preliminary experimental work on the destruction of the larvae of *Oestrus ovis* of sheep with the intramuscular injections of the organic phosphate compound Dimethoate (CL 12,880, Lederle, 0,0'-dimethyl S-a-mercaptop-N-methylacetamido-dithiophosphate). As a result of these preliminary experiments on at least 70 adult range sheep that were naturally infested with larvae of *Oestrus ovis*, an effective non-toxic and easily administered dose of Dimethoate was determined. The effective dose of this compound was 25 mg./kg. using a 50 percent injectable solution given intramuscularly. This compound was found highly effective, especially so for the first instar larvae where a 98 percent kill was consistently obtained, but slightly less effective for second instars (97 percent kill) and for the third instars (92 percent kill) of the larvae of *O. ovis*. These figures indicated an over-all kill of 96 percent in the experiments that were conducted during the month of November when numerous first instar larvae were still present in the nasal cavities before their migration into the frontal sinuses where some second and third instars were present together with a very few first instars that had already reached the frontal sinuses. The treated and control sheep were autopsied at 72 and 120 hours, respectively, after treatment to determine the effectiveness of Dimethoate.

The results of the several successive experimental trials conducted locally were most gratifying and sufficiently indicative to warrant mention of the progress of this research project to the Association at this time.

This method of intramuscular administration of Dimethoate in a 50 percent injectable solution seems more practical than the previously advocated methods, especially so where large numbers of sheep are to be treated. This compound is generally less irritating to the animals than the injection of harsh chemicals directly into the nasal cavities or directly into the frontal sinuses through previously made surgical perforations.
Opinions differ somewhat as to the economic and pathological significance of larval infestations of the *Oestrus ovis* in sheep, as well as the value of some rather drastic forms of treatment heretofore employed. Observing the heavily infested sheep ("snotty-nosed sheep") especially on the ranges, leaves little doubt in my mind as to the economic significance of such infestations. In connection with the Dimethoate treatment, it should be mentioned that the margin of safety for all sheep, regardless of their age or physical condition, will require more extensive work before safely making specific recommendations for its general use. Furthermore, Dimethoate is at present released for experimental purposes only; hence, at the present time, we can make no recommendations for its general use.]

In reviewing the activities of the past year, your Committee took note of the successful campaign by the Florida Livestock Board and the United States Department of Agriculture to eradicate an outbreak of cattle fever ticks. It seems particularly appropriate to call attention to this activity as we are meeting here in Miami.

In April, 1957, cattle fever ticks (*Boophilus microplus*) were found at the Okeechobee Livestock Market at Okeechobee, Florida, by a State inspector. The last outbreak of cattle fever ticks in Okeechobee County was in the winter of 1945, when quarantines were placed on that county as well as on Glades and Highlands and parts of Osceola and Polk Counties.

Since the last infestation in Florida, cattle passing through all auctions throughout the State have been inspected routinely for ticks by experienced inspectors, and all cattle except those being sold for slaughter have also been dipped. It was as a result of this routine search for fever ticks that the ticks were found on cattle from two Okeechobee County ranches. Steps were taken immediately to treat the infested and exposed herds and place a state quarantine on the areas involved. Experienced State and Federal tick inspectors were assigned to the eradication project. Action was taken also to trace movements of animals to and from the infested herds during the last two to three years, to place the premises involved under state quarantine, and to investigate the possible source of the ticks. In all, more than 100 ranches in 10 Florida counties were placed under quarantine. State quarantines were placed on premises to which cattle had been moved from ranches where ticks were discovered and on premises that had supplied cattle to ranches known to be infested. Altogether cattle on a total of eight premises in Okeechobee County, one premises each in Highlands and Dade Counties, and three premises in Broward, and two premises in Palm Beach Counties were found infested. The Florida Livestock Board quarantined all of Okeechobee, Broward, and Palm Beach Counties and parts of Dade and Highlands Counties. During September, 1958, the last quarantine on a part of Highlands County was released. Quarantines on other areas had been lifted previously. No cattle ticks had been found for more than a year. The most recent infestation was found on September 6, 1957, in Highlands County. The ticks were eradicated by the customary systematic inspections and dippings.
Florida was the last State, with the exception of Texas where a narrow buffer zone along the international border is still infested, to be freed of fever ticks. The last Federal quarantine was lifted in December, 1943.

Deer, which also served as hosts for the tropical cattle-fever tick, delayed the final eradication, and it was only after the Florida legislature provided proper authority to eliminate this host that the vector was finally eradicated. More than 20,000 deer were slaughtered to accomplish this end. During the 1957 outbreak, ticks from deer were examined but none were fever ticks.

During fiscal years 1947, 1948, and 1949 cattle fever ticks were found in a number of Florida counties. Systematic inspections and dippings brought the outbreaks under control, and all remaining Federal quarantines were removed in December, 1950. It was not until April of last year that any additional infestation was found.

Danger of reinfestation is constantly present because of the close proximity of Florida to tick-infested countries and islands of the West Indies with which commerce is active.

Your Committee also took time to reflect on the cattle fever tick situation along our border with Mexico.

All territory adjacent to the international boundary along the lower Rio Grande River is tick infested, and reinfestations in Texas by ticks carried by animals illegally entering the United States occur regularly. The river serving as a boundary is not an effective barrier against such illegal movements. A buffer area under Federal and State quarantines extends from Del Rio to the Gulf of Mexico—approximately 500 miles. This zone is constantly patrolled by Department inspectors who, in cooperation with Texas livestock sanitary authorities, work diligently to reduce the introduction and prevent the dissemination of the ticks. The area under quarantine includes parts of Cameron, Hidalgo, Kinney, Maverick, Starr, Val Verde, Webb, and Zapata Counties.

The fever tick was eradicated from California many years ago; however, this State also has a common border with infested areas in Mexico, and animals illegally entering the United States reintroduce ticks from time to time.

Your Committee wishes to encourage the United States Department of Agriculture, in cooperation with the States of California, Florida, and Texas, to continue the active efforts to keep cattle fever ticks from spreading into this country.

The Association’s Committee reviewed the status of psoroptic sheep and cattle scabies.

During the past year, psoroptic scabies was diagnosed in two herds of cattle in Colorado, two herds in Kansas, and one herd each in Iowa and Illinois.

The goal of complete eradication of psoroptic cattle scabies seems to be within reach. Your Committee urges that livestock sanitary officials not spare any effort to locate all infected animals and to trace cattle moved from and to infected herds in order to find all foci of the disease. They felt that nothing short of completely satisfactory methods of treatment should be ac-
accepted in the eradication program—dipping being the preferred method and perhaps where the situation warrants it a spray-dip type of machine being considered.

Psoroptic sheep scabies is reported regularly in almost half our states. Official records indicate that during the past four years, the number of infected flocks reported, and the number of counties in which infected flocks were found has virtually doubled. Last year more than 200 lots of sheep were found to be infected by inspectors at public stockyards.

In making a recommendation regarding sheep scabies, this Committee wishes to review recommendations made by your previous committees and reiterate and bring before this body the fact that sheep scabies can be eradicated. Methods and procedures for accomplishing this are well established. We recommend that a Scabies Eradication Program be vigorously pursued in order to eliminate this disease from the United States.

Your Committee wishes to make particular mention of the outstanding progress being made in the Screwworm Eradication Program. This program was described in detail in a paper presented this morning to the Association.
NEW RESEARCH RESULTS WITH SYSTEMIC INSECTICIDES

R. C. BUSHLAND

Entomology Research Division
Agriculture Research Service, United States Department of Agriculture

Last year, at your sixty-first annual meeting, Doctor Lindquist (1958) reviewed research in the United States Department of Agriculture leading to the development of systemic insecticides for animal treatment. He covered progress from the first promising laboratory test with rabbits (Lindquist et al. 1944) to practical control of cattle grubs (Hypoderma lineatum (de Vill) and H. bovis (L.)) by oral administration of Dow ET-57 (McGregor and Bushland 1957, Roth and Eddy 1957) or spraying with Bayer 21/199 (Brundrett et al. 1957, Graham 1958). After extensive field trials by state and industry workers, the Entomology Research Division (1958) recommended these treatments, and they are now being used by American livestock producers under the trade names Trolene (Dow ET-57) and Co-Ral (Bayer 21/199).

Another compound, Am. Cyanamid 12880 (Dimethoate), which Doctor Lindquist mentioned last year as being promising for intramuscular injection, did not turn out so well in nation-wide field trials. It seems that there is not a sufficient margin of safety between the minimum dose effective against first-instar larvae and the dose toxic to cattle. Under some conditions cattle were sickened by the treatment. Although this compound probably cannot be recommended for use against first-instar larvae, it may prove practical for controlling second- and third-instar grubs after they migrate to the back. Industry and state workers have reported that those older grubs are killed by a lower dose, which may not be injurious to cattle. If Am. Cyanamid 12880 can be developed for late-season treatment, it should be a valuable addition to our recommendations.

Many growers can be expected to neglect treatment of their cattle until grubs are already in the back and it is too late for best results with Bayer 21/199 or Dow ET-57. Then a simple, single intramuscular injection would be preferred to repeated spraying of rotenone during the winter. We are awaiting with interest the results of practical field tests that will be made by other workers over the country this winter.

SCREENING TESTS WITH NEW COMPOUNDS

At our Keerville, Texas, laboratory we have discontinued emphasis on the three systemic insecticides mentioned above in favor of evaluation of new chemicals. In the first place, although the two systemics now recommended have many advantages over the old rotenone spray, the treatments are still expensive, costing roughly from 50 cents to three dollars per animal. Through the facilities of the Division’s Pesticide Chemicals Research Laboratories we obtain new compounds from their synthesis program. In addition,
we are currently supplied with new chemicals from the laboratories of 10 chemical companies. From these abundant sources we hope to find safe compounds which will be more effective and less expensive than the present chemicals.

Bayer 21/199 is recommended as a 0.5 percent spray and Dow ET-57 as an oral treatment at approximately 100 mg./kg. When properly applied at these rates, cattle grub control ranges from 80 to 100 percent. There is not a sufficient margin of safety to recommend higher rates, which might invariably give 100-percent control. Complete kill with one treatment would be highly desirable for ordinary use by the livestock grower and even more important to a control program aimed to achieve cattle grub eradication.

Finally, as ultimate goals for livestock-insect control we should like to find compounds so safe that they could be mixed with salt or put into feed.

Thus far this discussion has been limited to cattle grub control. The three insecticides mentioned are effective against other parasites, but we do not yet have a chemical so inexpensive, effective, and safe that it can be used for repeated treatments to protect animals from such pests as ticks and biting flies which attack them over the entire season. The organic phosphorus insecticides seem to be rapidly metabolized and quickly eliminated from the treated host. A systemic insecticide for cattle to ingest whenever they take salt should be highly toxic to the pests but low in mammalian toxicity so that it can be administered safely through an entire tick or fly season. However, the treatments should be terminated in time for residues to disappear from the edible tissues before slaughter.

Thus we have the close-range objective of finding safer, cheaper, and more effective cattle grub systemics and the long-range goal of systemic control of many species of ticks and insects that attack livestock.

The entomological techniques for testing systemic insecticides have been described by McGregor and Bushland (1956). Recently some of the screening procedures have been improved and further described by Drummond (1958). Table 1 shows the results that he obtained with Am. Cyanamid

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Screw-worms</th>
<th>Stable Flies</th>
<th>Lone Star Ticks</th>
<th>Toxic Dose (Mg./kg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Am. Cyanamid 12880</td>
<td>10</td>
<td>10</td>
<td>25</td>
<td>NT*</td>
</tr>
<tr>
<td>Oral</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Subcutaneous</td>
<td>5</td>
<td>25</td>
<td>50</td>
<td>NT</td>
</tr>
<tr>
<td>Bayer 21/199</td>
<td>25</td>
<td>25</td>
<td>Failed</td>
<td>25</td>
</tr>
<tr>
<td>Oral</td>
<td></td>
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<tr>
<td>Subcutaneous</td>
<td>75</td>
<td>50</td>
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<td>100</td>
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<tr>
<td>Dow ET-57</td>
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<td>Oral</td>
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<td>Subcutaneous</td>
<td>50</td>
<td>50</td>
<td>do</td>
<td>NT</td>
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</tbody>
</table>

* NT indicates that the guinea pig showed no symptoms of poisoning when treated at 100 mg./kg.
12880, Bayer 21/199, and Dow ET-57 by the current technique using guinea pigs infested with day-old screwworms (*Callitroga hominivorax* (Cqrl.) and nymphs of the lone star tick (*Amblyomma americanum* (L.)). Starved stable flies are fed on the host four hours after administration of the insecticide. By the same technique he has found several promising new compounds. The Entomology Research Division has the policy of accepting newly synthesized compounds for tests and holding the data in confidence for one year (while company patent applications are pending); therefore, some of these new materials cannot be specifically designated here.

**PROGRESS IN DEVELOPING NEW TREATMENTS**

Cattle are too expensive and the life cycle of grubs is too long for us to do screening tests with infested cattle. All the chemicals that we have found to kill grubs in cattle also killed screwworms in guinea pigs, sheep, goats, and cattle. There are differences in efficiency of systemic activity in the different animals and some of the screwworm systemics failed on cattle grubs. However, the guinea pig is a cheap experimental animal, and effectiveness against screwworms in them can be detected within 24 hours of administration of the insecticide. Therefore, we use the guinea pig to find a few promising insecticides from the hundreds of new chemicals submitted for evaluation.

One of the difficulties in developing livestock systemics is that farm animals vary in their ability to tolerate insecticides. Treatments that do not injure guinea pigs may be highly toxic to one or more species of livestock. Chemicals tolerated by sheep may be toxic to cows, and *vice versa*. Some treatments that are harmless to mature animals are toxic to the young of the same species. Also, sick or emaciated animals, or those under stress from weaning or shipping, may be unusually susceptible to insecticide poisoning. All these conditions must be considered in the preliminary research, so that field tests may be made with chemicals selected for safety and tried out at dosages that reasonably healthy animals should be able to tolerate under the various conditions of farm and ranch practice.

The problems of safe treatments are the concern of cooperating veterinarians of the Animal Disease and Parasite Research Division. Radeleff *et al.* (1955, 1956) and Radeleff and Woodard (1956, 1957) have described these problems and have indicated the procedures used to assure that we entomologists experience a minimum of livestock poisoning in our field trials and that final recommendations represent safe treatments.

Following the guinea pig test, we check on systemic activity in Government-owned sheep and goats. The entomologists apply the insecticides and observe their effects on screwworms, stable flies, and ticks, and at the same time the veterinarians observe the livestock for symptoms of poisoning. Treatments tolerated by sheep and goats are then tried on a few baby calves, since such animals are usually the most susceptible to chemical poisoning. The next step is for entomologists to treat a few grub-infested Government-
owned cattle with the maximum dose that the veterinarians observed to be tolerated by the most susceptible farm animal. The veterinarians observe these cattle for any symptoms of poisoning, including cholinesterase activity in the blood before and after treatment.

Then follows a waiting period of several months to allow the first-instar larvae to complete their growth within the body of the test cattle and migrate to the back. During the grub season, which lasts three to five months, the entomologists make monthly counts in the backs of treated animals and untreated controls.

A large proportion of the selected insecticides are eliminated by this practical test, but those that continue to show promise are still not ready for field tests, since only a few Government cattle can be risked on each chemical in the first practical cattle grub trial, and the results on so few animals do not justify risking treatment of a cooperator's livestock. Therefore, larger numbers of Government cattle must be treated for statistically significant results and to give the veterinarians more evidence of toxicological safety. Sometimes chemicals that look promising on a few cattle fail in the larger test.

If the results of the larger tests support the findings with the first few animals, the veterinarians make more detailed studies on all kinds of farm animals to indicate more definitely the toxicological safety of a proposed treatment.

After a new treatment looks good on Government cattle, and before it is tried on privately owned livestock, investigations are made on residue hazards to assure that a cooperator's cattle will not be contaminated at the time of slaughter. The primary responsibility for determination of residues in meat and milk rests with chemists of the Entomology Research Division. H. V. Claborn, H. D. Mann, and M. C. Ivey are stationed at Kerrville to work with the veterinarians and entomologists.

In addition, at the Kerrville and Corvallis (Oregon) laboratories entomologists trained in radio-isotope techniques study the metabolism and insecticidal activity of compounds labeled with P32 to learn more about their mode of action, duration within the body of the host, method of elimination, and, incidentally, much about the residue hazard. Such studies were made on Dow ET-57, Bayer 21/199 and Am. Cyanamid 12880 by W. E. Robbins at Corvallis and by J. N. Kaplanis at Kerrville. W. C. Chamberlain also worked with radioactive 12880 at Kerrville. Doctor Radeleff participated in all the veterinary aspects of the radioactivity studies at Kerrville and worked with Mr. Claborn on radiochemical detection of residues in various tissues.

After several months of entomological work with guinea pigs, sheep, and goats and two years' work with Government cattle to indicate efficiency, the supporting toxicological observations by the veterinarians to indicate safety, and chemical and radiometric determination of residues, we are ready to try new chemicals in large-scale tests on privately owned cattle at cooperating farms and ranches. We publish our results as promptly as possible, but to assure simultaneous tests by state workers we present our preliminary information at annual systemic-insecticide conferences where state, Canadian,
Federal, and industry representatives meet to exchange research data. In 1956 and 1957 the meetings were held at Kerrville and in 1958 at Kansas State College.

From these investigations it is apparent that the development of a systemic insecticide for livestock is a difficult, expensive, and time-consuming task. It requires the best cooperation of chemists, veterinarians, and entomologists. We at Kerrville feel fortunate that the Agricultural Research Service has placed us together, where we can work as a team in the development of treatments through the preliminary stages.

Only the preliminary work can be accomplished at one location. By close cooperation with the Entomology Research Division's Corvallis laboratory, progress is expedited and a better idea of the utility of a new treatment can be obtained. The independent observations at two widely separated locations also help to assure the validity of preliminary conclusions as to the value of a new treatment.

However, our experimental resources are limited and final conclusions depend upon the critical evaluations and independent observations of many entomologists, parasitologists, and veterinarians who do research on cattle grubs at state experiment stations and Canadian field laboratories. Similar scientists employed by industrial organizations have also worked in many states to evaluate systemic insecticides, and their observations form a vital part of the performance record on new chemicals. Specialists in animal nutrition at the state experiment stations have made available the cattle in their feeding experiments, and their notes on reactions of treated animals and their weight-gain studies have been invaluable in final appraisal of the treatments. Veterinary parasitologists in the Animal Disease and Parasite Research Division at Beltsville and its field laboratories make important records on insect control and safety of treatments, as well as observations on effects on internal parasites.

Cooperation with industry is essential to success. The two treatments now recommended would not have been available for research investigation, were it not for the cooperation of the companies that synthesized the first experimental samples. Those companies supplied the radioactive insecticides as well as abundant quantities of field-test formulations. In addition, they defrayed, through grants-in-aid, part of the cost of the elaborate toxicological evaluation required to establish safety for livestock and freedom from insecticide residues in meat from treated animals. Many other chemical companies have cooperated similarly in studies on other livestock insecticides. This pattern of industry cooperation is expected to continue as new systemics are developed.
NEW RESEARCH RESULTS WITH SYSTEMIC INSECTICIDES

LITERATURE CITED


REPORT OF COMMITTEE ON STOCKYARDS, MARKETS AND TRANSPORTATION

UNITED STATES LIVESTOCK SANITARY ASSOCIATION

R. Cuff, Chairman, Kansas City, Missouri; A. Z. Baker, Cleveland, Ohio; T. W. Cole, Florida; A. G. Pickett, Topeka, Kansas; E. P. Ryan, Grand Island, Nebraska; D. H. Voltz, Omaha, Nebraska; F. B. Wheeler, Baton Rouge, Louisiana.

Our Committee feels that the establishment of a better working relationship and understanding between state and Federal regulatory officials with operators of stockyards, livestock markets and transportation agencies would create conditions favorable for the general good of the entire livestock industry. Closer understanding will aid in the freer interstate movement of clean, healthy animals. We mention a few such tried, practical programs that benefit livestock growers, stockyards, markets and transportation agencies. We suggest this type of activities be increased.

ANIMAL DISEASE ERADICATION INTERSTATE REGULATIONS

We wish to commend the Animal Disease Eradication Branch of the United States Department of Agriculture for its foresight in issuing C. F. R. Amendment 56-40 with special reference to Federal requirements governing the interstate movement of cattle. For many years the industry has been handicapped in the control of interstate shipments, as Federal regulation has not covered the interstate movement of livestock by all forms of transportation. This regulation requires that a Way Bill accompany all shipments, by truck as well as by rail.

We recommend the strict enforcement of this regulation to control disease by preventing the illegal interstate movement of cattle.

VISIBLE IDENTIFICATION HELPS INTERSTATE MOVEMENT

We recommend the use of the United States Department of Agriculture officially adopted, orange-colored eartag as a supplement to ear tattooing and registered brands for identifying commercial, beef-type heifers officially vaccinated against brucellosis.

These trade-marked, ready-to-go heifers are in increasing demand at central markets, as they can be moved as feeder cattle into many states without further unnecessary, costly tests and delays. Market operators are aiding in selling the calfhood vaccination program to growers by demonstrating the added sales value of such trade-marked cattle.

Market and transportation agencies can assist in the sale and more prompt interstate movement of officially calfhood-vaccinated heifers by informing...
growers of the importance of attaching their vaccination certificates to bills of lading.

We further recommend that state regulatory officials consider the advisability of adopting regulations, conforming as nearly as possible to those of the Animal Disease Eradication Branch of the United States Department of Agriculture, governing the interstate movement of cattle of the beef breeds for feeding or grazing purposes only.

SHEEP SCABIES CONTROL

As some sheep in several states are still infested with scabies; and as one thorough dipping in an approved dip such as Lindane or BHC (benzene hexachloride), in most instances, rids sheep of scabies, keds and lice in addition to protecting animals from flies for some time after dipping; and as many sheep and lambs are sold through public markets for feeding and grazing purposes; and as some livestock markets are now rendering a real service to sheep growers by dipping sheep under supervision at prices as low as ten cents (10¢) per head;

We recommend that for the general good of the sheep industry all markets that are not now equipped for dipping be apprised of the need and desirability of furnishing such dipping services.

Since increasing numbers of informed growers are requesting that feeding cattle and sheep purchased at supervised markets be treated for worm and coccidia control,

We recommend that state and Federally supervised markets offer the optional service of treatment of cattle and sheep for worm and coccidia control.

CATTLE GRUB CONTROL

As grubby slaughter cattle are now docked an average of $1.00 per hundredweight or around $10.00 per head; and as the running of pasture cattle during the egg-laying season of the heel fly causes an added loss; and as in a sizable test of 453 feedlot cattle in Kansas in which 225 cattle treated with a phosphate systemic made an added average gain of nearly one-quarter pound per day over a 97-day feeding period with an added net profit of $5.71 per head; and since there are now on the market two licensed systemic products that will kill the larvae of the cattle grub before they damage the loins, ribs and sides; and as one thorough summer or fall treatment will control grubs before they do their damage as well as controlling horn flies, lice, ticks and screwworms for an approximate three-weeks period;

We recommend that public livestock markets offer these systemic treatments as an optional service on outbound stocker and feeder cattle.

Since observations at the Kansas City Market on some 1,000 outbound feeder cattle, many of which were either dehorned, branded or castrated during the fly season, definitely showed that a single thorough skin wetting by spraying both top and underline of cattle with the new phosphate systemic
during July and August controlled screwworms for about three weeks or until ordinary wounds were healed; and as the treatment of stocker and feeder cattle at public markets before shipment to feedlots is an important factor in decreasing economic losses from external parasites and in increasing gains and profits; and as the yearly introduction of screwworm-infested animals from Southern areas where screwworms "over-winter" causes a large annual loss in screwworm-free areas.

We recommend that state and Federal regulatory officials request the supervised markets to furnish optional services for treatment of cattle with licensed systemic for screwworm control.

The adoption of these simple, practical, workable practices by supervised livestock markets will be a factor in increasing growers’ profits, in controlling parasites and diseases and in facilitating the interstate movement of livestock.
INFECTIOUS SYNOVITIS CONTROL 10: CHLORTETRACYCLINE IN CHICKS INOCULATED AT ONE DAY OF AGE*

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Infectious synovitis (IS) was first recognized as a separate disease entity of chickens in 1954 by Olson et al. (1) in West Virginia, Wills in Texas (2), and Cover and Galeta in Delaware (3) and of turkeys by Snoeyenbos and Olesiuk in Massachusetts (4). The disease has been recognized in all the major broiler producing areas of the United States. Its occurrence appears to be variable but not related to the season of the year. Cosgrove (5) showed evidence that IS was the second most important poultry disease to chronic respiratory disease.

The etiology of the disease has been classed as that of a large particle virus or virus-like agent by Wills (6), Lecce et al. (7), Cover et al. (8), and Olson et al. (9). The agent has been propagated in the allantoic cavity, chorioallantoic membrane (CAM), and yolk sac of embryonating eggs. The latter route of inoculation has given the most consistent results. Some strains produce plaque-like lesions on the CAM (10). Olson (11) found that two of nine strains produced plaque-like lesions on the CAM. The extent of the lesions varied from an occasional nodule to extensive proliferation.

Chickens are susceptible to the IS agent by all routes of inoculation except orally (6, 7, 8, 9). Cover (12), following intravenous or intramuscular inoculation of chickens, found the IS agent in almost all body tissues. It was not found in the intestinal contents, bile, or the synovial membrane. A viremia was produced in 48 hours and was still present on the tenth day post-inoculation when the experiment was terminated.

The disease has been characterized by Olson et al. (9) by anemia, pale comb, leg weakness, droopiness, emaciation, enlarged hocks and foot pads, breast blisters and dehydration; at necropsy by creamy or caseous exudate involving the synovial or bursal membranes, swollen kidneys, occasionally enlarged and discolored livers and occasional splenomegaly; and hematologically by reduced hemoglobin, lower erythrocyte and higher leukocyte counts, decrease in the percentage of lymphocytes and increase in the percentage of monocytes, heterophils, and “preforms” of white blood cells. Histologically, Sevoian et al. (13) found primarily a hyperplasia of the reticular cells of the reticuloendothelial system of the liver, heart, lungs and gizzard.

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The disease has been called synovitis rather than arthritis since the joint surfaces are not eroded (6, 9). One strain of IS, however, caused ossification of the tendons and tendon sheaths with a marked erosion of the joint surfaces (11).

Hofstad (14) mentioned the following bacteria as a cause of arthritis: *Streptococcus gallinarum*, *Streptococcus zooepidemicus*, *Staphylococcus pyogenes*, *Bacterium arthropypogenes*, *Escherichia venezuelensis*, *Escherichia coli*, and *Salmonella pullorum*. Olson (9) isolated pleuropneumonia-like organisms (PPLO) from swollen hocks and breast blisters. A PPLO or bacterial joint infection frequently is a secondary condition following some other disease and must be distinguished from the cause of synovitis. Infectious synovitis is not caused by a PPLO or bacteria.

Fowl pox inoculated into the foot pad of chickens will cause a synovitis-like condition. No relation between fowl pox and IS has been demonstrated (11).

Transmission of IS has not been completely elucidated. Thayer et al. (10) gave rather conclusive evidence that IS is egg transmitted. Wills and Delaplane (15) found only one chick out of 543 with IS that were hatched from dams which were artificially infected. Egg transmission appears to be variable and this may account for the variable occurrence of the disease in the field. Shelton et al. (16) demonstrated a marked increase in the gamma globulin fraction in the blood of chicks with IS. It is possible that birds develop a resistance to the disease and then egg transmission ceases. Evidence of immunity to IS was observed in hens (15).

No definite information is available on how the infection spreads from bird to bird. However, since the viremia stage is present up to 10 days and possibly longer, insect vectors should be considered. One of 12 chicks became infected following wing web stab with the IS agent (11). The disease spreads by contact, probably by the respiratory route with an incubation period of 24 days or longer (9).

Considerable information is available on the use of antibiotics and furazolidone (nf-180) to control IS. Many of these reports are not in agreement. Washko et al. (17) reported from field data that a single injection of 200 mg. of streptomycin controlled IS. Shelton et al. (18) and Munro et al. (19) found 25 mg. per lb. of body weight of streptomycin effective if injected at the time of experimental inoculation with the IS agent. If the disease was allowed to develop for four to eight or 12 days before injection, even 200 mg. per lb. was not very effective. Cosgrove (20) reported nf-180 effective; however, Shelton et al. (21), Thayer et al. (10) and Wills and Delaplane (15) reported a low rate of efficacy for nf-180. Bletner et al. (22) found that continuous chlortetracycline (CTC) medication was effective for IS control when the treatment was started at the time of inoculation. If CTC treatment was delayed two or four days after inoculation and then given for 26 days, IS was controlled; however, when the medication was removed, approximately 50 percent of the birds again showed signs of IS. Delay of the treatment four to eight days resulted in a lowering of the efficacy of CTC.
This is not surprising since the agent is apparently encapsulated in the synovial membranes. When the antibiotic is given, spreading and further development is stopped and there is a marked reduction in the severity of the disease. This was clearly shown by Munro et al. (19) when CTC was fed intermittently (five days on and five days off).

Olson et al. (23) found CTC more efficacious than oxytetracycline (OTC) against the IS agent. Shelton et al. (24) found this difference to be related to the absorption from the intestinal tract since CTC and OTC were equally efficacious when given by daily intraperitoneal injections.

Many growers have objected to the high cost of continuous medication at the rate of 50 to 100 grams of antibiotic per ton of feed. Shelton and Olson (25) worked with the potentiating effect of terephthalic acid on CTC for the control of IS. They found that 0.5 percent terephthalic acid in the ration increased the efficacy of CTC by four to five times. This would reduce the cost of medication approximately one-half.

Strain differences of the IS agent have appeared in terms of their sensitivity to antibiotics. Cover et al. (3) found one strain was resistant to antibiotics in ova. Olson et al. (26) found two relative non-pathogenic strains in foot-pad inoculated birds resistant to CTC (200 gm./ton of feed). There is also a difference in the efficacy of antibiotics depending on the amount of inoculum used (27). Sevoian et al. (28) reported a considerable difference among strains of the IS agent in their sensitivity to antibiotics. There is also a variation of a given strain which occurred in different pools of the virus (11). The cause of this variation is not known, but it may be related to the route of inoculation, pathogenicity of the culture, or age and susceptibility of the chicks.

The work of Thayer et al. (10) and Sevoian et al. (28) gave evidence of egg transmission of IS. This raises these questions: Will CTC fed for a three-week period at high levels and then removed control IS? What effect does high level feeding of CTC have once the infection becomes established? Can CTC be fed continuously to day-old inoculated chicks for a seven-week period and then withdrawn, without additional birds showing signs of IS? Once infection has become established, what effect does a 10-week feeding of CTC have on the control of IS?

MATERIAL AND METHODS

This experiment was conducted with White Rock-Vantress chicks. The birds were either placed in batteries in an air-conditioned room using 15 birds per battery or in a broiler house using 200 birds per pen. Suitable controls were maintained. All the chicks except the controls were inoculated with IS agent 1,853 at one day of age by the following routes and amounts: intraperitoneally (IP), 0.25 ml.; intramuscularly (IM), 0.10 ml.; or via the foot-pad (FP), 0.10 ml.

In the battery reared groups all chicks were placed on 0, 50, 100, 200, 500 or 1,000 grams of CTC per ton of feed starting at day-old or at eight-days old.
The chicks were maintained on the respective medication for three weeks. The medication was then removed and the birds observed for an additional three weeks.

Among the field reared birds all chicks were placed on 0, 50, 100 or 200 grams of CTC per ton of feed starting at day-old or seven days old and continued for seven weeks or nine weeks respectively. Observations were made daily and all birds that died were necropsied. Detailed observations were made at seven and 10 weeks. Two hundred birds per pen were maintained on each treatment and inoculated as follows: 50 IP, 50 IM, 50 FP and 50 contact controls. Two hundred birds were maintained as untreated, uninoculated controls in a separate pen in the same house and are referred to as isolated controls.

RESULTS

The results and design of the experiment in battery-reared birds are shown (Table 1). Too few IP inoculated birds became infected to permit one to draw any conclusions. When medication was started at time of chick inoculation at one day of age, CTC (50 gm. per ton of feed) controlled the infection in IM inoculated birds. When medication was removed, CTC (200

<table>
<thead>
<tr>
<th>Route of Inoculation</th>
<th>Number of Birds Infected or Showing Extension</th>
<th>Gms Chlortetracycline/ton of Feed From 1 to 21 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0   50  100  200  500  1000</td>
</tr>
<tr>
<td>Number Infected*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>0/0</td>
<td>0/0  0/0  0/0  0/0  0/0</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>1/3</td>
<td>0/1  0/1  0/0  0/0  0/0</td>
</tr>
<tr>
<td>Foot-Pad</td>
<td>14/14</td>
<td>9/11 9/11 0/4 0/1 0/0</td>
</tr>
<tr>
<td>Number Extended†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot-Pad</td>
<td>8/10</td>
<td>0/5  0/1  0/0  0/0  0/0</td>
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</table>

<table>
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<tr>
<th>Route of Inoculation</th>
<th>Number of Birds Infected or Showing Extension</th>
<th>Gms Chlortetracycline/ton of Feed From 8 to 29 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0   50  100  200  500  1000</td>
</tr>
<tr>
<td>Number Infected*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>0/1</td>
<td>1/2  0/0  0/1  0/0  0/0</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>4/4</td>
<td>1/3  1/1  0/3  0/0  0/0</td>
</tr>
<tr>
<td>Foot-Pad</td>
<td>14/15</td>
<td>13/14 14/14 1/11 0/8 0/5</td>
</tr>
<tr>
<td>Number Extended†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot-Pad</td>
<td>10/10</td>
<td>2/4  3/6  0/4  0/3  0/1</td>
</tr>
</tbody>
</table>

* Number of infected birds after 3 weeks medication/number of infected birds 3 weeks after removal of medication.
† Number of foot-pad inoculated birds showing extension of infection in other joints after 3 weeks medication/number of such birds 3 weeks after removal of medication.
INFECTIOUS SYNOVITIS CONTROL

205

gm. per ton of feed) was effective in preventing reappearance of IS. In FP inoculated birds CTC (50 gm. per ton of feed) prevented extension of infection but did not prevent signs of IS. The 100 gram level of CTC prevented signs of IS in the birds when the drug was being fed. When CTC was removed from the ration IS did not appear in those birds previously fed CTC (500 gm. per ton of feed).

When medication was started eight days after inoculation 200 and 500 grams per ton of feed were required to prevent signs of IS in IM and FP inoculated birds respectively. Three weeks after removal of medication, signs of IS were not apparent in the IM inoculated chicks given CTC (500 gm. per ton of feed); however, in the FP inoculated birds at the 1,000 gram level of CTC, five of 15 chicks developed signs of IS.

The number of infected chicks at seven and 10 weeks in the field reared birds, inoculated with IS at day-old by the IP and IM route, is shown (Table 2). Among the inoculated non-medicated controls, deaths occurred

TABLE 2

Chlortetracycline Treatment of Intraperitoneal and Intramuscular Inoculated Day-old Chicks Reared on the Floor (Fifty Birds Per Treatment)

<table>
<thead>
<tr>
<th>Route of Inoculation</th>
<th>Number Deaths/Number Infected Birds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gms Chlortetracycline/Ton of Feed From Day-old to 7 Weeks</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7 Wks</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>6/8</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>12/16</td>
</tr>
<tr>
<td></td>
<td>From 1 Week to 10 Weeks</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>---</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>---</td>
</tr>
</tbody>
</table>

in 12 and 24 percent and 24 and 36 percent became infected in the IP and IM inoculated groups respectively. No deaths occurred and there was a significant reduction in the number of infected birds when CTC (50 gm. per ton of feed) was given starting at one day of age. Removal of medication resulted in a slight increase in the number of chicks showing signs of IS. At the 100 and 200 gram levels of CTC, none of the birds showed signs of IS. Delay of CTC (50 gm. per ton of feed) until seven days after inoculation also resulted in a decrease in the number of birds with signs of IS when compared to the inoculated controls, but an increase when compared to the group given CTC starting at one-day of age. None of the IP inoculated birds given CTC (100 or 200 gm. per ton of feed) showed signs of IS; however, in the IM groups six and two percent showed signs of IS at the 100 and 200 gram levels respectively. Deaths as a result of IS occurred in the IP (two percent) and IM (eight percent) groups given CTC (50 gm. per ton of feed). This was a marked reduction in the number of deaths when compared to the untreated inoculated controls.
The number of infected birds, number showing extension of infection to other joints, and the number of deaths at seven and 10 weeks of age in foot-pad inoculated chicks, isolated controls, and contact controls are shown (Table 3). The foot-pad route of inoculation was a more sensitive test of the efficacy of CTC since the progress of the disease could be observed by the extension of infection or occurrence of swellings in joints other than the inoculated leg.

The CTC (50 gm. per ton of feed) when started at one day of age was effective in localizing the infection as shown by the lack of extension and lack of deaths as a result of IS. It was not very effective in preventing signs of IS. Forty-six percent as compared to 74 percent of the untreated birds showed signs of IS at the end of seven weeks when medication was removed. Only six percent of those receiving the 100 gram level and none of those receiving the 200 gram level of CTC showed signs of IS at the end of seven weeks. There was a 10 percent increase in the number of birds showing signs of IS at the end of 10 weeks when CTC (100 gm. per ton of feed) had been removed at seven weeks.

When CTC was given starting seven days after infection, even the 200 gram level of CTC allowed the infection to develop in 30 percent of the birds. There was a significant reduction in the number of deaths and the number of chicks with extended infection at all levels of CTC.

It is interesting to note that no infection occurred in the uninoculated contact controls when the birds were given CTC; however, 10 percent of the contact controls to which no CTC was given showed signs of IS. In the

<table>
<thead>
<tr>
<th>Degree of Infection</th>
<th>Seven-Week Observation</th>
<th>Ten-Week Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Day Old*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Infected</td>
<td>37/38</td>
<td>23/26</td>
</tr>
<tr>
<td>Number Showing Extension</td>
<td>29/30</td>
<td>0/2</td>
</tr>
<tr>
<td>Number Deaths</td>
<td>19/22</td>
<td>0/0</td>
</tr>
<tr>
<td>Seven Days Old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Infected</td>
<td>—</td>
<td>31/32</td>
</tr>
<tr>
<td>Number Showing Extension</td>
<td>—</td>
<td>4/6</td>
</tr>
<tr>
<td>Number Deaths</td>
<td>—</td>
<td>1/2</td>
</tr>
<tr>
<td>Uninoculated Controls</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Contact—Number Infected</td>
<td>0/5</td>
<td>0/0</td>
</tr>
<tr>
<td>Isolated—Number Infected†</td>
<td>1/2</td>
<td></td>
</tr>
</tbody>
</table>

* Medication removed at 7 weeks.
† 200 Birds.
isolated controls one percent of the birds showed signs of IS. The source of this infection is not known; however, it is possible that it may have been from contact since the so-called isolated controls were reared in the same house as the infected birds.

Approximately two percent of the birds died from unknown causes and these losses were classified as non-specific. Most of these deaths occurred in the first 10 days of brooding and were not included in any of the tables. The IS in these studies was not complicated with intercurrent infections.

**DISCUSSION**

The results of this experiment confirm previous observations that continuous medication is needed to control infectious synovitis (IS) (22). In previous experiments, with birds inoculated at two weeks of age CTC (50 gm. per ton of feed) gave good control (29); however, the results of these trials indicate the need for higher levels during the first two to three weeks. This difference may be due in part to the small amount of feed eaten during the first one or two days allowing IS to become established in foot-pad inoculated birds. This is confirmed in part by the greater efficacy of CTC in IP and IM inoculated birds where the incubation period of IS is longer.

Where the treatment was delayed seven or eight days CTC (200 gm. per ton of feed) was needed to prevent further spread of IS. In battery reared birds even a 1,000 gm. per ton of feed did not prevent the appearance of signs of IS when medication was removed. It is felt, therefore, that it would not be practical to give a high level of CTC for a short period of time to control IS. The practical applications of these results are that CTC (100 gm. per ton of feed) for the first three weeks and CTC (50 gm. per ton of feed) for the next four weeks should provide satisfactory control of IS in broiler flocks. These levels should prevent the spread of IS, but would not prevent the disease in those chicks infected prior to medication. Where complications with other diseases occur and feed consumption is reduced, higher levels of CTC should be given. Once an outbreak of IS has occurred at least 200 grams of CTC per ton of feed should be given.

It should be recognized that the ultimate goal is the eradication of IS. This is not possible with our present knowledge of the disease and where trouble has occurred medication should be used to reduce losses.

**SUMMARY**

Chlortetracycline (CTC) was effective in controlling infectious synovitis (IS) in day-old inoculated chicks if given at the time of inoculation. The CTC (50 gm. per ton of feed) reduced the amount of IS, localized the infection and prevented further spread. The CTC (200 gm. per ton of feed) if given at time of inoculation for a three- or seven-week period prevented the recurrence of the disease when medication was removed. Levels of CTC (500 to 1,000 gm. per ton of feed) given eight days after infection eliminated the signs of IS when given for three weeks, but allowed the recurrence of IS when medication was removed.
REFERENCES


INFEKTIOUS SYNOVITIS CONTROL


REPORT OF THE COMMITTEE ON
TRANSMISSIBLE DISEASES OF POULTRY

R. A. BANKOWSKI, Chairman, Davis, California; A. CHRISTIE, Kingston, New Hampshire; H. M. DEVOLT, College Park, Maryland; E. MILTON DICKINSON, Corvallis, Oregon; R. F. GENTRY, University Park, Pennsylvania; HARRY E. GOLDSTEIN, Columbus, Ohio; N. O. OLSON, Morgantown, West Virginia; R. E. OMOHUNDRO, Washington, D. C.; B. S. POMEROY, St. Paul, Minnesota; CHARLES S. ROBERTS, Auburn, Alabama; ROBERT H. SINGER, Frankfort, Kentucky; WILLIAM L. SIPPEL, Kissimmee, Florida; J. FRANKLIN WITTER, Orono, Maine.

Although no problem of single major importance developed in the field of poultry pathology during the past year, considerable progress has been made in the evaluation, control and prevention of diseases affecting chickens and turkeys. However, some attention should be focused on the gradual but progressive recognition of variants or changes in the immunological characteristics of poultry viruses which have been recently isolated from field infections. The occurrence of immunologically distinct types of a virus may seriously complicate the already overburdened vaccination schedules in our present methods of controlling specific infections in poultry. Most of the present-day vaccines are composed of living, virulent virus suspensions which are capable of introducing an infection in susceptible or partially-immune chickens or result in a more serious respiratory disease complex when superimposed on another infection. Multiple infections of the respiratory tract of poultry suffering from a respiratory disease are also reported more frequently. Because of these circumstances, without envisioning an early eradication of some of the diseases with the present-day vaccines, it is the opinion of the Committee that the responsibilities of the veterinarian to the poultrymen during the next few years may be considerably increased. Judicial recommendations of application of the various present vaccines will be necessary as well as a concentration of the efforts of the investigators on the improvement of the immunizing agents.

RESPIRATORY DISEASES

Newcastle Disease.—One severe outbreak of Newcastle disease (ND) was reported during the past year. Four unvaccinated flocks in Masaryktown, Florida, were affected resulting in a 74 percent mortality in one flock with paralysis involving some of the remaining birds. The outbreak was controlled by vaccinating birds with B1 water ND vaccine in a two square mile surrounding area. A number of reports appeared relative to preparation and improvement of vaccines. Sullivan, Gill and Somer (1) observed that Beta propriolactone (BPL) inactivated vaccines produced higher immune re-
sponses in chickens than similar products prepared from formalin-killed agents. Furthermore, the GB Texas strain of BPL vaccine was more immunogenic than products prepared from the Manhattan, Kansas, or Roakin NJ strains. Administration of a second or booster dose resulted in an equivalent and significant anamnestic response by all three strains.

The tissue culture attenuated ND vaccine prepared from the California 11914 strain of NDV which can be propagated in Hela, bovine kidney, and chick embryo cells, was shown to be apathogenic to susceptible chicks of any age when given intramuscularly or intravenously (2, 3, 4). The virus, however, was virulent for chicken embryos but did not regain its pathogenicity for chickens following 50 serial passages through the embryonic tissue. Five-day-old susceptible chicks were immunized for at least 13 weeks by a single intramuscular dose. A distinct advantage of the live tissue culture attenuated NDV was that there was no evidence of spread of the virus from vaccinated to susceptible penmates. Two intramuscular doses of the vaccine, protected laying hens at (78 weeks of age) against signs of disease and a drop in egg production following contact and intramuscular exposure to the GB NDV (5). In a limited number of field trials, the vaccine produced no apparent stress or other untoward reactions nor aggravated respiratory symptoms of chickens actively infected with CRD and IB. Susceptible pullets did not show any signs of disease nor drop in egg production following an intramuscular injection of the TC virus (3, 5).

An investigation of "breaks" following B1 vaccination of flocks in Mexico was attributed mainly to the loss of active virus in commercially available vaccines distributed in that country. The author suggested establishment of a method of standardization of this type of vaccine on a national or international basis to offer greater guarantees to the poultry farmer in the fight against Newcastle disease (6).

