REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY AND OTHER AVIAN SPECIES

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The Committee met on October 27, 2008 from 1:00 to 5:30 p.m. and October 28, 2008 from 12:35 to 5:30 p.m. at the Sheraton Greensboro Hotel in Greensboro, North Carolina. There were 65 Committee members and 54 guests in attendance, for a total of 119. Chair John A. Smith presided, assisted by Vice-Chair Julie D. Helm. The Chair welcomed the Committee, summarized the 2007 meeting, and reported on the responses to the 2007 Resolutions and Recommendations.

2007 Resolution 53, Amendment of the National Organic Program Section 205.239 to Make Access to the Outdoors Optional for Poultry, was approved. The United States Department of Agriculture (USDA) Agricultural Marketing Service responded that the program contains provisions for temporary confinement to protect animal health and safety. The Committee has made several attempts to convince the National Organic Program to change this dangerous requirement, with little success.

2007 Resolution 54, Movement Protocols for Eggs, Egg Products, and Day-old Chicks within, out of, and into Disease Control Areas, was approved. USDA, Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) responded with a series of meetings and conference calls with the United Egg Producers, egg industry officials and veterinarians, the University of Minnesota Center for Animal Health and Food Safety, the Iowa State University College of Veterinary Medicine, and state animal health officials to develop continuity of business preparedness and response planning. The Committee is encouraged by and appreciative of the progress made by APHIS and the egg industry in working cooperatively on these issues.

2007 Resolution 55, Inclusion of Swine and Poultry Workers in Pandemic Influenza Planning, was approved. This Resolution was directed to the United States Department of Health and Human Services Assistant Secretary for Preparedness and Response and to the Centers for Disease Control and Prevention Advisory Committee on Immunization Practices. No response was received.

2007 Resolution 56, Low Pathogenicity Avian Influenza Program Funds, was approved. USDA-APHIS-VS responded with a review of the increasing enrollment, increasing funding, and success of this program.

2007 Resolution 57, Need for Ongoing Funding for Development of Additional Methods for Depopulation of Poultry and Livestock, was approved. USDA-APHIS-VS responded with a review of currently funded projects at the University of Georgia, the University of Delaware, Texas A&M University, the Mississippi Board of Animal Health, and the North Carolina Department of Agriculture, as well at the Avian Influenza Coordinated Agricultural Project and Cooperative Research, Extension, and Education Service activities.

Dr. Eric Jensen, Aviagen, Inc., and Chair of the Mycoplasma Subcommittee, gave the subcommittee report. The report was approved by the Committee and is included at the end of this report.

Dr. Sherrill Davison, University of Pennsylvania, and Chair of the Infectious Laryngotracheitis Subcommittee, gave the subcommittee report. The report was approved by the Committee and is included at the end of this report.

Dr. David Swayne, Southeastern Poultry Research Laboratory (SEPRL), Agricultural Research Service (ARS), and Chair of the Avian Influenza and Newcastle Disease Subcommittee, gave the subcommittee report. The report was approved by the Committee and is included at the end of this report.

Dr Bruce Stewart-Brown, Perdue Farms, Inc., and Chair of the ad hoc Subcommittee on State/Federal Reportable Diseases Harmonization presented the subcommittee report. Dr. Stewart-Brown reported that the National Animal Health Reporting System (NAHRS) and the National Association of State Animal Health Officials (NASAHO) are also addressing this issue. The Chair appointed Dr. Stewart-Brown as the new Committee liaison with NAHRS, replacing Dr. Stanley Kleven who is retiring. The Committee agreed that Dr. Stewart-Brown in his capacity as NAHRS liaison would maintain contact with NAHRS and NASAHO and offer the assistance of this Committee in addressing the reporting of poultry diseases. The ad hoc subcommittee on disease reporting harmonization will remain active for at least another year to maintain contact with and be responsive to the activities of NAHRS and NASAHO. These activities are expected to fulfill the objective of this ad hoc subcommittee.

Dr. Scott Westall, Pilgrim's Pride Corporation, and President of the Association of Veterinarians in Broiler Production presented the annual disease status report for the broiler industry. The report was approved by the Committee and is included at the end of this report.

Dr. Eric N. Gingerich, University of Pennsylvania, delivered the annual disease status report for the table egg industry. The report was approved by the Committee and is included at the end of this report.

Dr. Steven Clark, Alpharma Animal Health, gave the annual disease status report for the turkey industry. The report was approved by the Committee and is included at the end of this report.

Dr. Charles S. Roney, Veterinary Services, USDA-APHIS, presented the annual status report for the National Poultry Improvement Plan (NPIP) for the Senior Coordinator, Mr. Andrew H. Rhorer. The report was approved by the Committee and is included at the end of this report.

Dr. Matthew Erdman, National Veterinary Services Laboratory (NVSL), USDA-APHIS-VS, delivered the annual NVSL Diagnostic Bacteriology, Mycoplasma, Pasteurella, and Salmonella report. His report was approved by the committee and is included at the end of this report.