In the study to determine routes of infection with NDV, Kohn reported that the alimentary infective dose of a virulent strain of NDV, was found to be of the same order of magnitude as the vaccination dose of the B1 strain administered in the drinking water. It was concluded that the infection by the alimentary route is initiated in the intestines below the gizzard by the fraction of virus which survived the acid inactivation in the gizzard (7). A viral agent believed to be the B1 strain of NDV was isolated from a case of shipping fever in cattle (8).

Sinha studied the influence of temperature of incubation of embryonating eggs inoculated with six strains of Newcastle disease virus (NDV) and found that in all cases, the embryos died much earlier at 99° than at 95° F. (9).

The Canadian goose (Branta canadensis) was added to the list of susceptible bird species to NDV (10). Zuschek et al. demonstrated that the Roakin GB and B1 strains of NDV could be propagated in chorio-allantoic membranes of embryonated chicken eggs suspended in Tyrode's solution (11).

Frederickson and Chute in their studies of chicken blood demonstrated that the white blood cell and leucocyte differential count were of considerable diagnostic value in Newcastle disease. The most apparent change in the WBC
count were a leucopenia on the eleventh day and a leucocytosis (monocytes and heterophiles) on the fourteenth day after infection (12).

Boyd and Hanson (13) demonstrated that a rapidly dried suspension of NDV persisted for a longer period than one exposed to slow drying. Aeration and lack of salts or organic matter in water adversely affected its survival. The virus also appeared to be destroyed by the digestive or assimilative processes of the earthworm (Helodrilus) and planaria (Planaria maculata).

Although the literature on Newcastle disease is voluminous, not all phases of the disease have yet been studied. Sullivan described the degenerative changes associated with the clinical manifestations caused by the neurotropic California 11914 strain of NDV (14).

Infectious Bronchitis.—Although vaccination against infectious bronchitis has been satisfactory, the disadvantage with the present-day vaccines is its spread from vaccinated to susceptible chickens. Christian and Mack demonstrated that Beta-propriolactone destroyed the infectivity of IBV for embryonating eggs but that the virus retained part of its ability to elicit specific antibodies in chickens. Although the inactivated IB vaccine did not protect 100 percent of the birds, considerable protection was offered with the vaccine (15). This initial experiment may lead to studies on the improvement of infectious bronchitis vaccines.

Studies on the evaluation of the immunogenicity of IB vaccines which were conducted by Chang et al. demonstrated that respiratory symptoms, gross and microscopic lesions in the trachea, and recovery of the virus from tracheal exudates were valid as criteria for only a limited period (16). None of the criteria used, however, was effective over the total test period. The virus was readily isolated from the trachea and lungs of susceptible birds, but from immune individuals, isolation from the trachea was more likely than from lung tissues. Hofstad demonstrated that IB isolates from field infections yielded strains of varying antigenicity and that three of the 14 isolates were found to be immunologically distinct (17).

Raggi and Lee demonstrated that in spite of a high level of passive antibodies in six-day-old chicks approximately 50 percent responded to an IB vaccine with respiratory signs and were immune to challenge 50 days later; chicks which were refractory to the vaccine were susceptible to challenge. The response to challenge did not always correlate with the SN titer. Sixty percent of the birds with insignificant SN titers were immune to an intratracheal challenge exposure of IBV. Vaccination of susceptible chicks resulted in a regular and satisfactory immunological response (18). More recently, Raggi demonstrated that a commercially-available IB vaccine prepared from the Connecticut A5968 strain differed serologically and immunologically from the egg adapted DA 138 and the Massachusetts LG43 strains of IBV. Under experimental conditions the vaccine protected only 25 to 40 percent of the birds to an intratracheal challenge of the Massachusetts strain seven weeks later (19).
Laryngotracheitis.—In spite of competent veterinary advice and adequate vaccines available to poultrymen, losses due to LT have been reported. Kingsbury and Jungherr (21) described an enzootic of LT disseminated by human carriers, which occurred within a 20-mile radius. Although properly advised, only a few poultrymen followed the recommendations despite a combined loss of more than $18,000 in mortality alone. In a preliminary report, Click (20) demonstrated that the bursa of Fabricus plays a prominent role in the production of antibodies.

As increasingly more attention is focused on the respiratory diseases of birds, there is an increasing need for practical tests that can be used for identification of a virus and a determination of an immunity by means other than challenge. Two reports described a serum-neutralization test and its application. Both techniques applied the serum-virus mixtures on dropped CAM of embryonating eggs (22, 23). Hitchner et al. demonstrated that virus-neutralizing antibodies were detected in birds vaccinated with laryngotracheitis vaccine one week after vaccination and reached their maximum by the third week (22). Although these tests are an improvement, neither is rapid since both methods require from five to 10 days for completion. The tests, however, are suitable for survey work and studying various aspects of infection and immune responses to LTV.

Mixed Infections of Respiratory Tract.—In an attempt to selectively isolate a specific strain of agent from a mixture, Quiroz and Hanson demonstrated that certain mixtures of nine strains of five avian viruses could be resolved by physical and chemical treatment of the inoculum (24).

In another study, Reid et al. demonstrated that parasitism by Ascaridia galli combined with IB infection resulted in a higher mortality and less weight gains than in broiler flocks infected only with IB or infested with the worms alone (25).

Chronic Respiratory Disease and Infectious Sinusitis.—The etiology of chronic respiratory disease has been reviewed by Adler et al. (26), Yamamoto and Adler (27), and Stafseth (28). It was emphasized that the role of the various strains of pleuropneumonia-like organisms now known as Mycoplasma (MP) as the cause of disease must be clarified. Since both pathogenic and non-pathogenic strains have been found, procedures for their identification need to be reevaluated and should include the use of bird inoculation (26). It has been reported that L forms of bacteria were present in many cultures of the so-called MP (29). It has been suggested (30) that these may account for many of the non-pathogenic strains and that only the pathogenic strains are true MP. L-forms may have originated from bacteria which were present as part of a secondary infection. None, however, have been identified as being related to Escherichia coli which has often been associated with this disease (31). The differentiation of respiratory infections caused by MP and those produced by combinations of other bacteria and viruses remains as a major problem.
MP are capable of producing disease in avian species other than the chicken and turkey. The presence of MP in the chukar partridge has been reported (32) and was apparently associated with respiratory infection. They were also found to be capable of producing encephalitis as well as aerocystitis in turkey poult (33) and arthritis in chicken embryos (34). The relationship of these findings to CRD was not established.

Working with purified cultures, Domermuth (35), described a method for the direct enumeration of MP but he pointed out that due to morphological variations this was only possible with certain strains.

Although bird to bird transmission must still be regarded as most important, transovarian infection is considered to be the principal route for perpetuating CRD from one generation to the next (36). Artificial infection in chicks by the inoculation of fertile eggs with pathogenic MP is readily accomplished. However, the insemination of hens with semen containing these organisms failed to demonstrate transmission of the infection to the resulting progeny (37). Therefore, the source of infection appears to be the hen.

The possibility of transmission through vaccination must be considered since MP are capable of surviving the freeze-drying procedures used in vaccine production (38), but this mode of transmission has never been conclusively demonstrated.

The control of CRD by breaking the cycle of transovarian infection has been attempted by the administration of high levels of antibiotics to laying birds. In many cases this did not inhibit the elimination of MP via the egg (36) and cannot be depended upon to adequately control the disease (39). The ultimate aim for the control of CRD is the eradication of MP from laying flocks. Serological testing gave variable results and served only as an indication of the relative degree of infection within the flock (40). Also the presence of other bacterial and viral infections intensified and prolonged the MP infection which seriously hampers the development of an adequate control program.

Infectious sinusitis in turkeys presents many of the same problems as are found with avian CRD. The mode of transmission is again through the egg (41) with the progeny having both sinus and air sac infection. Also, antibiotic treatment fails to inhibit this transmission. In certain instances the presence of infection apparently does not effect the growth and development of the birds (42). The national coordination of work has greatly helped advance the knowledge of this condition but control is dependent upon a method for the detection of carrier birds (43).

CRD and infectious sinusitis are still the most prevalent of our poultry diseases and cost poultry farmers approximately $60,000,000 annually (44).
AVIAN ENCEPHALOMYELITIS
(Epidemic tremors)

AE is known to be widespread throughout the United States and is recognized as egg-borne. Reports from 14 diagnostic laboratories along the Atlantic seaboard indicate that the importance of AE is increasing in three areas, diminishing in three areas, and remaining about the same in 11 areas. Consequently, with the increasing size of hatching-egg flocks, the potential economic importance of AE is growing. Iritis has been recognized as a possible sequel to AE (45).

Experimentally, the use of known immune breeding flocks has proved successful in curbing the incidence of AE. However, as far as is known, vaccination of laying stock is practiced by only a few large breeders on the western coast, and they are using home-produced vaccine for breeder hens. There are no practical control measures. In some states, attempts are made to trace the source of infection to specific supply flocks which are then temporarily suspended. Some hatcheries endeavor to anticipate the spread of AE in eggs by closely observing egg-production records. Unexplained declines are carefully investigated, and during such periods, the eggs are not used for hatching.

INFECTIOUS SYNOVITIS

Olson et al. (46) attempted unsuccessfully to differentiate by hematological studies synovitis caused by the infectious synovitis agent from that caused by MP and a combination of the two. They noted that the lesions produced by the infectious synovitis agent were more severe than seen in MP synovitis.

Olson et al. (47) recovered three synovitis producing agents from field flocks; the infectious synovitis agent, MP and an agent pathogenic for chicken embryos but relatively non-pathogenic for chickens. Chlortetracycline (aureomycin), (200 gm. per ton of feed) was effective in treating all three agents, but withdrawal relapses were common. Furazolidone (NF-180) was ineffective in these in vivo trials, differing from in vitro results. Shelton and Olson (48) have shown an enhancing effect of terephthalic acid (TPA) on the utilization of chlortetracycline (aureomycin) equivalent to a fourfold increase. By using a ration containing 25 grams of chlortetracycline and 0.5 percent TPA, the disease was completely controlled in their experimental birds.

Thayer et al. (49) found extreme variability in the pathogenicity of the various agents incriminated in field outbreaks of IS. They found the chorioallantoic membrane of nine-day-old embryonated chicken eggs to be a satisfactory method of isolating the agent and also found definite evidence of hatching egg transmission of a synovitis.
AVIAN HEPATITIS

The clinical picture and pathology has been well described by Sevoian et al. (50). They describe a protracted course of the disease through a flock with as much as 35 percent drop in egg production and a cumulative mortality of up to 15 percent. Sevoian (51) states that in young birds the heart lesions are more prominent than the liver lesions. Chicks appeared more susceptible to experimental infection than did older birds. Lesions, similar to those seen in natural infection, appeared in five to 15 days in experimentally infected older birds as compared to two days in chicks. Organs affected are the liver, heart and spleen.

These same authors (52) found the disease to propagate readily in six- and 10-day-old chicken embryos inoculated via the yolk sac. Furazolidone (NF-180) and dihydrostreptomycin sulfate, in that order, proved most effective as prophylactics and therapeutic agents for the disease. Sevoian (51) recommends 300 gms. of furazolidone per ton of feed as treatment. The tetracycline group also had a favorable effect, but relapse tendencies were noted on withdrawal. They found the agent to pass filters of 450 millimicron size, but not 300 millimicrons. It was sedimented by 15 to 20 minute centrifugation at 27,500 r.c.f. Antibody response following injection, or convalescent immunity, were not demonstrated. Turkey poult’s and four- to five-week-old rabbits were successfully infected with the agent. The agent withstood storage at −56° C for 28 days. Moore (53) studied an hepatitis agent with many similarities to that described by Sevoian. The latter concludes his agent “to be similar, if not the same, as the ones described by Delaplane et al. (Moore) and Tudor.” In addition, Moore found the agent to rise to the top in ultracentrifuged material, indicating a possible lipoprotein nature. Moore was able to infect three-week-old rabbits, as was Delaplane (54).

Peckham (55) and Hofstad (56) reported isolation of a vibrio from the gall bladder of hepatitis affected birds with which they had been able to reproduce the disease.

ERYSIPELAS

During the breeding season of 1957-58 erysipelas in breeder flocks in California reached an all-time high. Large numbers of hens, as well as toms were affected, but it is believed that artificial insemination may be responsible for this increase. Vaccination of all birds in a flock has been suggested because of last winter’s problems.

*Erysipelothrix rhusiopathiae* infection in poultry does not appear to have had much attention in scientific reports this past year. The proper use of an *E. rhusiopathiae* bacterin in turkeys, as previously reported, has appeared to provide reasonably good protection. It is recognized that the length of immunity is short and that two inoculations of bacterin 60 to 120 days apart gives the best protection.
TRANSMISSIBLE DISEASES OF POULTRY

*E. rhusiopathiae* infection in chickens has been recognized (57, 58, 59) more often than usual. It is possible that strains of this organism are adapting to chickens. It is urged that diagnosticians be alerted to this condition as a possibility. The ubiquitous nature of *E. rhusiopathiae* provides excellent opportunity for this to occur.

**ORNITHOSIS**

Ornithosis viruses were isolated from turkeys during acute outbreaks of the disease in three states during the past year: Oregon, California and Texas. Two outbreaks were diagnosed in Oregon, one in December, 1957 and one in January, 1958. Although the breeder flock in the second outbreak was treated with 500 grams of oxytetracycline per ton feed for two weeks and 200 grams per ton for two more weeks, the virus was nevertheless isolated from tissues taken at slaughter. In California, a virus of low virulence for pouls was isolated from turkeys during an outbreak in February in a breeding flock which underwent treatment at 400 grams chlortetracycline per ton of feed for five days and then was processed. No virus was recovered from slaughtered birds. An ornithosis virus was also isolated from sparrows nesting on the ranch.

Davis and Delaplane *et al.* (60, 61, 62) concluded that 200 grams or more of chlortetracycline per ton feed for three weeks was effective therapeutically. Furazolidone at 100, 200, and 300 grams per ton of feed given for a two-week period was ineffective as a prophylactic agent against ornithosis.

Benedict (63) proposed the use of a simplified, direct-complement-fixation (DCF) method for the sero diagnosis method in place of the standard more cumbersome indirect-complement-fixation (ICF) method of Karrer and Eddie (1950). Benedict's test, which utilizes a detergent-extracted antigen, has an over-all correlation of 88.5 percent with the ICF test. A similar antigen may be used as an allergen in an intradermal test in turkeys or chickens, but the test is considered more effective as a flock test than one for detecting infected individuals.

The DCF, ICF and a macroscopic agglutination method were compared by Neal and Davis (64) on 923 turkey serums. Agreement between all three tests was poor; however, the ICF and DCF tests exhibited 85 percent agreement. The use of low serum dilutions (1:2 to 1:8) produced a high rate of nonspecific reactions with the agglutination test. Unpublished observations by the California Experiment Station indicated that a Giemsa-stained agglutination antigen prepared by D. M. Mason of the Hooper Foundation in San Francisco was more sensitive for detecting ornithosis antibodies in a capillary tube agglutination (CTA) method than the ICF test. The CTA test is thus far the simplest and most rapid method available.

Two reports on ornithosis which may be of interest to investigators of this disease appeared during the past year (65, 66).
Neoplasms account for a high proportion of the mortality among domestic chickens. This is primarily due to the incidence of visceral lymphomatosis which appears to be the only one of several infectious neoplasms of the chicken which is also contagious. Its occurrence gradually increased so that it has become the most important disease problem in adult poultry. Most infections remain in a latent state and certain water fountains (reservoir type) may play an important role in the mechanism of transmission in the brooder unit (67).

Burmester (68) also reported that the three forms of lymphomatosis in chickens—ocular, neural, and visceral—have certain similarities in their histopathological manifestations, but appear to be caused by distinctly different viral agents.

Beard (69) demonstrated that the virus of myeloblastosis differs from that of erythroblastosis by enzymatic studies. With the agent of myeloblastosis there is associated a pronounced activity to dephosphorylate adenosine triphosphate in contrast to an extremely low level of activity or the essentially complete lack of activity on the part of the agent of erythroblastosis. The same author (70) also presented findings encountered in studies on the isolation and identification of tumor viruses including the agents of lymphomatosis and leukemias of chickens.

Burmester et al. (71) in a recent study of the disease response in chickens experimentally exposed by different methods to various sources of virus causing visceral lymphomatosis also produced erythroblastosis. A variation in the dose of virus had a marked effect on the type of response. High doses caused primarily erythroblastosis which occurred in less than four months; low doses resulted in a response that was primarily visceral lymphomatosis with death occurring after four months.

**SALMONELLOSIS**

*Pullorum Disease*

Continued progress has been made in the reduction of the incidence of Pullorum Disease. The voluntary programs of the National Poultry and Turkey Improvement Plans have been responsible for the progress in the control of pullorum disease. However, in the past few years the number of hatcheries participating under the Plans have changed very little but egg capacity of participating hatcheries has increased. The percentage of participating hatcheries qualifying for United States Pullorum-Typhoid Clean has increased to 89.3 percent of chicken hatcheries and to 99.5 percent of the turkey hatcheries.

The results of the program are indicated in Table 1.
Chickens and turkeys officially tested for pullorum disease, number and percent of reactors 1955-1958.

<table>
<thead>
<tr>
<th>Year</th>
<th>Chickens Tested Number</th>
<th>Chickens Reactors Number</th>
<th>Chickens Percent</th>
<th>Turkeys Tested Number</th>
<th>Turkeys Reactors Number</th>
<th>Turkeys Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1955</td>
<td>39,288,860</td>
<td>40,316</td>
<td>0.10</td>
<td>2,918,037</td>
<td>1,007</td>
<td>0.03</td>
</tr>
<tr>
<td>1956</td>
<td>36,112,781</td>
<td>25,529</td>
<td>0.07</td>
<td>3,310,470</td>
<td>1,014</td>
<td>0.03</td>
</tr>
<tr>
<td>1957</td>
<td>40,614,440</td>
<td>18,369</td>
<td>0.045</td>
<td>3,828,755</td>
<td>2,197</td>
<td>0.06</td>
</tr>
<tr>
<td>1958</td>
<td>36,756,760</td>
<td>11,423</td>
<td>0.03</td>
<td>3,509,580</td>
<td>640</td>
<td>0.018</td>
</tr>
</tbody>
</table>

* For the year ending June 30.

During the past year there has been very little change in the number of states that have regulations concerned with importations of hatching eggs, chicks, poults and other fowl shipped into the state (72). At the National Plans Conference June 24-26, 1958 at Louisville, Kentucky, a recommendation was passed to encourage the development of an inter-state regulation by Animal Disease Eradication Division, United States Department of Agriculture (73). Also the number of states that have a compulsory pullorum control program has not changed. The interest at the Plans Conference in the eventual development of an eradication program for pullorum program has increased but the present pullorum program of the National Plans is based on voluntary participation.

Several investigators (74, 75, 76) have studied the influence of furazolidone on the agglutination reactions and recovery of S. pullorum from experimentally and naturally infected birds. Carrier birds, negative to the agglutination test, may result when treated with furazolidone. The use of furazolidone in breeding flocks before the completion of the pullorum and paratyphoid testing programs is not generally recommended.

Further studies by Wright et al. (77) on pullorum disease in turkeys indicated that the whole blood test was not reliable for the detection of S. pullorum in turkeys beyond eight to 10 weeks after infection.

**Paratyphoid**

At the National Plans Conference considerable discussion was given to the development of an official S. typhi-murium testing program. Six states have an extensive typhi-murium testing program involving turkey flocks. The program is voluntary and no official recognition is given to the tested flocks. The Plans Conference postponed any action on the proposed plan until more information was available.

Burr et al. (78) reported on S. heidelberg infection as a problem in certain chicken breeding flocks. Egg transmission and fecal contamination in artificially infected birds were demonstrated.

Sieburth (79) discussed the use of indirect hemagglutination test in avian salmonellosis. Indirect hemagglutinins appeared earlier, persisted longer and were approximately 10 times higher than agglutinins.
Pomeroy et al. (80) found that furazolidone had value in reducing the mortality in experimental Arizona paracolon infections of poults.

Bigland and Quon (81) described outbreaks of paracolon infections of poults, chicks and adult hens in Alberta, Canada.

Over 400 Salmonella Serotypes have been isolated from man and various species of animals. In United States approximately 100 serotypes have been isolated from 12 species of fowl (82).

**PARASITIC DISEASES**

*Coccidiosis.* Coccidiosis continues to be one of the important diseases of poultry in spite of the many effective coccidiostatic agents that are available. There appears to be a never ending supply of new coccidiostats being discovered that are effective against these parasites in poultry. Gardner (83) reports that a combination of aureomycin and sulfamethazine greatly enhances the coccidiostatic effect of sulfamethazine against *Eimeria tenella* infection.

Because of the common occurrence and striking pathology produced by *E. tenella* most reports of work on coccidiosis in poultry deals with this species. Morehouse and McGuire (84) have recently drawn attention to the severity of the pathology and mortality produced by *Eimeria acervulina* infection in young chicks. In older birds mortality is not usually encountered; however, the severe effect of a heavy infection of this species on adult birds has been demonstrated (85). It is important that greater attention be directed to those species of coccidia that infect the small intestine. Many of these infections cause severe depression in production although there is only limited or no mortality. Further, the pathology in marginal clinical cases is definitely not of the spectacular variety. It is not always a simple procedure to arrive at an accurate diagnosis in many of these cases.

*Helminthiasis*

Edgar et al. (86) reported that 250 mg. (per half-grown chicken) and 500 mg. (per mature chicken) of piperazine hexahydrate, salts of piperazine or half that amount of pure piperazine usually resulted in 95 to 100 percent elimination of mature *Ascaridia galli* and 75 to 100 percent elimination of immature worms when consumed in capsule form or as 0.4 or 0.8 percent in the drinking water.

It was determined by Shumard (87) that 90 percent or more of *Ascaridia galli* were removed from chickens by 125 mg. of the active ingredient of the piperazincarbon disulfide complex whether administered as the powdered or liquid products (568 mg. of the powdered and 0.47 ml. of the liquid) per kilogram of body weight.

Worley et al. (88) examined a number of piperazine compounds regarding their ability to eliminate the lumen larvae of *Ascaridia galli* and found piperazine dihydrochloride and piperazine hexahydrate to show considerable promise.
A comparison of the mortality in three-week-old chicks suffering from a combined infection of Infectious Bronchitis and *Ascaridia galli*, with the mortality from the bronchitis infection alone and the worm infection alone, was made by Reid *et al.* (89). They found an average mortality of 3.1 percent in the case of the combined infection while none of the chicks having the single infections died.

A natural outbreak of *Tetramereres americana* in domestic pigeons was reported by Raggi *et al.* (90). The proventriculus of the birds was enlarged and thickened and there were numerous dark red bodies of the parasites observed on autopsy.

**External Parasites**

Complete control of heavy infestations of the northern fowl mite (*Ornithonyssus sylviarum*) was reported by Furman *et al.* (91) by the use of one application of 0.36 grams of 1½ percent malathion dust applied by puff duster per hen, or one application of 0.5 percent malathion spray applied at the rate of one gallon per 100 hens either as a suspension or an emulsion.

**Histomoniasis**

A pathological examination of experimentally produced Histomoniasis was performed by Malewitz *et al.* (92). Microscopic lesions, in which the parasites were demonstrated, were found in the ceca, livers, kidneys and spleens which were characterized by hyperemia, hemorrhage, lymphocytic infiltration, macrophages, multinucleated giant cells, necrosis and often a serous exudate.

Levis *et al.* (93) reported a level of 0.10 percent of 2-amino, 5-nitrothiazole fed continuously to turkey males from eight weeks of age to have a delaying effect on spermotogenesis. Following the onset of semen production, administration of this drug had no deleterious effects on testis function.

**Trichomiasis**

In a study of *Trichomonas gallinae* infection (Canker) in pigeons, Stabler *et al.* (94) reported soluble 2-amino, 5-nitrothiazole to have prevented death in 61 treated birds and rendered 53 of these Trichomonas-free, while all 28 controls died of canker.

**miscellaneous**

At the 1957 American Veterinary Medical Association convention a committee was formed to organize the American Association of Avian Pathologists. The constitution and by-laws were accepted in principle; officers were elected and standing committees appointed. Officers for 1958-59 are: President, Dr. Benjamin S. Pomeroy, Minnesota; Vice-President, Dr. Edwin L. Jungherr, Connecticut; Secretary-Treasurer, Dr. Morris S. Cover, Delaware. Membership includes both veterinarians and non-veterinarians who have made distinct contributions to, and have an interest in, avian diseases. The objectives stated in the constitution are:
1. To provide an organization for the promotion of mutual interests of those persons engaged in the field of avian diseases. 2. To stimulate scientific progress in avian pathology. 3. To encourage adequate training in poultry diseases and management in schools of veterinary medicine. 4. To encourage graduate and other forms of advanced training in avian diseases. 5. To encourage the publication of the scientific journal “avian diseases.”

Since 1955 work has been going steadily forward on the preparation of a “Manual of Methods for the Evaluation of Poultry Vaccines” with the cooperation of the agricultural experiment stations, the Agricultural Research Service, and producers of veterinary biologics, especially members of the Veterinary Biological Licensees Association. This manual will provide suggested standard techniques for testing and examination of poultry biologics and for diagnostic procedures relative to infectious diseases of poultry. This work was supported by regional research funds on avian respiratory diseases and coordinated by an interregional advisory committee. In May, 1958, 33 investigators attended an intensive editorial and subject matter workshop at Michigan State University to reexamine and revise the chapters of the manual. The manual, tentatively titled “Methods for the Examination of Poultry Biologics,” was revised, edited, and a steering committee was selected to arrange for publication and revision of the manual.

In view of the current tendency in some states for agencies supervising pullorum-typhoid programs to enter into the field of other diseases of poultry and adopt programs intended for the control of these diseases, the Committee suggests that the duly constituted regulatory officials be on guard that the control of such diseases remain in proper hands. It might also be desirable for the regulatory officials to investigate and study the feasibility of some of the programs which are being proposed.

Further, in view of the introduction of a variant strain of infectious agent in the form of a living virus vaccine into a state in which the agent was not previously demonstrated, this Committee proposes that when such changes are made in biologics that the Federal authorities or licensed laboratories bring such changes to the attention of the state regulatory officials.

REFERENCES


45. Personal communication.


TRANSMISSIBLE DISEASES OF POULTRY


The outbreak of Anthrax in Oklahoma in 1957 posed many problems and probably, our biggest problem was the tendency of people to panic at the word “Anthrax.” The outbreak occurred in an area where the disease had not been endemic. People became very excited and in some instances difficult to handle. The answer to the problem was partially solved by the holding of well attended community meetings, at which we attempted to explain the disease and what we were doing about it and to give them the opportunity to ask questions. Local news coverage was also a help. The County Agents and Vocational Agriculture Instructors also helped by giving out accurate information. Consumer resistance, as a result of panic, influenced the quarantining of products as well as the animals. When Anthrax appeared, the Oklahoma State Department of Health having the authority over fluid milk and the Dairy Division of the Oklahoma State Board of Agriculture having authority over manufacturing dairy products, placed the responsibility of handling the products in the State Veterinarian’s office. Milk and cream were not allowed to be marketed for the period of quarantine with a minimum of 14 days following vaccination and last death loss. Prior to accepting milk, the equipment was inspected by a representative of the proper agency under our supervision.

STERILIZATION PROCEDURE FOR EQUIPMENT FOLLOWING AN ANTHRAX QUARANTINE PRIOR TO RELEASE OF MILK OR CREAM

Cream Separator

1. Use a milkstone remover to remove all milkstone from the surfaces which come in contact with the milk.
2. All removable parts which contact the milk in any way such as the discs, milk and cream conductor spouts, flow regulator, float and rubber bowl ring must be sterilized in a pressure cooker under a steam pressure of fifteen (15) pounds maintained for 45 minutes.
3. The storage bowl, strainers, milk pails and other such parts or utensils are to be submerged for fifteen (15) minutes or longer in 300 parts per million chlorine solution.

Milking Machines

1. Metal parts of the milking machine which come in contact with the milk should have all milkstone removed by the use of a milkstone remover.
2. Milker pails or any other parts too large to be placed in a pressure cooker to be submerged for 15 minutes or longer in a 300 p.p.m. chlorine solution.
3. All rubber parts such as teat cups, milk hoses, air hoses are to be boiled in a three percent lye solution for 10 to 20 minutes. This should be done in an open type of agateware utensil such as will not be damaged by the action of the lye. Allow rubber parts to cool four to six hours, then brush and clean.

4. All plastic milk hoses must be destroyed.

5. Use milkstone remover to remove all milkstone from milk contact surfaces of inside of milk cans. Thoroughly clean outside of can and lid using a good detergent. Submerge all milk cans for fifteen (15) minutes in a chlorine solution of a strength of 300 p.p.m. for 15 minutes or longer in a 300 p.p.m. chlorine solution.

Other Utensils and Equipment

1. All milk cans and milk pails or utensils with open seams must be discarded or repaired by solder with all resultant seams smooth and easily cleanable. Any repaired utensil is then to be submerged in the 300 p.p.m. chlorine solution.

2. All pails or utensils used in the cleaning of udders having open seams must be discarded or repaired by solder with all resultant seams smooth and easily cleanable. Any repaired pail or utensil is to then be cleaned inside and out, using a good detergent. Any repaired and cleaned pail or utensil is then to be submerged in a chlorine solution of a strength of 300 p.p.m. for 15 minutes. All cloths used for washing cows' udders must be burned.

3. All open boxes of strainer pads and all such equipment not hermetically sealed must be destroyed.

Milk Stools

1. All metal milk stools must be thoroughly cleaned using a good detergent, then submerged in a chlorine solution of a strength of 300 p.p.m. for 15 minutes.

2. All wooden milk stools must be destroyed by burning.

Other

1. Manure and manure piles must be hauled away and spread. This procedure must be complied with before any milk or cream can be released to be received by a milk plant or other.

Eggs were quarantined to premises and released after 72 hours on inspection, to see that the chickens were put up and not allowed to run the barnyard or in proximity to animals. We realized this was a rather severe quarantine and hard to justify but consumer resistance was developing and it was necessary. (Feeder cattle were released on permit from place of destination after 21 days following vaccination and no death loss.) The Federal Meat Inspection Service requirement which states that animals immunized for Anthrax shall not be slaughtered for six weeks were conformed to. Animals were not released from this area for slaughter until they could comply. People were cautioned not to attempt to post or handle dead ani-
Public Health Aspects of Anthrax

Animals but to report to us, which they complied with and by a network of reporting from Sheriffs, Highway Patrols, Crime Commission personnel and personnel from the Fish and Game Division and from the Department as well as the Agricultural Research Service; veterinarians were dispatched to ascertain if the animals died from Anthrax or some other cause and to see that burning procedures were properly supervised.

The disposition of dead animals posed a problem that could be classified under this category. Again this same reporting system was utilized, as well as an airplane to locate carcasses. All premises were inspected to see that carcasses were properly burned. In some instances it was necessary to return several times to accomplish adequate burning. We are very happy to report that we did receive good cooperation and that people did comply with our wishes. As far as we can ascertain, there were no carcasses left unburned.

At the very beginning of the outbreak the picking up of animals by animal disposal wagons was stopped, and we feel very fortunate that this was accomplished in time to prevent some workers in one of the inedible rendering plants from contracting the disease.

As it has been previously outlined, I think it becomes evident to you that this was a coordinated effort by many departments of state government and included a number of persons from Animal Disease Eradication, Agricultural Research Service, United States Department of Agriculture. The direction of the entire operation was left to the state veterinarian, even though it was the various agencies' personnel that carried out the instructions. This was very satisfactory from our standpoint. We were pretty busy and certainly, to have the authority to act at a time like this, was paramount.

The use of Anthrax vaccine could pose a public health problem. We were fortunate, in the state of Oklahoma, that we do control the sale and use of Anthrax vaccine by law. In very few instances did the illegal use of vaccine give us any difficulty. When we consider the magnitude of this outbreak, having the authority of the Health Department and the Dairy Division vested in us, we believe kept down a great deal of confusion and accomplished a job with less difficulty than would have been possible otherwise.
The terminology of infections contracted by man through contact with birds—wild or domestic, living or dead—has followed the irregular course of discovery, and the fairly recent finding of related viruses in domestic and wild mammals makes it seem inadvisable to attempt to settle the classification yet. For the time being several official agencies have agreed to call the infection in man psittacosis, no matter what the avian source, and to distinguish in birds whether or not they are psittacine, by calling the infection in psittacine birds psittacosis and in other birds ornithosis. Virus isolations are constantly insisting on a broader and broader view of this infection, and it is important that the epidemiologist not allow the terminology to curb his imagination in considering possible sources. Viruses of this kind have been found in wild and feral birds, captured wild birds, exotic cage pets (psittacine or not) raised in captivity, fancy and racing pigeons and poultry.

During the early 1930s the parrot-man infection chain seemed to constitute the sole important problem and the psittacine birds to be the principal reservoir. Restriction of the movements of imported parrots and supervision of locally bred and raised parakeets and parrots were deemed adequate to prevent human psittacosis. That this assumption was ill founded was learned when the infection was identified in young seashore birds—in the fulmar, or petrel, used as food by the inhabitants of the Faroe Islands between Iceland and the Shetland Islands (Haagen and Mauer, 1939). This discovery explained the 186 cases of pneumonia with 38 deaths that the district health officer, R. K. Rasmussen, had observed beginning in 1933, principally among women who eviscerated and salted these birds for use as food during the winter. Human disease was stopped when killing and preservation of these seashore birds was discontinued. Interest elsewhere was then shifting to

Pigeons

Human infection was traced to this source in the United States in 1942 (Meyer et al., 1942), to racing pigeons and to small flocks raised for food or exhibition in back yards. The virus isolated from the lungs of the patients was identical to that isolated from the tissues of the apparently healthy pigeons. Cases were soon reported from Europe and to date, according to a tabulation recently prepared from the published records and the records of the Hooper Foundation, over 460 human infections (19 deaths) have been attributed to contact with pigeons (Table 1). It has become evident that not
ORNITHOSIS: A PUBLIC HEALTH PROBLEM

TABLE 1

Reported Incomplete Incidence of Human Psittacosis Contracted From Poultry Pigeons 1931-1956

<table>
<thead>
<tr>
<th>Place</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>152</td>
<td>8</td>
</tr>
<tr>
<td>Canada</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Argentina</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Czechoslovakia</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Denmark</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Great Britain</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Greece</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Israel</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Italy</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>1 (16)</td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Switzerland</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>U. S. S. R.</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>449 (16)</td>
<td>19</td>
</tr>
</tbody>
</table>

Numbers in parentheses are suspected additional cases.

Birds of solitary habits, but those that congregate in flocks are more likely hosts of the ornithosis virus.

Next it was found that the blood serum of some squab raisers had significant titers in the complement fixation test with psittacosis antigen. Careful studies at the Hooper Foundation of a few self-contained flocks showed that 60 (18 percent) of 332 so-called “wet squabs” submitted for examination during one year suffered from ornithosis. Occasionally there were deaths and diarrhea, and the tested birds yielded the ornithosis virus. Although salmonellosis coexisted in the flock it was not responsible for the lesions in all cases. At least 36 (60 percent) of the ornithosis-positive carcasses presented lesions in the form of exudates on the air sacs in the peritoneal cavity and on the pericardium. Both in the United States and in Great Britain (Hughes, 1947) poultry pigeons under 16 weeks of age have been found to be affected, and as many as 20 percent have died. During processing for the market infected squabs may become a source of human infection.

Most of the ornithosis viruses isolated from pigeons have been of low virulence in the test animal, the laboratory mouse. It seems likely that if the illness in man were also mild it would go unrecognized. It may be entirely inapparent and yet responsible for the high percentage of positive serum reactors among workers on poultry pigeon farms.
An infection rate as high as 84 percent has been found among adult pigeons. Many shed the virus in the droppings. The latently infected adult pigeons transmit the agent in the nests during feedings of the young, or their infective droppings contaminate the breeding establishments. Infection may be transferred from loft to loft as adult breeders are exchanged among flockowners. The infection reaches no serious proportions until the population of young susceptible birds reaches a certain proportion of the flock. When breeding is discontinued and the average age of the flock rises, the incidence of ornithosis declines. Thus most of a flock becomes infected in the squab stage, when they are most likely to be harvested for the market, and, depending on the environment, varying numbers succumb. Of the surviving birds, some recover completely, but others remain infected, and they then give the disease to their nestlings or to other birds in the next generation. Latently infected pigeons may eventually die from chronic lesions, or the infection may relapse due to unfavorable environment or improper nutrition.

It must be understood that not only the infected pigeon itself, but also the persistence of the infective agent in the loft or squab farm is a potential risk to human and pigeon health. In one epidemiologic investigation of a barnyard pigeon loft held unoccupied for over a month, cleaning of the loft evidently stirred up infective dust or aerosols, because a person in the loft at the time of the cleaning, who had had no contact with the original flock, contracted psittacosis. On commercial farms, as a rule cleaned only once a year, the accumulated discharges and soil contaminate the environment and continuously expose the entire flock to infection. The existence of this problem in commercial poultry pigeon farms is not generally known.

Ornithosis in pigeons is usually brought to the attention of official health agencies in connection with a severe human infection. The flockowner expects losses among his squabs, and he usually does nothing about them unless he becomes alarmed when these losses exceed his expectations.

There is no reason to doubt that infected squabs, even sometimes in the active stage of ornithosis, do reach the consumer. But since statistics are not available on human infections acquired through the handling of poultry pigeons, health authorities have made no effort to calculate or eliminate the risk of disease from this source. On the other hand, the United States Department of Agriculture and the Food and Drug Administration forbid the movement in interstate commerce of all birds from flocks proven infected by the ornithosis virus in isolation tests. In miscellaneous surveys in 20 of the United States, 25 percent of the tested poultry pigeons gave positive serologic reactions, and 18 percent harbored virus in their tissues. This suggests that if a regulatory agency were to undertake control of ornithosis in poultry pigeons it would encounter no difficulties in proving its existence, providing it had the funds, facilities and personnel to make the necessary field and laboratory studies. If the agency were to enforce the regulations, the trade in squabs and in breeding stock would probably come to an abrupt standstill. A dilemma would then have to be faced, because methods have not been found to control the infection in the pigeon reservoir. By offering
antimicrobial drugs in the drinking water the losses have been promptly reduced, but complete suppression of the prevailing infection has not been accomplished.

The ornithosis problem in all types of pigeons—wild, feral, fancy, racing, homing and poultry pigeons—cannot be solved by restrictive regimentation. It goes without special emphasis that to combat ornithosis among pigeons would serve as a prophylactic measure for man. In a few European countries ornithosis in pigeons seems to be the chief source of human infection. But, as pointed out by J. Jansen, in the Netherlands with a population of 10 million people in fairly close contact with one million pigeons, the number of reported human infections has not exceeded 54 a year. As the director of the Institute for Infectious Diseases of the Veterinary Faculty of the State University at Utrecht, Jansen has had the opportunity to examine 20,000 racing pigeons in the department called the "Pigeon Health Service," which was organized in his clinic at the request of the pigeon fanciers in his country. In his exceedingly informative report, made to the seventh International Congress of Comparative Pathology in 1955, he took issue with the public health authorities who had recommended that all pigeons excreting virus or with high complement fixation titers, in fact pigeons in intensively infected flocks, be slaughtered. He justly pointed out that the administration of this recommendation would be exceedingly costly. The slaughtering of thousands of pigeons would give no guarantee that ornithosis would not crop up again at some time or another among seronegative pigeons. One must agree with Jansen that the only approach capable of limiting the chances of pigeons infecting human beings lies in educating pigeon fanciers and breeders. It seems worthwhile to quote from his report because it is not readily available:

"Pigeon owners should be informed of the clinical symptoms of ornithosis in pigeons and man while stressing the imperativeness of examination as soon as there is the slightest suspicion. The owner should be advised to allow all his birds to undergo this disease (en bloc) as quickly as possible (i.e. don't separate them, no flying, take special care of them, and possibly make use of antibiotics). During this period, as few people as possible, and certainly no elderly persons, should be allowed to come in contact with the pigeons. The pigeon lofts should be disinfected, together with all the accessories, with NaOH (one to two percent). One should always avoid too intimate contact with the pigeons, something which is often carried to quite incredible extents, such as feeding them out of one's own mouth, a sin, incidentally, of which not only the weaker sex is guilty. If all these recommendations are adhered to, it will not mean that all the dangers of human beings being infected with pigeon ornithosis will have become a thing of the past, but it is reassuring to know that, in spite of the very large number of pigeons suffering from ornithosis, the number of human patients is still very low, that the human patient does not run such a great danger as is the case with psittacosis, and that really effective antibiotics are available for the treatment of those who are unfortunate enough to catch this disease. In this latter connection too the public at large should be instructed in such
a way that, should they fall ill, they will not forget to mention possible contacts with pigeons in their history. This is vitally important, as an early diagnosis and treatment will lead to an early recovery. I consider the proposed slaughtering scheme to be absolutely unacceptable. We, humans, must accustom ourselves to the fact that there are pigeons suffering from ornithosis, just as there are cows, sheep and goats suffering from brucellosis, a disease which can cause febris undulans to human beings, not to mention the hens, ducks, geese and other poultry infected with salmonellosis, a possible source of paratyphus. Just stop and think for a moment what it would be like if we tried to eliminate all this by means of a slaughtering system.”

The participation of wild and feral pigeons in disseminating psittacosis is difficult to know. Davis (1955) and Shaughnessy (1955) listed the recorded sporadic cases and even localized outbreaks of psittacosis due to close contact with sick or dead pigeons, or among persons exposed to dust of this bird. Feral pigeons have constituted a very definite hazard in Germany (Weyer and Lippelt, 1956), Italy (Babudieri, 1956) and England (Semple, 1956). Because of the filth wild pigeons create in cities, warehouses and elsewhere, many health officers think these nuisances should be eradicated. Anyone who undertakes such an ambitious program should know in advance of two obstacles he will encounter. First, the pigeon has been a symbol of love and peace for centuries. A great deal of consideration will be vociferously demanded by a large segment of a city population. Many city dwellers regard the street pigeons as their personal friends. Second, a method of complete painless destruction has not been worked out. Although chlorazol has been used in bait with some success, attempts in Liverpool have not seemed so encouraging. Trapping and subsequent gassing have been used by the Liverpool health department, legally authorized to destroy pigeons, homing doves or starlings in excess of such number as the department or the legal act considers reasonable (Semple, 1956). Such legal perquisites are, as far as it is known, rarely in force in the cities in which feral pigeons may be considered a hazard.

**CHICKENS**

Natural infection in chickens was identified in 1939, after serologic tests for psittacosis were made in a case of atypical pneumonia in New Jersey. Since then a few additional sporadic human infections have been attributed to contact with chickens in the United States and Argentina. Isolates of low virulence have been obtained from emaciated chickens or in one instance from chickens with pericardial exudates. As far as it is known the infection has been only subacute. Nothing is known about the clinical disease until the poultry pathologist examines the organs of barnyard fowl by inoculation of mice. The incidence of ornithosis in this species and its economic importance in the poultry industry are unknown. In serologic surveys of a limited number of flocks the incidence was negligible. In experiments chickens have seemed to be inherently resistant to artificial infection, though
some have retained the inoculated virus in spleen and liver for many months. As long as the present benign trend continues control measures seem unnecessary.

DUCKS

In 1942 serologic evidence of naturally acquired ornithosis in ducks was provided by a survey of these fowl in Michigan (Eddie and Francis, 1942). Within three years human infections due to contact with ducks were discovered in California (Meyer and Eddie, 1952) and in New York (Wolins, 1948; Korns, 1955). The Long Island experiences are of interest in several respects. It was the first poultry outbreak in which an extensive epidemiologic and serologic investigation was made. These revealed clinical and subclinical infection in duck handlers. Hence the first proof of the occupational importance of the infection in poultry workers. It showed that even only brief contact may result in subclinical infection. An ornithosis agent was isolated from sick and well ducks, and the incidence of positive seroreactions was high (40.2 percent). Clinically normal ducks had no gross anatomical lesions, but virus was isolated from their organs. Concurrent and secondary infections are probably quite common, and they may be more important in epizootics on duck farms than the ornithosis virus is.

There was recently an interesting incidental observation of latent infection in ducklings from a hatchery in Michigan. The birds used in experiments on duck malaria revealed exudate on the air sacs and pericardium and typical elementary bodies; these were then tested on mice and a weakly pathogenic psittacosis virus was found. This year Dr. R. E. Kissling wrote to me that he has isolated an ornithosis virus from three to six individual ducks and from a pool of tissue from ducks involved in a human outbreak in Virginia in the fall of 1957.

In Europe ornithosis in ducks has attracted attention in the last few years. Several hundred cases of human psittacosis in eastern Bohemia (Strauss, 1957) and single cases in Austria (Fürst et al., 1957), Germany (Mumme, 1955) and the Soviet Union have been reported. The infection is suspected in Great Britain among employees on farms where ducks are raised and in departments where they were being plucked, and this has focused attention on the possibility that poultry products such as feathers and barnyard waste may also be a menace to man. Despite the low death rate in Czechoslovakia (1.3 percent in 150 cases) the disease has been serious because of the long convalescence in the age group 40 to 60 years and among 70 percent of the women because of involvement of the cardiopulmonary system (Strauss, 1957). Among employees on duck farms the reactor rate in the complement fixation test has been as high as 80 percent. Young ducks in particular have been sources of infection in man. In one epizootic in a flock of 2,000 ducklings 600 died. Transmission through eggs or through contact with infected black-headed gulls roosting near fish ponds used for the breeding of the ducks are considered possible sources. As preventive measures the Czechoslovakian
workers have concentrated their efforts on teaching the employees on poultry farms and hatcheries to restrict avoidable contact infection. The veterinary control measures are in the exploratory stage and aim at breaking up the infection chain between the old and the new generation.

In view of the isolation of a virus similar to the duck isolate from gulls efforts are being made to place breeding farms in areas not visited by wild water birds. How this is to be accomplished is not detailed in the available reports.

No systematic follow-up studies have been made to explain the observation on Long Island that the organs of two ducklings of a group of six, four days after hatching already harbored the virus (Meyer and Eddie, 1952). The significance of this observation, coupled with the increasing number of reports that newly hatched ducklings are particularly dangerous to man, was not grasped until another incubator-hatched poultry bird, the turkey, was encountered as a host of the ornithosis virus. Due to the greater susceptibility of the young, nest infection maintains the parasite in psittacine birds, seashore birds and pigeons, but in incubator-hatched poultry this mode of transmission cannot operate. Egg transmission is suspected but has not been proved.

It seems probable that the infection could be maintained through contact between adult flocks and young. Even though contact were not direct the virus could probably survive in the environment through desiccated droppings. Research on this subject is indicated.

Proper sterilization by heat or chemicals of feathers and quills from infected poultry flocks before they reach the trade channels has received attention in Germany. It deserves some consideration in the United States, particularly in endemic areas.

TURKEYS

At the Fifty-Ninth Annual Meeting of the United States Livestock Sanitary Association in New Orleans in 1955, Dr. D. E. Davis and the late Dr. John P. Delaplane reviewed the problem of ornithosis in turkeys in Texas. They discussed treatment and control. Since then several serious outbreaks have occurred among processors in Oregon, New Jersey, British Columbia, Texas and Wisconsin. According to the incomplete records of these episodes (Table 2), between 400 and 500 employees in processing plants, rendering plants, truck drivers, home processors and others have been infected. Not included in the tabulation are the infections among two veterinarians and eight laboratory workers. The frank to mild clinical attack rate among the occupationally exposed workers has varied from 20 to 70 percent.

The most common form is the pneumonia, but an influenzal type has been encountered. It is not always benign, as the case fatality rate (2.6 percent) indicates. Delayed treatment with ineffective antimicrobial drugs and complications in the form of hepatitis or myocarditis in older people were
**TABLE 2**

*Psittacosis in Man Contracted Through Contact With Infected Turkeys*

*November, 1948—December, 1957*

(Hooper Foundation Records)

<table>
<thead>
<tr>
<th>Date</th>
<th>Place</th>
<th>Cases Officially Reported</th>
<th>Unofficial Estimate of Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Texas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>November, 1948</td>
<td>Giddings</td>
<td>22</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>December, 1951—January, 1952</td>
<td>Giddings</td>
<td>48</td>
<td>48</td>
<td>4</td>
</tr>
<tr>
<td>April-May, 1952</td>
<td>Giddings</td>
<td>19</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>November-December, 1952</td>
<td>El Campo</td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>April, 1954</td>
<td>Corsicana</td>
<td>48</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>April, May, June, 1954</td>
<td>Lampasas</td>
<td>40</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>April, May, June, 1954</td>
<td>Brady</td>
<td>71</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>May, 1954</td>
<td>Taylor</td>
<td>24</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>May, 1954</td>
<td>Comanche</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>May, 1954</td>
<td>Austin</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>September, 1956</td>
<td>Houston, Marlin</td>
<td>—</td>
<td>34</td>
<td>2</td>
</tr>
<tr>
<td><strong>New Jersey</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>December, 1954</td>
<td>Dutch Neck</td>
<td>17</td>
<td>17</td>
<td>1?</td>
</tr>
<tr>
<td><strong>Oregon</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January-May, 1956</td>
<td>Portland, Salem</td>
<td>86</td>
<td>86</td>
<td>2</td>
</tr>
<tr>
<td>August-December, 1956</td>
<td>Wisconsin</td>
<td>9</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>1956</td>
<td>Washington</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><strong>British Columbia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May-June, 1957</td>
<td>Vancouver</td>
<td>27</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>December, 1957</td>
<td>Oregon</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>430</td>
<td>487</td>
<td>12 (13?)</td>
</tr>
</tbody>
</table>

responsible for the deaths. Inadequate treatment favored relapses. Some workers have been infected more than once. Persistent or significant rises of the complement fixation titer in the well personnel of turkey-processing plants is attributed to subclinical infection. Workers on farms and home processors of diseased turkeys, employees of rendering plants handling dead birds and offal, butchers eviscerating packaged New York-dressed poultry even many weeks after processing have been the victims of psittacosis.

These observations emphasize that ornithosis of turkeys when caused by highly toxic and virulent strains is an important and highly contagious zoo-
anthroponosis. The suppression of infection requires the institution of preventive public health measures. The procedures to be instituted may be approached after a concise summary of the present-day knowledge of the disease in turkeys has been presented.

Since 1952 over 25 percent of sera collected from turkeys on certain suspected breeding farms has reacted in the indirect complement-fixation test in significantly high titers. The significance of these tests was not understood until many thousands of sera from pouls and adult birds from many farms in 12 states or the serologic pattern of birds on infected and uninfected farms had been compared. Flocks that had never yielded any psittacosis virus in isolation tests invariably yielded either no reactors or sera with titers rarely exceeding 1:2. In areas where ornithosis has been proven by isolation of the virus in some flocks the reactor rate has reached 20 percent, with titers of 1:8 to higher than 1:128. In one acute epizootic the reactor rate rose within a few weeks to 80 percent. The titers may persist for months, but it may be difficult or impossible to isolate the virus, in some cases because the innate immunity mechanism of the turkey has removed most or all of the virus and in others because in sampling the larger organs of the turkey the infected portion may be missed. In systematic serologic studies on turkeys artificially infected with different ornithosis viruses, antibodies demonstrable in the agglutination test (Mason, 1957) or indirect and direct complement-fixation test with detergent antigen (Benedict and O’Brien, 1956) usually appeared in the sera between the tenth and twentieth days. The titers gradually rose, sometimes remained for a time at a high level and then declined. Although isolation of the infective agent will remain the final conclusive method in individual birds, the indirect complement-fixation test provides evidence of active infection in flocks relatively simply and rapidly.

The clinical course of epizootic turkey ornithosis is influenced by the virulence of the agent. When it is rapidly fatal to mice and an intravenous injection kills these rodents in a dilution of 1:1,000 within 12 hours, a daily mortality of many (up to 50 birds in a flock of 2,000 has been recorded) calls attention to the existence of an epizootic. Sick turkeys with manifest respiratory distress, inflamed conjunctiva, soiling and matting of the vent and loss of feathers from the breast and back are obvious. The grounds of the pens are heavily blotched with soft or liquid yellowish droppings, some of which are blood tinged. Egg production may be far below the usual. Autopsies of sick birds show cachexia, inflammation of the air sacs, serosal surfaces and fibrinous exudates over the liver and pericardium. Diffuse pulmonary edema or localized marginal pneumonia have been noticed.

At processing after the acute epizootics are arrested by the use of tetracycline compounds, residual lesions in the form of thickened pericardium and adhesions with fibrinous exudations over the heart, liver, air sacs and pericardium may require that the birds be condemned by the inspecting veterinarian. In some instances 80 percent of the livers and hearts have been condemned. The ornithosis agent may be readily isolated through mouse passage, or some have had success with isolation in embryonated eggs. The processing
ORNITHOSIS: A PUBLIC HEALTH PROBLEM

of turkeys with these lesions may cause human infections. According to C. F. R. Amendment 57-14, Agriculture Research Service prohibits the interstate movement of packaged turkeys in this state of infection.

Another form of ornithosis was brought to light in at least three instances in California. In the first observation a poultry pathologist examined two turkeys brought to him for examination because of their low weight. The birds had lesions suggestive of ornithosis. Subsequently the microscopic findings were confirmed by virus isolation and serologic tests. About 83 percent of the sera of 289 turkeys from the same flock with residual lesions gave serologic evidence of infection. The infective agent was of low toxicity, and although fully capable of producing distinct anatomical lesions in turkeys, it was of low virulence for mice and guinea pigs. In this respect this agent and similar isolates from other flocks in California and Michigan (Meyer, 1959) and in Minnesota (Pomeroy et al., 1957) differed fundamentally from those isolated from turkeys in Texas, New Jersey, Oregon and British Columbia. Processing of the former did not cause infections in workers of the processing plants. The sera of none of the residents and employees of the turkey ranch where the infected flock was raised gave complement-fixation reactions. It must therefore now be recognized that ornithosis may be present on ranches as a mild unrecognized largely subclinical infection. The two other instances, again discovered by the poultry diagnostic laboratory, were quite similar.

Although in rare cases this mild form may be brought to the attention of the regulatory agencies through the poultry inspection services, more commonly it probably continues as an epizootic with a very low mortality and clinical manifestations are readily overlooked in ante-mortem examinations. Residual lesions may persist, but the virus can be obtained only from the spleens or kidneys. This form of latent ornithosis has been found in some turkey-raising ranches in California, Michigan, Wisconsin, Arizona and Massachusetts. Its incidence is unknown.

Primarily a poultry disease, not diagnosed until quite recently, it requires systematic study to explain the sudden appearance of the malignant form that menaces the flock and public health. When the owner of diseased turkeys depopulates his ranch in order to protect himself against losses, he initiates a chain reaction: The existence of ornithosis is recognized on the processing line, as a rule when already the defatherers and eviscerators have been heavily exposed to the infective agent clinging to the outside of the birds and in the viscera. Little can then be done, except when diseased turkeys have been retained on the farm for future processing or for selection of breeders. Unfortunately the infection may not be grossly manifest at the time of ante-mortem examination.

The turkey industry is of course interested in making the bird acceptable for processing by means of chemotherapy. In the early studies of Davis and Delaplane (1958), chlortetracycline in the concentration of 100, 200, 400, 600 or 800 gm. per ton of feed effectively prevented mortality among artificially infected turkey poults after two weeks of treatment. But treatment
of three-week-old infected turkeys with the same drug even in the concentration of 800 gm. per ton of feed failed to eliminate the virus. When the tetracycline drugs were used in a naturally occurring severe epizootic there was clinical improvement, but the birds were not safe for processing (Osgood et al., 1957). Many treated birds had gross anatomical lesions, harbored the virus and infected the personnel in the processing plant.

In carefully controlled experiments and field trials with parakeets a daily intake of 100 mg. of antibiotic per kilogram body weight for not less than four weeks has eliminated the virus from the tissues. At this dose the concentration of antibiotic in the blood has ranged between 0.5 and three micrograms per milliliter. It was a great disappointment to learn that in 40 naturally infected turkeys maintained on pellets containing 400 gm. of terramycin per ton (0.32 to 0.39 mg. of drug per gram of feed) for 108 to 118 days, antibiotic could not be found in the blood at all. These birds eliminated the drug in the high concentration of up to 480 micrograms per gram of cloacal content. Absorption of the antimicrobial drug from the intestinal tract of the turkeys was very slight. It has been increased when a chelating agent was added to the feed. In a few adult turkeys offered medicated mash containing Versene, the antibiotic levels in the blood reached as high as seven micrograms per milliliter. In this preliminary pilot experiment on artificially infected turkeys the therapeutic effect of the drug was visibly enhanced. Field trials, which would impose much more difficult conditions, have not been carried out. For the time being all that can be claimed for chemotherapy is that it reduces morbidity and mortality, and hence reduces the risk to human health, but if the flock is heavily infected with a vicious virus it will not protect the processors and for most practical purposes it is prohibitively expensive.

The ecology of the ornithosis virus in the turkey ranch niche is inadequately understood. The infection in a flock and the infinite possibilities can never be appraised by mere visual inspection. On ranches where ornithosis has been proved it is imperative that simple morbidity and mortality records on turkey flocks be kept by the flockowner and studied carefully by the supervising agency. At frequent intervals the blood sera, preferably of the entire flock, but at least of a sample of 10 to 20 percent, should be submitted to the indirect complement-fixation and agglutination test, under the supervision of the State Department of Agriculture. As soon as reactors are found the virulence of the agent responsible for the infection must be determined, and if necessary the infection rate in the flock must be established by serologic tests.

If the infection rate is high, but the morbidity and mortality rates are low, such a flock might be used to extend the preliminary laboratory work on treatment with feed containing antibiotics and the chelating agent. This would require cooperation among all concerned, careful supervision, financing and observation in order to extract the needed information from the field trial. The experiments suggest that it would be necessary to give the feed for at least four weeks and then to wait for an additional two months in
order to allow elimination of any remaining virus to proceed. Under the supervision of the agency in charge of the project and others who share the responsibility for control of this infection, an adequate sample of the flock might then be processed and particular care would be taken to observe whether the lesions on the heart and liver had healed. If there were no reason to suspect active infection, with the permission of any necessary authorities, the remaining birds might then be processed, with the approval and under the supervision of the state health department. This project would be undertaken with the hope that a method of effective treatment can be worked out.

Maintenance of reacting, virus-shedding birds unquestionably constitutes a continuous potential menace to the uninfected susceptible turkey poults. How far these virus shedders contribute to contamination of the environment and maintenance of the infection on the premises is not known. Until more is learned about the possibility of success of treatment and about the details of such treatment, from the economic and the veterinary control point of view the prompt disposal of flocks will prove more effective than the wasteful expensive attempts to salvage the investment by haphazard chemotherapy.

Early recognition of epizootic ornithosis is critical. Ideally the flockowners would submit sick and dead turkeys to qualified poultry laboratories, and efforts must be made to inspire them to take this cooperative step. They should be informed that nonprofessional advice may prove more costly than the minimal charges made by poultry pathologists for a thorough etiologic diagnosis of a few sick or dead birds. Again ideally, a diagnostic laboratory qualified to test birds for ornithosis would be available to all flockowners, particularly in known epizootic and enzootic areas.

Since over 50 percent of the malignant ornithosis outbreaks causing occupational human infections have developed in breeding flocks, it is reasonable to suspect that the stock of hens and toms originated from latently infected poults. The serum testing programs introduced into prevention and control programs of salmonellosis lends itself admirably to the selection of breeding stock free from ornithosis. Small preliminary studies have shown that even the testing with the indirect complement-fixation test of 10 percent of the blood samples submitted for bacterial tests can furnish valuable information. If this 10 percent sample yields sera that give complement-fixation reactions in dilutions of 1:4 and above, it must be concluded that the flock has at least latent ornithosis. To use such a flock for breeding may lead to disastrous epizootics, as has been amply proven. The turkey raiser should always use only seronegative birds as breeding stock. For the time being this testing program for the breeding stock must be a basic prerequisite in the control programs. Here again, a properly qualified laboratory able to accurately interpret the results is essential.

As an adjunct to this testing program, active immunization may prove valuable in regions where reservoirs other than turkeys may play a role. In exploratory laboratory studies an inactivated psittacosis egg-propagated
antigen in an oil adjuvant has stimulated antibody production and protected 24 adult turkeys against clinical disease and death when artificially infected with a highly virulent strain. But here too virus was recovered from vaccinated birds, but the anatomical lesions were minor and could be expected to be definitely less dangerous for man than the full-blown disease in the unvaccinated. The cost of vaccination and its effectiveness under field conditions have not been determined.

Finally, the role of eggs in the transmission of the ornithosis virus from naturally infected turkeys remains a mystery. This fairly urgent problem must be solved. It seems advisable, until it is, to restrict the distribution of eggs from ranches where the infection is known to exist. The eggs should be hatched under supervision, if at all, and the poults serologically examined when they are three months old. Turkey poults younger than three months rarely respond with antibodies to experimental infections.

Ornithosis is of growing interest to large groups—the consumer of poultry, the flockowners, the poultry industry, agricultural agencies, poultry processors, labor unions, insurance companies, health agencies and biologists.

REFERENCES


ORNITHOSIS: A PUBLIC HEALTH PROBLEM 243


REPORT OF THE COMMITTEE ON PUBLIC HEALTH

ROBERT J. SCHROEDER, Chairman, Los Angeles, California; A. L. BRUECKNER, College Park, Maryland; G. H. GOOD, Cheyenne, Wyoming; R. J. HELVIG, Silver Spring, Maryland; H. J. ROLLINS, Raleigh, North Carolina; OSCAR SUSSMAN, Princeton, New Jersey.

Two hundred and thirty million population is forecast for the United States by 1975.

To maintain our high standard of living and preferably to better it, the production of food animals must keep pace.

The number of household pets including birds kept by man will also undoubtedly rise correspondingly. This great increase shall result in closer and more complicated man-animal relationship, and the problems of veterinary public health will become more demanding.

The Public Health Committee is of the opinion that there is a great need for increased coordination between livestock sanitary agencies and public health agencies. The duties of each are often necessarily interwoven and efficiency would be enhanced by better communication between the two groups.

Joint committees should be established at the Federal, state and local levels, with both livestock sanitary and public health officials represented. We realize that this arrangement has undoubtedly existed in some instances but feel that an over-all, nationwide plan should be instituted.

These committees would be responsible for the study of mutual issues. No doubt improved relationships would result from better understanding through conference. The responsibility for the organization of these groups should be designated as follows:

Federal level—United States Department of Agriculture and the Veterinary Public Health Section of the United States Public Health Service.
State and local level—Chief Livestock Sanitary Official and Federal counterpart of each state and territory and the corresponding official in the Veterinary Public Health Section of the state or territory.

We note that the 1958 American Veterinary Medical Association Directory lists only 32 state public health veterinarians. In those cases where there is no veterinary representation in the state public health service, we recommend that every possible effort be instituted to obtain such representation. Undoubtedly, letters from the United States Livestock Sanitary Association, urging the appointment of veterinarians to public health services which do not have them would be of help. State officials wishing such letters are asked to direct their requests to the Public Health Committee.

It is further suggested that during the ensuing year a survey be made of the entire United States to determine whether or not the suggested committees have been set up and are operating.
It has been brought to the attention of the Committee that, although most milk ordinances and codes contain general requirements concerning the health of the dairy cow used for the production of milk, the specific procedures by which they can be accomplished need clarification.

A proposal to prepare a procedure manual outlining the methods for use in health inspections and examinations of dairy cattle is under study.

It would be the purpose of this manual to provide public health and livestock sanitary agencies with workable procedures in determining the health status of dairy cattle used to produce milk for human consumption. The desired objectives are:

1. Promote disease control on a herd basis.
2. Improve the health of the individual dairy cow in the production of milk.
3. Provide the basis for more economical as well as increased milk production.

To be workable, this manual must provide procedures which are acceptable to the many agencies and individuals involved. Above all, it must have the support of the dairyman and provide him with a system which is economically sound and practical, and which will allow him to operate with a minimum of restriction.

Preliminary considerations by the Public Health Committee indicate that the development of this manual merits further investigation. It is the opinion of the Committee that these studies should be continued.

ARTHROPOD-BORNE ENCEPHALITIDES

A considerable amount of research has been carried on recently, particularly in California, on the arthropod borne encephalitides.

Of particular interest is the study of winter survival of the encephalitis viruses in mosquito vectors as described by Reeves et al. (1) and the presence of the virus in resident and migratory birds found by Dr. H. N. Johnson (2) of the Rockefeller Foundation. Doctor Johnson also describes the isolation of the Rio Bravo virus of bats (3). This is a new virus of group B. arthropod-borne viral agents. The mode of transmission of the virus is not known and human illness has been observed only in the laboratory.

The California State Health Department Viral and Rickettsial Disease Laboratory reports the presence of western equine encephalitis in squirrels (4).

It is apparent that much more will become known concerning the possible reservoirs of the encephalitides as these studies continue.
REPORT OF COMMITTEE

REGULATIONS GOVERNING THE INSPECTION
OF POULTRY AND POULTRY PRODUCTS

This organization has been extremely active in recommending the development of regulations governing the inspection of poultry and poultry products.

Under the authority of the Poultry Products Inspection Act (7) Stat. 441: U.S.C. 451 et seq., these regulations have been promulgated after discussion in 12 regional meetings. They shall be administered by the Agricultural Marketing Service, and are designated as Subchapter D “Regulations Under the Poultry Products Inspection Act” Chapter 1—Agriculture Marketing Service, Title 7—Agriculture (5).

The requirements apply to the inspection of poultry and poultry products in interstate or foreign commerce and in designated major consuming areas. They will become mandatory on January 1, 1959, but inspection has been provided since May 1, 1958 to those qualified applicants voluntarily applying for it.

As this report is being prepared additional regulations covering such phases of the program as record keeping, imports, and exports are under development.

The Public Health Committee has examined these poultry inspection regulations. We find them to be quite complete and are of the opinion that the United States Livestock Sanitary Association should consider their adoption as representing national standards.

THE PUBLIC HEALTH VETERINARIAN AND AIR POLLUTION

The potential threat of air contamination to the health and well-being of livestock merits serious consideration. This is particularly true in certain areas, the names of which I shall not mention. Of course, the fact that I live in Los Angeles has nothing to do with my reticence.

Veterinarians have now entered the field of air pollution research and are attempting to determine the effects of environmental contamination on animals. This type of project is of great value in the further understanding of effects upon human beings.

Dr. Earl J. Catcott, of the United States Public Health Service, notes that with exception of a relatively few acute episodes serious health effects due to air pollution have not been recognized. However, more subtle effects may be associated with prolonged exposure. He further states that complete elimination of all man-made air pollution is unlikely.

It is considered prudent that the efforts to define the specific injurious effects of the various pollutants be continued (6).
RECOMMENDATIONS

1. The activation of joint livestock sanitation—Public Health Committees, on the Federal, state and local levels.
2. The development of a suitable procedure manual for the health inspection of dairy cattle.
3. The adoption by this Association of the regulations governing the Inspection of Poultry and Poultry Products.

REFERENCES

5. Regulations Governing the Inspection of Poultry and Poultry Products, Poultry Division Agricultural Marketing Service, United States Department of Agriculture.
PRESENT STATUS OF BAT RABIES IN THE UNITED STATES

ERNEST S. TIERKEL, A.B., V.M.D., M.P.H. AND PAUL ARNSTEIN, D.V.M.

The transmission of rabies by bats was first established in Brazil in 1916 (Haupt and Rehaag, 1921) during an outbreak of a severe paralytic disease of cattle. In Trinidad, between 1929 and 1935, 89 persons died of a disease characterized by an ascending paralysis. The early cases were diagnosed as bulbar poliomyelitis. At the same time an epizootic of a fatal paralytic disease in cattle was occurring which was thought at first to be botulism. The work of Pawan and Hurst (Hurst and Pawan 1931 and 1932; Pawan 1936a, 1936b) is a well documented epidemiological classic in the establishment of this outbreak of fatal paralytic disease in man and cattle as rabies caused by the bites of infected bats. The principal vector involved in these outbreaks was the vampire bat (Desmodus rotundus murinus) and this species remains as the most important vector of rabies in many parts of Latin America today. Vampire bats are found from northern Argentina and southern Brazil northward to about 100 miles south of the Rio Grande River in Mexico. The vampire bats of Trinidad came over from the northern coast of South America. They are not known to occur in the United States or in the Eastern Hemisphere. The vampire bat is hematophagous in nature, i.e., it depends for its existence on the ingestion of blood. With their highly adapted sharp incisor teeth, they inflict small crater-like wounds in the skin of their victims and lap the escaping blood with a long, well-developed tongue. They have been shown on occasion to transmit rabies as a true symptomless carrier. Vampire bat rabies continues to be a great problem in most of the South American countries and Mexico. Malaga (1958) states that vampire bat rabies is the most frequent cause of death in South American native cattle and has estimated the losses during 1956 to be about one million head of livestock constituting an economic loss of about 80 million dollars. The hazard of human attacks by vampire bats is present when persons sleep out of doors or in unscreened buildings in enzootic areas; the vampire bat will feed on man when livestock sources for blood meals are not readily available. A total of 117 human deaths have been reported from Trinidad, Mexico and British Guiana (Pawan 1936b; Malaga and Campillo 1957; Nehaul 1955). Programs for the destruction of vampire bats by dynamiting and gassing caves, bat-proofing buildings and tree hollows have proved temporarily successful in some countries; these measures seem to have been most successful in Trinidad but almost impossible to carry out in some of the rugged mountain areas of the Sierra Madre mountain ranges in Mexico. The most effective method for controlling the disease in livestock in recent years has been immunization of cattle with high egg passage (HEP) Flury chicken embryo vaccine. In Mexico, alone over five million head of cattle have been vaccinated with the HEP Flury vaccine in mass immunization programs; these
programs have reduced paralytic cattle rabies to occasional sporadic cases in the enzootic areas. (Malaga 1958; Camargo 1955.)

Over the years, investigators have found evidence of rabies in a variety of non-hematophagous bats in the vampire bat rabies areas of Latin America; several species of fructivorous and insectivorous bats have been found infected during outbreaks of bat rabies. Bat rabies in the United States was
unknown until June, 1953 when the virus was isolated from a yellow bat
(*Dasypterus floridanus*) which had bitten a child near Tampa, Florida. This
episode aroused great interest, prompting surveys and surveillance activities
throughout the country. Since the first reported case in Florida, about 200
cases have been diagnosed in bats from 19 states in widely diverse geograph-
ic areas of the United States (Figure 1) and from one province in Canada
(British Columbia) and recently from Yugoslavia and Turkey. Four species
of tree living or solitary bats and 14 species of colonial or cave-dwelling bats
have been implicated thus far. All are of the insectivorous variety. The
greatest number of isolations have been made from the Mexican free-tailed
bat (*Tadarida braziliensis-mexicana*) in southwestern United States. The
largest number of examinations have been made in Texas (approximately
2,500 with 70 isolations), New Mexico (2,856 with 61 isolations), Okla-
homa (two positives out of 1,071) and Florida (33 out of 5,570). The re-
main ing rabid bats were reported from smaller survey areas and isolated
cases diagnosed in public health laboratories and reported through the usual
channels reflecting an increasing awareness of the presence of bat rabies
among disease control authorities and the general public.

About 75 of the positively diagnosed cases of bat rabies in the United
States were reported as episodes involving the biting of human beings. In
two instances rabid bats had bitten vaccinated dogs. As yet there has been
no direct and definite evidence of natural transmission of rabies from bats
to wild or domestic animals in this country. One human rabies death which
occurred in a woman in October, 1951 in Big Springs, Texas, was investi-
gated retrospectively and attributed on anamestic evidence to bite of a rabid
bat. The biting occurred when the woman picked up a moribund bat along
the roadside. Another human rabies death occurred in January, 1956 in a
state health department scientist who had been working in the field on the
bat rabies project there. In spite of the patient’s close association with in-
fected bats in several Texas caves, no history of a bat bite could be elicited.
The first case of rabies in the United States which could be unquestionably
attributed to the bite of a known rabid bat occurred in Butte County, Cali-
ifornia, in October, 1958. In this case a woman was bitten when she picked
up a moribund silver-haired (*Lasionycteris noctivagans*) bat in her back
yard. Rabies virus was isolated from the bat. Two months later the woman
became ill and died after symptoms strongly suggestive of rabies. Rabies
virus was isolated from her brain at autopsy by the California State Health
Department.

Investigations are now being carried out to discover the epidemiological
significance of the bat rabies findings. A few observations from the early
phases of these studies may be of interest here. It has been possible experi-
mentally to infect several species of animals, including bats, with virus iso-
lated from naturally infected bats by peripheral routes, but in most instances
this has been accomplished with difficulty (Stamm, et al. 1956; Enright, et
al. 1956; Burns, 1956; Kleckner, 1958). It is of interest to note that experi-
mentally infected bats often exhibit furious signs of the disease. All attempts
thus far to transmit the disease by inducing known rabid bats with infectious saliva to bite other susceptible animals in the laboratory have failed (Burns, et al. 1958; Constantine, 1957).

One of the many problems in the epizootiological puzzle of rabies in insectivorous bats is the question of asymptomatic transmission of the disease. It is well established that their chiropteran cousins, the vampire bats in Latin America, are capable of transmitting the disease for long periods of time without showing signs of illness. Some had suggested that this symbiotic host-virus relationship may be present in the insectivorous bats. During current studies being carried out by the Communicable Disease Center in caves of southwestern United States, rabies virus has been isolated from 0.5 percent of a sample of 2,478 normal bats tested in pools of three and four and 13.3 percent of 203 individually tested bats which were collected in flight. Rabies virus was collected from the salivary gland and none from the central nervous system in four of the 13 rabid bats in the first sample and 12 of the 27 rabid bats in the second sample; these bats remained clinically normal until sacrificed for testing. One bat kept alone in captivity yielded virus from a sample of its saliva obtained 16 months after capture. The data thus far, though suggestive, is not sufficiently conclusive to prove asymptomatic carriers in United States insectivorous bats; these must be corroborated by tests which demonstrate continuous yield of salivary virus over long periods of time from clinically normal bats.

In sharp contrast to the infection rates of 0.5 percent and 13.3 percent found in clinically normal bats, infection rates in the respective samples of moribund and abnormally behaving bats were 8.7 percent and 56.5 percent. A majority of the moribund bats were collected in midst of die-offs which lasted from 10 days to three weeks. During these die-offs some of the ill bats exhibited convulsions and spasmodic contraction of the abdominal muscles. Vicious biting of one bat by another was observed in one instance; however, most of the sick bats showed signs of paralysis. Monthly samples of bat sera were tested for S N antibodies and an average of 21.3 percent of a total of 1,588 bats were found to have significant levels of antibody, with a range that fluctuated from 14 percent to 40 percent.

Of interest in methodology is the significant advance made in the development of an efficient automatic device for capturing large numbers of bats for carrying out studies in bat rabies. The trap developed by Constantine during the course of the current CDC bat rabies studies consists of an aluminum frame with fine, taut, stainless steel wires arranged vertically and spaced one inch apart. It was found that many species of bats in flight are not able to avoid this obstacle with their supersonic echo ranging phenomenon and will fly into the wires, slide down them and drop into the cloth catch funnel thence into the receptacle. The bats thus collected are unharmed and may be banded and released for migration studies or taken to the laboratory for experimental work. Most important of all, the device traps bats automatically since it does not require attention during the collecting operation (Constantine, 1958).
REFERENCES


REPORT OF COMMITTEE ON RABIES

E. S. TIERKEL, Chairman, Atlanta, Georgia; V. D. CHADWICK, Jackson, Mississippi; M. J. CEROSALETTI, Albany, New York; H. R. COX, Pearl River, New York; E. M. DWYER, Boston, Massachusetts; T. J. GRENNAN, Jr., Providence, Rhode Island; J. W. MANN, Atlanta, Georgia; L. E. STARR, Atlanta, Georgia.

Rabies Morbidity Trends—1957

Increased effectiveness of state-wide and local rabies control programs and the public acceptance of canine anti-rabies vaccination in the United States continues to bear fruit. The strides we have made are reflected in the reported national cases over the 10-year period 1946-1956 and in the cases for the last complete calendar year, 1957 (Table 1). It can be seen that the decreases given in the last report of this Committee for the 10-year period of control program development has been sustained in 1957. Thus, in one year the total cases have dropped from 5,846 to 4,802, a decrease of 17 percent, dog cases from 2,592 to 1,758, a decrease of 32 percent in spite of a steadily growing dog population. By the same token it is heartening to note the decline in human cases from 22 in 1946 to 10 in 1956 and six in 1957. Interestingly enough, the cases in livestock have decreased by 10 percent and, for the first time, reports of wildlife rabies have declined by six percent in spite of increasing awareness of the problem of sylvatic rabies over most of the country.

States contributing most to this decline are: Texas (from 744 to 536), Louisiana (from 414 to 179), Alabama (from 484 to 213), South Carolina (from 314 to 199), California (from 289 to 190), Indiana (from 256 to 120), Pennsylvania (from 99 to 21), and New York (from 306 to 202). States showing a sizable increase over last year's incidence are: Minnesota (from 176 to 363), Kentucky (from 320 to 420), Florida (from 63 to 117), Oklahoma (from 11 to 50), and North Dakota (from five to 55). The Minnesota and North Dakota cases are mostly in wildlife (skunks). Florida cases are mostly in foxes, followed by cattle and dogs in frequency. In Kentucky and

TABLE 1
Reported Cases of Rabies in the United States
(ARS-USDA; CDC-USPHS)

<table>
<thead>
<tr>
<th>Year</th>
<th>Dogs</th>
<th>Wildlife</th>
<th>Cats</th>
<th>Livestock</th>
<th>Man</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1946</td>
<td>8,384</td>
<td>956</td>
<td>452</td>
<td>1,055</td>
<td>22</td>
<td>10,872</td>
</tr>
<tr>
<td>1956</td>
<td>2,592</td>
<td>2,079</td>
<td>371</td>
<td>794</td>
<td>10</td>
<td>5,846</td>
</tr>
<tr>
<td>1957</td>
<td>1,758</td>
<td>1,942</td>
<td>382</td>
<td>714</td>
<td>6</td>
<td>4,802</td>
</tr>
</tbody>
</table>
Oklahoma somewhat under half the cases are in dogs, followed by wildlife, cats and cattle.

The New England and the Rocky Mountain States remain essentially free of rabies with the following exceptions: one horse in Wyoming, one dog in Utah, and three cases in Colorado, including one bat and one dog.
Three new states have reported bat rabies during 1957: Wisconsin, Colorado and Arizona, and one state in 1958, Nebraska. The total number of rabies positive bats diagnosed since 1953 is about 200 distributed among 19 states: Florida, Pennsylvania, Georgia, Alabama, New York, Texas, Oklahoma, Utah, California, Montana, Ohio, Michigan, Louisiana, New Mexico, Minnesota, Wisconsin, Colorado, Arizona and Nebraska.

Human Rabies—There were six human rabies deaths reported to the United States Public Health Service during calendar year 1957. Four of the cases occurred in children under 15 years of age.

Four of these persons are known to have been bitten by rabid dogs, the fifth, in California, is presumed to be a dog exposure because of the victim’s habit of befriending stray dogs.

It is interesting to note that only one case, the one in California, was due presumably to exposure by a stray dog. Four cases resulted from bites by owned unvaccinated dogs. Three of the patients were bitten by their own family dog.

Three fatalities received no antirabies treatment. Case No. 3, a 10-year-old boy, received local wound cleansing and a series of 14 vaccine injections starting on the day of exposure. This child developed rabies on the day following completion of the series, after a very short incubation period of 15 days. One of the bites received was on the cheek, a site often associated with short incubation periods and high mortality in spite of vaccine prophylaxis. Case No. 5, a young woman in Texas, died late in 1957 after an incubation period of five months. She was the wife of an Army Lieutenant stationed in El Paso and was bitten by her own unvaccinated dog. Serum and vaccine treatment were begun five days after exposure. It is interesting to note that this tragedy prompted the Armed Forces to reinstitute antirabies vaccination for pets of all service personnel, a program which had been halted by administrative orders from former Secretary Charles Wilson in his attempt to restrict veterinary activities in the services.

The sixth case, reported in an infant in Indiana, was reported on the basis of a wound on its finger and demonstration of Negri bodies in the brain at autopsy. Investigation of the case revealed no information on the time or nature of exposure.
The number of rabies cases reported to the United States Public Health Service during the first six months of 1958 totals 2,500 (Table 2). This is 50 cases less than those reported for the same period in 1957. The hot spots in rabies incidence thus far this year have been in Wisconsin, Iowa, Minnesota, Kentucky, Indiana, Ohio and Texas. Thus far there have been four human deaths, one each reported from South Carolina and Georgia and two from Ohio.

**Wildlife Rabies**

The fox and the skunk continue as the principal sylvatic vectors of rabies in the country. During the past three years, there has been a slight shift in the geographic distribution of sylvatic rabies. In 1955 fox and skunk rabies were quite discrete in their distribution (Figure 2) with fox rabies being...
limited to eastern United States and skunk rabies to the broad areas of the upper Mississippi and Missouri valleys and to the central valley of California. In 1958 there has been evidence of expansion of both these large areas with overlapping of skunk and fox rabies in Kentucky, Ohio, Indiana and Illinois (Figure 3).
Regional Control and Training Activities

Experience has shown that the regional approach to the development of rabies control programs has led to free flow of information and the use of uniform control practices in groups of states having similar rabies problems.

During the past year, four major regional rabies conferences were held throughout the country under the sponsorship of the Communicable Disease Center, United States Public Health Service. These were at Berkeley, California, for the Pacific Coast States, Alaska, Hawaii and British Columbia; New Orleans, Louisiana, for the South Central States; Chicago, Illinois, for the Great Lakes States and Kansas City, Kansas, for the Missouri Valley States.

Two regularly scheduled refresher courses in the diagnosis and control of rabies were held at the Communicable Disease Center in Atlanta with 31 students in attendance from the United States and abroad.

A new manual was published by CDC on Laboratory Methods in the Diagnosis of Rabies. This is now available from the United States Government Printing Office as Public Health Service Publication No. 568.

Investigational Activities

Major break-throughs have been accomplished in the rabies research program of the Communicable Disease Center. The use of fluorescent antibody technique has been developed for the detection of rabies virus in animal brains and rabies virus isolates have been adapted to tissue culture.

Studies in sylvatic rabies have shown that 75 percent of rabid foxes contain virus in their salivary glands, that there is no evidence of true or symptomless carriers in foxes and that the transmitting potential is quite large in foxes as shown by demonstration of virus in saliva for long periods of time. Virus surveys of over 1,500 small wild rodents have shown that these animals do not serve as reservoirs of the disease in nature. Preliminary results of SN antibody studies at the Communicable Disease Center Southeastern Rabies Station has shown evidence of natural immunity of wild foxes in nature.

The Communicable Disease Center has established two new wildlife rabies investigations stations during the past year. One at Montgomery, Alabama, will be concerned primarily with fox rabies studies in the Southeast and one at Las Cruces, New Mexico, primarily for research in bat rabies in the Southwest. Plans are now being completed for a midwestern CDC Rabies Station at Poynette, Wisconsin for sylvatic rabies studies in the enzootic skunk rabies area.

Field studies in human immunization with HEP Flury vaccine are now being carried out in volunteers among veterinarians and veterinary students. Preliminary results of these studies show that this vaccine will serve an excellent purpose as a pre-exposure prophylatic measure in high risk groups such as veterinarians. The schedule consists of a primary series of three intradermal inoculations of 0.2 ml. of HEP vaccine followed by a booster of the same dose two to six months later.
Your Committee recommends:

1. That health departments and regulatory agencies continue to encourage widespread immunization of dogs, preferably with chicken embryo vaccine.

2. That local health departments strengthen programs for collection and disposal of strays. This can be done by elevating qualifications and positional responsibilities for personnel engaged in these activities, by developing training programs for dog wardens and animal shelter personnel, by improving and expanding physical facilities and equipment for these activities.

3. That predator control programs be developed in areas of enzootic and epizootic sylvatic rabies for reduction of fox and skunk populations.
OVINE VIRUS ABORTION*

E. A. TUNNICLIFF, D.V.M., M.S.

Montana Veterinary Research Laboratory
Bozeman, Montana

I wish to review briefly for you some pertinent facts concerning ovine virus abortion, also known as enzootic abortion in ewes. This information was obtained from the literature and by Dr. Hadleigh Marsh's contact with the workers consulted in Scotland, England and France. Then, I would like to give you a short account of the first recorded outbreak in the United States and some of the relevant facts and related information.

ETIOLOGY

Ovine Virus Abortion is caused by one of the viruses of the psittacosis-lymphogranuloma group.

DISTRIBUTION

The reported incidence has been limited to sheep in Scotland, England, Sardinia, Germany, France, Hungary and the United States.

SYMPTOMS AND PATHOLOGY

Abortions occur from mid-gestation through late pregnancy, with the majority in the latter part of pregnancy. The incidence usually does not exceed five percent, but may run to 25 to 30 percent. Placental retention is common. A vaginal discharge usually occurs for several days. The ewe may lose weight during the process. The cotyledons are dull-clay to dark red in color with or without necrosis, the consistency appearing more solid than normal.

Experimentally infected ewes have shown a body temperature rise three days after inoculation, the temperature returning to normal by the eighth day. Maximum temperature ranges were 106.4° to 107.0° F.

The aborted fetus may be mummified, degenerated or normal, or the lambs may be born full-term alive or dead. One lamb of twins may be normal, the other dead and/or mummified, or degenerated. Subsequent fertility of the ewe does not seem to be affected.

TRANSMISSION

Experimental. Pregnant ewes in mid-gestation have been experimentally infected with bacterial-free fetal membranes, fetal tissues and infected chick embryo yolk sacs. Non-pregnant ewes, although less susceptible than preg-
nant ewes, may develop the disease during their first gestation period following oral, subcutaneous, intravenous or intradermal inoculation of the virus.

**Natural.** Normal ewes grazing during pregnancy on pasture where abortions occurred the previous season did not abort. Chick embryo inoculation may demonstrate the virus from the stomach contents, and the lung, liver, spleen and kidney tissues of aborted fetuses. Ewes that aborted and had infected membranes lambed normally the following year with no evidence of infection. Ewe lambs and unbred ewes, because of their association at lambing time with aborted fetal and maternal tissues, may become infected and carry the infection without outward evidence of it until the next pregnancy, at which time they may abort. Thus the infection may be transmitted to the subsequent susceptible ewe generation.

There is no evidence, either from field observations or experimental work, to show that the disease can be transmitted by the ram.

**DIFFERENTIAL DIAGNOSIS**

In virus abortion and vibriosis, the clinical manifestations are practically identical, and the pathologic changes in both fetus and fetal membranes are the same in each disease.

However, in the case of viral abortion, inoculation of chick embryos, guinea pigs, mice or ewes is the final and most positive diagnostic method. Microscopic examination of stained smears from the cotyledons usually identifies the elementary bodies.

Complement-fixation or agglutination tests give positive confirmation of viral infection.

Bacteriological cultures should be prepared to rule out vibriosis or other possible bacterial etiologic agents.

**IMMUNITY**

Infected ewes rarely abort more than once. In Scotland, a single injection of the vaccine currently in use, a formalin inactivated chick embryo yolk sac suspension, is considered capable of producing lifetime immunity. First lambing ewes or previously unexposed ewes in infected flocks, should be vaccinated before breeding. A vaccine will be available commercially in the United States at an early date.

In infected flocks, the rate of abortion decreases rapidly following annual systematic vaccination of all unexposed and first lambing ewes.

**CONTROL**

The disease may be completely controlled by vaccination of all unexposed first lambing ewes. The situation is not quite so encouraging in flocks previously exposed to the infective agent. Some of the ewe lambs, or older ewes that did not abort from the previous year’s exposure may be infected before vaccination. In such case, the vaccine may not protect. In other words, vaccinated ewes may abort if infected prior to vaccination.
Since the infection is spread predominately at lambing time, all of the ordinary preventive precautions should be followed.

The successful control of the disease by vaccination in Scotland, England, France, Germany and Italy has made regulatory control measures unnecessary. In those countries it is not classed as a reportable disease.

**HOW AND WHERE THE VIRUS WAS FOUND IN THE UNITED STATES AND WHAT IS BEING DONE**

One of the most active research projects at the Montana Veterinary Research Laboratory is the study of ovine vibriosis. While engaged in this study it became apparent that vibriosis was not the only infectious type of abortion present in Montana sheep. During the lambing seasons of 1956, 1957 and 1958, 61 separate outbreaks of abortion among sheep with an infectious disease history were investigated in Montana. *Vibrio fetus* was the causative organism in 62 percent of these outbreaks. However, the aborted tissues from 37 percent of the affected premises were bacteriologically negative. These negative bacteriological findings worried us and suggested that we must have overlooked the etiological agent. However, it was not until early in 1958 that we were equipped for virological studies. In March-April, 1958, aborted tissues from ewes were available for study and we had an opportunity to examine thoroughly material from four of these outbreaks and the tissues from three of these were bacteriologically sterile. Elementary bodies were found in the cotyledons of aborting ewes from all four outbreaks. From two of these, the virus of ovine abortion was demonstrated (2). Inoculation studies proved the virus to be indistinguishable from enzootic abortion described by Stamp (1). From one of these ranches, three aborted fetuses were received. Elementary bodies were demonstrated in the cotyledons of a ewe that had aborted and the tissues were vibrio-free. From the other two fetuses, *V. fetus* was isolated but no inclusion bodies could be found.

Confirmation of the diagnosis of ovine virus abortion presented quite a serious problem, since the Foreign Animal Disease Handbook (United States Livestock Sanitary Association, 1954) lists the disease as foreign to our continent.

The occurrence of infection on widely separated premises and our experience covering several years of negative bacteriological findings in some outbreaks of obviously infectious abortion, appeared to justify the opinion that the presence of the virus was not new or localized.

Another fact which strengthened this opinion was that the Rocky Mountain Laboratory of the National Institute of Health has run complement-fixation tests with psittacosis-lymphogranuloma antigen on sera from a considerable number of sheep in Utah and Idaho and found seven to eight percent showing positive reactions. We do not know that the antibodies were those of ovine virus abortion, yet we know of no other disease of sheep present in those areas that stimulates such antibodies. Consequently, we suspect the disease has been present in at least three Intermountain states for some years.
Since transmission of infection is seasonal, occurring at lambing, the disease could not be declared an emergency and therefore handled by the routine eradication methods followed in the case of such conditions as foot and mouth disease.

As a result of the information presented, the United States Department of Agriculture, Agriculture Research Service authorities very wisely decided to learn all that was known about the disease before reaching any conclusions regarding control. Consequently, a mission was appointed, composed of Drs. H. Marsh, E. E. Saulmon and P. D. Delay, charged with the responsibility of visiting Scotland, England, France and other European countries if necessary. They were to learn as much as possible about enzootic ovine abortion and then report back to the Agriculture Research Service administration. All arrangements and appointments were made most efficiently in England and Europe by the Agriculture Research Service. The tour began July 26 and ended August 8, 1958.

We are heavily indebted to Dr. J. T. Stamp and staff of the Moredun Institute, Edinburgh, Scotland, for their generous reception and for the wealth of information on the problem. Incidentally, our diagnosis was confirmed on the spot from tissue material and serum samples taken by Doctor Marsh from the Montana outbreaks. Much helpful information was supplied by other veterinary research workers and control officers of Scotland, England, and the Pasteur Institute in Paris. Each of the people contacted on the trip was most helpful in assisting our group to obtain the maximum from their trip. We wish to express publically our deepest gratitude to all who made the mission successful and worthwhile.

Upon the mission’s return, a meeting was called in Washington, D.C., where all available information on the problem was placed before a group of Agriculture Research Service research workers, regulatory and administrative officials, and several state veterinarians from interested states, and representatives of the sheep industry.

CONFERENCE CONCLUSIONS

The group concluded that many undiagnosed cases of ovine abortion in this country may be due to the virus, and it is suspected to be widespread.

The disease does not compel drastic eradication measures and the Animal Disease Eradication Division will not participate in the control unless conditions warrant it in the future.

State and Federal regulatory officials were urged to review flock histories of undiagnosed cases of ovine abortion within their states and report the incidence to the respective state and Federal disease control authorities. This will be helpful in developing the disease survey now underway.
PROPOSED DISEASE SURVEY

With the cooperation of the Agriculture Research Service, Animal Disease Eradication Division, the Montana Veterinary Research Laboratory is prepared to make a serological survey of flocks in Montana and other Western Regional Vibriosis Project states that had an abortion problem in 1958, in which no vibrio or other bacterial agent was incriminated as the etiological agent. Since antibodies for the ovine abortion virus usually persist for at least nine months, there is still time to gain some valuable information on the 1958 outbreaks.

In as many cases as possible, flock owners of the Intermountain and Pacific Coast states where abortion losses occur will be contacted during the 1959 lambing season. In addition to the serological survey, tissues will be collected for bacteriological and virological studies in an effort to determine the distribution of virus abortion among the sheep of the areas under study.

REFERENCES


Suwannee County, Florida, was designated as a hog cholera pilot test eradication area during 1955 in compliance with recommendations by the Committee on Nationwide Eradication of Hog Cholera of our Association.

Control of swine movement for this area is simplified by the fact that Suwannee County is bounded on three sides by the Suwannee and Santa Fe Rivers. There are 25 points of access into the county but only 12 major roads. Guard stations are located at five of these 12 roads, with 24-hour truck inspection enforced. The inspectors at these guard stations require veterinary certification that all hogs entering Suwannee County have been properly vaccinated against hog cholera. Also, all hogs passing through public-auction markets in Suwannee County, except those for immediate slaughter such as feeders or breeding animals, must be vaccinated for hog cholera with a modified live virus vaccine and serum before being transported to the farm.

All vaccinations are performed by the three practicing veterinarians living in Suwannee County. The products used are restricted to the modified live virus vaccines and serum. These are supplied free of charge by the Florida Livestock Board to the swine raiser through his veterinarian, who makes a small service charge to the owner. All vaccinations by the veterinarians are reported to us. These reports include the name and address of the owner, the condition of the premises, the health of the pigs, the temperatures of at least 10 percent of the pigs to be vaccinated, the type of vaccine used and the serial number, the serum used, the serial number of the serum, and the total amount, and the average amount of serum per pig. A minimum dose (15 cc.) of serum is used, unless there are factors such as known or suspected exposure to hog cholera or intercurrent conditions which require larger dosages. In such cases, the dose of serum is increased at the discretion of the administrating veterinarian. In addition to the above information, the veterinarian also procures from the owner some breeding information, as well as information regarding the previous occurrence of any abnormality, especially hog cholera in his own herd or in any of his neighbors' herds. The owner is then required to sign an agreement that he will sell to us two percent but not less than two of the vaccinated animals for virus-challenge purposes.

At the time of vaccination, the veterinarian notches an ear of each animal according to a prearranged system by which the serial number or numbers of the vaccine used can be determined. The Florida Livestock Board supplies us with samples of all serial numbers of vaccine and serum used in the county. This is for the purpose of investigation in the event that a postvac-
cinal reaction or immunization failure occurs and it is desirable to determine whether or not the vaccine or serum is culpable.

There are approximately 60,000 swine on 1,500 farms in Suwannee County. The swine have been vaccinated on 884 (58.9 percent) of these farms and challenged on 384 (43.4 percent) of the 884 farms.

**TABLE I**

*Vaccinations Since September 1, 1956*

<table>
<thead>
<tr>
<th>Modified Live Virus Vaccine</th>
<th>Herds Number</th>
<th>Herds Percent</th>
<th>Pigs Number</th>
<th>Pigs Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine Origin</td>
<td>816</td>
<td>35.0</td>
<td>24,598</td>
<td>36.8</td>
</tr>
<tr>
<td>Tissue Culture</td>
<td>793</td>
<td>33.9</td>
<td>20,903</td>
<td>31.2</td>
</tr>
<tr>
<td>Lapine Origin</td>
<td>725</td>
<td>31.1</td>
<td>21,415</td>
<td>32.0</td>
</tr>
<tr>
<td>Total</td>
<td>2,334</td>
<td>100.0</td>
<td>66,916</td>
<td>100.0</td>
</tr>
</tbody>
</table>

In order to carry out the recommendations of our Association, a Hog Cholera Research Station was constructed by the United States Department of Agriculture at Live Oak, Florida. It was completed in April, 1957, and the first virus challenge of farm-vaccinated pigs was started on April 25, 1957.

The vaccinated hogs to be virus-challenged are purchased from the owners, delivered to our Station, and penned on Wednesday afternoon of each week. Observations are started immediately. Prechallenge temperatures are taken Wednesday afternoon, Thursday morning, and Thursday afternoon. The challenge virus injection is made on Thursday afternoon. One cc. of Station virus serial No. 1 is used for each challenge.

Station virus s.n. 1 was prepared at our Station from three susceptible hogs. These hogs were locally procured and were all apparently normal. Each of the hogs was injected with two cc. of BAI hog cholera virus s.n. 7,183 procured from Dr. L. O. Mott, Head of the Viral and Rickettsial Diseases Section of the Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland. The hogs developed symptoms usually associated with hog cholera and were exsanguinated and the blood defibrinated on the seventh day after injection. At necropsy, the lesions observed were those usually seen in hog cholera at this stage of the disease. The total volume of defibrinated blood collected was 5,320 cc. This volume of blood was prepared in order to have a supply adequate for future needs and to obviate changing the virus during the course of the work. As a result of the titration of the blood, it was found that the minimum lethal dose of the virus was $2 \times 10^{-7}$ cc. The blood was bacteriologically negative. The blood-virus was bottled in small volumes and stored at $-40^\circ$ F.

At the time of the challenge of the vaccinated hogs each week, a susceptible virus control pig also is injected with the same amount of the same virus to demonstrate its virulence.
Observations of the challenges are conducted for two weeks. The reactions during the two-week challenge observation period are recorded mainly on the basis of appetite and temperature. A hog with a slight loss of appetite is charged with one point for that day. A hog which is slow in eating, seems to be sick, and has an elevated temperature is charged with two points for the day. A hog which is anorectic and is obviously sick is charged with three points for the day. Table II is a guide which is used for determination of the severity of the reaction following virus challenge and the percentage of protection imparted by the vaccine. Variability of these reactions makes it necessary to consider the numerical values and verbal descriptions in the table as more suggestive than definitive.

In a determination of whether or not the herd from which the challenges originated was adequately protected, the average of the percentage of protection of the challenges is taken. If this figure is 70 percent or more from any one herd, it is assumed that that herd is adequately protected; if it is less than 70 percent, the contrary is assumed.

Table III gives the number of hogs vaccinated and subsequently challenged

<table>
<thead>
<tr>
<th>Number of Points</th>
<th>Reaction</th>
<th>Degree of Protection</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Negative</td>
<td>Adequate</td>
<td>100</td>
</tr>
<tr>
<td>1-10</td>
<td>Slight</td>
<td>Adequate</td>
<td>80</td>
</tr>
<tr>
<td>11-20</td>
<td>Severe</td>
<td>Inadequate</td>
<td>60</td>
</tr>
<tr>
<td>21-30</td>
<td>Severe</td>
<td>Inadequate</td>
<td>40</td>
</tr>
<tr>
<td>&gt;30</td>
<td>Death</td>
<td>Inadequate</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE III

<table>
<thead>
<tr>
<th>Modified Live Virus Vaccine</th>
<th>Average Number of Days Post-Vaccination</th>
<th>Number Vaccinated</th>
<th>Herd Number Challenged</th>
<th>Percent</th>
<th>Number Vaccinated</th>
<th>Herd Number Challenged</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine Origin</td>
<td>218.8</td>
<td>816</td>
<td>284</td>
<td>34.8</td>
<td>10,492</td>
<td>536</td>
<td>5.1</td>
</tr>
<tr>
<td>Tissue Culture</td>
<td>177.6</td>
<td>793</td>
<td>274</td>
<td>34.6</td>
<td>9,872</td>
<td>526</td>
<td>5.3</td>
</tr>
<tr>
<td>Lapine Origin</td>
<td>352.0</td>
<td>725</td>
<td>302</td>
<td>41.7</td>
<td>12,119</td>
<td>524</td>
<td>4.3</td>
</tr>
<tr>
<td>Total</td>
<td>252.4</td>
<td>2,334</td>
<td>860</td>
<td>36.8</td>
<td>32,483</td>
<td>1,586</td>
<td>4.9</td>
</tr>
</tbody>
</table>
and the number of herds from which they originated from the beginning of our challenge program to September 30, 1958.

Table IV is a record of our challenge results.

### Table IV

**Result of Challenge**

<table>
<thead>
<tr>
<th>Modified Live Virus Vaccine</th>
<th><strong>Adequate Protection</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine Origin</td>
<td>Number*</td>
<td>Herds</td>
</tr>
<tr>
<td></td>
<td>264</td>
<td>513</td>
</tr>
<tr>
<td>Tissue Culture</td>
<td>251</td>
<td>497</td>
</tr>
<tr>
<td>Lapine Origin</td>
<td>276</td>
<td>492</td>
</tr>
<tr>
<td>Total</td>
<td>791</td>
<td>1,502</td>
</tr>
</tbody>
</table>

* Numerator—number adequately protected. Denominator—number challenged.

Twenty-eight suspected outbreaks of hog cholera were reported by practicing veterinarians in the test area during the 17-month test period. One positive case of hog cholera in a vaccinated herd was confirmed by animal-inoculation tests. Eight suspected cases of hog cholera in non-vaccinated herds were negative on animal-inoculation tests, and there were 19 unconfirmed cases of suspected hog cholera, for which specimens were not submitted.

There is no record of suspected hog cholera cases on farms where veterinarians were not called; however, it is believed their number would be almost insignificant.

The problem of eradicating hog cholera from Suwannee County remains a thorny one. Until July 1, 1958, there was no control of movement of non-vaccinated swine from public markets back to a farm. As the result of a study of suspected cases of hog cholera in Suwannee County, it was found that in a majority of cases the hogs had originated from public markets. In order to correct this situation, the Florida Livestock Board passed a resolution, effective July 1, 1958, making it mandatory that all non-vaccinated swine passing through any public market in Suwannee County and not destined for immediate slaughter be vaccinated before being transported back to a farm.

Prior to July 1, 1958, most of the reports of cases of suspected hog cholera were not accompanied by specimens by which the diagnosis could be con-
firmed. As reported above, only nine such specimens were submitted and tested. The results of these tests have been given above. In fiscal year 1959, greater emphasis is being placed on the submission of specimens, suitable for testing, from suspected cases of hog cholera. Immediately upon receipt of these specimens, the Florida Livestock Board is notified and the Board quarantines the herd. Our Station makes animal-inoculation tests of the specimens in susceptible pigs with and without serum and in a cholera-immune pig, in order to demonstrate the presence or absence of hog cholera virus. At the completion of our tests, the Florida Livestock Board is notified, and, depending on whether or not the diagnosis is confirmed, the Board acts accordingly in regard to the raising of the quarantine.

As stated above, the hogs on 58.9 percent of the farms in Suwannee County have been vaccinated. It is generally believed that in a national eradication program, it would be practically impossible to obtain 100 percent vaccination coverage. Therefore, in order to simulate the national picture, it was thought desirable to leave some herds unvaccinated in Suwannee County. However, due to the large number of variable conditions to be considered, it is impossible to determine the percentage of swine that would need to be immunized to conform with a hog cholera eradication program that would be successful. Some have expressed opinions that as many as 80 percent of the swine population would need to be vaccinated. But others believe that 80 percent would be far more than is required. In order to stimulate interest in vaccination, publicity through various public communication media by the Florida Livestock Board is being used. The local veterinarians and county agricultural officials are also encouraging vaccination, and it is generally felt that some progress in this connection is being made.

The challenge program is being carried on as usual, but priority is being given to investigation and testing of specimens from hog cholera cases in unvaccinated swine. However, in the purchase of challenge hogs, more emphasis is being placed on the selection of herds from which the hogs originate. Hogs are being selected for challenge from herds which were assumed to be inadequately protected after challenge and from herds in which hog cholera was confirmed.
Death losses in farm herds after vaccination have indicated that in many instances the pigs failed to develop proper immunity.

A paper presented to this Association in 1955 (1) pointed out that all of the modified live-virus vaccines on the market had failed to immunize some farm herds. In 1956 (2), a paper presented to this Association on the vaccination of farm herds with crystal violet-glycerol vaccine also pointed out that there was a marked variation in the protection of different herds when the same serial of vaccine was used for immunization. It therefore seemed expedient to determine how prevalent this condition is among farm herds and, if possible, to determine what factor or factors are involved in immunization failures. A program of vaccination of farm herds was begun in 1956 to study the immunization response. This is a report on two years’ study of this problem.

**MATERIALS**

Crystal violet-glycerol vaccine was selected for this study because those herds that did not develop immunity would not be exposed to a live hog cholera virus. The crystal violet vaccine was prepared by the method reported in 1956 (2). Pigs were purchased from a hog cholera-free area where no vaccination was practiced. These pigs were tested for susceptibility to hog cholera before being used for making vaccine by the injection of two pigs with two cc. of a virus that had been previously tested for variant characteristics and for which the MLD had been determined. Vaccination of farm herds was done at the requests of the farmers without their being solicited or selected for any reason. Vaccination was carried out on 96 farms the first year with 11,784 pigs and on 82 farms the second year with 13,117 pigs. Spring and fall litters were vaccinated and are designated as separate herds. All farmers do not raise two litters a year; therefore, only 125 herds of pigs were vaccinated each year. There were no purebred herds in the program. For each year, five herds were full-blooded Hampshires and the remaining herds were hybrids or two-, three-, or four-way crosses of the major breeds of hogs.

**METHODS**

Pigs were vaccinated about two weeks after weaning. The ages varied from six to 15 weeks, the majority of pigs being eight to 10 weeks old at time of vaccination. Five cubic centimeters of crystal violet-glycerol (CVG)
vaccine was injected subcutaneously. At one month, three months, and six months after vaccination, pairs of pigs from each herd were delivered to the Hog Cholera Research Station at Ames, Iowa. Two cubic centimeters of virulent hog cholera virus was injected subcutaneously into each pig as a challenge dose. Daily observations were made and the condition of the pig was recorded by a point system. No points were recorded if the pig remained normal; one point, if the pig did not eat a normal daily ration and was somewhat slow in eating; two points, if the pig ate only a small amount of feed and soon went back to the nest; and three points, if the pig refused to eat and stayed in the nest. All pigs with no points were marked "normal (N)"; those with one to 10 points were marked "slight (SL)"; and those with over 10 points were marked "severe (Sv)." In order to evaluate the degree of protection in each pig, the total number of points given a pig was assigned one of the following percentages:

- 0 points — 100 percent protection
- 1-10 points — 80 percent protection
- 11-20 points — 60 percent protection
- 21-30 points — 40 percent protection
- 31-40 points — 20 percent protection
- 41-50 points — 10 percent protection
- 51-60 points — 5 percent protection
- Died — 0 percent protection

Eight different lots of CVG vaccine were used the first year (1956) for treating farm herds. The amounts of vaccine in the lots varied from 4,000 to 25,000 cc. The larger lots of vaccine were made by combining smaller sublots that had been tested on pigs to find the percent protection. One lot of vaccine and one lot of challenge virus were used the second year (1957) so that the vaccine and virus as variable factors might be eliminated and that more uniform results might be obtained. All lots of vaccine were tested once, and in some instances twice, on susceptible test pigs before being used on the farms. Six test pigs were used to test each sublot of vaccine and to test each lot after the sublots were pooled. Two pigs were given one cc. each of vaccine subcutaneously, two pigs were given 2-1/2 cc. each, and two were given five cc. each. The six pigs were challenged 21 days after vaccination with two cc. of virulent hog cholera virus. The percentage protection was evaluated by the method described.

RESULTS

There are many variable factors involved in the immunization of pigs against hog cholera. The pigs, the vaccine, and the administration of the vaccine, are variable. The immune response of the pigs is influenced by general farm conditions, by farm management, by the weather, by other stress factors such as parasites and diseases, and in some instances by the breeding. The variables in the production of vaccine are influenced by the viruses used for production and the viruses used for challenging. The pig from
<table>
<thead>
<tr>
<th>Vaccine Serial Number</th>
<th>Number Vaccinated</th>
<th>Number Tested</th>
<th>Percent Survival</th>
<th>Percent Died</th>
<th>Percent Immunity</th>
<th>21-Day Challenge</th>
<th>LABORATORY TEST PIGS</th>
<th>Percent Immunity</th>
<th>21-Day Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-Month Challenge</td>
<td>3-Month Challenge</td>
<td>6-Month Challenge</td>
<td>Total Percent Immunity</td>
<td>Test 1</td>
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<tr>
<td>102</td>
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<td>74</td>
<td>73.0</td>
<td>27.0</td>
<td>67.0</td>
<td>59.2</td>
<td>41.7</td>
<td>56.0</td>
<td>82.5</td>
</tr>
<tr>
<td>103</td>
<td>1,096</td>
<td>64</td>
<td>86.0</td>
<td>14.0</td>
<td>66.3</td>
<td>72.7</td>
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<td>70.3</td>
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<td>107</td>
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<td>122</td>
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<td>83.0</td>
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<td>100.0</td>
</tr>
<tr>
<td>110</td>
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<td>79.6</td>
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</tr>
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<td>80.0</td>
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<td>75.1</td>
<td>56.1</td>
<td>59.3</td>
<td>64.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>
which the blood is obtained for vaccine production and the pigs used to test
the vaccine influence the results obtained in vaccination. Some of these vari-
ables were apparent before the experiment was begun; others were not evalu-
ated until after the experiment was underway.

The first year eight different serials of vaccine and four challenge viruses
were used. Chart I gives the results of testing these eight vaccines on labora-
tory test pigs and on farm pigs. Laboratory tests of the eight vaccines on
susceptible pigs were quite variable. One vaccine, No. 109, gave 100 percent
protection on one lot of pigs and 40 percent protection on another lot of
pigs. Another vaccine, No. 112, gave good protection to two different lots of
pigs. Vaccine No. 107 gave poor protection on two lots of pigs. Vaccine No.
110 gave good protection on one lot of pigs and poor protection on two lots
of pigs; one of these lots of pigs proved to be hypersusceptible to hog cholera
virus. Vaccine No. 108 was made from a variant virus. The percent protec-
tion was good in laboratory test pigs and in farm herds when a regular virus
was used for challenge. The vaccine gave no protection when its homologous
virus was used for challenge.

When these vaccines were used in farm herds, the percent protection was
not always comparable to that in the test pigs. Some vaccines gave better
results in farm herds, and some gave less favorable results. Vaccine No.
107, for example, tested very poorly in the laboratory, with practically no
protection. When it was used in farm herds, the percent protection was 63.4.

The second year only one lot of vaccine, No. 112, and one challenge virus
were used (see Chart I). This vaccine gave good protection on two lots of
laboratory test animals, the percent protection being 100 and 80; however,
the percent protection on farm pigs was 64.0. The survival percent on farm
pigs was high (93.3).

The percent protection of the nine vaccines at one-, three-, and six-month
challenge of pigs after vaccination was variable (see Chart I). The one-
month protection varied with the different vaccines from 63.5 to 95.0 per-
cent; the three-month protection, from 56.1 to 95.8 percent; and the six-
month protection, from 41.7 to 88.4 percent. Of the nine vaccines tested by
challenge, six gave the greatest protection at one month, two at three months,
and one at six months.

The results of the comparative study for 1956 and 1957 of the vaccination
of farm herds on a basis of reaction to challenge are shown in Chart II. The
percent of animals having no reaction or very slight reaction was 65.0 in
1956, and 48.59 in 1957. The survival rate, however, was 87.25 percent in
1956, and 93.4 percent in 1957. The death loss in 1956 was 12.75 percent;
in 1957 it was 6.59 percent. There is very little difference in the percent-
ages of death loss of the one-month, three-month, and six-month vaccinated
pigs within each year. This would seem to indicate that this percentage of
pigs did not become immune when vaccinated.
<table>
<thead>
<tr>
<th>Year</th>
<th>Herds</th>
<th>Vaccinated</th>
<th>Challenged</th>
<th>Reaction (Number of Pigs)</th>
<th>Percent Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>S</td>
</tr>
<tr>
<td>1956</td>
<td>96</td>
<td>125</td>
<td>11,784</td>
<td>697</td>
<td>1 mo.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 mo.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 mo.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>1957</td>
<td>82</td>
<td>13,117</td>
<td>747</td>
<td>1 mo.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 mo.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 mo.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
</tbody>
</table>

N—normal; S—slight; Sv—severe; D—died.
A comparison of protection according to breeds of pigs was made. The farm herds are of such mixed breeding that it is difficult to classify them, and the protection after vaccination is as variable as the breeding.

A comparison was made of the protection in spring and fall litters of 16 herds when the same dams and sires were used. The percent protection was better in the fall in seven herds, and in the spring in eight herds. One herd had the same protection in both seasons.

A study of the pairs of pigs, with a comparison of the pairs in the two years, is as follows:

<table>
<thead>
<tr>
<th></th>
<th>1956</th>
<th>1957</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of pairs tested</td>
<td>355</td>
<td>340</td>
</tr>
<tr>
<td>Pairs with 100 percent immunity</td>
<td>43.2%</td>
<td>22.3%</td>
</tr>
<tr>
<td>Pairs with both pigs having same percent protection</td>
<td>53.2%</td>
<td>64.7%</td>
</tr>
<tr>
<td>Pairs in which one pig was protected and the other died</td>
<td>17.1%</td>
<td>11.7%</td>
</tr>
<tr>
<td>Pairs with neither pig protected</td>
<td>4.2%</td>
<td>1.76%</td>
</tr>
<tr>
<td>Pairs with varying degrees of protection</td>
<td>25.5%</td>
<td>23.84%</td>
</tr>
<tr>
<td>Herds with 1-month pigs not protected and 3- and 6-month pigs protected</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

If the pairs of pigs are indicative of the herd immunity, there is a percent of the herds in which the pigs are adequately protected, there is a percent in which the pigs have no protection, and a percent in which the pigs are mixed; that is, some of the pigs may have good protection and others do not.

**DISCUSSION**

Two years' study of farm herds after vaccination with CVG vaccine gave evidence that the pig is the most variable factor in immunization against hog cholera. A comparison at this time can be made only on the results of study for two different years. When the herds on the same farms have been tested for a longer period of time, the results can be better evaluated.

The challenge of all the pigs in a herd would be the best way to determine the herd immunity, but since this is not practical the protection must be determined by challenging a representative number of pigs from the herd. The testing of pigs at one, three, and six months determines how good the immunity was early in the life of the pigs and the decrease or increase in this immunity in later months. There are herds in this study which demonstrate that both of these conditions existed. More than 50 percent of the herds were solidly immune and remained so for the six months. Only five to six percent were not immune at all. The remaining percentage of the herds were variable in their immunity, and the immunity changed in the six-month period.

Hog cholera or postvaccination sickness did not occur in any of the 250 vaccinated herds under study.
SUMMARY

Pigs on 83 widely scattered farms were vaccinated two consecutive years with crystal violet vaccine to study the immune response of different herds. The first year 125 herds, composed of 11,784 head, were vaccinated and 5.9 percent of the pigs were challenged. The second year 125 herds, composed of 13,117 head, were vaccinated and 5.7 percent of the pigs were challenged. Pigs were challenged at one, three, and six months after vaccination. There was marked variation in both years of the study in the immunity produced at the different challenge periods. When one vaccine and one challenge virus were used on all pigs tested, the variations were similar. Over 50 percent of the herds were adequately protected each year. Six to 12 percent were not protected and 30 to 40 percent had varying degrees of protection. The pig seems to be the most variable factor in the immunization of pigs against hog cholera.

Hog cholera did not occur in any of the herds vaccinated.

REFERENCES


RECENT STUDIES ON THE PROPERTIES OF A NONVIRULENT LIVING HOG CHOLERA VACCINE


Indianapolis, Indiana

The purpose of this report is to present a resume of experimental and field data obtained during the development and refinement of a modified, living virus hog cholera vaccine.* These data support the view that this living virus is completely nonvirulent, while retaining the highly antigenic properties of the original, unmodified strain. Certain details of the experiments considered here either have been or are to be reported elsewhere (1, 2).

EXPERIMENTAL

Previous publications (3, 4) have outlined the method by which modification of the virus was accomplished. The original strain was adapted to rabbits by making alternate passages in pigs and rabbits until, after several such passages, it could be maintained in rabbits without further transfer in pigs. During subsequent passages it gradually lost its virulence for pigs without loss of antigenicity. It currently is maintained at a level of more than 400 consecutive rabbit passages.

Antigenicity

Table I shows that the vaccine initiates a rapid immune response. Of various groups of pigs challenged with virulent virus at daily intervals following vaccination, 43 percent (three of seven) of the pigs were protected following vaccination, 43 percent (three of seven) of the pigs were protected

TABLE I

Rapidity of Development of Immunity Following Vaccination

<table>
<thead>
<tr>
<th>Number of Pigs Tested</th>
<th>Days Between Vaccination and Challenge</th>
<th>RESULTS OF CHALLENGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Remained Well</td>
<td>Died</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

* Swivax, Pitman-Moore Co.
against challenge as early as two days following vaccination, 87 percent (six of seven) by the third day, and 100 percent (seven of seven) by the fourth day and thereafter.

As shown in Table II, a remarkably high degree of immunity is induced

### TABLE II

**Degree of Active Immunity Produced by the Vaccine With and Without Serum**

Demonstrated by Challenge After 78-91 Days With Various Amounts of Virulent Virus

<table>
<thead>
<tr>
<th>Number of Pigs</th>
<th>Dose of Vaccine (cc.)</th>
<th>Dose of Serum (cc.)</th>
<th>Dose of Challenge Virus</th>
<th>Number of Pigs Receiving</th>
<th>Results Number of Pigs</th>
<th>Remained Well</th>
<th>Sickened</th>
<th>Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>2</td>
<td>None</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>15</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>30</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>None</td>
<td>15</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>16</td>
<td>None</td>
<td>None</td>
<td>16</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>15</td>
<td>14</td>
</tr>
</tbody>
</table>

* This dose in 200 lb. pigs equals 1,000,000,000 lethal doses.

with a standard two cc. dose of vaccine. In one instance, 12 pigs, averaging 200 lb. each were inoculated intravenously with five cc. per lb. body weight of virulent virus (1,000 cc.) 78 to 91 days following vaccination. All of these pigs withstood this challenge which approximated 1 billion lethal doses of virulent virus.

Results of the experiments summarized in Table II and III show that the

### TABLE III

**Efficacy of the Vaccine at Different Intervals Following the Administration of Antiserum**

The doses were as follows: vaccine, 2 cc.; serum, 35 cc., and virulent challenge virus, 2 cc. All pigs were challenged 75 to 83 days after administration of serum.

<table>
<thead>
<tr>
<th>Interval Between Serum and Vaccine</th>
<th>Number of Pigs Tested</th>
<th>Number of Pigs Remaining Well</th>
<th>Sick and Recovered</th>
<th>Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simultaneous</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 Week</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>10</td>
<td>9</td>
<td>0</td>
<td>1*</td>
</tr>
<tr>
<td>3 Weeks</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4 Weeks</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vaccine Only</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serum Only</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>No Treatment</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

* Died six days after challenge; few lesions suggestive of cholera; hemolytic-streptococcus isolated from spleen and heart blood.
vaccine is equally effective when used with, without or following hog cholera antiserum.

Deliberate attempts were made to determine whether the vaccine might induce untoward clinical effects or fail to induce an immune response in pigs under conditions of artificial or naturally-occurring stress. For this experiment, one group of pigs given cortisone and two other groups with bacterial infection and verminous pneumonia, respectively, were used. The results (Table IV) show that no change in clinical attitude developed in any of the

TABLE IV

<table>
<thead>
<tr>
<th>Number of Pigs</th>
<th>Stress Factor(s)</th>
<th>Following Vaccination</th>
<th>Following Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Cortisone</td>
<td></td>
<td>Susceptible</td>
<td>Immune</td>
</tr>
<tr>
<td>2 Bacterial infect.</td>
<td></td>
<td>Susceptible</td>
<td>Immune</td>
</tr>
<tr>
<td>2 Verminous pneumonia</td>
<td></td>
<td>Susceptible</td>
<td>Immune</td>
</tr>
</tbody>
</table>

*Vaccinated pigs received 2 cc. of vaccine. All pigs received 2 cc. of virulent virus 7 days later.

various pigs following vaccination and that all of those vaccinated developed a satisfactory immune response. By contrast, all of the control pigs proved to be susceptible when challenged with virulent virus.

SAFETY

Field Evaluation

During early field use of the vaccine, under special license, an attempt was made to evaluate its effectiveness by circulating inquiries which, when completed, were sent to the United States Department of Agriculture for tabulation. Table V is a summary of these reports concerning 2,301 herds in which a total of 175,765 pigs were vaccinated. Reported losses from all

TABLE V

*Field Use of the Vaccine in 2,301 Herds*

Calculated from reports to United States Department of Agriculture during period of special license.

| Total pigs vaccinated          | 175,765 |
| Losses diagnosed as other than cholera | 1,433 (0.815%) |
| Losses from undiagnosed causes | 641 (0.364%) |
| Total losses from all causes   | 2,074 (1.179%) |
causes after vaccination totaled 2,074 or only 1.179 percent of all the pigs vaccinated. These reports concerned earlier use of the vaccine when the number of passages in rabbits was about 280, compared with the present level of more than 400. Although these results were entirely satisfactory, the number of rabbit passages has been increased to more than 400 to assure the safety of the vaccine.

Histopathology

A technic for histopathologic diagnosis of hog cholera has been described and evaluated in detail by Helmboldt and Jungherr (5) who pointed out that virulent hog cholera virus almost invariably causes nonpurulent, disseminated inflammatory lesions in the brain of susceptible pigs. These inflammatory changes occur also in the spinal cord. Similar microscopic lesions are not associated with common infectious diseases of swine, such as erysipelas, pasteurellosis and salmonellosis, although the gross lesions seen in these diseases frequently are identical with those found in hog cholera.

In our laboratory this method has been found experimentally to be remarkably accurate (2). It is now used routinely to support a differential diagnosis of hog cholera, particularly when virus isolation attempts in pigs are not made. The characteristic lesions of encephalomyelitis are illustrated by Figures 1 and 2 which are photomicrographs made from the brain and spinal cord of a pig with hog cholera. Figure 3 is a photomicrograph from a normal pig, showing the same area of the brain that is depicted in Figure 1.

Fig. 1. Photomicrograph of inflammatory lesions in the medulla of a pig with hog cholera. Note marked perivascular cuffing (upper left) and gliosis (lower center). Stain, H and E. x 80.
FIG. II. Photomicrograph of inflammatory lesions in lumbar spinal cord of a pig with hog cholera. Note perivascular cuffing and diffuse infiltration of lymphoid cells in the ventral horn. Stain, H and E. x 80.

FIG. III. Photomicrograph of a section of brain from a normal pig. Compare with Figure I. Stain, H and E. x 80.
With the knowledge that virulent hog cholera virus regularly produces disseminated aseptic encephalomyelitis, attempts were then made to determine whether similar lesions might occur in pigs killed at progressive intervals following vaccination with the modified, live virus vaccine. No lesions were found in a series of clinically normal vaccinated pigs. Following this, a series of two groups of pigs under conditions of naturally occurring stress (bacterial infection and verminous pneumonia) were deliberately used in a similar manner to determine whether stress might enhance production of microscopic lesions in the central nervous system (CNS). As is shown in Table VI, no histologic lesions were demonstrated in the CNS of any of 24 pigs killed variously between four to 16 days following vaccination. By contrast, lesions associated with hog cholera were demonstrated in each of the four controls challenged with virulent virus.

Hematology

Since leukopenia has long been considered a characteristic feature of hog cholera, the absence of leukopenia might be regarded as a significant criterion in demonstrating lack of virulence of a hog cholera vaccine. To determine this point, three groups of susceptible pigs were used. One group was clinically normal. Two other groups, deliberately chosen because of conditions of naturally occurring stress (bacterial infection, verminous pneumonia), were used to determine if stress per se might enhance production of leukopenia. In the experiments summarized in Table VII, leukopenia did not develop after vaccination, either in the clinically normal pigs or in those
TABLE VII
Hematologic Studies of Pigs Vaccinated under Conditions of Naturally Occurring Stress, Compared with a Normal Group

<table>
<thead>
<tr>
<th>Group I Stress Factor</th>
<th>V (21 pigs)</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg. WBC in thousands</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Days postvaccination</td>
<td>-6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group II Stress Factor</th>
<th>V (20 pigs)</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verminous pneumonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg. WBC in thousands</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Days postvaccination</td>
<td>-7 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group III Stress Factor</th>
<th>V (2 pigs)</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg. WBC in thousands</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Days postvaccination</td>
<td>-6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
</tbody>
</table>

V = vaccinates
C = susceptible controls

under stress. However, in all three groups the nonvaccinated control pigs developed marked leukopenia after administration of virulent hog cholera virus. The vaccinated pigs in groups one and three as well as those in group two, proved to be immune upon subsequent challenge with virulent virus.
Absence of transmission of the virus vaccine from vaccinated to susceptible pigs in contact is particularly important from the standpoint of safety. As shown in Table VIII, the vaccine was not transmitted from vaccinated to susceptible pigs during a period of contact of 42 to 91 days.

TABLE VIII

Nontransmissibility of the Vaccine During a Period of 42 to 91 Days of Contact Between Vaccinated and Susceptible Pigs

<table>
<thead>
<tr>
<th>Number of Pigs</th>
<th>Number Receiving Vaccine</th>
<th>Number Receiving 15 to 35 cc. Serum</th>
<th>Results of Challenge With 2 cc. Virulent Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>126</td>
<td>126</td>
<td>84</td>
<td>126</td>
</tr>
<tr>
<td>54</td>
<td>None</td>
<td>16</td>
<td>54</td>
</tr>
</tbody>
</table>

Failure of the virus vaccine to revert to virulence is an especially important point. Since modification of the virus to its present nonvirulent form was accomplished by passage in rabbits, it is reasonable to question whether several reverse passages in the pig, the definitive host, might not induce reversion to virulence. To determine this point, 12 deliberate back-passages in two series of pigs were made. Two pigs, held in isolation, were used for each passage. As shown in Table IX, the virus did not revert to virulence during these two series of 12 back-passages each.
TABLE IX

Back-Passage of the Vaccine in Susceptible Pigs
(2 Pigs Used at Each Passage Level)

<table>
<thead>
<tr>
<th>Back-Passage</th>
<th>Days Between Vaccination and Bleeding</th>
<th>Days Between Vaccination and Challenge</th>
<th>Results of Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Series</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>9</td>
<td>Immune</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>12</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>7</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>12</td>
<td>&quot;</td>
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<tr>
<td>5</td>
<td>7</td>
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<td>8</td>
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<td>9</td>
<td>7</td>
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<td>12</td>
<td>&quot;</td>
</tr>
<tr>
<td>12</td>
<td>7</td>
<td>13</td>
<td>&quot;</td>
</tr>
<tr>
<td>Second Series</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>9</td>
<td>Immune</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>10</td>
<td>&quot;</td>
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<tr>
<td>3</td>
<td>7</td>
<td>13</td>
<td>&quot;</td>
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<tr>
<td>4</td>
<td>7</td>
<td>9</td>
<td>&quot;</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>16</td>
<td>&quot;</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>18</td>
<td>&quot;</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>17</td>
<td>&quot;</td>
</tr>
<tr>
<td>8</td>
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<td>&quot;</td>
</tr>
<tr>
<td>9</td>
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<td>11</td>
<td>&quot;</td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>15</td>
<td>&quot;</td>
</tr>
<tr>
<td>12</td>
<td>7</td>
<td>18</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Virulence of the challenge virus and susceptibility of the test pigs were demonstrated by challenging part of each herd.

Absence of persistence of the virus vaccine in the pig is another point in evaluating its safety. To determine if this high rabbit-passage virus might be harbored in the pig, two young susceptible litters were used. In one test using six-week-old pigs, four of a litter of nine were inoculated with two cc. of vaccine. Four weeks later blood and urine were collected from the vaccinated pigs and injected into two additional groups of susceptible pigs. The pigs which were injected with blood and urine remained well and were susceptible to challenge with virulent virus six weeks later, indicating that neither modified nor virulent virus was present in the blood or urine of the vaccinated pigs. When all of the original nine pigs were challenged with virulent virus 10 weeks after the beginning of the experiment, the four vaccinated pigs remained well, whereas the controls were susceptible.
STUDIES ON LIVING HOG CHOLERA

In a second experiment with a litter of five-week-old pigs, identical results were obtained.

Safety and potency of the vaccine in pigs raised in a cholera-free area were determined using a drove of pigs obtained from Canada, where eradication of hog cholera is practiced. Fourteen pigs were used to conduct a test for safety and potency in accordance with the rules and regulations of the United States Department of Agriculture. In this test, 10 of the 14 pigs selected at random were vaccinated and observed closely for seven days, at which time the entire group of 14 was challenged with virulent virus. As is shown in Table X, all the vaccinated pigs remained well from the time of vaccination until the test was concluded two weeks after challenge, except for one which developed an erratic fever. This pig survived throughout the test period and was then killed for necropsy. Abscesses were found in the lungs and gross or microscopic lesions characteristic of hog cholera were not demonstrated. All of the controls developed symptoms of cholera following challenge, and either died or were killed when moribund. Three of these were examined in detail and all showed gross and microscopic lesions associated with hog cholera. The test was then judged by a United States Department of Agriculture Veterinary Inspector to have satisfactorily demonstrated the safety and potency of the vaccine.

**TABLE X**

<table>
<thead>
<tr>
<th>Number of Pigs</th>
<th>Vaccine</th>
<th>Clinical Attitude Following Vaccination</th>
<th>Clinical Attitude Following Challenge With 2 cc. Virulent Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2 cc.</td>
<td>No change</td>
<td>No change*</td>
</tr>
<tr>
<td>4 (controls)</td>
<td>None</td>
<td>---</td>
<td>Sickened and died</td>
</tr>
</tbody>
</table>

*One pig had an erratic fever following challenge. Necropsy showed lung abscesses, but no gross or microscopic lesions of hog cholera.

**DISCUSSION**

For many years regulatory officials and others have sought the ideal instrument with which to effect the control and ultimate eradication of hog cholera. The highly contagious nature of hog cholera, its rapid spread in unprotected swine, and general uncertainty concerning possible reservoirs of the virus in nature are well recognized. In view of these factors, programs employing quarantine and slaughter have not been generally favored in this country. Therefore, creation of an immune barrier to the disease by means of vaccination would offer a more realistic alternative to such drastic measures. This particular approach has been given considerable thought in this association by a special committee on the nation-wide eradication of hog cholera (6).
It is generally agreed that a nation-wide vaccination program designed to eradicate hog cholera would need to exclude the use of virulent virus which is a major source of infection. While a killed vaccine is a safe vaccine, it has the disadvantage of inducing only short-term immunity and then only after a considerable lag-phase (7). Also the killed vaccines in general probably are ineffective when used with serum. This has been clearly demonstrated with the crystal-violet vaccine (8). Consequently, a vaccine combining the immunologic properties of a living virus with the safety of a killed virus appears to be ideal for the control and eradication of hog cholera.

It seemed worthwhile to evaluate the data presented in this report by comparing the results with a hypothetical vaccine that represents a theoretically ideal immunizing agent. This would be a modified, living hog cholera virus having the following properties:

A. **Antigenic properties**

1. Produces a rapid immunity.
2. Produces a high degree of immunity.
3. Produces a long-lasting immunity.
4. Produces immunity under conditions of naturally occurring stress.
5. Produces immunity with a single small dose.
6. Produces immunity when used with, without or following hog cholera antiserum.

B. **Safety properties**

1. Does not cause clinical signs of disease.
2. Does not cause histopathologic signs of disease.
3. Does not cause hematologic signs of disease.
4. Does not spread from vaccinated to susceptible animals.
5. Does not revert to virulence when deliberately “back-passaged” in the definitive host.
6. Does not produce a carrier state.
7. Does not produce abscesses, anaphylactoid reactions, isohemolytic disease, or other untoward effects.

The data submitted in this report satisfy the criteria for such a theoretically ideal hog cholera vaccine, all the properties of which are considered to be important. Of special importance, perhaps, is the ability of the modified vaccine to produce an immune response when given with or without, or following antiserum, since the use of antiserum would be necessary in controlling epizootics. Equally important, however, are the features of non-transmissibility from vaccinated to susceptible animals, and failure of the modified virus to revert to virulence when deliberately back-passaged in susceptible pigs. A word of caution may be in order, lest this report of the deliberate vaccination of pigs under conditions of stress be misinterpreted as a recommendation for using the vaccine routinely in herds of pigs which are in a state of general debility. These experiments sought to demonstrate
certain characteristics of the viral vaccine that had not been clearly defined previously, and were performed in the known absence of virulent hog cholera virus, a condition that cannot, with certainty, be duplicated in the field. The evidence showing that the modified live virus produces no clinical, hematologic, immunologic, or histopathologic changes either in clinically normal or debilitated pigs is extremely important. It demonstrates conclusively the nonvirulent properties of this live virus vaccine.

SUMMARY

Extensive laboratory and field studies demonstrated that a modified live virus hog cholera vaccine was safe and effective. These properties were more clearly defined by experiments which showed that even under conditions of stress the vaccine caused no clinical symptoms or gross or microscopic lesions. Neither serum nor stress interfere with its immunogenic properties; it does not spread by contact, and does not revert to virulence upon deliberate backpassage. Comparison with hypothetical standards for an ideal vaccine shows that the vaccine virus combines the immunogenic properties of a living virus and the safety of a killed-virus vaccine and is, in fact, nonvirulent.

REFERENCES

REPORT OF THE NATIONWIDE COMMITTEE
ON HOG CHOLERA ERADICATION


Your Committee wishes to again call to your attention the recommendations made before this Association in the proceedings of 1956, with special emphasis directed to the ten (10) points setting forth the necessary measures in establishing a sound program for the control and eradication of hog cholera.

Your Committee endorses the resolution of the National Association of State Departments of Agriculture of 1958, which urges those states which have not passed hog cholera legislation to enact laws which will prohibit the sale of virulent hog cholera virus as soon as possible.

Your Committee appreciates the efforts of Livestock Conservation Incorporated in stimulating interest in a national hog cholera eradication program.

Your Committee submitted to the respective state regulatory officials a questionnaire on hog cholera; its incidences, its control, and its economic significance. The results of this questionnaire indicate a serious lack of information relative to these points. It is therefore recommended that those states which do not maintain disease reporting programs give serious consideration to the institution of such procedures that more factual and comprehensive statistical analyses can be made for the benefit of the swine industry.

In compliance with the recommendations of the 1951 report, your Committee has reviewed and edited the material prepared by the sub-committee appointed in 1953 and herewith submits "What Is Known About Hog Cholera" for its acceptance and publication.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE


The Committee on Transmissible Diseases of Swine mailed questionnaires to the livestock sanitary officials of the 49 states and 29 answers were received.

A kind of record is kept in every state of the number of swine which cross state lines for purposes other than slaughter. But this does not mean that those records can be evaluated with a reasonable effort. Some states feel that it is important to know how many swine enter and leave the state. These individual states have very good records on the movement of swine. Other states for various reasons do not have available records. The Committee wishes to encourage the latter states in their effort to obtain adequate facilities for record keeping.

Only two-thirds of the states reporting had knowledge of the number of swine that had entered for purposes other than slaughter. In three states this transportation of swine had resulted in serious sickness among swine. In 24 states the transportation of swine across state lines resulted in swine health problems that were not serious but would have been so without constant vigilance. In 16 states swine transportation with resulting disease spread created moderate problems in swine health.

Of the 29 states that reported, there were five (mostly southern and western) that stated hog cholera was prevalent during the past fiscal year while 24 stated the disease was not prevalent. Of the five states that reported hog cholera to be prevalent, only one had had an increased number of outbreaks during the past year. Two southeastern states found that there was an increase in outbreaks of hog cholera within their borders during the last fiscal year while 10 states found the situation unchanged over the previous years and 17 reported a decrease. Thus, the over-all situation was apparently a decrease in outbreaks as compared to previous years. Causes for the decreased number of outbreaks of hog cholera were suggested. Discontinuing the use of virulent virus in vaccination was suggested by nine states as a major cause of the decrease and garbage cooking was suggested by five states. Four states reported that better hog handling procedures, restrictions on imports and at markets, better informed producers and compulsory vaccination of garbage fed hogs accounted in part for the decrease of hog cholera outbreaks.

Presently there are 18 states (Alabama, Arkansas, Connecticut, Florida, Georgia, Illinois, Kentucky, Louisiana, Michigan, Mississippi, Montana,
Nevada, New Mexico, North Dakota, South Carolina, Tennessee, Utah and Wisconsin) which prohibit the use of virulent virus in the vaccination of swine against hog cholera. Of these 18 states, Michigan is a new one where virulent virus was prohibited in 1958. Oklahoma can prohibit such use of virulent virus whenever the industry makes a request. In Oregon one must obtain approval by the state veterinarian before using virulent virus. No report was obtained on the effectiveness of this restriction on the use of virulent virus in Oregon. It might be very effective, but, in Wisconsin a similar law was not effective.

Of the 18 states that do not allow the use of virulent virus in vaccination and the others that use very little virulent virus, an increase in hog cholera was reported in one, South Carolina. The increase in this state was due to complacency among producers—very few herds were vaccinated when for some reason largely unknown, there was some exposure. One state that allows the use of virulent virus had an increase in hog cholera. The increase was due to a large outbreak among herds in a single county.

Of the states that do not allow the use of virulent virus, none anticipates a change back to virulent virus—all are well satisfied. Of the reporting states that allow virulent virus (15 states), one, New York, is considering a change to no virulent virus. Again Michigan is the state that outlawed virulent virus in 1958.

Swine erysipelas is a problem in each of the 29 states reporting. It is considered a serious problem in seven states, and of less serious nature in 22 states. It was found to be increased in nine states, decreased in five states, and to have remained about the same in 14 states, when the cases during the last fiscal year were compared to previous years.

Atrophic rhinitis was considered to be a serious problem in one state, moderately serious in 13 and a rather significant disease in 14. The Committee is most happy to have this type of evaluation by states of such an occult disease. It may be pointed out that the term “occult” applies to the effect of the disease on swine. It has been pretty well established that affected pigs grow about seven percent slower than do comparable unaffected pigs and that they fail to convert feed by about the same amount. This is truly a substantial difference but one that cannot be detected by observation. Whether or not atropic rhinitis is a significant disease in an area depends on the percentage of pigs affected. It is a significant disease in every affected pig. The disease increased in incidence during the past year in five states, decreased in one and remained unchanged in 20.

During the past year the incidence of swine leptospirosis increased in nine states, decreased in none and remained unchanged in 16. Some of the states recognized that the so-called increased incidence of this disease was a “false-increase”—the increased recognition of the disease was due to improved means of diagnosis and increased awareness of the disease. The state of Michigan which has put much study on the disease was unable to account for the increased incidence in the state. The following reasons for increases seem valid, 1) greater animal populations, 2) greater amounts of rain so
TRANSMISSIBLE DISEASES OF SWINE

that there were floods and also because of the greater growth of grasses there was more grazing with greater contact between animals and 3) too much dependence was placed on vaccination.

Enteritis was a serious problem in six states and not serious in 23 states. There was increased incidence in one state, decreased incidence in one state and no change in 23 states. Transmissible gastroenteritis was about the same as enteritis. It was a serious problem in three states and not serious in 21 states. There was increased incidence in three states and no change in 21 states when the records of the last fiscal year were compared to previous years. Multiple housing and feeding were largely responsible for the increased incidence of transmissible gastroenteritis in one state. In another the increase was attributed to the rapid increase in the swine population.

Respiratory diseases of swine remained stationary. These conditions were considered serious in nine states and not of serious nature in 20 states. There was increased incidence in three states and no change in 21 with decreases nowhere.

Eperythrozoonosis remained even more stationary. Nineteen states reported no change in incidence of the disease, three states had seen fewer clinical cases and two thought there might have been an increase.

The Committee is aware of the swine brucellosis problem and recommends that the individual states set up means for the certification of swine herds as brucellosis-free. Otherwise, we are leaving the discussion of this disease to the Committee on Brucellosis.

The Committee also recommends that the various states pass laws which control the use of virulent hog cholera virus in the vaccination of hogs and plan toward eventual hog cholera eradication.

The Committee recommends that a division of swine diseases be set up within Animal Disease Eradication.

The Committee recognizes that we lack information pertaining to diseases of swine and recommend that this Association lend its efforts, where possible, to promote research on the cause and control of transmissible diseases of swine.
THE EFFECT OF CHEMICAL AND PHYSICAL AGENTS ON THE
VIRUSES OF VESICULAR STOMATITIS AND
VESICULAR EXANTHEMA

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S. R. HOPKINS, B.S., AND J. R. SONGER, B.A.*

Beltsville, Maryland

Research on the effect of chemical and physical agents on vesicular exanthema virus (VEV) has been materially retarded because of the restricted host range of this disease. On the other hand, vesicular stomatitis virus (VSV) with a wide host range has been studied to a greater extent.

Control of these diseases has been dependent in a large measure on eradication, quarantine, and disinfection. The disinfectants of choice have been two percent lye (sodium hydroxide) and four percent soda ash (sodium carbonate).

Reported in this paper is the efficacy of some of the commonly used disinfectants on VSV, the inactivation of VSV and VEV in various hydrogen-ion concentrations, and the results of heat-treating virus suspensions and tissues containing active VEV.

Part I. The Inactivation of Vesicular Stomatitis Virus by Various Chemicals

Studies of the effect of certain chemical agents on VSV were undertaken in an effort to determine the efficacy of certain commonly used disinfectants. Shahan (10) has reported on the effect of phenol on VSV as used in the preservation of serums. Manthei and Eichhorn (5) have reported on the use of sodium bifluoride and sodium silicofluorides in the disinfection of hides, using VSV as the test virus.

MATERIALS AND METHODS

The VSV was of the New Jersey-type Concan strain, fifth passage in eggs. A pool was prepared by macerating one part of chorio-allantoic membranes (CAM) with four parts of allantoic fluids from infected embryonating hens' eggs and stored in five-ml. aliquots in a dry-ice chest. When needed, an aliquot was thawed and centrifuged at 2,500 r.p.m. in an International centrifuge for 10 minutes. The LD$_{50}$ titer, as calculated by the method of Reed and Muench (8), of the virus suspension was 10$^{-8.6}$ for nine-day chick embryos.

* Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.
The chemical agents tested were proprietary compounds commonly used as disinfectants, as follows:

   - Calcium hypochlorite 50%
   - Inert ingredients 50%

2. Roccal (Winthrop Laboratories, New York, N. Y.)—
   - Active ingredient: alkyl \((C_8H_{17}-C_{18}H_{37})\)
   - Dimethyl-benzyl-ammonium chloride (benzalkonium chloride) 10%
   - Inert ingredients 90%

3. Wescodyne (West Disinfecting Co., Long Island City, N. Y.)—
   - Active ingredients:
     - Polyethoxy polypropoxy ethanol-iodine complex 7.75%
     - Nonyl phenyl ether of polyethylene glycoliodine complex 3.75%
   - Inert ingredients 88.5%

   - Active ingredients:
     - Cresylic acid and soap
   - Inert ingredients:
     - Water, not over 22.5%
     - Glycerin, not over 2.5%
   - 25.0%

5. Formalin (Fisher Scientific Co., New York, N. Y.)—
   - Analysis:
     - Formaldehyde gas 36.3%
     - Nonvolatile matter 0.02%
     - Acidity 0.03%

6. Septisol (Vestal Laboratories, Modesto, Calif.)—
   - Active ingredients:
     - Hexachlorophen* 0.75% (2% of soap)
     - *Bis-(2-hydroxy-3,5,6-trichlorophenyl) methane

   - Active ingredients:
     - Alcohol (10% by volume), soap, terpineol, safrol, thymene, napthalene,
       thymol, bis (4 hydroxy-2,3,5-trichlorophenyl) methane 38%
   - Inert ingredients:
     - Water 61%, alcohol by volume 18%

8. Lye (Bolgianos, Washington, D. C.)—
   - Sodium hydroxide flakes

In preliminary trials, it was found that in the lower dilutions all the chemicals tried were toxic to the embryos and caused their death. At slightly higher dilutions the embryos survived, showing that the virus was inactivated and that these dilutions of the chemicals were not toxic to the embryo. At higher dilutions the virus killed the embryos, as proved by the complement-fixation test. The pH of the virus-chemical mixtures, other than lye, at the nontoxic levels varied from pH 6.6 to 7.6, which were well within the stability range of VSV.
Nine-day-old embryos were used in the test. The route of inoculation was into the allantoic sac. The amount of inoculum was 0.15 ml. per egg. Data from previous trials determined the dilutions to be used for each disinfectant. Equal volumes of the disinfectant and the virus were mixed to give a concentration of $10^{-3}$ virus suspension in a predetermined concentration of the disinfectant.

After 10 minutes and after two hours of exposure at room temperature, 0.5 ml. of the mixture was removed and added to 4.5 ml. of sterile distilled water for lye and B-K Powder. All the other agents were diluted in a similar manner with sterile buffered saline. The final dilution which was injected into the eggs was a $10^{-4}$ dilution of VSV suspension and had a minimum toxic effect on the embryo. Five eggs were used per dilution of the virus-disinfectant mixture, and an equal number without the virus for toxicity control.

Incubation was continued at 96° F., and observations were made daily for five days. All dead embryos were checked by the complement-fixation test for the presence of VSV.

RESULTS

The results of this test are recorded in Table I. The following disinfectants inactivated the virus within 10 minutes at the dilution noted: B-K Powder 1:1,000, Roccal 1:200, Wescodyne 1:200, Crestall Fluid 1:100, Formalin 1:100, Septisol 1:50, and Therapogen 1:5. Three percent lye failed to inactivate the virus after an exposure of two hours.

DISCUSSION

In this study the criterion of the effectiveness of several chemical agents against the VSV was the survival of the embryos during a five-day observation period after injection with a mixture of virus and different dilutions of the disinfectant tested. The exposure times were 10 minutes and two hours. With the exception of those solutions which contained soap, increased inactivation was negligible when the exposure time was lengthened from 10 minutes to two hours.

In this experiment all dilutions of B-K Powder inactivated the virus. In another similar experiment a 1:5,000 dilution of B-K Powder failed to inactivate the virus when it was exposed to the virus for three hours.

With the exception of lye, all the disinfectants used inactivated the virus in 10 minutes in dilutions greater than the lowest dilution recommended by the manufacturer. Lye, which has been used for years at a two-percent concentration as the standard disinfectant for vesicular viruses, failed in both the two-and three-percent concentrations to inactivate the VSV in two hours.
TABLE 1

The Inactivation of Vesicular Stomatitis Virus by Various Chemicals

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Dilution</th>
<th>Number of Embryos* Dying Within 5 Days After Injection With—</th>
<th>Chemical Alone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Virus Exposed to Chemical For—</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 min. 2 hrs.</td>
<td></td>
</tr>
<tr>
<td>B-K Power</td>
<td>1:100</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1:500</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1:1000</td>
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<td>0</td>
</tr>
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</tr>
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<td></td>
<td>1:200</td>
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</tr>
<tr>
<td></td>
<td>1:300</td>
<td>2 2</td>
<td>0</td>
</tr>
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<td></td>
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<td></td>
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<td>1:200</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>1:300</td>
<td>5 4</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Crestall Fluid</td>
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<td>1† 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1:200</td>
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<td>0</td>
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<td></td>
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<tr>
<td>Formalin</td>
<td>1:20</td>
<td>2† 0</td>
<td>1†</td>
</tr>
<tr>
<td></td>
<td>1:50</td>
<td>1 2†</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Septisol</td>
<td>1:10</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1:50</td>
<td>1† 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
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<td>0</td>
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<td></td>
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<td>Lye</td>
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</tr>
<tr>
<td></td>
<td>2%</td>
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</tr>
<tr>
<td></td>
<td>1%</td>
<td>5 4</td>
<td>0</td>
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</tbody>
</table>

* In all cases 5 embryos were injected.
† Dead embryos which were negative to the complement-fixation test; all others were positive.

SUMMARY

The dilutions at which various commonly used chemical disinfectants inactivated the VSV as tested in embryonating hens' eggs were found to be: B-K Powder 1:1,000, Roccal 1:200, Wescodyne 1:200, Crestall Fluid 1:100, Formalin 1:100, Septisol 1:50, and Therapogen 1:5. Three percent lye failed to inactivate the virus after an exposure of two hours.
Part II. The Inactivation of Vesicular Stomatitis and Vesicular Exanthema Viruses in Various Hydrogen-ion Concentrations

Several reports have been published concerning the stability of VSV in various pH values. Olitsky (6), in 1927, reported the optimum hydrogen-ion concentration for this virus as pH 7.5 to 7.6, and that the virus was inactivated in 52 hours at pH 6.8 and at pH 8.0 when held at 37° C. during the test period. Later, Pyl (7) suggested a method for differentiation between VSV and foot-and-mouth disease virus (FMDV) based on the greater stability of the VSV. He observed that when these two agents were treated with HCl to a pH of 3.0 and immediately neutralized with NaOH, only VSV remained infective. This is termed a realkalization test. Reppin and Pyl (9) attempted to use this realkalization procedure to identify an unknown virus causing a vesicular condition among swine in California and concluded that the virus should be regarded as VSV rather than FMDV because it was still viable after realkalization. That unknown virus is now known to have VEV.

Subsequently, an extensive report by Galloway and Elford (4) generally confirmed these findings but showed that VSV would lose its infectivity at pH 3.0 when held at this pH for a longer period of time. The zone of stability was limited to pH 4.5. The times of exposure of the virus to the different pH levels varied from two to 48 hours. Fong and Madin (3) confirmed the instability of VSV below pH 4.0 but placed the actual end point for complete inactivation at pH 2.0 for one hour. They also reported that VSV was not completely destroyed at pH 12.5 for one hour.

MATERIALS AND METHODS

The VSV was of the New Jersey-type, Concan strain, second passage in eggs. A pool was prepared by macerating the CAM from infected hens' eggs. The CAM pool was diluted 1:5 with a 1/75 M phosphate buffer saline (PBS) at pH 7.6 and centrifuged at 2,500 r.p.m. for 20 minutes, and the supernatant stored in five-ml. aliquots in a dry-ice chest. Prior to use, an aliquot was thawed and centrifuged at 3,000 r.p.m. for 10 minutes. The LD50 of this virus pool was 10^-6.6 for eight-day chick embryos.

The VEV was type B51. A 10-percent suspension of ground vesicular material, harvested from experimentally infected swine, was diluted with tryptose broth. It, too, was centrifuged at 2,500 r.p.m. for 20 minutes, frozen in five-ml. amounts in a dry-ice chest, and centrifuged again at 3,000 r.p.m. prior to use. The ID50 titer of VEV was 10^-4.0 for swine.

The pH was adjusted by the addition of NaOH (lye), Na2CO3 (soda ash), HCl (hydrochloric acid), or CH3COOH (acetic acid) to an equal part of a 10-percent virus suspension. The virus was allowed to remain in the acid or alkaline suspension for 15 minutes.

The suspensions with VSV were returned to approximately pH 7.5 by the addition of 1.5 M PBS and further diluted to a virus concentration of 10^-3 with 1/75 M PBS. They were inoculated immediately into eggs or guinea pigs. The VEV suspensions were not restored to a neutral pH, but were
inoculated immediately into swine. Inoculations with VSV were either in eight-day embryonating hens' eggs by the allantoic route or in guinea pigs by tunneling the metatarsal pads. Swine were inoculated by scarification of the snout. Guinea pigs and swine were examined daily for evidence of vesicular lesions at the sites of inoculation. Inoculated eggs were examined up to 72 hours, and all dead embryos were tested by complement fixation for the presence of virus.

### TABLE II

**The Inactivation of Vesicular Stomatitis Virus in Various Hydrogen-Ion Concentrations**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration (Percent)</th>
<th>pH</th>
<th>Positive Reactions in—</th>
<th>Percent Positive In—</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Guinea Eggs</td>
<td>Guinea Pigs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Eggs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pigs</td>
</tr>
<tr>
<td>NaOH</td>
<td>2.0</td>
<td>12.6</td>
<td>3/12*</td>
<td>0/6*</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>12.4</td>
<td>13/36</td>
<td>2/6</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>12.2</td>
<td>19/36</td>
<td>2/6</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>12.0</td>
<td>25/36</td>
<td>3/6</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>11.7</td>
<td>27/30</td>
<td>6/6</td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>2.0</td>
<td>10.9</td>
<td>9/12</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>10.6</td>
<td>10/12</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>10.4</td>
<td>12/12</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>10.1</td>
<td>12/12</td>
<td>NR</td>
</tr>
<tr>
<td>HCl</td>
<td>0.025</td>
<td>3.0</td>
<td>0/12</td>
<td>5/12</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>2.3</td>
<td>0/12</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>3.8</td>
<td>0/6</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>0.0125</td>
<td>6.0</td>
<td>18/18</td>
<td>6/6</td>
</tr>
<tr>
<td>CH₃COOH</td>
<td>1.0</td>
<td>3.3</td>
<td>1/12</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>3.7</td>
<td>7/12</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>4.0</td>
<td>3/6</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>6.0</td>
<td>12/12</td>
<td>12/12</td>
</tr>
<tr>
<td>Controls</td>
<td>—</td>
<td>7.3</td>
<td>6/6</td>
<td>—</td>
</tr>
</tbody>
</table>

* Numerator = number of positive reactors; denominator = number of eggs or guinea pigs inoculated.

NR = Not run.

All virus exposures were for 15 minutes.

### RESULTS

Table II summarizes the results obtained when VSV is treated with various concentrations of acids or bases. The greatest concentration of base used two percent NaOH (pH 12.6), failed to completely inactivate all VSV, even though the concentration of virus was greatly reduced, as shown by the death of three out of 12 embryonating eggs. The presence of virus was confirmed in these eggs by complement fixation, using the amnionic-allantoic fluids.
from the dead eggs as antigen. The table shows that increasing amounts of virus survive as the pH drops from pH 12.6 toward neutrality. On the acid side, Table II shows that there were no deaths in eggs at pH values below 4.0 with HCl and no guinea pig lesions at pH values below 3.0. There was virus survival in eggs down to pH 3.3 when acetic acid was used to adjust the pH. In no instance was virus recovered below pH 3.0. Between the pH values 4.0 and 11.0 the VSV seemed fairly stable.

**TABLE III**

*The Inactivation of Vesicular Exanthema Virus in Various Hydrogen-Ion Concentrations*

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration (Percent)</th>
<th>pH</th>
<th>Positive Reaction in Swine</th>
<th>Percent Positive</th>
<th>Result of Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH</td>
<td>2.0</td>
<td>13.0</td>
<td>0/2*</td>
<td>0</td>
<td>2/2*</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>12.8</td>
<td>0/6</td>
<td>0</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>12.3</td>
<td>0/2</td>
<td>0</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>11.9</td>
<td>2/4</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>6.0</td>
<td>10.9</td>
<td>4/4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>10.6</td>
<td>4/4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>10.5</td>
<td>4/4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>10.3</td>
<td>4/4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>HCl</td>
<td>0.05</td>
<td>2.7</td>
<td>0/4</td>
<td>0</td>
<td>4/4</td>
</tr>
<tr>
<td></td>
<td>0.025</td>
<td>4.3</td>
<td>2/2</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>—</td>
<td>7.4</td>
<td>2/2</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

* Numerator = number of positive pigs; denominator = number of pigs inoculated.

The VEV is less stable in the alkaline pH range than the VSV. Table III shows that there was no instance of VEV survival above pH 12.0, whereas VSV was not completely destroyed up to 12.6 (Table II). VEV also failed to survive at 2.7 but did survive at pH 4.3.

**DISCUSSION**

It should be emphasized that pH values obtained under the experimental conditions employed were altered by the buffering action of the diluent (PBS) used for virus suspension. Similar concentrations of chemicals prepared under field conditions would have somewhat different pH values from those reported in Tables II and III.

As shown in Table II, VSV when subjected to pH 12.6 (two-percent NaOH) for 15 minutes was not completely inactivated. The fact that VSV shows marked resistance to inactivation in the alkaline range at pH values of 12.6 and below for 15 minutes casts some doubt on the value of NaOH as a disinfectant for this virus. The concentration of NaOH usually recommended is two percent, and the average pH of a fresh solution at this concentration is 12.8. In addition a solution of NaOH exposed to the atmosphere will react with CO₂ present to form Na₂CO₃, with a resultant drop in pH.
The drop in pH which occurs in a four-liter solution of two-percent NaOH exposed to the atmosphere was recorded. The initial pH of the solution was 13.1. The pH value dropped to 12.6 by the fifth day and was below 11.0 by the thirteenth day. This is a drop of 0.1 pH unit per day through the first five days and 0.2 pH unit per day thereafter. Such a drop rapidly lowers whatever value NaOH may have as a disinfectant due to its pH. In actual practice a lowering of the pH value may be accelerated by contamination with organic and inorganic materials and by dilution with rain water.

The VEV showed less stability at pH values above 12 and was rendered inactive by subjecting it to this pH range for 15 minutes. NaOH is, therefore, a satisfactory disinfectant for VEV under these conditions.

Although acids are generally too caustic to be used as disinfectants, it is interesting to note that VSV and VEV are inactivated at about the same pH in the acid range.

The pH of boned and unboned hams from freshly killed hogs was determined over a 48-hour period with the hams at room temperature (22° C.) and for a one-month period at refrigerator temperature (4° C.). In no instance did the pH value fall below 5.4, which is insufficient to inactivate VSV and VEV.

Soda ash (Na₂CO₃) has no application as a disinfectant for VSV and VEV from the standpoint of pH values obtainable from this chemical. A 30-percent solution prepared at 23° C. has a pH of 11.6, which is well below the pH values required for inactivation of VSV and VEV.

SUMMARY

Experimental data confirm the report by Fong and Madin (3) that VSV shows a marked stability in the alkaline range. Data reported above indicate that VEV is less stable in the alkaline range than VSV. The two viruses show about the same resistance in the acid range. Soda ash (Na₂CO₃) has no value as a disinfectant for VSV and VEV. Two percent lye (NaOH) has doubtful value as a disinfectant for VSV, but is effective for VEV.

Part III. Heat Inactivation of Vesicular Exanthema Virus

The inactivation of the virus of vesicular stomatitis in the presence of bovine, equine, or procine serum at 58° C. (136.4° F.) and 60° C. (140.0° F.) for 30 minutes has been reported by Shahan (10). Dimopoulos et al. (2) inactivated VSV suspensions of bovine tongue epithelium at 56° C. (132.8° F.) and 60° C. (140.0° F.) for 30 minutes.

Only recently has Shahan (1) reported that fractional portions of FMDV populations survived 80° C. (176° F.) for six hours. Survival could be demonstrated only by use of massive doses.

Published data on temperatures required for the inactivation of VEV are not available. Reported in this paper are the results of several methods of treating virus suspensions and tissues containing active VEV by heat. While it was not possible to utilize normal meat-curing procedures, various tech-
niques were employed in an effort to determine heat requirements necessary for virus inactivation.

1. Heat Inactivation of Virus Suspensions in Water Bath

MATERIALS AND METHODS

The virus material used in the following tests was VEV, type B51, identified as a sixth passage of an original field outbreak harvested June 17, 1952, at a biological plant in Grand Island, Nebraska. This material was a virus pool of vesicular material harvested from 36 swine at 48 and 72 hours after intravenous inoculation with five cc. of a five-percent suspension. The vesicular material was placed in sterile bottles, sealed, and stored in a dry-ice chest at $-70^\circ C$. Twelve days after harvest, the frozen epithelial tissue was thawed, pooled, minced with scissors, and ground in mortars, using sterile ground glass as an abrasive. A 20-percent suspension was prepared by adding buffered saline at pH 7.6. The virus suspension was centrifuged in 250-ml. bottles at 1,800 r.p.m. for 40 minutes, after which the supernatant was drawn off and 10 mg. of dihydrostreptomycin per ml. was added. This 20-percent suspension was placed in sterile tubes, sealed, and stored in a dry-ice chest at $-70^\circ C$. Tittrations were made on this pool, using a series of four 100-fold dilutions. Each of these dilutions was inoculated intradermally on the snout of four pigs. Prior to freezing, the 50-percent infecting titer (calculated by the method of Reed and Muench, 8) was $10^{-5.47}$, and after freezing and storage of one month’s duration, the titer was $10^{-5.032}$.

Procedure followed in the inactivation of tissue suspensions were to thaw the virus slowly at room temperature, centrifuge at 2,500 r.p.m. for 20 minutes, pour off the supernatant, and inactivate. For inactivation, five-ml. amounts of 10-percent virus suspension were placed in sealed test tubes, which were then placed in a precision water bath (accurate to $\pm 0.5^\circ C$). The tubes were completely immersed in water and gently agitated during the period of inactivation. The inactivation time was calculated from the time the virus suspension reached the temperature of inactivation. The amount of time required in each case to raise the temperature of the suspension to that which was to be used in a given trial was predetermined. This time was added to the period of inactivation. After removal from the water bath, the suspensions were immediately immersed in ice water and then placed in a refrigerator ($45^\circ F.$) until inoculated into test pigs. Six different thermometers were placed in rubber-stoppered test tubes containing five-ml. each of saline, and temperatures during the periods of inactivation were recorded. All thermometers showed less than $\pm 0.5^\circ C$. variation from the desired inactivation temperature during the heat-treatment period.

Test pigs averaged 100 lb. each, and each group of four pigs was completely isolated from the other groups. All personnel wore rubber clothing which was disinfected between pens.

One-ml. amounts of virus suspensions were inoculated intradermally on the snouts of test swine. The temperatures of the test pigs were taken twice a day, and the pigs were inspected daily for vesicular lesions.
EFFECT OF CHEMICAL AND PHYSICAL AGENTS ON VIRUSES 303

RESULTS

The results of four experiments using a single uniform virus suspension are tabulated in Table IV. Techniques employed were the same in all experiments. Where the identical temperature and time factors were used in several experiments, results were fairly consistent. An exception was noted in the 60°C (140.0°F.)-60-minute group where both pigs were positive in Exp. 3 and negative in Exp. 4. Fully negative results were achieved only in the 62°C (143.6°F.)-60-minute group.

Although fully negative results were not accomplished in all groups, results plotted on a graph (Figure 1) will indicate probable end points. For example, with 60-minute time intervals, noninfectivity will be attained at 62°C (143.6°F.). Using 30-minute time intervals, 66°C (150.8°F.) would be required for inactivation.

### TABLE IV

**Heat Inactivation of Vesicular Exanthema Virus Suspensions**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
<th>Exp. 4</th>
<th>Total</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>56°C (132.8°F.)</td>
<td>30 min.</td>
<td>2/2*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2/2</td>
<td>100.0</td>
</tr>
<tr>
<td>58°C (136.4°F.)</td>
<td>30 min.</td>
<td>2/2</td>
<td>3/4</td>
<td>-</td>
<td>2/2</td>
<td>7/8</td>
<td>87.5</td>
</tr>
<tr>
<td>58°C (136.4°F.)</td>
<td>45 min.</td>
<td>-</td>
<td>4/4</td>
<td>-</td>
<td>-</td>
<td>4/4</td>
<td>100.0</td>
</tr>
<tr>
<td>58°C (136.4°F.)</td>
<td>60 min.</td>
<td>-</td>
<td>4/4</td>
<td>2/2</td>
<td>2/2</td>
<td>8/8</td>
<td>100.0</td>
</tr>
<tr>
<td>60°C (140.0°F.)</td>
<td>30 min.</td>
<td>-</td>
<td>3/4</td>
<td>2/2</td>
<td>2/2</td>
<td>7/8</td>
<td>87.5</td>
</tr>
<tr>
<td>60°C (140.0°F.)</td>
<td>60 min.</td>
<td>-</td>
<td>-</td>
<td>2/2</td>
<td>0/2</td>
<td>2/4</td>
<td>50.0</td>
</tr>
<tr>
<td>62°C (143.6°F.)</td>
<td>30 min.</td>
<td>-</td>
<td>1/4</td>
<td>2/2</td>
<td>1/2</td>
<td>4/8</td>
<td>50.0</td>
</tr>
<tr>
<td>62°C (143.6°F.)</td>
<td>60 min.</td>
<td>-</td>
<td>-</td>
<td>0/2</td>
<td>0/2</td>
<td>0/4</td>
<td>0.0</td>
</tr>
<tr>
<td>64°C (147.2°F.)</td>
<td>15 min.</td>
<td>-</td>
<td>-</td>
<td>1/2</td>
<td>-</td>
<td>1/2</td>
<td>50.0</td>
</tr>
<tr>
<td>64°C (147.2°F.)</td>
<td>30 min.</td>
<td>-</td>
<td>-</td>
<td>1/2</td>
<td>0/2</td>
<td>1/4</td>
<td>25.0</td>
</tr>
<tr>
<td>Control</td>
<td>2/2</td>
<td>4/4</td>
<td>2/2</td>
<td>2/2</td>
<td>10/10</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

* Numerator = number of positive pigs; denominator = number of pigs in group.

![FIG.1. HEAT INACTIVATION OF VESICULAR EXANTHEMA VIRUS SUSPENSIONS](image-url)
2. Inactivation of Virus by Cooking Hams.

MATERIALS AND METHODS

In this experiment an attempt was made to simulate temperatures to which hams are subjected during the normal meat-curing process. Hams to be heat-treated were placed in the oven of a conventional domestic electric stove which was modified with a thermostat which allowed only ± 2.0-degree fluctuation in oven temperature. The temperature of the oven was adjusted so that a 145° F. internal temperature of the ham would be attained after cooking for 18 hours. The final internal temperatures varied somewhat, depending on the exact location of the thermocouple leads and variation in size of hams.

Two thermocouple leads were placed in each ham. The first lead was placed in the bone cavity of the proximal end of the femur by drilling a small hole into the bone. The second lead was placed in the heavy musculature, so that it might be deeply buried, but not in contact with the bone. Temperatures were recorded by a Brown multi-point strip chart temperature recorder (potentiometer).

In test groups 1, 2, and 3, the hams used were from reacting pigs which had been inoculated intravenously with five ml. of a five-percent virus suspension (type B51) and destroyed at 48 hours after inoculation.

In the first test group of pigs, one ham was cooked for approximately 18 hours, at which time the internal temperature of the meat was 145° F. A control ham from the same donor pig was held in a refrigerator at 45° F. while the other ham was being heat-treated. When removed from the oven, the ham was cut into pieces, and the bones crushed to expose all marrow. Each of two susceptible test pigs was fed 10 lb. of heat-treated meat and bone. The uncooked control ham was similarly fed to two susceptible test pigs. In all instances where two test pigs were fed meat scraps, the snout and feet of one animal were scarified so that actual breaks in the skin existed. Test groups of pigs were placed in complete isolation and temperatures were taken twice daily.

A second group of test pigs was a repetition of the first test group.

A third group of test pigs was fed heat-treated meat prepared from reacting pigs as described above, the only difference being that the infected ham was heat treated for 20 hours until the internal temperature of the meat was 152° F. A control ham was similarly fed to a group of two pigs.

A fourth group of test pigs was fed heat-treated hams which had been made infective by injecting the meat with virus suspension. A normal ham was injected with 10 ml. of a 10-percent virus suspension (one ml. in each of 10 sites). After heating for 19 hours, with an internal meat temperature of 148° F., the ham was removed from the oven, the meat was cut into chunks, the bones were crushed, and the meat and bones were fed to two susceptible test pigs. A control ham was similarly injected and placed in a refrigerator at 45° F., while the other ham was being heat treated. Two control pigs were fed scraps from the control ham.

The fifth group was identical to group 4, except that the ham was cooked for 17 hours to an internal temperature of 145° F.
The sixth group of test pigs was inoculated on the snouts with virus material which had been heat-treated. Epithelial tissue harvested from unruptured and freshly ruptured vesicles was placed in a piece of gauze which was, in turn, buried in the heavy muscle of a normal ham. This ham was cooked until the internal temperature of the meat reached 158°F. A paste was then prepared from the epithelial tissue and inoculated into the snouts of two susceptible pigs by scarification. Two control pigs were inoculated with epithelial tissue which had similarly been wrapped in gauze and placed in the muscle of a normal ham. This ham was held at 45°F. while the other ham was being heat treated. A second set of control pigs was inoculated with epithelial tissue which had been held in a sealed glass test tube at 45°F. during the heat treating of the first ham.

The seventh and eighth groups were inoculated with virus pastes made from virus material which had been heat-treated in separate hams. In both cases, the epithelial tissue was wrapped in gauze and buried in the muscle tissue, and the ham was cooked to an internal temperature of 145°F.

A ninth group was similar to groups 6, 7, and 8, except that the final internal temperature of the cooked ham was only 142°F.

RESULTS

Results of heat inactivations in hams are indicated in Table V. VEV was inactivated in all tests. The virus in the hams from reacting pigs was inactivated when final internal meat temperatures were 145°F and 152°F. Un-

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Description</th>
<th>Temperature</th>
<th>Method of Expose</th>
<th>Initial Challenge</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ham from reacting pig</td>
<td>145°F.</td>
<td>Feeding</td>
<td>Neg.</td>
<td>2/2</td>
</tr>
<tr>
<td>2</td>
<td>Control on above</td>
<td>None</td>
<td>Feeding</td>
<td>1/2*</td>
<td>0/2</td>
</tr>
<tr>
<td>3</td>
<td>Ham from reacting pig</td>
<td>152°F.</td>
<td>Feeding</td>
<td>Neg.</td>
<td>2/2</td>
</tr>
<tr>
<td>4</td>
<td>Control on above</td>
<td>None</td>
<td>Feeding</td>
<td>Neg.</td>
<td>0/2</td>
</tr>
<tr>
<td>5</td>
<td>Ham inoc. with 10 cc. virus</td>
<td>148°F.</td>
<td>Feeding</td>
<td>Neg.</td>
<td>2/2</td>
</tr>
<tr>
<td>6</td>
<td>Control on above</td>
<td>None</td>
<td>Feeding</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Virus tissue cooked in ham</td>
<td>158°F.</td>
<td>ID</td>
<td>Neg.</td>
<td>2/2</td>
</tr>
<tr>
<td>8</td>
<td>Control on above</td>
<td>None</td>
<td>ID</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Virus tissue cooked in ham</td>
<td>142°F.</td>
<td>ID</td>
<td>Neg.</td>
<td>2/2</td>
</tr>
</tbody>
</table>

* Numerator = number of positive pigs; denominator = number of pigs in group.
ID = intradermal.
cooked control hams from reacting pigs failed to produce lesions when fed to test pigs in all cases except one. However, all control pigs were immune on challenge, indicating active virus was present in the hams, but in low infecting doses.

Virus suspensions injected into hams and subjected to heat treatment likewise were inactivated. Internal temperatures of 145° and 148° F. were used.

Pieces of vesicular material containing virulent virus were inactivated when placed in hams which were heated until final internal temperatures reached 158°, 145° and 142° F. Control virus maintained in an uncooked ham for 24 hours at normal refrigeration temperature (45° F.) remained infective in all test groups.

Where scarified and nonscarified susceptible pigs were fed infective meat scraps, the scarified pig was either the first of the two test pigs to react or the only pig to show lesions.

DISCUSSION

Apparently, VEV is more durable than VSV, but not so resistant to heat as FMDV. Temperatures of 56°, 58°, and 60° C. for 30 minutes were not sufficient to inactivate VEV. The majority of research work reported in this paper was conducted before tissue culture was used as a working tool for VEV. Therefore, the only test animal available was the pig. This meant that the numbers of animals in each test group were restricted. It should be pointed out that with FMDV the most critical measurement of virus infectivity has been found to be the natural host animal (1).

The term "inactivation of virus," as used in this paper, refers to destruction of virus below the level of infectivity under the test conditions used in a given experiment. It does not mean necessarily the complete elimination of the last active virus particle in a given suspension. The possibility exists that, were more critical measurements available to detect active virus, higher temperatures and/or longer inactivation periods would be required to destroy the agent. This deficiency can best be overcome by adding adequate margins of safety in the form of higher cooking temperatures required in garbage-cooking regulations.

The rate of inactivation has been shown in Figure 1 as being linear with respect to time and temperature inactivating a given concentration of virus. As mentioned previously, if more critical tests were available, the 30-minute exposure line might well be represented by a curve with 68° or 70° C. being the end point.

In the processing of tenderized hams, the meat is subjected to hot smoke and steam for a period of approximately 18 hours. At the conclusion of the smoking process, the internal ham temperature is about 145° F. Experimental work indicates that these conditions are sufficient to inactivate the virus of vesicular exanthema. Initially, hams from artificially infected pigs were used for test purposes. Because of the difficulty of obtaining a reaction in control swine, two alternate test conditions were employed using hams injected with virus suspensions and hams implanted with vesicular tissue. In
all three methods the virus was inactivated, indicating that slow, dry heat
(170° F. for 18 hours) is adequate to destroy VEV in infected hams.

For comparative purposes, internal temperatures of hams were recorded
when placed in boiling water (212° F.) and in ovens at 212° F. In water an
internal temperature of 145° F. was reached in 60 minutes, whereas it re-
quired 3½ hours in an oven. As a result of heat trials in water and in dry
ovens, there should be no distinction made on cooking regulations between
boned hams and hams with the bone in. In general, the bone conducted
heat more rapidly than meat and thus reached inactivation temperatures
more quickly than muscle tissue.

SUMMARY

(1) Vesicular exanthema virus (type B51) suspensions were heat treated
at 56° C. (132.8° F.) to 64° C. (147.2° F.) for periods of time varying
from 15 minutes to 60 minutes. Treatments of 62° C. (143.6° F.) and
64° C. (147.2° F.) for 30 minutes did not completely inactivate the virus,
whereas inactivation was obtained in the 62° C.-60-minute group.

(2) The cooking of hams in a dry oven at 170° F. for 18 hours with a
final internal ham temperature of 145° F. was sufficient to inactivate VEV
under a variety of techniques.

ACKNOWLEDGMENT

Acknowledgment is made to Dr. J. N. Geleta and Dr. Harry Higa for the
technical assistance given in carrying out certain phases of this work.
REFERENCES


FERAL SWINE AS A RESERVOIR OF VESICULAR STOMATITIS VIRUS IN SOUTHEASTERN UNITED STATES

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The feral pig in southeastern United States is a descendant of domestic hogs that have returned to the wild and live and breed in swamps. A trace of the swine of the early Spanish explorers may linger in his blood but more evident are characteristics that come from recent crossings with domestic stock. Like his ancestry, his ownership is often a question argued between landowners on whose property he trespasses and hunters who seek his flesh. The feral pig should not be confused with the wild pigs of Europe and Asia; animals that have never been domesticated.

Even the casual transient along the Federal highways in southern Georgia may see bands of animals on the marshes in the early morning. Scattered over the marshlands the visitor may see areas, sometimes several acres in extent, that have been eaten and trampled into wallows by the feral pigs. If he stops to talk, local hunters will tell of shooting and trapping young animals.

![Map of Feral Pig Range](image-url)

Fig. 1. Range of the Feral Pig in Southeastern United States (from Hanson and Karstad, J. Wildlife Management, 1960).
On back roads and on small waterways, he may come across bands of feeding pigs. Or even if the pigs remain unseen, he may hear sounds of their fighting in the brush. It is very apparent even on superficial study that feral pigs greatly exceed deer in numbers and in their effect upon the land.

Men, who are acquainted with wildlife problems in southeastern United States, have recently reported the prevalence of feral pigs in their states (Hanson and Karstad, 1959). The range extends from North Carolina south to Florida along the lower coastal plain and from Florida west to eastern Texas along the Gulf Coast (Fig. 1). The feral swine population on this relatively narrow strip lies in the vicinity of two million. The range of the feral pig is probably defined by at least three factors: a mild climate (average frost penetration of less than one inch), swamps and marshland (much of it wild, unfenced and uncultivated), and profuse, succulent vegetation.

The feral pig appears to be a most important factor in the perpetuation of vesicular stomatitis in southeastern United States. No other species of animal except horse which is becoming rare has a greater number of infected individuals as measured by the presence of antibody (Table I). No other species of animal has a range so closely fitting the boundaries of the enzootic area in the United States. These two observations in particular justify serious consideration of the role of feral pigs in the perpetuation of vesicular stomatitis.

**TABLE III**

*Helminth Parasites that are Shared by Swine and Four Other Mammals*

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Swine</th>
<th>Cow</th>
<th>Deer</th>
<th>Wild Carnivora*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasciola hepatica</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Diphyllobothrium mansoni</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Taenia hydatigena</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Taenia solium</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Multiceps multiceps</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Echinococcus granulosus</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Macracanthorhynchus hirudinaceus</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Stephanurus dentatus</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Trichostrongylus axie</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Cooperia punctata</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Dictyocaulus viviparus</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Metastrongylus apri</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Metastrongylus pudendotectus</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Protostrongylus rufescens</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Physostephalus sexualis</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Gongylonema pulchrum</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Trichinella spiralis</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Dioctophyma renale</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

* Bobcat and raccoon.
FERAL SWINE AS A RESERVOIR

The differences and similarities of feral and domestic pigs should be clarified. Both have come from the same stock, but the domestic pig has been bred for type and conformation, undesirable characteristics having been eliminated as they arose. The feral pig, on the other hand, is a mongrel that has survived in the woods because he has successfully evaded predators and catastrophes and because he has been capable of foraging for himself. Natural selection played a dominant part in the determination of his characteristics. Generally, the feral pig is leaner, has longer legs, longer snout, and longer hair, than breeds of domestic swine. His coloring is varied. Although unconfined the feral hog does not wander indiscriminately. He has feeding grounds, wallows and a bed to which he repairs on a schedule of his own selection. Often he travels with a band of six to a dozen other pigs. The range or territory of each band is probably determined by the territories of other bands of pigs in adjoining areas.

The female produces a small litter, once or twice a year. In one sow examined six fetuses out of 12 were being resorbed. Such a fetal mortality, the result of disease or malnutrition, may be common. Some of those born fall prey to predators. It is not uncommon to see sows with no more than two or three young following them. In spite of the sometimes low productivity, populations increase rapidly if food supplies are abundant and disease and predators ease their toll.

The diet of the feral pig differs from the balanced ration of his well-bred domestic cousin. His staples are the roots and tubers of marshland plants such as the Wapota. During the winter, the diet of hogs on coastal marshes appears to be almost exclusively tubers of the Wapota which are high in carbohydrates and roughage and low in protein. In warmer months, protein is introduced by the consumption of earthworms, fiddler crabs and sometimes larger animals. Wildlife conservation personnel in southeastern Georgia believe that death of the feral pigs which occurred during the winter of 1957-58, a time when dozens of carcasses were counted on some of the coastal islands, could be ascribed to malnutrition and exposure. As the feral and domestic pig differ to considerable extent in conformation, behavior and diet, they probably also differ in their physiology and rate of maturation.

The direct and indirect contact between feral swine and domestic swine or cattle is a matter of importance in the epizootiology of vesicular stomatitis. Feral swine invade barnyards and feral boars mate with domestic sows. In some areas, domestic swine as well as cattle mingle with feral animals in the pasture.

Opportunities for indirect contact are even greater. All feral animals examined during the past winter were heavily infected with lice, *Haematopinus suis*, and in the summer many had ticks, *Dermacentor variabilis*, *Amblyomma maculatum*, and *Amblyomma americanum*. The lice are strictly parasitic on swine, but ticks will pass to cattle, deer and to many other species. Most of the diptera are rather omnivorous. Screwworms attack feral and domestic swine alike as well as cattle and deer. The constant fighting among feral swine provides sites for the reproduction of screwworms. Horseflies and
yellowflies, *Tabanus*, and *Chrysops* spp., have been observed feeding on feral swine. Their vicious attacks on horses and cattle are well-known. Less is known about the species of mosquitoes and midges which feed on feral swine. We believe that swine like horses are attacked by them as (35 percent of some 30) feral swine tested had antibodies to eastern equine encephalomyelitis. It is believed that this disease is transferred only by the agency of mosquitoes. Three of six groups of possible vectors of vesicular stomatitis have been found on the coastal plain of southern Georgia in every month of the year (Table II). In some years, species in these groups have become locally abundant during the winter season (Fletcher and Flanagan, 1957).

**TABLE II**

Relative Seasonal Abundance of Possible Arthropod Vectors of Vesicular Stomatitis in South Georgia

<table>
<thead>
<tr>
<th>Month</th>
<th>Mosquitoes (2)</th>
<th>Culicid (2)</th>
<th>Horselles (2)</th>
<th>Yellowflies (2)</th>
<th>Hippelates (2)</th>
<th>Horn Flies (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>x</td>
<td>xx</td>
<td>—</td>
<td>—</td>
<td>x</td>
<td>—</td>
</tr>
<tr>
<td>February</td>
<td>x</td>
<td>xxx</td>
<td>—</td>
<td>—</td>
<td>x</td>
<td>—</td>
</tr>
<tr>
<td>March</td>
<td>xxx</td>
<td>xxx</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>April</td>
<td>xxx</td>
<td>xxx</td>
<td>x</td>
<td>xxx</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>May</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
<td>xx</td>
<td>xxx</td>
</tr>
<tr>
<td>June</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
</tr>
<tr>
<td>July</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
<td>xxx</td>
</tr>
<tr>
<td>August</td>
<td>xxx</td>
<td>x</td>
<td>xx</td>
<td>xx</td>
<td>xxx</td>
<td>xxx</td>
</tr>
<tr>
<td>September</td>
<td>xx</td>
<td>xx</td>
<td>xx</td>
<td>x</td>
<td>xxx</td>
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<tr>
<td>October</td>
<td>xx</td>
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<td>xx</td>
<td>x</td>
<td>xxx</td>
<td>xxx</td>
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<tr>
<td>November</td>
<td>xx</td>
<td>xx</td>
<td>x</td>
<td>—</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>December</td>
<td>xx</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>x</td>
<td>—</td>
</tr>
</tbody>
</table>

(1) Estimate of abundance based on light-trap records of (Milton, 1956) U. S. Army entomologists at Fort Stewart, Georgia.

(2) Estimate of abundance based on experience of entomologist of Georgia State Department of Health (Fletcher, 1958).

Abundance indicated by, — none, x scanty, xx common, xxx abundant.

Helminths are another possible means of indirect contact between feral swine and livestock. Virtually all of the feral pigs that we have examined have had swine lungworms *Metastrongylus apri*. Most of them have the kidney worm, *Stephanurus dentatus*, while the large roundworm, *Ascaris lumbricoides*, a common parasite of domestic swine, is relatively rare in the populations studied. Several helminth parasites of swine whose prevalence is still unknown in the feral swine of southeastern United States may occur in cows, deer and raccoon as well (Table III). These include *Echinococcus granulosus*, *Taenia hydatigena*, *Macracanthorhynchus hirudinaceus*, *Gongylonema pulchrum*, and *Trichinella spiralis*. 
TABLE 1

Presence of Antibodies of NJ-Type Vesicular Stomatitis in Species of Mammals of Southeastern Georgia*

<table>
<thead>
<tr>
<th>Species</th>
<th>Number Tested</th>
<th>Present Positive</th>
<th>Population/Square Mile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td>10</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Feral Swine</td>
<td>58</td>
<td>79</td>
<td>37**</td>
</tr>
<tr>
<td>Deer</td>
<td>25</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>Cattle</td>
<td>104</td>
<td>49</td>
<td>14</td>
</tr>
<tr>
<td>Raccoon</td>
<td>77</td>
<td>45</td>
<td>52**</td>
</tr>
<tr>
<td>Domestic Swine</td>
<td>52</td>
<td>44</td>
<td>23</td>
</tr>
<tr>
<td>Bobcat</td>
<td>8</td>
<td>37</td>
<td>1</td>
</tr>
<tr>
<td>Man</td>
<td>200</td>
<td>25</td>
<td>26</td>
</tr>
</tbody>
</table>

* Data for ten representative counties (Appling, Brantley, Camden, Glynn, Liberty, McIntosh, Pierce, Tatnall, Ware and Wayne). Domestic animal populations from 1954 Agricultural Census. Human population from 1950 census. Deer population estimate for southeastern Georgia, Allan, 1948.

** Fifty percent of estimated populations of feral swine in good Georgia habitat (Myers, 1958) and of raccoon in good Mississippi habitat (Yeager, 1937).

The seasonal occurrence of vesicular stomatitis is not clearly established. The disease is usually first seen early in May among pastured swine in southeastern Georgia. It is felt by some (Randall, 1957) that cattle are infected secondarily. The last cases in swine or cattle are observed in late September or early October. In the summer of 1957, an attempt was made to obtain vesicular stomatitis susceptible feral swine from the Altamaha Wildlife Management Area. Most of the mature animals were found to be immune when captured and the young susceptibles although separated from the older animals became immune during the summer. The continued presence of vesicular stomatitis virus in marshy or swampy areas isolated from land inhabited by domestic livestock has been definitely established by this work. Susceptible young domestic pigs were released into a 23-acre tract of marshland during the fall of 1957 and did not become infected during the winter as judged by their failure to develop antibodies. The winter of 1957-58 was unusually severe. Whether the disease might remain active during a mild winter has not been tested. The question remains as to whether vesicular stomatitis may persist among feral swine by vector facilitated transmission, in a mild climate, such as exists in Florida every winter and on the lower coastal plain or adjoining states during many winters, or whether the disease has a yet undiscovered reservoir host into which it goes on the advent of the winter season. A low level of virus activity during the winter in the warmer portions of the southeastern states, may explain the apparent absence of the disease during this season and its reappearance in the same areas each spring. If this occurs, it would seem that feral swine may be the host most commonly infected as disease among them would go unnoticed. Insect vectors may assist in virus transmission.
SUMMARY

The free-ranging feral pig population of the coastal counties of seven of the southeastern states exceeds the domestic pig population.

Antibodies to vesicular stomatitis were found in the sera of 79 percent of the feral swine tested from southeastern Georgia as compared to 44 percent of the domestic swine from the same area. In 1957, the disease was found to persist throughout most of the year in captive feral swine by transmission among young susceptibles maintained in their swampland habitat and isolated from domestic animals. Avenues of direct and indirect contact between feral swine and livestock are discussed. Programs for control of vesicular stomatitis or other diseases of swine would appear to be unrealistic if the feral swine are not taken into consideration.

REFERENCES


President Milligan: Thank you, Doctor Hanson.
Are there any questions that you would like to direct to these people about these papers?

Doctor Moncrum: Moncrum, of Beltsville.
I would like to ask Doctor Hanson: There was something about the distribution of the people. There were 200 people tested. Did these represent farmers, or farmers’ wives, or families, or children, or what about the distribution?

Doctor Hanson: We have not completed a study of this particular aspect. It is true that on farms, where the disease has been diagnosed in the livestock, the incidence is far higher than 25 percent. In many instances it has included most of the individuals on such farms.
This 25 percent is a random population of individuals that come into the Waycross Southeastern Station of the Georgia State Board of Health for consideration of some febrile infection as yet undiagnosed.

We have attempted to collect information on what percentage is rural population, and what percent is city, but this becomes rather complex. For example, one individual having stomatitis antibodies lived in Waycross, apparently was engaged in business there, but we found out that he spent every weekend in the woods fishing. So you have to know about the hobbies and avocations as well as the business.

**Doctor McCrome:** I am Doctor McCrome, the Assistant State Epidemiologist in Georgia.

Perhaps I can add just a little bit to what Doctor Hanson said.

These people have been screened. The serum has been submitted for diagnostic purposes and it has been screened against typhoid, tularemia, and brucellosis, so these were negatives left over, to which we could assign no illness, so they were then suitable to be suspected as possibly having been infected with vesicular stomatitis.

They were scattered widely over a considerable area, and I think you can truthfully say that they are really selected at random in every sense except that they were all ill.
COMMITTEE ON VESICULAR DISEASES


It has been two years this month since the last case of vesicular exanthema was reported. Nevertheless state and Federal inspectors are still looking for the disease as they carry out monthly inspections.

Federal funds are being reduced annually, thus making it necessary to reduce the number of Federal inspectors that will be available each year. It is strongly urged that states seek funds to employ state inspectors to compensate for the reduction in Federal inspectors.

At least 95 percent of swine fed garbage are receiving cooked garbage. New Jersey, the last state to pass a garbage cooking law is doing a splendid job in getting feeders to cooperate with their state law. Some of the better cooking installations in the country can now be found in New Jersey.

The bill to repeal the Massachusetts garbage cooking law is still in Committee. It was to be turned over to a Committee to visit other states to determine if they were enforcing their laws and if, because of the low incidence of V. E., it was still necessary to cook garbage in Massachusetts. So far, funds have not been made available for the Committee to carry out its investigation.

Prospects at this time look promising for a garbage cooking law in Connecticut when the next session of their legislature convenes. Someone said we lost ground last year because we now have two states without garbage cooking laws, but after investigation it is our understanding that Alaska prohibits the feeding of raw garbage by regulation.

This year the Defense Department strengthened the clause in military installation garbage contracts by requiring the cooking of garbage if it was fed to any livestock rather than that being fed to swine only. Also, the clause requires the feeder to permit inspection by Agricultural Research Service personnel at any time.

In reviewing last year's goals for vesicular exanthema, we did very well except that very few states changed their authorization to require cooking in order to prevent the spread of all garbage borne diseases rather than limiting it to vesicular exanthema as most of them do.

In some states we have a certain percentage of swine being fed raw garbage, even though it is prohibited by law. Unfortunately, some state legal advisors are not receptive to taking action based on present purposes of their laws, since vesicular exanthema has not been reported within their state for some time. Let's not turn our back on this development—it can become a trend whereby we will lose all that has been gained in the control of garbage borne diseases.

316
In Canada the garbage feeders willingly accept the cooking requirements. They have had a national garbage cooking law since 1917. However, violators are prosecuted whenever located.

Goals for next year:

1. A garbage cooking law in Connecticut.
2. Urge states to increase number of inspectors to carry out monthly inspection of garbage feeding premises.
3. Once again urge states to change justification for cooking laws in order to prevent the spread of all garbage borne diseases rather than V. E. alone.
4. States to prosecute violators of garbage cooking laws.
5. Must continue to recall that vesicular exanthema was not reported in California for a 42-month period, only to return in larger numbers of outbreaks than it had been during previous years.

VESICULAR STOMATITIS

Much progress was made towards meeting our goals. More practitioners are reporting the disease. Movements of animals from infected premises are not being made until the outbreak has subsided. We are getting better cooperation from practitioners and feel that a fairly good coverage is being obtained in the endemic areas by the number of samples being submitted.

Personnel were sent into endemic areas where outbreaks occurred in order to conduct an epizootiological study on outbreaks as they occur. We hope that such an approach will eventually provide a break-through on how this disease is perpetuated and spread.

From a survey of diagnosticians, their observations have been:

a. Acute cases are usually seen from May through October.
b. More teat lesions in cattle are being seen than during previous years.
c. Outbreaks seem to occur when insect population in the area is high.
d. It is not possible to differentiate clinically between other types of stomatitis and vesicular stomatitis when the vesicular stage is passed.

Due to the past severe winter, cases were reported later than usual in the Southeast. Also, the total incidence of the year is much lower than previous years. No cases were reported in Canada. A vesicular disease condition was reported in Mexico. Harvested material submitted to date was positive for New Jersey Vesicular Stomatitis.

Feral swine in some areas of the enzootic zone exceed in population the number of domestic swine. It is suspected that this type hog is playing an important role in the epizootiology of vesicular stomatitis—chiefly because it has been shown that within the enzootic area they have a greater percentage of serological positive individuals.

Goals for Vesicular Stomatitis:

Continue to send a team into endemic areas as outbreaks occur to study factors present in the hope of finding reservoirs of the disease.
FOOT-AND-MOUTH DISEASE

Foot-and-mouth disease has been reported in most countries of the world during the year. However, striking increases were reported in Italy, Great Britain, Belgium, Portugal and Spain. A flare-up was reported in France during August and September.

Asia 1, the most recent type isolated, was reported in Israel.

VESICULAR DISEASE RESEARCH DEVELOPMENTS

During the past year, safety tests were completed in the newly constructed laboratory building at Plum Island and research is in progress with foot-and-mouth diseases virus (FMDV). Biochemical, cytological and immunological investigations are being carried out in the building, supplementing diagnostic investigations which are conducted in another building. Microbiological studies are scheduled to begin this Fall in the newly completed structure.

All three of the vesicular disease viruses (FMD, VE, VS) have been adapted to tissue cultures of a variety of types of cells. Such cultures have been used for primary isolation of virus from experimentally infected animals and are considered useful in diagnostic studies and neutralization tests.

Methods for precise determination of the concentration of FMDV in suspensions have been developed at Plum Island. Briefly, this is done by placing a thin layer of agar on cultures of outgrowths of bovine kidney cells following their inoculation with virus. After gelation, the cultures are incubated for 72 hours when the numbers of plaques, or areas of virus activity, may be counted. On the basis of titrations in cultures, each plaque is considered to be caused by one infective unit of virus. Similar methods have been developed at other laboratories for the viruses of VS and VE. It has been shown that antigenic strains of VE virus produce different plaque types and that these plaque types are related to the virulence of the virus. This is true for all seven types.

Studies at the Research Institute, Pirbright, England (reported in 1956), have demonstrated the complexity of the vesicular stomatitis virus (VSV) system. Four components have been identified with sedimentation co-efficients of 625S, 330S, 20S, and 6S. These differently sized particles are considered to be distinct and separate products of virus multiplication or to arise from disintegration of the infective particles. It has also been revealed in precipitation and centrifugation studies at the Research Institute, Pirbright, England, that the infective particle in the VSV system is rod shaped and measures 165 x 65 mu. The next larger particle is 62 mu in size and is spherical. It is thought that this particle is not infective. The two smaller particles may contribute toward infectivity; however, they appear to be most active as complement-fixing particles.

The multiplicity of components in the VSV and FMD virus systems, as determined by physical separation and electron microscopic techniques re-
VESICULAR DISEASES

quires further study to determine the relationship between physiochemical structure and biological activity.

Biophysical studies at Plum Island, following isolation and photography of FMDV, have been continued and workers there have succeeded in partial purification of a smaller particle in the virus system. It is about 8 mu in size and represents most of the complement-fixing factor in the system. Unpublished reports indicate that progress has been made with VE virus.

It has been further reported from the British foot-and-mouth disease laboratory and elsewhere that several of the seven known types of FMDV have been adapted to embryonating chicken eggs. While none of the types have thus far been rendered completely innocuous for cattle, there are indications that some of the viruses may be altered in the course of serial passages to permit their use as an immunizing agent.

Four additional types of VE virus have been typed, the details of which will be published in the near future. Two of these were isolated as early as 1943 and the other two in 1954 and 1955.

IMPORTANT RESEARCH FINDINGS

A vesicular exanthema (VE) hyperimmune horse serum has been developed by immunization procedures and is being used routinely in the complement-fixation test to differentiate VE swine viruses of any type from vesicular stomatitis (VS) viruses. Vesicular exanthema and foot-and-mouth disease antisera have also been produced in rabbits and such sera may be used in serological tests.

Swine serums have been successfully tested by the complement-fixation test. These serums were passed through ion-exchange resins, which removed the pro-complementary substances which interfered with the test.

Cooperative field and laboratory studies of VS by the University of Wisconsin, Agricultural Research Service and various state agencies are continuing. These investigations are developing increasingly substantial information on incidence, mode of transmission, reservoirs and human infections. One-fourth of the human sera tested from persons in the enzootic area have been found to contain VS antibodies.

It has been found and reported at this meeting that VSV and VE have a marked stability in the alkaline range. The two viruses are much less stable in the acid range. Soda ash (Na₂CO₃) has been found to have no application as a disinfectant for VS and VE. Ordinary laboratory disinfectants, especially those with a pH of 3 or less have been found effective for VSV and VE.
RECOMMENDATIONS FOR RESEARCH DEVELOPMENT IN VESICULAR DISEASES

1. Continue studies in VSV enzootic areas at the time of infection to define methods of transmission and reservoirs.
2. Initiate more detailed and intensive "natural history" investigations to ascertain factors common to widely separated vesicular stomatitis outbreak areas.
3. Project and carry out more basic studies of the biochemical and biophysical properties of the vesicular viruses.
4. Continue studies to find more suitable disinfectants for the destruction of vesicular viruses to develop improved measures for the control of VS.

Electron-microscope photograph of foot-and-mouth disease virus. The virus is spherical in shape and about one-millionth of an inch in diameter. This is one of the first electron micrographs of the virus, taken by Agricultural Research Service scientists at United States Department of Agriculture’s Plum Island Animal Disease Laboratory at Plum Island, N. Y. The foot-and-mouth disease organism is the smallest of any of the viruses affecting animals—even smaller than polio virus. The Plum Island laboratory is the only place in the United States where research is permitted on foot-and-mouth disease, a potential threat to the United States beef and dairy cattle industries and a livestock disease of great economic importance throughout most other areas of the world.

BN-4817 Credit USDA Photo September, 1957
REPORT OF THE NOMINATING COMMITTEE

COMMITTEE ON NOMINATIONS

R. L. West, Chairman, St. Paul, Minnesota; C. L. Campbell, Tallahassee, Florida; K. J. Peterson, Salem, Oregon; R. W. Smith, Concord, New Hampshire; K. F. Wells, Ottawa, Canada.

Doctor West: Mr. President and members of the Association: The names of the Nominating Committee were published in the proceedings of the last meeting and are listed on your program. Unfortunately, Doctor Peterson of Oregon was unable to be present, and for that reason your President selected Dr. F. L. Schneider of New Mexico to represent the western states.

Your Committee on Nominations has met, and we are pleased to place in nomination the names of the following members for the positions designated. In addition to the unquestioned ability of each of these men to represent this Association and to uphold its traditions, you will note that their places of residence are widely distributed throughout the country, thus carrying out the long established policy of this Association of fair and equal representation of all regions.

The names and the positions for which they are nominated are as follows: For President, Mr. Francis G. Buzzell, of Maine. For First Vice-President, Dr. J. R. Hay, of Ohio. For Second Vice-President, Dr. A. P. Schneider, of Idaho, and for Third Vice-President, Dr. W. L. Bendix, of Virginia.

This has been approved by all members of the Committee.

President Milligan: Will you give those to Doctor Hendershott now, so that he can write them down?

You have all heard the report of the Nominating Committee. Are there any further nominations?

Doctor Geyer: Mr. President, I move that the nominations be closed.

[The motion was duly seconded.]

President Milligan: All in favor of the motion, let it be known by saying "aye."

[General response: "Aye."]

President Milligan: All opposed, same sign.

[No response.]

President Milligan: The new officers for this Association are: President, Mr. F. G. Buzzell; First Vice-President, Dr. J. R. Hay; Second Vice-President, Dr. A. P. Schneider; Third Vice-President, Dr. W. L. Bendix.

I will ask Doctor Bruckner to escort Mister Buzzell to the rostrum.

I would ask Doctor Geyer to escort Doctor Hay, and Doctor Thompson to escort Doctor Schneider, and Doctor Estes to escort Doctor Bendix.

Doctor Bendix, being a new member to the official family here, I want to congratulate you and tell you that we in the south are proud to have you represent us as an officer in this Association.
DOCTOR BENDIX: Mr. President, I thank you very kindly.

PRESIDENT MILLIGAN: Doctor Schneider, it is indeed a pleasure to see you move up, and I know you will continue to do the job that you have done in the past.

DR. A. P. SCHNEIDER: I thank you, sir.

PRESIDENT MILLIGAN: Doctor Hay, I think you have the roughest position in the entire organization. Having served in all the places coming up the line, I can realize what you have before you next year.

And Mister Buzzell, I know that you can depend on these men to help you carry on as you have and they have helped me.

I now turn the Presidency of the organization over to you.

PRESIDENT F. G. BUZZELL: I wish to say that I appreciate this honor that you have bestowed upon me, and that we will try to carry out the work of this organization in such a way that it will be a credit to the industry we represent.

Is there any further business to come before this meeting?

If not, we will stand adjourned.
1st ANNUAL MEETING
CONFERENCE OF VETERINARY LABORATORY DIAGNOSTICIANS

November 4, 1958
Miami Beach, Florida

Paul Bennett, President, Ames, Iowa
William L. Sipple, Secretary, Kissimmee, Florida

1. A Recommended Immunization Program for Veterinary Laboratory Personnel, M. L. Weil, M.D.
2. Poultry Laboratory Assession Forms, D. G. Draper.
4. Reports to the Veterinarian and the Owner, H. Elliott.
5. Anerobic Bacteriology Techniques, E. M. Ellis.
6. The Diagnostic Laboratory and Parasitological Diagnosis, J. S. Andrews.
7. A Serological Service for Dogs, J. A. Baker et al.
8. Techniques for Evaluating Poultry Vaccines, R. P. Hanson
9. Hepatopathies in Chickens, R. W. Wichmann and R. A. Bankowski
A RECOMMENDED IMMUNIZATION PROGRAM FOR
VETERINARY LABORATORY PERSONNEL

MARVIN LEE WEIL, M.D.

Protection of the Veterinary Diagnostic Laboratory worker continues to present an increasingly vexing and complicated problem to the physician charged with the care of these personnel. At the present time there are some 86 zoonoses which are a potential or present threat to man (1). Many of these are encountered by laboratory diagnostic personnel in a most intimate fashion. It is the purpose of this paper to present certain criteria for use by veterinary laboratory personnel in the choice of desirable vaccines, to discuss certain diseases are transmissible to man which should be considered by a veterinary group in the United States, and to treat in detail various diseases which have available prophylactic agents. Finally, recommendations as to immunization programs for Veterinary Laboratory Diagnostic Personnel are presented.

There are certain major considerations in the choice of vaccines for veterinary diagnostic workers. A disease must have sufficient incidence and virulence to warrant the need for protection. Vaccine should be given only to those at risk of catching the disease in question. The specific, effective, vaccine must exist and be readily available. It must be safe and should not cause disease itself, nor should it produce severe reactions of an allergic or isoallergic nature. For example, while Sylvatic Plague is widespread over a large area of the Western United States of America, only one human death was reported in the entire United States during 1956 (2). Newcastle disease in man produces a conjunctivitis which is not a serious disease. For this reason, even though the disease is transmissible to man, no major efforts in human prophylaxis are warranted. Chemotherapeutic agents which are 100 percent effective in achieving a prompt cure eliminate the need for a vaccine. Scarlet fever immunization has passed from the medical scene in this country with the advent of highly effective antibiotics against the beta hemolytic streptococcus. In the consideration of chemotherapeutic agents, it is appropriate to mention Rickettsial diseases. In this particular group, excellent chemotherapeutic agents are available which have reduced the mortality to a very low level. Chloromycetin and the tetracycline derivatives currently in use are all rickettsiostatic, and final recovery from these infections still depends on the evolution of immunity by the infected person (3). Recovery from Rickettsial infection is more assured by the presence of immunity developed by immunization than where no antecedent immunity exists.

There are many bacterial diseases which are potential hazards to veterinary diagnostic laboratory workers. Among these, suitable vaccines are available against tetanus, salmonellosis, tularemia, diphtheria, plague, and tuberculosis. Some protection is available against brucellosis, anthrax, and staphyloccosis,
but certainly these problems remain to be solved. Other diseases which present a hazard include streptococcosis, listeriosis, erysipelas, pseudotuberculosis, and glanders. Viral diseases for which some protective agent is available include rabies, equine encephalomyelitis (Eastern, Western, St. Louis, and Venezuelan types), dengue fever, yellow fever, Colorado tick fever, Japanese B encephalitis, Russian Spring-Summer encephalitis, psittacosis, ornithosis, and louping ill. Other viral agents which are of some significance but lack vaccines include B virus, cat scratch disease, Newcastle disease, lymphocytic choriomeningitis, Duran's disease, encephalomyocarditis, foot-and-mouth disease, bovine pustular dermatitis and infectious anemia of horses. Certain virus diseases of man can be prevalent and caught from other men rather than from animals. These include poliomyelitis, smallpox, influenza, adenovirus, Coxsackie virus and ECHO virus. The same recommendations which apply to mass immunizations of humans must apply to laboratory personnel. The Rickettsial agents, in particular Q fever, the typhus group (epidemic typhus including Brill's disease, endemic typhus), the spotted fever group (Rocky Mountain spotted fever; Rickettsialpox), Scrub typhus and trench fever are all potential threats to man. Fungal diseases such as histoplasmosis, favus, thrush, aspergillosis, and tinea corporis do not have appropriate immunologic agents and are not within the scope of this paper nor are the parasitic diseases of protozoan or spirochetal origin.

We would now like to turn to a detailed consideration of specific diseases for which good immunologic agents exist:

**Tetanus:** This vaccine is alum precipitated or fluid tetanus toxoid. The current recommended immunization against tetanus consists of alum precipitated toxoid two doses of 0.5 ml. subcutaneously given one to three months apart and a routine recall of 0.5 ml. is recommended one year after the primary course (4). Booster doses of 0.5 ml. are given every three years thereafter or when injury occurs. Tetanus toxoid is highly effective as has been proven by the Dunkirk experience of the British Army and is to be recommended in that there is neither immediate serum reaction nor risk of serum sickness.

**Salmonellosis:** This is a problem for all veterinary diagnostic workers, but in particular avian workers are at greatest risk. The current vaccine contains 1,000 million S. typhosa, 250 million S. paratyphi, and 250 million S. schottmuelleri in saline suspension. The total immunizing dose of 1.5 ml. should be given subcutaneously in not less than three injections one to four weeks apart in the following order: 0.3 ml., 0.5 ml., and 0.7 milliliter. Recall injections of either 0.1 ml. intradermally, or 0.5 ml. subcutaneously are recommended every one to two years depending on the exposure. In case of exposure to disease an immediate booster dose is recommended (4).

**Tularmia:** This represents a type of vaccine which is most interesting in that the interval between injections is critical. Vaccine should never be given without the use of a 1:1,000 dilution of vaccine as a skin test prior to use since severe reactions may occur. The dose of vaccine is 0.1 ml. subcu-
taneously followed in two days by 0.5 ml. subcutaneously and two days later a final dose of 0.5 ml. subcutaneously. Longer intervals between injections may result in sensitization and severe reactions (4, 5).

Diphtheria: The diphtheria immunization currently available is being developed into a highly refined, less allergenic type of toxoid. Dosage is 0.5 ml. repeated in one to four weeks subcutaneously. Those considered for vaccine should be previously screened by the use of a Shick test so that only susceptible patients are immunized. It is important that all persons over 12 years of age taking diphtheria vaccine have a Moloney test of 0.1 ml. of a 1:20 dilution of fluid diphtheria toxoid administered intradermally before receiving the vaccine. This is a sensitivity test.

Plague: This vaccine is available as the suspension of pasteurella pestis containing 2,000 million killed organisms per milliliter. 0.5 ml. is given subcutaneously followed by 1.0 ml. seven to 28 days later. Recall injection is 0.5 ml. any time within four years of the initial series or last stimulating dose. Boosters may be required not more often than every four to six months where plague is a hazard, but protection is incomplete with the best current vaccine.

Brucellosis: No effective vaccination is available, although vaccines are used as a therapeutic tool in the chronic disease (6, 7, 8). This disease warrants extreme effort for the development of a satisfactory vaccine. Brucella melitensis can be acquired from direct or indirect contact with bedding, corral dust, or even street dust which has been soiled with urine. Manual removal of the placenta exposes the veterinarian to considerable hazard, and infected milk or milk products are highly contagious. Thus the farmer, goat herder, and veterinarian are all at risk. Brucella suis can also be acquired by direct or indirect contact through soil contamination and water infected with urine. Procedures such as vaccination, castration and ringing, as well as the handling of vaginal discharges, fetuses, and fetal membranes place the veterinarian, the farmer and packing house employee at risk. Brucella abortus which infects meat and hides, as well as vaginal discharges of cattle, fetuses, placentas, and milk products, puts not only the veterinarian and the packing house employee at risk, but also the butcher, farmer and other urban populations.

Rabies: This disease is the most important viral illness to which the laboratory worker is exposed. It is 100 percent fatal when contracted and is only treatable by prevention. The presence of rabies in wild animals and vampire bats is well known, but over 150 cases of rabies have been reported from insectivorous bats in 15 diverse states. Development of vaccines derived from avian embryos, either chicken or duck, has changed the rabies vaccine problem completely by decreasing the risk of isoallergic encephalitis. All veterinary laboratory workers should be protected against rabies prior to exposure to the disease. An original course of the vaccine plus one booster dose seems to give sustained immunity against rabies over a number of years.
Recall doses of vaccine have elicited high antibody levels as long as 25 years after Pasteur treatment in some individuals. Mammalian nervous tissue vaccines give greater antigenicity than avian types, though both sources provide satisfactory vaccines (9). At the present time there are four types of vaccines available. The Semple vaccine which is formalinized mammalian spinal cord, the Harris vaccine which is a live fixed virus that does not reproduce in man, the HEP (high egg passage) Flury strain vaccine which is a partially clarified 40 percent to 80 percent suspension chick embryo vaccine, and the duck embryo vaccine. The duck vaccine can be used either as a suspension of duck embryo with live virus or virus killed with betapropriolactone. Many different vaccination courses have been investigated. At present, that series which seems to be most effective is the HEP Flury strain vaccine given as three doses of 0.2 ml. intracutanously five to seven days apart followed by a booster dose of 0.2 ml. intradermally two to six months later (10). One or two recall doses are given at time of exposure, depending on the seriousness of such exposure.

No discussion of rabies would be complete without touching on the passive immunization which is available after exposure to rabies infected material or after the bite of a rabid animal. Local prophylaxis with soap and water cleansing for a period of 10 minutes is just as effective as fuming nitric acid. Antirabies serum, refined, concentrated and standardized, is available from hyperimmunized horses. It is recommended for the immediate treatment of persons exposed to rabies, particularly bites about the head and neck should be supplemented by rabies vaccine prophylaxis. Some authors recommend the use of local infiltration with this antiserum. Experience of the WHO in its study of rabies in Iran can be cited as an example of the effectiveness of anti-serum. During this study, a rabid wolf bit 29 persons of which 18 were injured about the head or neck. This group of 18 was divided into two groups. Three of five who received vaccine alone developed rabies. One of 13 who received vaccine plus one or two doses of antiserum developed rabies. There is definite evidence that passively administered antibody interferes with the active antibody response to vaccine. In particular, there is a suppression of late antibody production where vaccine is instituted or administered two weeks after treatment with serum. Experiments in mice confirm this, and also demonstrate a reduction in subsequent immunity to intracerebral challenge (11). The value of hyperimmune serum is in the induction of early protection until a sufficient level of active immunity is achieved from vaccine.

Dosage of antiserum should be a single dose of 0.5 ml. (40 International Units) per kilogram of body weight (12). This should be followed immediately by a complete course of at least 14 daily doses of an approved vaccine. Best results are obtained if antiserum is administered within 24 hours of exposure.

Encephalomyelitis: Vaccine is available against the equine types Eastern (13), Western (13), Venezuelan (14) and St. Louis (15) type encephalomyelitis as formalinized chick embryo or mouse brain suspension. The chick
embryo vaccine or mouse brain vaccine are both given in two doses of one ml. subcutaneously at one week intervals followed by a third dose of one ml. after four weeks. A booster of one ml. is given every one to two years as needed. Laboratory personnel closely exposed to encephalomyelitis should receive this agent. It is important to realize that equine encephalomyelitis can be transmitted not only by mosquito, but also that the tick D. Andersoni has been known to pass the virus transovarily from generation to generation.

Several virus diseases have yielded attenuated virus vaccines. Dengue fever vaccine utilizes a mouse adapted strain of attenuated virus (16). Yellow fever vaccine utilizes the 17-D strain of chick adapted virus (17). Colorado tick fever vaccine utilizes a multiple passage, chick embryo attenuated virus, usually the 37th to the 74th passage, and produces a mild disease (18).

Psittacosis: Vaccination has been attempted using live virus which is given as a high dilution of active virus (19) or an ether-phenol treated suspension of yolk sac (20). This yolk sac vaccine requires repeated large doses.

Louping Ill: A formalinized vaccine is available for sheep, but no definite vaccine is available for man (21). Cross relationship between louping ill and Russian Spring-Summer encephalitis suggests that a vaccine similar to that used against Russian Spring-Summer encephalitis may be helpful.

Typhus: A Cox type vaccine (22) is available for epidemic typhus and its chronic form, Brill’s disease. Adults are given one ml. subcutaneously followed in 10 to 14 days by one ml. and a third injection of one ml. is given one year thereafter to people at risk. A similar vaccine is available against endemic or murine typhus. It is important to note that these vaccines are effective only against homologous strains of typhus. A polyvalent vaccine must be used to achieve protection against both agents. Effective antibiotics available at the present time are rickettsiostatic and not rickettsiocidal.

Q Fever: This is highly contagious to laboratory workers. The most common source of infection is air contamination, although wool, placentas, milk products, meat and blood have been indicted. The act of parturition seems to seed large quantities of R. burnetii into the air in a highly infectious aerosol. Patient to nurse infections have been recorded. A human carrier is said to have infected 0 patients in a hospital. An autopsy is reported to have infected two pathologists, 16 medical students and one physician. Man to man infections are rare, however, and the United States Army treated patients with Q fever in open wards. Laboratory infections have spread to people throughout entire buildings, and the infectiousness of this agent cannot be overemphasized. Vaccine is prepared from infected yolk sac (23) and is given in one ml. doses subcutaneously every five to seven days for three doses. A booster dose of one ml. is given once a year. Severe local reactions such as sterile abscesses have been reported following multiple injections of this vaccine. The intradermal inoculation of R. burnetii leads to a small local lesion with only fever in two to ten individuals, whereas the intramuscular inoculation of the same rickettsii leads to a local swelling, tem-
temperature of 104° F. after 48 hours, but no pulmonary lesion. Only intranasal or respiratory introduction of the rickettsii leads to a grippe like syndrome in 14 to 26 days with one to ten days of fever and a mild dry cough (24). The isolation of this agent is very dangerous and it is recommended that a complement-fixation or rickettsial agglutination test be used for diagnosis.

Rocky Mountain Spotted Fever: Two types of vaccine are available against this disease: the Cox type vaccine derived from the yolk sac of infected chick embryos (25) and a formalinized infected tick vaccine (26). These vaccines are given as three subcutaneous or intramuscular injections of one ml. at seven to 10 day intervals. Recall injections are given as annual doses of one ml. Infections by tick bite in which the tick has fed for a very short time leads to the evolution of immunity but no virulent disease. Upon biting man, approximately three hours are required before the tick bite can render the disease in its virulent form. The blood meal taken by the tick causes an activation of the rickettsii within the tick which then become infectious through the feces or through the tick content. It is important to note that the crushed tick on the hands may cause infection by contact with the conjunctiva.

The veterinary diagnostic laboratory worker should be protected against certain diseases which are transmitted primarily from man to man. Recommendations for immunization against these diseases apply to laboratory personnel in the same fashion that they apply to other members of the community.

Poliomyelitis: The Salk type vaccine contains type I, II, and III poliovirus prepared from formalinized tissue culture material. Dosage recommended at the present time is one ml. subcutaneously followed in four to six weeks by one ml. subcutaneously and a third dose of one ml. given subcutaneously seven to ten months following the second dose. While booster doses have not been definitely established as necessary, it is currently recommended by many groups such as the American Academy of Pediatrics and the Florida State Board of Health, that a booster dose be given one to two years following the third dose.

Smallpox: Immunization consists of scarification of the skin with live vaccinia virus obtained from calf lymph or chick embryo. Following primary vaccination, a repeat vaccination is given every five to seven years. It is imperative that extreme caution be exercised so that no false immune reactions occur in persons who have been previously immunized. This false immune reaction is elicited by the inoculation of dead virus, and gives a small papule which persists for several days and is difficult to distinguish from the true immune response (27).

Influenza: This vaccine is derived from infected chick chorioallantoic fluid. The current vaccine consists of a polyvalent killed mixture representing types A, A prime, B and Asian flu and is given as one cc. with a repeat dose recommended in two to four weeks. This vaccine is not recommended for
veterinary personnel who are exposed to many vaccines derived from chick sources because it represents unnecessary exposures to chick antigen for a minor illness.

DISCUSSION

The final decision as to recommendations for an immunization program for virus diagnostic laboratory workers depends upon availability of vaccines as well as upon the needs of the individual worker. While some of the vaccines recommended are available commercially, it is regrettable that a number can only be made available on a research basis or if individual laboratories elect to make the vaccine on their own initiative.* The development of an immunization program for any particular laboratory must always consider those diseases peculiar to the locale and function of the unit. Individual sensitivities to egg or nervous tissue may further modify this program for any one person. The following immunization program is recommended for all workers: tetanus, poliomyelitis, smallpox, typhoid fever including paratyphoid A & B, and rabies. Workers should also have protection against the following diseases if they are at risk of exposure: Q fever, typhus fever including the epidemic and endemic forms, Rocky Mountain spotted fever, tularemia and equine encephalomyelitis, particularly the Eastern and Western strains. At the present time there are no suitable vaccines against brucellosis, psittacosis, ornithosis, or B virus. Exposure to B virus warrants the injection of large doses of human gamma globulin because of its cross relationship with Herpes Simplex virus. This passive immunization procedure should be used only following monkey bites.

SUMMARY

A vaccine program for all veterinary diagnostic laboratory workers is outlined which recommends protection against tetanus, poliomyelitis, smallpox, typhoid fever including paratyphoid A & B, and rabies. It is further recommended that particularly workers who are at risk should be immunized against Q fever, typhus fever, Rocky Mountain spotted fever, tularemia, and equine encephalomyelitis of the Eastern and Western types.

* United States Department of Health, Education and Welfare, Public Health Service Publication No. 50 on Biologic Products lists all authorized products and their manufacturer. (Editor.)
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10. TIERKEL, ERNEST: (Personal Communication.)
The purpose of this paper was to evaluate the accession forms in use at a number of poultry disease diagnostic laboratories, take the best features of each, add an idea or two and by this means produce an accession form acceptable to all laboratories.

After reviewing the forms in use at the Maryland State Board of Agriculture, the University of Massachusetts, The University of Maine, the Georgia Poultry Laboratories, the University of Connecticut, the New York State Veterinary College, the University of Illinois, and the Ontario Veterinary College, it became evident that there was little unanimity among the various laboratories as to what constituted an accession form. It would, therefore, seem unlikely that one form could be devised that would suit the individual needs or circumstances of a large number of laboratories.

Generally, the opinion as to what constitutes an accession form depends upon the size of the laboratory or of the institution of which it forms a part, rather than upon any other factor. The larger poultry diagnostic laboratories tend to regard an accession form as a permanent record only. It might take the form of a small or large card, or a bound book, but regardless of the form it takes, such information as is printed upon it is in most cases limited to such headings as will serve to identify the case. All other information is copied onto the accession form from a variety of history questionnaires and other laboratory forms that have been completed by the various departments of the institution that have worked on the case. By this method there might be a separate history sheet, a pathology report, a histopathology report, a virology report and a bacteriology report, pertinent excerpts from which are transcribed onto the accession form along with the diagnosis.

On the other hand, the smaller field laboratories tend to combine everything onto one sheet which serves the purpose of all of the individual departmental forms mentioned previously and finally is filed as the permanent record of the case.

Along this general line of division, I would like to present two poultry disease laboratory accession forms for your consideration.

The accession forms which I think might be considered by the laboratory attached to or forming part of a veterinary college or other similar institute, I have borrowed intact from the Ontario Veterinary College.

This accession form is made up in the form of a punch card. The card is circulated to the various departments that are working on the case and their findings are filled in with different coloured ink, e.g., green for virology, red for bacteriology, black for histopathology. This of course does away
with the necessity of transcribing information from a number of individual forms to the final record. These cards also have the two prime requisites of any permanent record. They take up little space and lend themselves to ready reference since the card sorting machine will sort for disease, initial of the owner's last name, month, or accession number.

Small field laboratories are not likely to have access to a card sorting machine, nor do they have an abundance of clerical help to transcribe reports from one form to another. For these reasons, the accession form offered for consideration for use by the field laboratory has been designed to combine as far as possible all of the necessary information and observations into one permanent record.

This type of form can be divided into five parts:

1. Identification. Name of owner, address, etc.

2. History. There are many times when the history must be taken by someone other than the person making the diagnosis. In order to keep the method of taking the history uniform and to make sure that as much of the pertinent information as the diagnostician is apt to want is obtained, I like to see a fairly comprehensive list of questions printed of the form. I cannot agree, however, to the inclusion on this or on a separate form of a long list of symptoms to be checked off either by the owner or by the person taking the history. Under the best of circumstances, histories can be unreliable. Most people, and this includes most owners and all field service men, hate to say "I don't know," so that you usually get some sort of answer to any questions that you ask. I see no point in listing 30 or so symptoms upon which these people can use their imagination.

The increasing use of coccidiosis vaccination, at least in this area suggests that there should be a record of the type of coccidiosis control in use.

Under the heading "Vaccination," the word type might be better replaced with method. "Other" here refers to other ages at which booster shots have been given.

3. Post-mortem Findings. No comment necessary.

4. Laboratory Findings. The space allowed does not permit much detail, but it does group the various headings closely and tells at a glance the work that has been done. At added cost for printing, a more detailed outline could be put on the back of the sheet. However, I think that most of us find it advantageous to keep a separate record of bacteriology and virus work done and it seems unnecessary to go into more detail on these forms.

Under parasitology, I would record the results of intestinal scrapings, cecal scrapings, or other techniques used.

Under Bacteriology, I would simply list the organs cultured.

Virology would list the tissues used to inoculate eggs or susceptible birds.

Serology would cover HI tests or agglutination tests that might be carried out.

5. Diagnosis and Recommendations. I would like to squeeze in subheadings "Tentative Diagnosis" and "Final Diagnosis," but do not feel that they are imperative.
DIVISION OF LABORATORIES

Accession No.

Name of Laboratory

Owner's Name_________________________________________ Date____________

Address ___________________________________________ Phone__________ County________

Submitted by_________________________________________ Address____________

Laying Flock _______ Replacements _______ Meat Birds_______________

Breed_______ Age_______ No. In Brood_______ Source____________________

No. Birds Brought In_______ Live_______ Dead_______________

Type of Operation. Floor_______________ Cage__________

HISTORY

Vaccination NCD Type____ Age 1.____ 2.____ Other____________________

IB _______ _______ _______

FP _______ _______ _______

Lar _______ _______ _______

Coccidiosis Program________________________________________

Illness first seen________________ Losses since first seen______ Last 3 days______

Type of feed_____________ Consumption________________ Medication________

Symptoms Noted by the Owner____________________________________

POST-MORTEM FINDINGS

Parasites________________________

Lesions________________________

______________________________

LABORATORY FINDINGS

Parasitology_____________________

Bacteriology _____________________ Results________________

Serology _________________________ Results________________

Virology _________________________ Results________________

Diagnosis _________________________

Recommendations________________

______________________________

Reported to____________________ Date____________ By Whom_____________

Wa—water; Ww—wing web; Io—intraocular; In—intranasal; Cl—cloacal.
Departmental forms are an important adjunct of the diagnostic laboratory, and this part of the symposium will be spent in illustrating and briefly discussing their need and usefulness. Since such forms serve as a medium for communication in the laboratory, work sheets, and also as a permanent record of findings, it is especially important that they be reasonably detailed. The amount of detail depends primarily upon the diversity of the work routinely conducted in a laboratory and, to some degree also, the general methods employed.

That no form or set of forms can satisfy the requirements of very many different laboratories is well-recognized. Thus, the samples of forms presented here undoubtedly will appear to be overly complex to those diagnosticians conducting a small laboratory operation, whereas more data might well be desired by those engaged in combined diagnostic and research laboratory operations. Those individual forms provide us with records of the findings from the various units (i.e. serology, bacteriology, clinical pathology, tissue culture, etc.), which are then incorporated by a pathologist into a final report or summary. The final report is based on all available information concerning a particular use, and includes whenever possible the results of a histopathologic study made by the pathologist. All of the forms pertaining to a case are filed with a copy of the final report to serve as a permanent record.

The forms reproduced here are in routine use in our laboratory and are, in general, self-explanatory. Forms are printed in different colors so that it will be easy to identify at a glance the department from which any form comes. Each form (or color) thus represents a particular area of work. The preliminary history and other data are recorded by carbon replication on each form as an aid to the individual doing the work.

*Form 1* is white and is used primarily to summarize the case on the basis of all available evidence, and to indicate whether the case is terminated or follow-up information will be sought.

*Form 2* is white and is used to record necropsy findings.

*Form 3* is blue and is used to record bacteriologic findings and the results of routine animal inoculation studies.

*Form 4* is green and is used for clinical pathologic findings, including fecal examinations and routine pregnancy examinations.

*Pathology Research Laboratory, Pitman-Moore Company.*

335
Form 5 is pink and is used for recording results of routine serologic tests. It might be mentioned here that a separate form explaining the interpretation given various serologic test results is used for reporting to the referring veterinarian.

Form 6 is dark orange and is used to record tissue culture studies. Several different tests may be recorded on one sheet, if desired, and space is provided for a general statement concerning the significance of the results.

Form 7 is light orange and is used for special serologic tests and virus isolation studies in embryonating eggs. Many of these cases are those of a follow-up nature.

Form 8 is yellow and is used to record histopathologic findings.

All of these forms are 8½ x 11.

DISCUSSION

With this fairly wide variety for different purposes, it is possible to fit these forms into almost any situation that arises. Most routine procedures such as standardized serologic tests will require only one. By contrast, certain cases of a complex or doubtful nature might involve the use of all the forms. Thus, many different combinations are possible.

As was pointed out earlier, the data recorded on the various forms are used in the formation and support of a final opinion, which is then reported by personal letter to the referring veterinarian.

If any part of the information I have given here proves to be useful in the operation of your own individual laboratory, then whatever effort I have made to bring this before you has been more than justified.

I thank you.
PATHOLOGY LABORATORIES CASE RECORD

Date

Lab. Record No.

Source: Veterinarian

Address

City State

Owner: Address

Specimen: Date Rec'd

Species Condition

Request:

History:

Remarks:

Preliminary Reports

By Date

Chart Letter Phone Wire

Final Report

By Date

Disposition of Correspondence

Circulate Complaint File Follow-Up File General File Hold

Charge Ticket No.

Remittance Rec'd
PATHOLOGY LABORATORY GROSS NECROPSY RECORD

Date_________________ Veterinarian_________________ L.R. No.__________
Prosector___________ Species No.________ Animals Owner____________________
Clinical History:

Clinical Diagnosis:

POST-MORTEM FINDINGS

Circulatory:

Digestive:

Endocrine:

Genitalia:

Hemapoietic:

Locomotor:

Nervous:

Respiratory:

Sensory:

Urinary:

Remarks:

Necropsy Diagnosis: _______________________________________________________

Signature: _______________________________________________________________
# PATHOLOGY LABORATORIES CASE RECORD

**Date:**

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<th>Microbiology No.</th>
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**Source:** Veterinarian

<table>
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**Owner:**

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**Specimen:**

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<th>Condition</th>
<th>Date Rec'd</th>
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**Request:**

**History:**

**Gross:**

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**Animal Inoculation:**

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**Examined by**

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2V-531 B
**PATHOLOGY LABORATORIES CASE RECORD**

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Examined by: ___________________________ Date ___________________
SYMPOSIUM ON LABORATORY FORMS

(Pink)

PATHOLOGY LABORATORIES CASE RECORD

Date

Lab. Record No.
Serology No.

Source: Veterinarian
Address
City State
Owner: Address

Specimen: Date Rec'd
Species Condition

Request:
History:

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Examined by: Date

2Y-821 D
PATHOLOGY LABORATORIES CASE RECORD

Lab. Record No.________________________

Tissue Culture No.______________________

Source: Veterinarian

Address

City ___________________________ State__________

Owner: __________________________ Address________________________

Specimen: __________________________ Date Rec'd_________________________

Species __________________________ Condition __________________________

Request: __________________________

History: __________________________

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Summary: __________________________

Examined by: __________________________ Date __________________________

ZV-231 F
**SYMPOSIUM ON LABORATORY FORMS**

(Yellow)

**PATHOLOGY LABORATORIES CASE RECORD**

<table>
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<th>Lab. Record No.</th>
<th>Serology No.</th>
<th>Egg Culture No.</th>
<th>Source: Veterinarian</th>
<th>Address</th>
<th>City</th>
<th>State</th>
<th>Owner:</th>
<th>Address</th>
<th>Specimen:</th>
<th>Date Rec'd</th>
<th>Species</th>
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**Request:**

**History:**

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**Neutralization Results:**

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**Summary:**

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Examined by ___________________________ Date ____________

ZV-231 G
REPORTS TO THE VETERINARIAN AND OWNER
HERB ELLIOTT, D.V.M.
W. E. ANDERSON LIVESTOCK DIAGNOSTIC LABORATORY
Baton Rouge, Louisiana

1. All reports are typed on letter head stationery.

2. Distribution: Person sending or bringing specimen receives a copy, or original, or both whether veterinarian, county agent or owner. In poultry cases this person is usually a field serviceman.

Other copies are distributed as dictated by the circumstances.

(a) District veterinarian receives copies of all anthrax cases when first diagnosed in any given area and for several cases thereafter. If epidemic develops (anthrax) they are kept informed throughout but as epidemic subsides usually such copies are omitted.

(b) Veterinarian receives copy or original of all reports of animal cases other than poultry and he will get such here if he has directed specimen to laboratory or if owner requests it.

(c) Veterinarian receives original and extra copy in all cases where he directed specimen to laboratory unless he requests that the original be mailed to owner. This happens occasionally where owner lives in a city some distance from the premises of origin, or in adjacent state.

(d) Owner receives original if he brings specimen in person and mentions the veterinarian only casually, especially if his reason for giving veterinarians name is because of our present policy of requiring a veterinarian on each history (other than poultry). Here the owner may request that the veterinarian receives all copies with original as he (the owner) doesn't care to maintain such a record file, or wouldn't understand letter, or he has the veterinarian on a retainer fee and expects him to keep all records and take proper action in each problem with or without his consultation.

(e) Owner receives copy regardless of origination of specimen, on all serious infections of highly communicable nature. This primarily refers to anthrax, leptospirosis and anaplasmosis. We have had a few embarrassing misunderstandings where various people, practitioner, district veterinarian, state veterinarian and county agent each receiving copies with each assuming that one or more of the other persons had notified the owner. These usually occur in the rush events of an epidemic where the district veterinarian
is working overtime attempting to keep the state veterinarian current on vaccination progress and the practitioner is going day and night vaccinating and/or treating.

(f) Where owner brings specimen and refuses to give the name of a practitioner due to personal dislike, we use the name of the area veterinarian, unless this too is distasteful to the owner, in which case we use the district veterinarian, Doctor Wheeler or myself. Such is quite rare however.

(g) Copy sent to owners whether requested or not when veterinarian is particularly careless about notifying owner of laboratory results, especially negative results. Owners consider them very important whereas some veterinarians feel disappointed or unimpressed by negative results.

3. All highly communicable diseases are reported by phone and letter, and any disease may be, especially when it is the first occurrence of the season of unusual condition; where the disease is not endemic to the area; in “vaccinal breaks”; where owner or veterinarian will not understand or will probably be very surprised at the occurrence of any given condition; where veterinarian is not expected to understand the cause, nature or course of action in any given disease and will end up calling us for clarification. This happens especially with older veterinarians and the occurrence of recently identified disease such as adema disease, leptospirosis, phylogenic pulmonary emphysemae, etc.

4. We have intentionally avoided reporting forms where body of report is set and results are typed in. These we feel are too impersonal and poor economy in public relations and disease control prestige.
AEROBIC BACTERIOLOGICAL TECHNIQUES
EDWIN M. ELLIS, D.V.M., PH.D.*

Tifton, Georgia

Aerobic bacterial cultures differ in only one respect from aerobic ones; namely, in that they require conditions under which oxygen is excluded. In spite of such a minor difference, they require an entirely different approach for their isolation.

It is my feeling that because of the special techniques and effort necessary to grow aerobes, many laboratories may tend to slight this group of organisms. Aerobic cultures need not be done as a routine practice, but should depend on the history, ante-mortem, and post-mortem examination.

Many attempts to culture aerobes fail when improper techniques are used. Kitasato, one of the first workers to successfully isolate the tetanus bacillus, succeeded because he fulfilled two important requirements; (1) he used anaerobic methods and (2) he eliminated non-spore bearing contaminants by means of heat. These two basic procedures plus biochemical tests comprise the necessary tools for the isolation and identification of aerobic bacteria.

Our procedure begins with a direct smear and gram stain of all suspicious material. Muscle, spleen and liver are included along with any other tissue selected. The surface of the tissue to be cultured is seared with a red hot knife or spatula and a portion removed aseptically. This is placed in a sterile Ten Broeck tissue grinder to which has been added two ml. of thioglycollate broth (Difco). Other types of broth may be used for certain species of clostridia. One-tenth ml. of each tissue suspension is inoculated into two tubes of thioglycollate broth and two tubes of Difco heart-infusion semi-solid agar. Media of this nature is either made up fresh or is placed in boiling water for 10 minutes before using. One tube of each type of media inoculated is heated to 70° C. and held for 10 minutes. One tube of each is not heated. The reason for culturing tissue in media lacking thioglycollate is that some strains of certain species, chiefly Clostridium chauvoei, do not grow well in the presence of thioglycollate. If such a strain is encountered the brain-liver-heart semi-solid media is used in all subsequent differential tests. Such semi-solid media contain 0.2 percent agar rather than the 0.1 percent agar contained in media with thioglycollate added.

After incubation overnight, the cultures are examined and smears are prepared for gram stains. The heated cultures are preferred since they are more likely to contain pure cultures. Smears of all cultures are examined carefully to determine whether there are organisms resembling more than one species of Clostridia. The unheated tubes are never overlooked since some

* Georgia Coastal Plains Experiment Station, Agricultural Experiment Stations, University of Georgia College of Agriculture.
species of Clostridia do not sporulate readily and if no spores were present the culture would be killed by heat.

After the above preliminary observations are made a culture is selected and inoculated intramuscularly into a guinea pig. This procedure, besides providing information for a tentative diagnosis, aids in determining pathogenicity and provides, in many cases, a pure culture for further study. Injection of the guinea pig is more successful if the tissue of the leg is traumatized prior to injecting the culture (chauvoei). A sterile two percent solution of CaCl$_2$ injected along with the culture will accomplish essentially the same thing. If one is dealing with pathogenic strains the guinea pig will be dead within 24 hours following injection of the muscle.

Necropsy of the guinea pig is carried out in the same manner as that performed on the suspected animal. All lesions are observed and smears and cultures are prepared from the liver. A preliminary diagnosis can usually be made on the basis of the smears and lesions at this time, which is usually about 48 hours after receiving the specimen.

If it is essential that a preliminary diagnosis be made as soon as possible the guinea pig can be inoculated with the original tissue suspension after heating it for 10 minutes at 70° C. Obviously this procedure is not carried out routinely because of the possibility of death due to contaminating organisms or that the desired organisms have not sporulated and have been killed in heating. In the latter case the guinea pig is not affected.

Our next step is to select one of the original cultures that appeared to be pure or had few contaminants. This culture is streaked on two blood agar plates, one to be incubated anaerobically and one aerobically for comparison. If one of the unheated tubes appears to contain a different Clostridium, it too is streaked on two blood plates and incubated as above. These are incubated two days.

We use a Brewer anaerobic jar and pyrogallic acid with sodium hydroxide: 10 grams of pyrogallic acid and 100 ml. 10 percent NaOH per qt. capacity. The disadvantages of this method should be kept in mind in order to assure good results. It should be made certain that an excess of alkali is added to the pyrogallic acid to insure neutralization. In this case all of the oxygen is removed and anaerobic conditions are produced. However, this action also absorbs carbon dioxide which is required by some microorganisms for initial growth from a small inoculum. Also it is known that alkaline pyrogallate produces a small amount of carbon monoxide, which can be somewhat inhibitive. This method is used at our laboratory regardless of these disadvantages. The cost of keeping an inert gas on hand to correct them has been considered unwarranted.

When it is necessary to observe subsurface colonies, a pure culture of the organism is diluted 1:10, 1:100, and 1:1000 in thioglycollate broth, depending on the amount of growth present, so as to have about 100 organisms (estimate). The diluted culture is inoculated into a tube of melted semisolid agar (Difco heart infusion) which has been tubed in 20 ml. amounts.
When used it is melted, placed in a water bath at 45° C, and inoculated with the diluted inoculum (100 organisms). It is then poured into a sterile Burri tube (10 mm. glass tubing, 10" long, the bottom stoppered with cork, top with cotton) and cooled under a cold water faucet to solidify the media.

Observations are made of the surface and subsurface colonies after two days incubation and one or more colonies are selected, if there appears to be more than one species of Clostridia present. These may be picked from the Burri tube or plate. In selecting from the Burri tube it is necessary to heat the tube under warm water or over a flame until the media slides out into a sterile Petri dish. The selected colony is removed with a sterile Pasteur pipette. The colony is placed in a tube of thioglycollate broth and incubated for several hours or overnight. The following media are inoculated from this colony: iron milk, dextrose, maltose, lactose, salicin, sucrose, proteose peptone for the determination of hydrogen sulfide, gelatin, tryptone for the determination of nitrate reduction, media for the production of indole and, in rare cases, media for milk agar digestion.

The biochemical changes can usually be read in 24 hours after inoculation and the diagnosis made on the basis of these changes: (1) hemolysis on blood plate media, (2) colony morphology, (3) individual morphology of stained organisms including spore form (if present), position of spore, and (4) pathogenicity.

If we are to get the best results from laboratory media for the isolation and identification of anaerobic bacteria, several media should be employed. First, for our fermentation reactions we have found that although thioglycollate media containing sugar and indicator can be purchased, better results are obtained by using thioglycollate broth that contains no indicator. Thioglycollate broth for general use contains dextrose. For fermentation reactions, we add our sugars to indicator-free media. We have observed that the indicator present during incubation is inhibitive. After sufficient incubation, indicator is added to each sugar tube.

Learning the reactions of the more common anerobes on ox blood agar is very important. One may be able to differentiate Clostridium welchii, Cl. septicum, and Cl. feseri (chauvoei) on such media if keen observations are made.

Cl. welchii produces colonies that appear round and smooth, having an entire edge. They are gray to yellow in color, not too unlike Staphylococcus aureus. They produce a double zone of hemolysis; the one close to the colony is alpha and the outer zone beta.

Cl. septicum on ox blood agar has colonies that are rather small and flat having a ground-glass appearance. They are irregular and translucent with a filamentous edge. Occasionally the colony will exhibit finger-like projections extending for a mm. or so out from the colony. Such colonies usually show a narrow zone of alpha hemolysis.

Cl. feseri grows very light on ox blood agar. One seldom finds many colonies present. The colonies are small and irregular with a granular center and are gray in color. They often give a chestnut-burr-like appearance. A slight zone of alpha hemolysis is usually formed around the colony edge.
Several points regarding _Clostridium welchii_ should be made. Colonies on ox blood agar are very distinctive in that colonies are surrounded by a narrow zone of complete hemolysis, due to theta toxin, and by a larger zone of incomplete hemolysis due to alpha toxin. Most strains found in animals show this typical reaction.

If strains are isolated from antibiotic treated animals, variation may be observed in the colony.

Strict anaerobic conditions are not required by _Clostridium welchii_. Growth is good even when a streaked plate is covered with a layer of agar three to four millimeters thick.

The “stormy fermentation,” reaction of milk cannot be relied upon for rapid identification of _Clostridium welchii_.

There are 12 toxins formed by _Clostridium welchii_ and we make no attempt to differentiate between them as they have no important bearing on the diagnosis. Agglutination studies are not dependable as cross agglutination between species occurs frequently.

To briefly summarize the disease conditions brought about by _Clostridium welchii_ we recognize the types A, B, C, and D. Type A strains are not of great importance in animals. Type B strains produce lamb dysentery which can be highly fatal for new born lambs. The disease can be prevented by immunization of the ewes several months before, and again at lambing. A toxoid is used as the immunizing agent.

“Struck” has been reported as a disease of sheep caused by _Clostridium welchii_ type C. Organisms, apparently in the intestine, pass out and involve many of the vital organs.

Type C strains also have been involved in a disease of young calves. The findings are a hemorrhagic enteritis which proves fatal.

The most numerous type seen at our station is probably type D. This strain produces the well-known enterotoxemia of sheep. We refer to the disease in lambs as “overeating disease” or “pulpy kidney” disease. These terms should be put aside in favor of “enterotoxemia.”

The lack of lesions in the intestinal tract and the fact that the largest and best looking lambs are affected is indication of this toxemia.

Fecal filtrates containing antibiotics injected intravenously into mice will cause death within a few minutes. The disease is prevented by injection of the toxoid.

Guinea pigs are highly susceptible to _Clostridium welchii_ and are used most commonly in our laboratory. Intramuscular injection of a culture usually causes death within 24 hours.

We do not culture for this species often as most cases are diagnosed by the history and symptomatology, especially at death. Culture of the organism is not difficult and the species is readily identified. Finding of the typical
ANEROBIC BACTERIOLOGICAL TECHNIQUES

club-shaped spores and a lesion on the animal in which destruction of tissue has occurred are indicative. The colonies tend to swarm and coalesce.

Injection into the muscle of the hind leg of a guinea pig produces an interesting and diagnostic “ascending” paralysis in which first one hind leg, then the other, followed by the muscles of the back, are paralyzed. The toxin appears to follow the motor nerve trunks to the cord and then ascends anteriorly.

**Clostridium botulinum**

This species is not easy to recover from suspected field cases of poisoning. In only one instance have we isolated and identified this species. Generally this is not necessary as the toxic effect in guinea pigs, when injected intraperitoneally, is quite characteristic. A dyspnea usually occurs with subsequent relaxation of the neck and abdominal muscles. Hypersecretion of saliva can be noticed quite early. Injection of laboratory mice can be carried out and is very practical. It is interesting that botulinus toxin is removed from a filtrate by passing it through a resin ion-exchange column.

**Clostridium septicum**

We see this species almost as often as we see Cl. chauvoei. When doing a necropsy on cattle it is important to skin the animal because of the small areas of necrosis and hemorrhage sometimes produced beneath the skin of the abdomen will yield typical appearing rods on impression smear. The isolation and identification of this species is quite easy by the appearance of the colonies, the morphology of the organisms, and the symptoms observed in injected guinea pigs.

Livers removed aseptically from guinea pigs are nearly always infected with the organism.

We have not seen a case of braxy in sheep due to this species. This organism is not easily confused with *Clostridium chauvoei* although it may be found in the crepetant swellings of black leg.

**Clostridium chauvoei** (feseri)

This species is somewhat more difficult to grow out than some other of the clostridia. However, we have little difficulty using the media already mentioned. The technique used has been given. The disease “black leg” is generally diagnosed by the practitioner so we do not get many cases that are typical. Occasionally where very small areas of the body are affected animals are referred to the laboratory. We have observed a relationship between this infection and animals in the best nutrition. It seems that the prime animals are affected early.

It is interesting to note that although immunity for this species is good and enduring, if a culture of Cl. sporogenes is injected into an animal along with *Cl. chauvoei*, death will result due to *Cl. chauvoei*, even when the animal
EDWIN M. ELLIS

has been immunized recently. (Ryff and Lee, 1947). It has been brought out that only antibacterial protection is afforded and not antitoxin.

In order to complete a discussion of this kind two anerobic forms should be mentioned, both of which are encountered with some regularity. *Sphero- phorus necrophorus*, which is a strict anerobe, but which does not form spores, can be cultured on the ordinary thioglycollate media with slight modification in procedure. The principal changes that we make are two in number. Since these organisms do not form spores the contaminates cannot be killed by heating. In order to obtain a pure culture the tissues must be cultured as soon after death as possible and extreme care must be used to avoid contamination in removing tissue for culture. Secondly, it is difficult to streak material suspected of containing Spherophorus because the contact with the air before anerobic conditions can be produced may damage the organisms so they will not grow. For that reason, if it is necessary to pick a colony in order to obtain a pure culture, it is best to use a dilute inoculum in semi-solid agar in a pour plate or Burri tube.

This organism produces acid and gas from dextrose, lactose, sucrose, maltose, and salicin. Techniques used and explained previously for anerobic bacteria apply here.

The second organism to be mentioned is *Actinomyces bovis*. This organism is one of several actinomycetaceae which are anerobic. Aerobic ones are called Nocardia. Some claim two species of this one organism, based mainly on whether the colony morphology is rough or smooth. In our experience only the smooth form is isolated from lesions in animals. Pus is usually the material presented to the laboratory for making an examination.

We usually look for typical “sulphur granules.” These are about one-half to three mm. in diameter and have irregular radiating structures quite characteristic of the species.

We transfer a typical granule to a clean glass slide, add a drop of saline, and crush under a coverslip. The typical radiating filaments are seen. After this inspection a Gram stain is applied on the same slide and the Gram-positive hyph, or fragments of them, observed. At the outer ends are club-shaper structures that do not take the Gram stain.

One must be cautious that similar appearing structures observed are not *Actinobacillus ligniersi*. These however are Gram-negative and should not be confused.

If the history and lesion is typical for *A. bovis* infection, culture need only be done to be sure that the organism in question does not liquefy gelatin or peptonize milk. If these are true, similar non-pathogens are ruled out.
THE DIAGNOSTIC LABORATORY AND PARASITOLOGIC DIAGNOSIS

John S. Andrews,* Sc.D.

INTRODUCTION

Laboratories for the study of animal diseases are basically of two types. One is designed primarily for research, and its staff usually is engaged in the study of specific problems in veterinary medicine such as those having to do with the development of vaccines and serums for the prevention of disease and new or improved drugs for the treatment of disease. Others in this category are concerned with increasing our basic knowledge of disease processes in domestic animals. Some research laboratories are supported by private industry, whereas others are supported by the Federal and State governments. The latter are usually associated with other research institutions or with universities and colleges having departments of veterinary medicine.

The other type is the diagnostic laboratory which is primarily concerned with ferreting out the causes of livestock diseases in the region which it serves. It is usually supported by the State government and may be located in veterinary schools where it plays an important part in the teaching of clinical medicine, or it may be some distance from these centers in an area where it can best serve the needs of the livestock industry of the region.

Although, both kinds of laboratories make important contributions to the science of parasitology, veterinarians who are located in diagnostic laboratories are often in a more favorable position to contribute to our knowledge of the pathogenesis, incidence, and geographical distribution of parasites and parasitic diseases of domestic animals than those in laboratories which are engaged in research on specific problems. This advantage is primarily the result of their being associated almost daily with a wide variety of pathological conditions in animals that are of undetermined or unconfirmed etiology which they are expected to investigate in an effort to ascertain their nature and cause.

Among the conditions often encountered are the diseases caused by internal parasites. In some instances the nature of these cases can be ascertained from the clinical history and physical condition of the animal, supported by the finding of parasites in the blood or in the exudate of the genital system, or their reproductive elements in the feces, urine, or mucous discharge of the respiratory system. In other instances the etiology may be very obscure because of the absence of external evidence of parasitism. Since the diagnostician must pursue the study of such cases until he can determine their etiology, he has a unique opportunity to obtain information not only on the species of parasites occurring in the area which the laboratory serves, but

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353
also on their incidence, relative economic importance, the clinical appearance and history of the condition for which each parasite is responsible, the manner in which they harm their hosts, and the relation of numerous environmental and climatological factors to the development of the disease.

From the point of view of the parasitologist, the maximum contribution can only be made if all animals and specimens of both animal and plant origin which are submitted to the laboratory are examined for parasites. It is only by taking a close look at all of the material that is remotely associated with parasites that new host-parasite and environmental relationships are discovered. However, it is recognized that, in many instances, the number of cases receiving parasitologic examination must be limited to those for which such studies are indicated.

It is the purpose of this paper to point out how these cases may be selected and to suggest procedures for diagnosing and evaluating parasitic infections in domestic animals and poultry. These procedures are (1) obtaining and evaluating the clinical history; (2) making the physical examination; (3) performing laboratory tests on feces, blood, and exudates of the respiratory and genital systems; (4) conducting the post-mortem examinations; (5) fixing, staining, preserving, and identifying the parasites recovered; and (6) determining the pathological significance of the findings.

ANTE-MORTEM DATA

Clinical History: A complete clinical history of the condition presented by the subject should be obtained. Special attention should be paid to the age of the animal, the date symptoms were first noted, the time required for the development of the condition, its incidence in the group to which the animal belongs, the extent and kind of losses sustained because of the disease, the number, kind, and effect of treatments administered and the date of each, the adequacy of the food supply, and results obtained from necropsies of affected animals. If the subject is a grazing animal, information should be obtained on the occurrence of early abortions, the stocking rate, the condition of the pasture, supplementation of the forage by other feed, whether or not the pastures were grazed continuously or were used in a rotation scheme, the rotation plan that was followed, the kind of drinking water supplied, and whether ponds, water holes, or streams were available to the animals. It is also important to know if the affected animals were native to the area or were imported from other geographical regions, and the number, species, age, and origin of the animals previously grazed on the pasture.

If the history discloses a loss of condition over an extended period of time in the presence of abundant feed of good quality, and infectious disease can be ruled out, or the breeding history has been marked by early abortions, internal parasitism should be considered as a possible cause of the trouble and a more thorough investigation should be made.

Physical Examination: The physical condition of the animal should be noted, and its body temperature taken. The presence or absence of a rough
coat, anemia, edema, ascites, diarrhea, and respiratory difficulty should be noted. The skin and hair of mammalian species and the feathers of birds should be examined for external parasites (lice, mites, ticks, and fleas) and their eggs. Lesions in the skin of cattle, sheep, swine, dogs, and cats should be scraped and the scrapings examined for mites. The skin scrapings from cattle and sheep should also be examined for the nematode, *Stephanofilaria stilesi* (12, 13, 15), and for the microfilaria of *Elaeophora schneider* (15), respectively. The skin of the back of cattle should be palpated for the grub of the heel fly, *Hypoderma* spp. The nematode, *Thelazia* spp., and Manson’s eyeworm may be found in the eyes of ruminants and poultry, respectively (39). Specimens of feces and blood from all animals, exudate or washings from the genital tract (if this is indicated), and urine from swine should be obtained for laboratory examination.

*Preparation and Examination of Fecal Specimens:* Eggs and in some instances larvae and adults of round worms, eggs of flukes, eggs and segments of tapeworms, oocysts of coccidia, trophozoites, and cysts of other protozoa are often found in the feces of parasitized animals.

Motile trophozoites and cysts of protozoa, as well as eggs of worm parasites, may be observed in fresh fecal smears made with physiological saline by examining them with a microscope. In instances where protozoan organisms cannot be demonstrated directly, the material can be cultured in Cysteine-Peptone-Liver infusion-Maltose media (CPLM) or any of its derivatives (9, 23, 25). The fecal smear may also be stained with iodine to increase the contrast between the parasites and the surrounding debris (2, 37). Eggs of round worms and tapeworms and oocysts of coccidia may be separated from the heavier debris by one of the flotation methods, incorporating saturated solutions of sodium chloride, or concentrated solutions of zinc sulphate, or sugar, with or without centrifugation (6, 18, 24, 26-28, 35, 36). Helminth eggs that are too heavy to float on the above-mentioned solutions, namely, eggs of liver flukes and the thorn-headed worm of swine, or protozoan cysts that may be damaged by these solutions may be sedimented by allowing the specimens, which have been thoroughly mixed with water, to stand for a few minutes or by centrifuging them. The supernatant containing the soluble opaque material may then be decanted and the specimen resuspended repeatedly, until the eggs or cysts are no longer obscured by it. The sedimentation technique is also useful for examining the urine of swine for kidney worm eggs.

The flotation and sedimentation techniques can be made roughly quantitative if the feces are weighed before processing and all eggs present in the samples are counted. However, these techniques are best adapted for fecal samples containing relatively few eggs or cysts.

If a large number of helminth eggs or protozoan cysts are present in the sample and a quantitative estimate is desired, the Stoll Dilution Method (34) or the McMaster Slide Technique (36, 38) may be used. These techniques require that a weighed portion or a measured volume of feces be shaken thoroughly with a definite volume of water, a solution of 0.1 N sodium hy-
droxide (34), or a saturated aqueous solution of ortho-dichloro-benzene (36) and that the eggs or cysts in an aliquot portion of the evenly distributed suspension be counted. According to Whitlock (36), ortho-dichloro-benzene prevents the development of worm eggs, thus permitting a delay of up to 24 hours even in hot weather between collection of the sample and enumeration of the eggs without materially changing the result. Australian workers reported that the fecal examination of sheep for parasite eggs provided useful information concerning the kinds of parasites present and to some extent the relative severity of the infections on a herd basis only. Their results were not dependable when applied to individual animals.

The eggs can usually be identified to genus by comparing them with published figures and descriptions, most of which may be found in the literature listed at the end of this paper. The eggs of the nematodes parasitic in ruminants can be readily differentiated as shown by Shorb (31).

If a more accurate determination of the genera of the gastrointestinal parasites of ruminants is desired, the eggs may be cultured by mixing the feces containing them with sphagnum moss or animal charcoal. The infective larvae which develop in approximately seven days may then be differentiated by means of the key prepared by Dikmans and Andrews (16) or by Keith (21). Such larvae can be separated from free-living nematodes by treating the mixture of the two with dilute hydrochloric acid (32).

Preparation and Examination of Blood: Thick blood smears stained with Romanowsky stain or similarly stained sections of hemopoietic tissue (10) should be examined microscopically for blood protozoa. Giemsa's stain and Wright's stain may also be used. Rothstein (30) has recently described a method for the vital staining of blood parasites in thick blood smears with a fluorescent dye (acridine orange), which appears to be very effective in facilitating the detection of parasites such as anaplasma, leucocytozoa, plasmodia, and microfilaria, especially when they occur in relatively small numbers.

Blood to be examined for trypanosomes should be fresh and fractionated by centrifugation before being subjected to microscopic examination, as described by Diamond and Rubin (11). If the organisms are not found, the processed specimen should be put in culture media N.N.N., Saline-Neopeptone-Blood (SNB-9) as suggested by Diamond and Herman (10) or in Saline-Tryptose-Blood (STB) as described by Diamond and Rubin (11).

If a protozoan disease is suspected and no organisms are found, the inoculation of the same host species or a laboratory animal with fresh blood from the suspect may reveal the organism (11).

Examination of the Genital Tract: In animals from herds having a history of early abortion, exudates or material from the genital tracts of females and from the preputial cavity of males of cattle, sheep, goats, and swine used for breeding purposes, obtained by flushing with physiological saline, as described by Bartlett (4), should be examined microscopically for Trichomonas foetus, directly or after centrifugation. If the organisms are not observed by direct examination, the material may be cultured by the method described by Dia-
DIAGNOSTIC LABORATORY & PARASITOLOGIC DIAGNOSIS 357

mond (9). In instances when this organism cannot be demonstrated in the washings from the preputial cavity and it is not practicable to culture the material, the suspected animal may be mated to a virgin female and washings from its genital tract examined microscopically for *T. foetus* two or three weeks later.

Although these techniques are useful in making parasitologic diagnoses, they have limitations in that they do not disclose parasites that have not yet attained the stage which produces eggs or oocysts. These techniques do not reveal male worms in the digestive tract nor parasites in other regions of the body that do not have access to the outside by way of the feces or urine. Since sheep, cattle, pigs, and chickens can harbor lethal infections of internal parasites without passing worm eggs or oocysts, it appears that the only accurate method for determining the extent to which these animals are parasitized is by performing a thorough and complete post-mortem examination.

**POST-MORTEM EXAMINATION**

Since there is no organ or soft tissue of the body that is not susceptible to invasion by parasites, the post-mortem examinations should be carried out as completely as possible and all abnormalities occurring from the lips to the anal sphincter should be investigated in an effort to determine whether or not they are parasitic in origin. The fact that one has not encountered a parasite in an organ or tissue of the animal's body should not be made the excuse for failing to examine that part carefully and painstakingly. It is only by looking in places where one least expects to find parasites that new discoveries are made. The joy of discovery and the additional knowledge acquired will more than repay the investigator for the time and effort he expends in making a thorough search.

**Digestive System:** Since gastrointestinal parasitism is one of the most common conditions in ruminants as well as in other animals, the procedures used in examining these organs will be described first.

The mucosa of the lips and mouth should be examined for nodular lesions which may contain parasites. The tongue should be excised and cut into thin slices to reveal possible hidden nodules. The esophagus should be opened, and its wall examined by transmitted as well as by reflected light so that the helminths that may be present within the wall may be seen. Suspicious nodules or raised areas should be excised and carefully opened to release any parasites that they may contain.

Each organ of the digestive tract should then be opened separately, and the contents washed out with water or physiological saline into a container large enough to hold the entire volume of fluid. Davis (8) described an apparatus for opening the intestine of calves that speeds up this operation considerably. The mucosa should then be washed clean and examined for attached worms and associated lesions. The rumen and reticulum should be examined for flukes (amphistomes). The mucosa of the abomasum should be scrubbed with a brush to remove worms that are embedded in the glands,
and the intestine should be stripped through the fingers or scraped with a
dull blade to remove the worms situated between the villi. These washings
and scrapings should then be sedimented in separate containers, the super-
natant poured off, and the material resedimented until the contents of the
jars are relatively clear. Instead of allowing the material to settle the wash-
ings may be poured through a series of screens having three to 200 meshes
per inch, thus speeding up the operation. The contents of the various screens
may be flushed into dishes and examined as described below. The worms
can then be distinguished from the plant fibers and other debris, and the
number recovered can be estimated by counting the parasites in one or more
aliquot samples of the evenly distributed suspension. A dissecting microscope
should be used in examining the aliquots because many of the parasites may
be too small to be readily seen by the naked eye (18).

The contents of the digestive tract should not be passed through screens
if a quantitative result is desired because the worms tend to cling to the wire
and are very difficult to remove. Furthermore, unless the screens are washed
after each use in very hot water, are brushed thoroughly, and are sterilized
by boiling in a two percent solution of sodium hydroxide for two or three
minutes, there is great danger of mixing specimens recovered from one ani-
mal with those from another.

Additional parasites may usually be recovered by incubating the mucosa
of the stomach and intestines with artificial gastric juice as described by
Herlich (20). Ciordia et al. (7) have demonstrated that the tissue to be di-
gested may be frozen and kept for several months if it cannot be processed
immediately, without affecting the results of the experiment.

The fact that mammals other than ruminants have but one stomach simpli-
fies the parasitological examination of their digestive systems. However,
domestic poultry and closely related wild fowl have a crop, proventriculus
or glandular stomach, and a gizzard, all of which complicate the parasitolog-
ical examination. As in ruminants, each organ should be opened and its
contents washed into a suitable container (19). The wall of the crop should
be examined by direct and by transmitted light. The glandular wall of the
proventriculus should be squeezed to express worms that may be hidden in
the ducts of the glands, and the horny layer of the gizzard should be lifted
off to expose helminth parasites that may be located beneath it.

**Abdominal Viscera other than the Digestive Tract:** The ducts and blood
vessels of the liver, the pancreatic duct, the ureters, urinary bladder, and
urethra should be opened and washed out, and the walls examined for
worms that may be attached or embedded in the duct walls. Lesions in the
parenchyma of the liver, kidney, and spleen should be excised and carefully
explored for parasites (22). The genital tract may also be more thoroughly
examined than was possible in the intact animal. A fluke may often be found
in the egg-forming organs of poultry (19).

**Filaria** spp., migrating larvae of kidney worms, large roundworms, hook-
worms, intestinal threadworms, and the dog ascarid may be observed free in
the body cavity or in various organs, and tapeworm larvae may be found
attached to connective tissue. Lungworm larvae may also be found in the lymphatic system of cattle.

Respiratory and Circulatory Systems: The air passages leading to the lungs of mammals and to the air sacs of birds should be opened completely and examined for mites, helminths, and nodular lesions. The spongy tissue should also be examined for nodules which should be teased apart and their contents checked for parasites. The lungworms of swine may be prevented from breaking up by allowing them to remain in the mucus of the air passages or by dropping them into a saline solution somewhat stronger than physiological saline.

The heart and circulatory system, particularly the large vessels, should be examined for roundworms and flukes, and gross lesions in the muscle of the heart or in the walls of the large vessels should be checked carefully since they may be caused by the larvae of metazoan parasites.

Miscellaneous Regions: The space between the skin and the body musculature may often harbor adult filarid nematodes whose microfilariae occur in the blood stream of carnivores, and the muscles of many species of animals may contain encysted trichinae, sarcosporidia, cysticerci, spargana of tapeworms, linguatulids, fly larvae, and other as yet unreported parasites.

The brain and spinal cord as well as the cranial cavity of birds (35), sheep, and swine may harbor nematodes, and the nasal and orbital sinuses of birds may contain flukes (5).

It is realized that the time necessary for such thorough examinations as have been outlined is not always available to the diagnostician. However, performing such an examination, even when not indicated, may lead to the discovery of information of inestimable value in the diagnosis, prevention, and control of parasitic disease.

THE FIXATION, STAINING AND PRESERVATION OF PARASITES

Many techniques for fixing, staining, and preserving parasites are described in detail in the books on general parasitology which are listed among the references at the conclusion of this paper. These, as well as shorter articles on special phases of this work, should be consulted. For present purposes, a few general statements on this subject should suffice.

All metazoan parasites should be washed free of mucus and body fluids before they are fixed. Tapeworms and acanthocephalids should be allowed to remain overnight in running tap water so that the musculature can become completely relaxed before the specimens are fixed in three to five percent formalin.

Nematodes should be fixed in hot (not boiling) 70 percent ethyl alcohol to which a few drops of formalin have been added. Flukes should be fixed in Gilson's fluid, of which the principal ingredient is mercuric chloride. Organs and tissue containing parasites or parasitic lesions should be cut into small pieces (not more than one centimeter in the smallest dimension) and
fixed in five percent formalin (33). Special fixatives may, of course, be used for special purposes, but many of these would not be needed in a laboratory where the chief interest was diagnosing disease.

Borax carmine is excellent for staining parasites for the study of internal structure. There are also many other stains that may be used for this purpose. In the hands of different investigators, some stains may be better than others. Any of the hematoxylin stains are excellent for staining parasites in the body tissues and organs.

For the preservation of parasites, 70 percent ethyl alcohol to which has been added glycerine and formalin in the amounts of five and three percent, respectively, is very satisfactory. Glycerine prevents the specimens from becoming brittle.

IDENTIFICATION OF PARASITES

A list of internal and external animal parasites of domestic animals in North America has been compiled by Dikmans (14). The mature worms and many of their larvae and the protozoan parasites encountered in animals may be identified by comparing the specimens recovered with descriptions published in the literature such as those found in Biester and Schwarte (5), Cameron (6), Kudo (24), Monnig (26), Morgan and Hawkins (27, 28), Neveu-Lemaire (29), and Yorke and Maplestone (40). A key to the immature parasitic stages of the gastrointestinal nematodes of cattle is available in the paper by Douvres (17). There are also many other general references as well as innumerable short papers from which this information may be obtained.

EVALUATION OF FINDINGS

In many instances the history, physical condition, and clinical symptoms presented by the sick animal, the history of the group to which it belongs, and the recovery of large numbers of internal parasites will all point to an undisputed diagnosis of internal parasitism. However, there is no exact relationship between the number of worms recovered and the physical condition of the animal that can be considered as indicative of the presence or absence of clinical parasitism because there are often many other factors that enter into the situation (1, 3). Among these are poor nutrition and the recent removal of parasites by the administration of anthelmintics or other drugs. In addition, one must realize that the parasite burden revealed at the post-mortem examination is representative of only a fraction of the total assault that has been made on the animal’s health up to the time it was brought to the laboratory. In order for the diagnostician to make a firm diagnosis of parasitism, the group to which the animal belongs should be under regular parasitological surveillance. If the evidence is limited to that obtained at the post-mortem examination it is obviously incomplete and in many instances would lead to an erroneous conclusion.
SUMMARY

The unusual opportunities for making important contributions to our knowledge of the pathogenesis, incidence, and geographical distribution of parasites and parasitic diseases of livestock offered to veterinarians located in laboratories established for the diagnosis of animal diseases were pointed out. Procedures were suggested for taking the clinical history, making the ante-mortem and post-mortem examinations, and for examining feces, blood, urine, and various animal exudates. The necessity for making thorough and complete post-mortem examinations of all animals brought to the laboratory was stressed and limitations placed on the interpretation of parasitological data obtained in this manner when the information on the past and present parasitological status of the group to which the animal belonged was not available. A few basic techniques for fixing, staining, and preserving parasites were described, and sources of information for obtaining further information on these procedures and the identification of parasites were appended.

ACKNOWLEDGMENTS

The writer wishes to acknowledge the assistance of Drs. L. A. Spindler, L. S. Diamond, E. E. Lund, and J. C. Lotze, and Miss Marion M. Farr, who provided information on the protozoa, and to express appreciation to Dr. K. C. Kates, who reviewed the paper and made helpful suggestions.
REFERENCES
DIAGNOSTIC LABORATORY & PARASITOLOGIC DIAGNOSIS 363


A SEROLOGICAL SERVICE FOR DOGS


Veterinary Virus Research Institute and Diagnostic Laboratory, New York State Veterinary College, Cornell University, Ithaca, New York

There are 26,000,000 dogs in the United States, but this figure includes mongrels as well as purebred dogs. Registration figures of the American Kennel Club show approximately 500,000 purebred dogs are registered each year and, with an average life of a dog calculated to be 10 years, there should be 5,000,000 purebred dogs. Ordinarily, mongrel dogs are not considered to have value except as consumers; therefore, if we assign an average value of $100 to each purebred dog, we have a $500,000,000 investment in dogs. Again omitting the mongrel, if we assign a consumer figure of $100 for each purebred dog, we have a $500,000,000 industry. Of course, the mongrel dog eats and many receive veterinary attention. This figure also is low. While this economic appraisal is incomplete, nevertheless in guarding the health of dogs, veterinarians have a tremendous responsibility for a significant element of America's economy.

Dog owners are not looking for government subsidy but can and will pay a reasonable fee for service. Any well conceived program, therefore, can be activated quickly without legislation. Furthermore, it is an area in which the individual animal counts and this makes serological and diagnostic service greatly appreciated. On this basis, a serological service for canine distemper, infectious canine hepatitis and leptospirosis is presented.

DISTEMPER VIRUS NEUTRALIZATION TEST

Materials. It is necessary to have in stock a virus preparation of known content. Stock virus can be prepared in the individual diagnostic laboratory or procured from another source.* For example, at present the Cornell Research Laboratory for Diseases of Dogs is supplying stock virus but it is hoped that a biological supply house will perform this service.

A good supply of fertile hen's eggs are necessary. These can be incubated at the laboratory or perhaps arrangements can be made with a hatchery for eggs already incubated for seven to eight days.

Serums, of course, are supplied by veterinarians for test purposes. Some instruction in bleeding dogs, preparation of serums and shipping directions will be necessary. Serums should be relatively free from contamination and not hemolyzed.

* If preparation of stock virus is to be done by an individual laboratory, a virus specimen and procedure will be supplied by a request addressed to Dr. James A. Baker, Veterinary Virus Research Institute, Cornell University, Ithaca, New York.
A SEROLOGICAL SERVICE FOR DOGS

Procedure. 1. Dilute stock virus to contain 300-500 egg ID₅₀ per 0.1 ml using BPSSP (buffered physiological saline solution with penicillin). Formula to make BPSSP: For stock solutions, dissolve 9.08 gm of KH₂PO₄ (potassium phosphate anhydrous monobasic) in a liter of distilled water, and autoclave. Dissolve 9.47 gm of anhydrous Na₂HPO₄ (sodium phosphate dibasic) in 1 liter of distilled water, and autoclave. Dissolve 8.5 gm of NaCl (sodium chloride) in one liter of distilled water.

For BPSS:  
NaCl solution .......................... 900 ml  
Na₂HPO₄ solution ..................... 80.8 ml  
KH₂PO₄ solution ..................... 19.2 ml  
*  
1000 ml  
Autoclave—pH should be 7.2

For BPSSP: Penicillin (10,000 units/ml) ............ 1 ml  
BPSS .................................. 100 ml

2. Dilutions of serum for test are prepared as follows:

<table>
<thead>
<tr>
<th>Serum Volume</th>
<th>Virus Volume</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 ml</td>
<td>0.8 ml BPSSP</td>
<td>1:5</td>
</tr>
<tr>
<td>0.1 ml</td>
<td>0.9 ml BPSSP</td>
<td>1:50</td>
</tr>
<tr>
<td>0.1 ml</td>
<td>0.9 ml BPSSP</td>
<td>1:500</td>
</tr>
<tr>
<td>0.1 ml</td>
<td>0.9 ml BPSSP</td>
<td>1:5000</td>
</tr>
</tbody>
</table>

Discard 0.1 ml from the last tube so that each tube contains 0.9 ml. Inactivates diluted serum in a water bath at 56°C for 15 to 30 minutes and then cool in the refrigerator at 4°C.

3. For serum-virus mixtures, add:

<table>
<thead>
<tr>
<th>Virus Volume</th>
<th>Serum Volume</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9 ml</td>
<td>0.9 ml of 1:5</td>
<td>10⁻¹</td>
</tr>
<tr>
<td>0.9 ml</td>
<td>0.9 ml of 1:50</td>
<td>10⁻²</td>
</tr>
<tr>
<td>0.9 ml</td>
<td>0.9 ml of 1:500</td>
<td>10⁻³</td>
</tr>
<tr>
<td>0.9 ml</td>
<td>0.9 ml of 1:5000</td>
<td>10⁻⁴</td>
</tr>
</tbody>
</table>

Shake mixture well and incubate in the refrigerator at 4°C for two hours.

4. Inoculation of eggs: Candle seven to eight day embryonated eggs and place those with viable embryos in racks with the air sac up. With swab, paint a small spot with iodine and, in the sterilized area, punch a hole through the shell. Meanwhile, the serum-virus mixtures have been placed in an ice water bath where they are kept while inoculating eggs. Using a two or three ml syringe with a 22 gauge 1" (1⅛" or 1½") needle, inoculate a group of five eggs each with 0.2 ml of a serum-virus mixture or, for virus control, 0.1 ml of virus by the method of Gorham (1). In doing so, the needle is held vertically and inserted through the hole in the shell only to cover the beveled end, after which inoculum is released, the length of the needle is plunged downward through the shell membrane and underlying membranes and then quickly withdrawn. Juxtaposition of the chorioallantoic
membrane to the shell membrane causes inoculum to spread between these membranes and over the CA membrane by capillarity. Incubate inoculated eggs for six to seven days at 37° C.

5. Reading test: Harvest eggs after six or seven days. To do so, break the shell at the small end, dump the embryo and yolk into a pail for discard and, using forceps, draw out the CA membranes. Wash the membranes in a pan of water and then place them in a dark pan. All eggs given a single test dilution are examined at one time. Spread out the membranes and look for lesions under a fluorescent lamp. A lesion or plaque will appear as a white opacity in the translucent membrane. One plaque is not considered evidence of infection, since it might be the site of inoculation, and two must be found for positive evidence of virus growth. Therefore, record as positive only those membranes with two or more plaques. Determine the ID$_{50}$ by the Reed-Muench formula. Naturally suppression of lesions indicates neutralization and titer of a serum is calculated on this basis. Titers are important only in the nomograph which is a test of antibody content of a bred female in order to predict the age at which to vaccinate her puppies. For a simple test of immunity, only a 1:100 dilution of serum is tested.

NEUTRALIZATION TEST FOR INFECTIOUS CANINE HEPATITIS

Materials. It is necessary to have a stock preparation of known virus content. Stock virus can be prepared in the individual diagnostic laboratory or procured from another source.* For example, at present the Cornell Research Laboratory for Disease of Dogs is supplying stock virus but it is hoped that a biological supply house will perform this service.

Dog kidney cell cultures can be prepared by the individual laboratory, although it is hoped that these can be supplied ready for use.

The same preparation of serum used for distemper tests applies to serum for infectious canine hepatitis.

Procedure. 1. From a previous titration of stock virus (usually 10$^{-6}$), calculate the dilution necessary to make 500-1000 TCID$_{50}$ per 0.1 ml. Make the prescribed dilution in buffered saline.

2. Serum dilutions: Prepared as for distemper, except BPSS is used instead of BPSSP.

3. Add 0.9 ml of diluted virus to each of the serum dilutions made above. Shake the tubes to insure mixing. This will give serum dilutions ranging from 1:10 to 1:10,000 (10$^{-1}$ to 10$^{-4}$) in a volume of 1.8 ml with each 0.2 ml containing 500-1000 TCID$_{50}$ of virus.

* If preparation of stock virus is to be done by an individual laboratory, a virus specimen and procedure will be supplied by request addressed to Dr. James A. Baker at the address previously given.
Virus controls are prepared as follows:

0.9 ml diluted virus is considered $10^{-0}$

$0.1 \text{ ml } + 0.9 \text{ ml BPSS} = 10^{-1}$

$0.1 \text{ ml of } 10^{-1} + 0.9 \text{ ml BPSS} = 10^{-2}$

$0.1 \text{ ml of } 10^{-2} + 0.9 \text{ ml BPSS} = 10^{-3}$

Incubate the serum-virus mixtures and virus controls for one hour at 37° C. in a water bath.

4. Inoculate a group of three tubes of dog kidney cells each with 0.2 ml of a serum-virus mixture or with 0.1 ml of control virus dilution. Three tubes must be left uninoculated to serve as cell culture controls. Incubate all tubes at 35° C.

5. The test is read at the time when there is complete degeneration in the virus control tubes inoculated with $10^{-2}$ dilution. If complete degeneration has not occurred in virus control tubes by the seventh day, the test is discarded, since after seven days spontaneous degeneration of cells may occur unless the maintenance medium is replaced. The test is also discarded if uninoculated tubes show degeneration before the seventh day. With satisfactory controls, any degeneration in a tube is regarded as negative (no neutralization). The titer of a serum is calculated as for distemper by the method of Reed and Muench. For a simple test for immunity, a 1-10 dilution of serum is considered positive if no degeneration occurs.

**AGGLUTINATION-LYSIS TEST FOR LEPTOSPIROSIS**

**Materials.** Cultures of *Leptospira pomona*, *L. canicola* and *L. icterhemorrhagiae* are maintained in Stuart's modified medium for use as antigens.

Serums are prepared as for a distemper test and should be uncontaminated. Hemolysis does not interfere with the test.

**Procedure.**

1. Cultures are incubated at 30° C. for seven days and held at room temperature for at least 14 days before use as antigens.

2. Dilution of serum:

$0.1 \text{ ml of serum } + 0.9 \text{ ml BPSS} = 1:10$

$0.1 \text{ ml of } 1:10 \text{ dilution } + 0.9 \text{ ml BPSS} = 1:100$

Further dilutions to determine serum titer are prepared in a similar manner except serum dilutions and saline are mixed in equal parts to make two-fold dilutions.

3. For serum-leptospira mixture, place 0.05 ml serum dilution in a spot plate and add 0.05 ml of antigen. Final dilution of serum is thereby increased twofold. Mixtures are then incubated at 30° C. for at least 30 minutes and readings completed within three hours after mixing.

4. Reading Test: One drop of mixture is removed to clear glass slide and examined under a dark microscope with 10X magnification. The 1:20 dilution gives preliminary results from which the 1:200 dilution can be examined.
critically. Agglutination-lysis in the 1:200 dilution is considered evidence for the presence of leptospiral antibodies. Titer is determined by similar examination with endpoint considered the greatest dilution that agglutinates and lyses leptospira.

The agglutination-lysis test as reported here indicates only the presence or absence of leptospiral antibodies. A positive test indicates a leptospiral infection which occurred at least seven to 10 days prior to obtaining the blood sample. The active disease might have occurred many months before, perhaps unnoticed, inasmuch as antibodies in this disease are known to persist for years.

Serological confirmation of acute leptospirosis depends on obtaining a blood sample at the time of acute illness (when the test is negative), and another sample at least seven to 10 days later (when the test is positive). The fact that the test changed from negative to positive during the period between tests indicates leptospiral infection.

DISCUSSION

The serological procedures presented here are not considered final. It is probable that modification will occur and certainly, if several laboratories become interested in offering this service, simplification should be expected as combined experiences are shared. Nevertheless, these procedures have been shown to be sufficiently accurate, especially the distemper test, to suggest their use and on October 15, 1958, a serological service to aid canine practitioners began in the Diagnostic Laboratory at the New York State Veterinary College. The service offered is presented in Table 1.
### Table 1

**SEROLOGICAL SERVICE FOR DOGS OFFERED BY THE DIAGNOSTIC LABORATORY AT THE NEW YORK STATE VETERINARY COLLEGE, ITHACA, NEW YORK**

<table>
<thead>
<tr>
<th>Laboratory Accession No.</th>
<th>Veterinarian</th>
<th>Owner</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Address</td>
<td>Address</td>
</tr>
<tr>
<td></td>
<td>Animal's name or number</td>
<td>Age</td>
</tr>
<tr>
<td></td>
<td>Date bred</td>
<td>Date expected to whelp</td>
</tr>
<tr>
<td></td>
<td>Date serum taken</td>
<td>Date rec'd at laboratory</td>
</tr>
<tr>
<td></td>
<td>Condition of serum when received at laboratory</td>
<td></td>
</tr>
</tbody>
</table>

(Hemolyzed and contaminated samples will be discarded)

**PREVIOUS VACCINATION HISTORY:**
- Distemper type used: (underline) Inactivated tissue, Modified live: (egg origin) (ferret origin)
- Hepatitis type used: (underline) Inactivated tissue, Modified live
- Date of last vaccination: Distemper___ Hepatitis___
- Has there been history of distemper within the last year?___

**TEST REQUESTED:** (underline)
- Nomograph (includes 30-day post-vac. immunity check on 2 puppies)___ $10.00
- Distemper immunity check ___ 2.50
- Hepatitis immunity check ___ 2.50
- Post-infection titer increment: Distemper Hepatitis ___ 10.00
- Leptospirosis: L. canicola ___ L. pomona ___ each ___ .35 L. icterohemorrhagiae ___

**LABORATORY FINDINGS:** Nomograph titer___ Age to vaccinate puppies___
- Immunity test: Distemper___ Infectious canine hepatitis___
- Post-infection titer increment: Distemper___ Hepatitis___
- Leptospiral results ___
- Date reported ___ By ___
The serological service for distemper was planned to give information on three phases of practice. For determination of immunity (2), a 1:100 dilution of serum is tested. If it fails to neutralize, then revaccination is recommended although some dogs with titers lower than 1:100 are immune. The 1:100 dilution was chosen since it has been shown to be the lowest titer that indicates complete immunity in all dogs.

In another use, serum is taken from a potential mother one month after she is bred in order to predict the age to vaccinate her puppies even before they are born (3). This is possible since it has been found that the antibody titer of a mother prior to whelping determines the amount and duration of colostral protection received by her puppies and, therefore, the age at which they can be actively immunized. A nomograph (Figure 1) has been constructed which permits an easy determination of the age to vaccinate after the serum titer of the mother has been determined. The nomograph was field tested with success on 57 dogs from 13 litters where the age of vaccination ranged from four to 12 weeks, and a statistical analysis indicated that use of the nomograph will result in at least a 95 percent vaccination success rate. This procedure is expected to supplant the practice of giving antiserum for temporary protection of young puppies. In order to have protection from antiserum, puppies must receive approximately 100,000 Cornell antibody units per pound of body weight, and serums have not been standardized on this basis as yet. The protective capacity of antiserum therefore is unknown. Furthermore, cost of the nomograph service should be no greater than serum

![Fig. 1. Nomograph for prediction of age to vaccinate puppies against distemper.](image-url)
and easier, since it does not involve individual handling of puppies. It would seem a most useful application of the serological service.

A final use was designed for diagnosis of dogs whose clinical signs do not permit a conclusion. For this test, serum should be procured from a sick dog during the acute phase of illness and again 10 to 14 days later. A rise in antibody titer against distemper is proof that the illness was due to distemper and not something else. However, failure to show an increase indicates the illness probably was not due to distemper.

A nomograph service has not been developed for infectious canine hepatitis as yet, although studies are underway. At present, a test of a 1:10 dilution of serum indicates immunity if there was neutralization (4). For diagnostic purposes, serum should be collected as for distemper and tested for increase in antibodies. The serological service offered for leptospirosis is useful as a diagnostic aid. Also, the test will detect any previous infection and this is important since dogs with a leptospiral titer should be treated with antibiotics to eliminate the carrier condition.

SUMMARY

Methods are presented for detection of immunity against canine distemper, infectious hepatitis and Leptospira canicola, L. pomona and L. icterohemorrhagiae. Quantity of distemper antibodies also can be determined for application of a nomograph which predicts the age to vaccinate puppies before they are whelped. Other applications of the serological service are discussed.

REFERENCES

The National Research Council will soon publish a manual entitled, "Methods for the Examination of Poultry Biologics." Over 30 people in the state experiment stations, Federal Government and privately owned laboratories have participated directly in its preparation. The subject is more extensive than the title would suggest. Most of the laboratory procedures that are employed in the study of infectious diseases of poultry are treated.

How did this manual come to be written? In 1952, the poultry pathologists of the state experimental stations, who had been organized into four regional technical committees on respiratory diseases of poultry, delegated to an Interregional Subcommittee the responsibility of suggesting a better approach to the evaluation of vaccines. This Subcommittee, after meeting with representatives of the vaccine producers and of the regulatory agencies, decided that there was a need for higher standards for potency and safety. However, the first step in setting up minimum standards was a definition of methods to be used in establishing these standards. This was a stumbling block. The Subcommittee then dropped the matter of minimum standards and tackled the definition of standard methods.

Information on methodology was scattered in the literature. Basic procedures were sometimes buried in journals that had been published several decades earlier and sometimes were found in the most recent issues. Useful, technical information was located in the station reports that had had very little circulation. Everyone on the Committee could cite instances in which the original report had been in error and the error had not been corrected, or in which the technique had been simplified and the simplification had not been published. We also knew of pitfalls that had beset most workers following a certain procedure and yet nowhere were there warnings of these pitfalls. The complete lack of information on many subjects was startling, particularly, of comparative information. Pleuropneumonia-like organisms, when injected into the infra-orbital sinus of a chicken, produced a marked swelling but what happened when other agents were injected: fowl pox or Newcastle disease virus for example. We obviously needed a thorough going, critical review of the literature by someone who had been actively engaged in poultry disease investigations. It was obvious that only one who had grown up with the field and had kept in contact with others that were active in it could be well-informed. Not all workers who must employ the diagnostic or evaluative procedures could be expected to have such a background. Nor did anyone active in the field have the breadth of experience in all
poultry infections and in both research and diagnostic laboratories to write authoritatively the manual that we needed.

The compromise was to get a group to prepare the manual. This approach is slower and more cumbersome but as the need was well recognized we found cooperation at every turn. The Subcommittee which represented the regional technical committees became the Editorial Board. It had several decisions to make: first, what to include and exclude. This was a difficult problem. Obviously, we were concerned with the four primary poultry vaccines: Newcastle disease, infectious bronchitis, fowl pox and infectious laryngotracheitis. We needed methods for identifying these viruses and for characterizing strains of these viruses in order to define their purity. We needed quantitative methods for determining the potency and safety of vaccines prepared for these viruses, but we needed also methods for detecting contaminants which might occur in the vaccines and methods for determining whether the eggs used in the preparation of vaccines and chickens used in the testing of vaccines were free of complicating diseases. It meant dealing with most of the techniques used for the study of infectious diseases of poultry.

Another basic problem concerned the status of the methods that would be described in the manual. We might place these in three categories: (1) proposed and untested methods (2) methods that have been used but may need improvement (3) methods which can be approved as tested and sound. If we limited the methods to be included in the manual to the last category, the manual would be very short and most incomplete. We decided to include many of the second category and even a few of the first, indicating when those of the first category were used that such methods were only proposed on the basis of limited experimentation.

With some agreement as to the contents of the manual and the approach to it, the format was worked out next.

The manual was divided into sections and into chapters. Matters of style, the question of references and how they should be cited was settled and the timetable of operation was set up. Most of you are aware that while committees can plan and rewrite a book an individual must do the writing. We selected to prepare the chapters individuals whom we felt to be particularly competent. The response was most satisfying. Only one individual turned us down and he was seriously ill. When the first drafts of the chapters were received by the Editorial Board (I hope you do not think that everything happened with such a logical sequence and precision), they were sent to referees, who had a background and viewpoint which differed considerably from that of the author. Most of the authors and referees were contacted by mail and correspondence among members of the Editorial Board was carried out in the same way. Last May, however, about 30 of the authors, referees and editors got together at East Lansing and in less than three days hammered out most of the differences which still existed. The Committee on the detection and identification of pleuropneumonia-like organisms, for example, consisted of Dr. H. Van Rockel of Massachusetts, Doctor Adler of California, and Doctor Gentry of Pennsylvania. The referee system elimin-
ated pet methods and introduced alternative methods of equal merit. It assured us that the chapter reflected the mature viewpoint of several competent individuals.

The contents of the manual can be described briefly. There are 10 chapters. The introductory chapter is concerned with definitions. What is the normal chicken? What conditions constitute adequate isolation? Should birds that are on potency tests receive coccidiostats? Under what conditions is an embryonated egg suitable for culture of virus? The second chapter is concerned with methodology. What is an adequate sample? How may a virus be titrated? How can the endpoint be evaluated? The next four chapters deal with the viruses for which the vaccines are now prepared: Newcastle disease virus, infectious bronchitis virus, fowl pox virus and infectious laryngotracheitis virus. The methods of identifying these viruses and their strains, of determining their potency, safety and purity are described. The remaining chapters are concerned with methods of detecting possible contaminants which may occur in vaccines or of detecting latent infections of chickens which would affect the evaluation of vaccines. The potential danger in each instance is discussed. These chapters deal with pleuropneumonia-like organisms, with Salmonella, with miscellaneous bacterial and mycotic infections and with miscellaneous viral infections.

The Editorial Committee feels that this manual will be very useful to diagnosticians as it will answer many of their questions dealing with techniques and procedures. We know that it will be used as a supplemental manual in classroom teaching and will be extensively used by research workers in training laboratory technicians. Most important, it has stimulated many of us to see the need for further research on techniques. Basic research is dependent on methodology. A new instrument and a new test frequently open the field which could not be explored previously. In the development of methods, closer cooperation is needed between diagnostic laboratories and research laboratories.

We need to consider three types of development. The first is a new procedure. This is the application of a new idea to diagnosis. A good example is the discovery of a method by Coons of conjugating fluorescent dye to an antibody. The fluorescent antibody can be used to reveal the presence and site of its antigen. This is an excellent research tool and it is also a practical diagnostic aid. For example, smears of vesicular vesicle fluids prepared in the field and air dried may be later stained in the laboratory with a drop of fluorescent conjugated antibody and then examined with an ultraviolet microscope. The staining and examination can be done in less than an hour. It is a rapid and simple test for vesicular stomatitis. Second is the simplification of a method. Simplification can transform a procedure from one that is useful only in a research laboratory to one that is also of great importance in a diagnostic laboratory. The development of a stable fluorescent conjugated antibody made the fluorescent-antibody technique practical for laboratory diagnosticians. The reagent may now be prepared in a central laboratory and stored until needed in the diagnostic laboratory. The third type of
TECHNIQUES FOR EVALUATING POULTRY VACCINES

development is that of logistic invention. This concerns ways and means of getting diagnostic material in a suitable form for testing and in suitable quantities from the farm to the laboratory. I can cite several examples. The discovery that the titer of antibody in the yolk of the egg parallels the titer in the serum of the chicken makes possible to submit eggs rather than bleed chickens. In many instances, eggs are more readily obtained and shipped by the lay cooperators than is blood. The discovery that blood may be absorbed on filter paper discs, dried and then hours or weeks later its antibody eluted from the disc for testing simplifies collection and shipment. We have been interested in finding the extent of certain livestock diseases among wild animals. Game wardens who carry a kit containing the paper discs and suitable envelopes have supplied us with samples of blood obtained from wild birds and animals killed along highways. Freed of the problem of packing and shipping blood tubes, veterinarians are more willing to supply materials needed for special serological surveys. Still another adaptation of the blood discs has been reported. Some viruses which are present in whole blood can be dried without complete inactivation and infective material recovered a week or more later. Both western equine encephalomyelitis and Newcastle disease viruses have been handled in this way. The possibility of making isolations of both viruses and bacteria from material collected several hundred miles away from the diagnostic laboratory is increased by simple collection and rapid shipping methods.

Any manual of diagnostic methods must be subject to amendment as new ideas are presented and accepted. The principal reason for selecting the National Research Council as a publishing agency of the manual of “Methods for Examination of Poultry Biologics” was because the National Research Council has several established handbooks which are periodically revised. One has only to look at the “Standard Methods of Official Agricultural Chemists” and of “Standard Procedures for Water Analysis” to see not only the value of a manual of diagnostic methods but also how the value and importance of such handbooks increase with revision.
HEPATOPATHIES IN CHICKENS

R. W. WICHMANN AND R. A. BANKOWSKI

The problem of hepatopathies in chickens has increased in importance with reports in the last few years of liver degenerations of infectious, toxic, and unknown etiology. Inflammatory changes due to infectious agents, often occurring secondarily to, or in association with, pathological changes in other organs, have long been recognized in poultry livers. Examples are: fowl cholera, Salmonellosis, tuberculosis, chronic pseudo-tuberculosis, Coli granuloma, listeriosis, erysipelas, spirochetosis, ornithosis, aspergillosos, visceral lymphomatosis, avian monocytosis, streptococcosis, histomaniasis and trichomoniasis. The differential features of these conditions are familiar to the average diagnostician, and hence not included in this report.

In 1954 Tudor (1) described a primary liver degeneration of unknown etiology that had been observed for many years in chickens in New Jersey. The following year Lucas (2), in California, described a hepatitis in chickens and described an infectious agent. In the same year Delaplane et al. (3) reported, in Texas, an infectious hepatitis that appeared to be distinct from those previously reported. In 1957 a hepatitis apparently similar to Delaplane's was reported in Massachusetts, by Winterfield and Sevoian (4).

Toxic degeneration of livers in chickens, caused by feeding toxic fats, was reported in 1958 by Schmittle et al. (5), Naber et al. (6), and Edgar et al. (7).

Liver pathology accompanying synovial inflammations in avian infectious synovitis was reported in West Virginia by Olson et al. (8), in Delaware by Cover et al. (9), and in Texas by Wills (10). The disease was recognized in California in the spring of 1955 (11).

Peckham (12) and Hofstad et al. (13) recently reported independently on isolating a vibrio from the livers of chickens with a primary liver infection.

The recent literature reveals that the history, clinical signs, gross pathology, and peripheral blood pictures are sufficiently similar to make very difficult a differential diagnosis among hepatopathies.

AVIAN VIBRIONIC HEPATITIS

It appears from the reports that the diseases described by Delaplane et al. (3), Winterfield et al. (4), Packham (12), Hofstad et al. (13), Moore (14), and Sevoian et al. (15) are caused by the same agent. *Avian vibrionic hepatitis* (Peckham's term) is used herein to differentiate this disease from hepatopathies due to other causes. AVH is discussed in detail, and the other diseases considered in relation to it.

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HEPATOPATHIES IN CHICKENS

Clinical.—AVH has been observed both in mature chickens and, less frequently, in the growing birds in replacement flocks. Both light and heavy breeds are affected. Onset is insidious, with only a small percentage of the birds affected at any one time, yet mortality over several weeks may reach 10 to 15 percent. Egg production lags in pullet flocks, and in laying flocks decreases as much as 35 percent. Shrunken, dry combs, lethargy, and often emaciation characterize affected birds.

Gross Pathology.—The most obvious lesions observed in the livers of older birds are degenerative and hemorrhagic changes. The severity of lesions may vary between birds and between liver lobes in the same specimen. Some livers are slightly enlarged and mahogany brown; others are markedly enlarged, with necrotic foci varying in diameter from one mm. to one cm. The larger lesions are irregularly round, and described as asterisk—or cauliflower-like. Hematocysts are occasionally present under the liver capsule. Hearts and kidneys are enlarged and pale, and ascites and hydropericardium may be present. A catarrhal enteritis is occasionally observed. In younger chickens the gross liver lesions are less evident, with the heart lesions predominating.

Histopathology.—The histopathology is characteristic of a chronic nonsuppurative hepatitis.

Microscopically, early infections are characterized by lymphocytic infiltration, occasionally with heterophiles in the portal areas, and accompanied by congestion, hemorrhage, and early degenerative changes. As the disease progresses, lymphocytic and granulocytic foci and focal necrobiosis are the predominant changes. Connective tissue proliferates around the portal and interlobular areas. Proliferation of bile duct epithelium and development of additional bile ducts are common. Granulomatous areas, with fibrinoid necrosis, may occasionally be seen. The liver capsule is sometimes edematous, and infiltrated with inflammatory cells. In advanced cases the spleen exhibits decreased lymphopoietic activity, with granulomatous lesions on occasion. The kidneys show focal areas of cellular infiltration, and occasional focal areas of degeneration. Large immature myelocytes increase in number in the bone marrow. Mononuclear cell infiltration of the epicardium and myocardium is common in young birds.

Hematology.—In blood studies of experimentally infected birds it was found that heterophiles, thrombocytes, and total leucocytes increased, while erythrocyte and lymphocyte counts decreased.

Diagnosis.—AVH can be diagnosed definitively by isolating the agent—from the bile by streaking blood agar plates and incubating them at 37.5 C. in an atmosphere of 10 percent CO2, or from the liver by inoculating five-to seven-day-old embryonating chicken eggs via the yolk sac. Primary isolations on artificial media show no colonies in 18 to 20 hours; however, a smear of the agar stained three minutes with safranin or basic fuchsin will reveal the organisms in large numbers. After further incubation, or serial
passage on blood agar, a more readily detectable mucoid growth or individual colonies of the organisms can be observed. The vibrio is sensitive to aureomycin, terramycin, thallium acetate, streptomycin, and, to a much lesser extent, penicillin; therefore, treatment of liver tissue with these antibiotics will prevent isolation of the agent. Although some variation was observed in strain susceptibility to the antibiotics, bacitracin (5,000 u/ml) did not destroy the agents tested.

Embryos inoculated by the yolk-sac route on the first passage usually die on the third or sixth post-inoculation day. The dead embryos exhibit cutaneous and subcutaneous congestion and hemorrhage. Embryo deaths occur later if the inoculation route is the allantoic cavity, and the lesions seen in these embryos consist of enlarged, pale spleens and greatly enlarged livers showing congestion and focal degeneration.

HEPATOPATHY CAUSED BY TOXIC FATS

Hepatopathy due to toxic fats (5, 6, 7) was studied in chickens fed the known toxic feeds. Egg production of laying hens decreased at two weeks, and dropped 65 percent by the third week. Hatchability decreased, and embryos from affected hens showed considerable edema. The lesions in birds were characterized by hydropericardium, ascites, and subcutaneous edema. Hearts and livers doubled in size as the disease progressed. The livers varied from normal color to a red and yellow mottled appearance. In the later stages of the disease the liver became shrunken and brownish, with a roughened surface. Microscopically, the parenchymal cells die and are replaced by connective tissue. There is a leucocytosis due to an increase of heterophiles in the peripheral blood.

The differential features in this condition are the absence of an infectious agent, the edematous embryos, retention of body fluids, lack of inflammatory cells in the liver lesions, and the leukocytosis in the absence of an anemia in the peripheral blood.

AVIAN INFECTIOUS SYNOVITIS

Avian infectious synovitis (AIS) is a disease of chickens that principally affects growing replacements and broiler chicks and is characterized primarily by a tendovaginitis. However, in addition to involving the synovial membranes, the disease often affects the liver, spleen, and kidneys, especially during the septicemic stage. During the early stages of the disease, the liver is swollen, rose-colored and mottled. As the disease progresses, the liver tends to become greenish and smaller. The absence of focal areas of necrosis in a diffusely involved organ helps differentiate this disease from AVH and pullets' diseases. However, chronically affected birds often show no apparent liver involvement.

Histologically, the lesions observed in the liver are those of an acute nonsuppurative hepatitis. In the early stages of the disease, congestion is soon followed by a mononuclear infiltration in the portal areas, which, in experi-
mentally infected birds, reaches its maximum by the fifth day. At this time some degenerative changes are seen, primarily cloudy swelling and fatty degeneration, with no widespread hepatic necrosis. Focal areas of predominantly mononuclear cells, with some heterophile infiltration, become disseminated throughout the organ. As the disease progresses, the liver appears to return to its normal state without evidence of cirrhosis.

Concurrent with these changes is the development of an anemia. Hematocrit readings in experimental infections may fall to as low as eight percent in 10 days. Buffy coats increase during this period, partially because of immature red cells and principally because of leucocytosis. Olson et al. (16) and Savoian et al. (17) have shown that the leucocytosis is mainly due to an increase in monocytes and heterophiles, while lymphocytes decrease. In the majority of instances, or in all, the birds present the typical and characteristic viscid exudate in the synovial bursae.

It has been stated that avian infectious synovitis is caused by a viral agent; however, the evidence has been conflicting. The infectious agent has not been grown on any artificial medium, though it can be cultivated in embryonating chicken eggs or in tissue culture. Electron photomicrographs by Lecce et al. (18) have shown the organisms to occur intracytoplasmically and to be cytopathogenic for tissue cultures. The period between yolk-sac inoculation of five- to seven-day-old embryos and embryo death will vary with concentration of the infectious agent. Deaths may occur between the fourth and thirteenth day; however, a straight-line relationship has been observed when the mean day of death is plotted against the log ELD50 concentration of the AIS agent. Lesions in early embryo deaths consist of cutaneous hemorrhages, whereas later deaths are characterized by enlarged, mottled livers, enlarged spleens, and hemorrhagic kidneys. In no instance has arthritis or synovitis been observed in the dead embryos.

Differential diagnosis is based on the absence of focal areas of necrosis in the liver involvement. The disease is usually found in young growing stock, and the tendons and joints contain a viscid, yellowish-white exudate that contains large numbers of heterophiles and mononuclear cells. Histologically, the liver lesions do not show areas of necrobiosis or the proliferation of connective tissue observed in AVH.

UNCLASSIFIED HEPATOPATHIES

The liver degeneration of chickens observed and described by Tudor (1) appeared, grossly and histologically, to be most like AVH. The flock history of the disease, too, is not unlike AVH. However, repeated attempts failed to isolate an infectious agent or to reproduce the disease experimentally. This fact, at present, appears to be the primary criterion for differentiation. The absence of gross or histological changes in the heart, as described in AVH, may also serve to differentiate these two diseases.

Avian infectious hepatitis, as described by Lukas (2), is caused by a fastidious, pleomorphic, Gram-negative, coccoid to yeast-like organisms. The course of this disease may be peracute to chronic. Peracute or acute cases
were observed in broilers that appeared in good flesh but showed signs of cyanosis of combs and wattles. Necropsy of these birds revealed dehydration, enlarged, soft, purple to blackish livers, and swollen spleens and kidneys. Histopathological sections of these livers revealed congestion, lymphoid infiltration, and in the portal areas, proliferation of connective tissue with degenerative changes in the adjacent hepatic cells. Chronic cases were observed in laying flocks in which the disease was most prevalent. Affected birds showed depression, progressive emaciation, and shrunken combs, and stopped laying. Morbidity in a flock reached 20 percent, with a daily cull rate of two to three percent. At necropsy, the spleen and kidneys often showed evidence of congestion, but the most obvious change was in the liver. The gross liver changes were of two types, ranging from soft, friable, enlarged livers with surfaces varying from smooth to granular and of variegated colors of red, green, and purple to bronze and chocolate, to cirrhotic livers, which were a dull gray and somewhat enlarged. Ascites was usually seen with the cirrhotic form. Histological sections of these chronically involved livers showed a proliferation of connective tissue, which usually initiated in the periportal areas and occasionally involved entire lobules. The infectious agent was demonstrated to grow on enriched blood agar plates, in brain-heart infusion broth with 20 percent avian serum, or in embryonating chicken eggs. Lukas said a tentative diagnosis may be made on the basis of flock history and gross pathology, with confirmation by isolation of the causative agent and histopathological findings.

**SULFAQUINOXALINE TOXICITY**

Many cases involving the liver that are presented for diagnosis do not fit the conditions described above. A condition frequently seen simulates in part the blue comb syndrome. The complaint is a sudden drop in feed consumption, decrease in egg production of laying flocks, listlessness, and shriveled and dried combs, accompanied in some cases with diarrhea or excess urates in the droppings. The daily morbidity and mortality rates are relatively low. In all instances the history preceding this complaint is one of treatment with sulfaquinoxaline (SQ) for an outbreak of coccidiosis, often followed by other therapeutic measures to combat the condition that develops after using SQ. SQ toxicity has been observed in broilers, replacement pullets, and young layers.

On examination, the birds appear weak and are often lame. There is a distinct pallor of the cutaneous tissues about the face, and in some instances the wattles may be edematous or engorged with blood. The legs are often dry and shriveled, showing red streaks along the shanks, probably caused by passive congestion resulting from the enlarged kidneys. On autopsy, the liver is swollen with varied discoloration due to congestion or anemia. The spleen and kidney may be swollen and congested, and in more chronic cases may exhibit white-grayish granulomatous foci 0.5 to one mm. in diameter. Petechial and ecchymotic hemorrhages are often seen in the myocardium, skeletal muscles, intestine wall, and proventriculus, simulating hemorrhagic
disease (19). The gizzard often contains feed discolored with hematin, and the lining may be severely eroded. The most conspicuous finding is a severe anemia. Hematocrit values of seven to 10 mm. are not uncommon, and the buffy coat may be barely detectable. Microscopic examination of the peripheral blood reveals a high percentage of immature red blood cells and an agranulocytosis. The bone marrow is distinctly hypoplastic.

Microscopically, the liver in acute cases reveals some areas of degeneration and necrosis. The sinusoids appear distended, and vacuolation of hepatic cells is observed. Delaplane and Milliff described distinct granulomatous lesions in the liver and other organs in chronic cases of SQ poisoning (20).

The differential diagnosis can be made on a history of SQ treatment, hemorrhagic tendencies, marked hypoplastic anemia, agranulocytosis, and the absence of an infectious agent.

BLUE COMB DISEASE

Jungherr (21) described blue comb as principally a disease of young laying chickens; however, it has been observed in birds still in the growing stages and also in old layers. The disease, usually occurring in the hot months, is characterized by depression, decreased feed consumption, diarrhea, darkening of the head, and a cyanotic comb. Layers show a severe drop in egg production. The morbidity is usually low (five percent), but may reach 50 percent. The lesions observed in the liver are sometimes congestion, but most typically an evenly spaced studding of round yellowish areas about one mm. in diameter, often with minute hemorrhagic centers. Lesions in other organs may consist of congestion of the skeletal muscles (often with a "fish-flesh" consistency), chalky spots in the pancreas, scattered punctiform hemorrhages on the serosal surfaces, and an enlargement of the kidneys.

Microscopically, the liver may show areas of necrosis without particular zonal orientation. The necrotic foci undergo secondary polynuclear infiltration, which is later replaced by regenerating liver cells. There is a biliary stasis, especially of the larger ducts.

The most characteristic feature of this disease is dehydration and hemoconcentration. Hematocrit readings may be higher than normal, and the differential count reveals an increase in monocytes in the peripheral blood.

Differential Diagnosis.—Differential diagnosis of this disease will depend on the presence in the liver of typical lesions differing from those in AVH, on the gross pathology in the pancreas, and on hemoconcentration and the relative and absolute increase of monocytes in the peripheral blood. No infectious agent has been demonstrated.

It is obviously impossible in a paper of this length to discuss fully all of the known causes of hepatopathies in chickens. The diseases selected for discussion represent those of recent interest and similarity which often require differentiation from each other. From the discussion it becomes clear
that a diagnosis based solely on gross observations is inadequate and that histological, hematological and microbiological techniques must be employed for the differentiation of these hepatopathic syndromes.

Our present knowledge of many of the hepatopathies is limited and inadequate with regard to the occasional idiopathic hepatopathies encountered. However, every effort should be made to correctly diagnose each disease so that the treatment recommended will be effective and not unnecessarily costly to the poultryman.

REFERENCES

HEPATOPATHIES IN CHICKENS


<table>
<thead>
<tr>
<th>Disease</th>
<th>History</th>
<th>Pathology</th>
<th>Other Organs</th>
<th>Blood</th>
<th>Etiological Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avian Vibrionic Hepatitis</td>
<td>Principally older birds</td>
<td>Hepatomegally, necrotic foci, hematocysts</td>
<td>Mononuclear infil. and proliferation; connective tissue proliferation</td>
<td>Enlarged heart and kidneys; occas. ascites and hydropericardium</td>
<td>Anemia, leucocytosis (＞ heterophiles and thrombocytes; ＜ leucocytes)</td>
</tr>
<tr>
<td>Toxic fats</td>
<td>All ages</td>
<td>Roughened surface, cirrhotic in later stages</td>
<td>Necrobiosis and connective tissue proliferation</td>
<td>Enlarged heart, hydropericardium, ascites, subcutaneous edema</td>
<td>Leucocytosis (＞ heterophiles)</td>
</tr>
<tr>
<td>Avian Infectious Synovitis</td>
<td>Principally younger birds</td>
<td>Diffuse hepatomegally, no necrotic foci</td>
<td>Mononuclear infil. and proliferation; no connective tissue proliferation</td>
<td>Enlarged spleen and kidneys; tendovaginitis</td>
<td>Anemia, leucocytosis (mainly monocytes and heterophiles, ＜ lymphocytes)</td>
</tr>
<tr>
<td>Liver degeneration of Tudor</td>
<td>Principally older birds</td>
<td>Hepatomegally, necrotic foci, hematocysts</td>
<td>Lymphocytic infiltration, hemorrhages, necrobiosis and connective tissue proliferation</td>
<td>Kidneys enlarged</td>
<td>Unknown</td>
</tr>
<tr>
<td>Hepatitis of Lucas</td>
<td>All ages</td>
<td>Hepatomegally and cirrhosis</td>
<td>Lymphoid infil. and connective tissue proliferation</td>
<td>Enlarged spleens and kidneys; often ascites</td>
<td>Anemia, agranulocytosis</td>
</tr>
<tr>
<td>Sulfquinaoxaline toxicity</td>
<td>All ages</td>
<td>Hepatomegally with occas. small granulomas</td>
<td>Necrobiosis, granulomas in chronic cases</td>
<td>Spleen and kidneys often enlarged; hemorrhagic lesions; hypoplastic bone marrow</td>
<td>Anemia, agranulocytosis</td>
</tr>
<tr>
<td>Blue Comb Disease</td>
<td>All ages</td>
<td>Hepatomegally often with necrotic foci containing hemorrhagic centers</td>
<td>Necrosis without zonal orientation; polynuclear infiltration</td>
<td>Enlarged kidneys, Zenneker's degeneration of breast muscle, necrotic foci in pancreas</td>
<td>Hemoconcentration, Monocytosis</td>
</tr>
</tbody>
</table>

＞ = Increase.
＜ = Decrease.
CONSTITUTION AND BY-LAWS
OF THE
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

ARTICLE I—NAME
The name of this Association shall be "The United States Livestock Sanitary Association."

ARTICLE II—PURPOSE
The purpose of this Association shall be the study of livestock sanitary science, milk and meat hygiene, and the dissemination of information relating thereto, the unification so far as possible of the laws, regulations, policies and methods pertaining to milk and meat hygiene, and to the prevention, control and eradication of transmissible livestock diseases; to maintain co-ordination among the various livestock regulatory organizations, and to serve as livestock sanitary science clearing house between this Association and the following: The livestock owner, the livestock sanitarian, the milk and meat hygienist, the veterinary practitioner, the transportation and stock yard companies, the milk and meat producing and distributing companies, and various other interested agencies. The word "livestock" as herein used shall be understood to include poultry.

ARTICLE III—MEMBERSHIP
There shall be three kinds of members—Official and Individual and Non-Voting Junior.

OFFICIAL MEMBERSHIP
The livestock sanitary departments of each state also the United States, and the Canadian, Cuban and Mexican governments, The Territories, Puerto Rico, the Virgin Islands and Los Angeles County, California shall be eligible to official membership in this Association and be represented on the Executive Committee by the livestock sanitary executive official.

INDIVIDUAL MEMBERSHIP
Any person engaged in livestock sanitary work for Federal, provincial, state, territory, county or municipal governments and any other person interested in livestock sanitation or milk and meat hygiene may be elected to individual membership.
31 JUNIOR NON-VOTING MEMBERSHIP

32 Students in agriculture, medicine, veterinary medicine, vocational agriculture or any 4-H Club member as well as future farmers under 21 years of age are eligible to election as junior non-voting members.

35 ARTICLE IV—MEETINGS

36 The meetings of this Association shall be annual and special.

37 ARTICLE V—OFFICERS

38 The officers of this Association shall be: President, First Vice-President, Second Vice-President, Third Vice-President, Secretary-Treasurer, and an Executive Committee.

41 EXECUTIVE COMMITTEE

42 The Executive Committee shall be composed of the executive officer representing the livestock sanitary departments of the various States and Territories, the Chief of the United States Bureau of Animal Industry, the Veterinary Director General of Canada, the executive regulatory officer of Cuba and Mexico, The Territories, Puerto Rico, the Virgin Islands, Los Angeles County, California and the elective officers of this Association.

49 The Executive Committee shall constitute the administrative body of this Association and shall determine its activities and policies.

51 All recommendations and reports of officers and committees shall be referred for consideration to the Executive Committee.

53 The First Vice-President shall be ex-officio chairman of the Executive Committee.

55 The Executive Committee shall elect yearly a Secretary-Treasurer for the Association. The Secretary-Treasurer shall receive such salary and allowance as may be fixed by the Executive Committee.

58 The Executive Committee shall cause to be audited annually or oftener if deemed necessary, the receipts and disbursements of the Secretary-Treasurer, and shall have authority to hear and determine all complaints filed before it in writing relative to the conduct of any member; and shall have authority to accept or reject applications for individual membership properly placed before them. Three negative votes shall disqualify for such membership.
ARTICLE VI—PROGRAM COMMITTEE

The President, the Chairman of the Executive Committee and the Secretary-Treasurer and the Chairman of the respective committees shall constitute the Program Committee. It shall be the duty of the officers of the Program Committee to make the necessary arrangements and provide the program for the annual and special meetings.

ARTICLE VII—DUTIES OF OFFICERS

1. President: It shall be the duty of the president to preside at all meetings of this Association; to appoint all committees excepting the Executive and Officer faction of the Program Committee; to call special meetings of the Association whenever he considers the holding of such meetings necessary for the good of the livestock industry or upon the written request of five members of the Executive Committee. The president shall be an ex-officio member of all committees.

2. First Vice-President: The first vice-president shall be chairman of the Executive Committee. In the absence of the president, he shall preside at the meetings of the Association. In the event of the absence, disability or resignation of the president he shall perform all duties of the president. He shall be an ex-officio member of the Executive and Program Committees.

3. Second Vice-President: The second vice-president shall assume the duties of the president in the event of the absence, disability or resignation of the president and first vice-president. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability or resignation of the first vice-president. He shall be an ex-officio member of the Executive Committee.

4. Third Vice-President: The third vice-president shall assume the duties of the president in the event of the absence, disability or resignation of the president, first vice-president and second vice-president. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability, or resignation of the first and second vice-presidents. He shall be an ex-officio member of the Executive Committee.

5. Secretary-Treasurer. The Secretary-Treasurer shall keep an accurate record of the proceedings of the Association. Whenever authorized so to do by the Executive Committee he shall publish said proceedings and distribute them to the members of the Association. The Secretary-Treasurer shall also keep an accurate record of the proceedings of the Executive Committee and shall furnish a copy to each member of said Executive Committee. He shall forward to each Executive Committee member a copy of each regulation approved by the Association.
CONSTITUTION AND BY-LAWS

He shall keep an accurate account of all Association moneys received and disbursed. He shall also present to the Chairman of the Executive Committee a list giving the name, occupation and address of each applicant for individual membership for the approval of the Executive Committee. He shall perform such other duties as may be authorized and prescribed by the Executive Committee. He shall be ex-officio secretary of the Executive Committee, also an ex-officio member and secretary of the Program Committee. He shall be bonded for not less than ten thousand dollars.

ARTICLE VIII—AMENDMENTS

The constitution of this Association may be amended by a two-thirds vote of the members of the Association present and voting at an annual meeting, provided that the specific amendment to be acted upon shall have been presented in writing at a previous annual meeting and further provided that the amendment has received the approval of the Executive Committee.

BY-LAWS

ARTICLE I—ORDER OF BUSINESS

Registration.
Call to Order.
Report of Secretary-Treasurer.
President’s Address.
Reading of Papers.
Committee Reports.
Discussion.
Unfinished Business.
New Business.
Nomination and Election of Officers.
Adjournment.

A suspension of the By-laws may be made by a two-thirds majority for the purpose of changing the order of business or to facilitate important business.

ARTICLE II—APPLICATIONS FOR MEMBERSHIP

Applications for individual membership shall be made in writing to the Secretary-Treasurer. The Application shall give the name, occupation and address of the applicant and shall be accompanied by a fee of five dollars ($5.00), which amount shall include the membership dues for one year. Applications shall be presented in proper form to the Secretary-Treasurer, who shall in turn submit them to the Executive Committee.

An individual member may be expelled for cause by the Executive Committee.
ARTICLE III—MEETINGS

The annual meetings shall unless otherwise determined not less than thirty (30) days in advance by a majority of the members of the Executive Committee, be held at Chicago, Illinois, during the time of the International Livestock Exposition. The place for holding the meetings in Chicago as well as the duration of said meetings shall be determined by the Officer Members of the Program Committee of the Association.

The place for holding special meetings shall be determined by the President with due regard to the wishes of the members of the Executive Committee, the subject matter to be considered, accessibility, and the information to be obtained. The notice of time and place of holding a special meeting shall be mailed to the members at least thirty days prior to the date fixed for the special meeting.

ARTICLE IV—QUORUM

Twenty-five members of the Association shall constitute a quorum.

Five members of the Executive Committee shall constitute a quorum.

ARTICLE V—DUES

The dues for individual membership in this Association shall be five dollars ($5.00) per annum, payable in advance (on or before January 1st of each year) to the Secretary-Treasurer of the Association.

The dues for non-voting junior members shall be three dollars ($3.00) per annum, payable (on or before January 1st of each year) to the Secretary-Treasurer of this Association.

The dues for official memberships shall be fifty dollars ($50.00) each per annum, payable in advance (on or before January 1st each year) to the Secretary-Treasurer of this Association.
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