Mr. Dennis Senne, NVSL, USDA-APHIS-VS, delivered the annual Avian Import Activities report for Dr. Peter Merrill, USDA-APHIS Import-Export Animals Staff. Mr. Senne also presented the NVSL Avian Influenza and Newcastle Disease diagnostic reports and a report on the North American Animal Health Laboratory Network (NAAHLN). The reports were approved by the Committee and are included at the end of this report.

Dr. Lindsey Garber, Centers for Epidemiology and Animal Health (CEAH), USDA-APHIS-VS, reported on the National Animal Health Monitoring System (NAHMS) Small Enterprise Chicken Study for 2007-2008, and requested input on the 2010 Needs Assessment. Dr. Garber's report was approved by the Committee and is included at the end of this report. Dr. Jose Linares, Texas Veterinary Medical Diagnostic Laboratory, pointed out the emergence of the "urban poultry" phenomenon, part of the local food movement, and suggested that some attention to the practices of this group is needed.

Dr. Eric Gingerich, University of Pennsylvania, gave a report on the activities of the USAHA Committee on *Salmonella*. The report of that Committee is found elsewhere in these proceedings.

Dr. John Smith, Fieldale Farms Corporation, delivered an update on the United States Poultry and Egg Association's (USPEA) research grants program for Dr. Charles W. Beard, USPEA. Since 1968, USPEA has disbursed $22,330,299.17 in research funds. Funds have been provided to over 50 colleges and universities, USDA, and private research institutions. Research has focused on diseases, food safety, production, environmental issues, processing, and other areas. A committee of industry experts reviews grant proposals twice yearly, and approximately 30 percent of proposals are funded. Information about the program and the submission of grant proposals can be found at the USPEA web site www.poultryegg.org.

The Monday session adjourned at this point, at approximately 5:30 p.m. The meeting reconvened at 12:35 p.m. on Tuesday, October 23, 2008.

Dr. Elizabeth Krushinskie, Mountaire Farms, presented some industry perspectives on the USDA Food Safety and Inspection Service (FSIS) Salmonella Initiatives Program. Her comments are included at the end of this report.

Drs. David Suarez and Mary Pantin-Jackwood, USDA-ARS-SEPRL, gave an update on Avian Influenza and other emerging and exotic disease research at SEPRL. Their report is included at the end of this report.

Dr. Michael David, Director of Sanitary International Standards, National Center for Import and Export, USDA-APHIS-VS, presented the annual update on the World Organization for Animal Health (OIE) poultry activities. His comments are included at the end of this report.

Dr. Jonathan Zack, USDA-APHIS-VS, gave an update on the USDA response plans for highly pathogenic Avian Influenza. His update is included at the end of this report.

Dr. Lee Myers, USDA-APHIS-VS, reported on the activities of the National Veterinary Stockpile. Her report is included at the end of this report.

Dr. Angela Pelzel, USDA-APHIS-VS, presented an update on the Live Bird Marketing System Working Group (LBMSWG) activities. The report is included at the end of this report.

Dr. Steve Weber, USDA-APHIS-VS, gave a presentation on Compartmentalization. His comments are included at the end of this report.

Dr. Clark Tibbets, TessArae LLC, delivered a presentation entitled "Modern, Precise Detection of Poultry Pathogens using Advanced Genomics", which is included at the end of this report.

Committee Business:

Dr. Floyd Horn, Dunkirk, MD proposed and the Committee approved a Resolution entitled Additional Resources for Validation of Genomics-based Pathogen Detection Technologies, urging the Congress to appropriate funds for USDA-APHIS-VS and ARS to validate multiplexed genome sequencing technology for rapid and precise diagnosis of dangerous animal diseases.

REPORT OF THE SUBCOMMITTEE ON *MYCOPLASMA*

Eric Jensen, Chair
Aviagen, Inc.

The subcommittee met at the Sheraton Hotel in Greensboro, North Carolina on October 26, 2008 with 35 attendees. Dr. C. Stephen Roney (NPIP) reported nearly a two-fold increase in the incidence of both *Mycoplasma synoviae* (MS) and *Mycoplasma gallisepticum* (MG) cases in meat-type parent stock chickens in the preceding year. Many of the MS positive parent stock flocks have not been depopulated because of the relatively low virulence of the field strains and economic considerations. Some in industry are considering the use of a live MS vaccine to reduce the susceptible populations. No live MS vaccines are currently available so this strategy would require obtaining a conditional license. The mycoplasma check test sera from the laboratory of Dr. Stanley Kleven, University of Georgia continues to be widely used and remains an invaluable asset for laboratory quality assurance. Dr. Naola Ferguson-Noel discussed the limitations of current diagnostic tests to identify mixed MG infections, specifically with finding wild-type strains in MG-vaccinated poultry.

REPORT OF THE SUBCOMMITTEE ON VACCINAL LARYNGOTRACHEITIS

Sherrill Davison, Subcommittee Chair
University of Pennsylvania

Contributing authors: Dr. George Boggan, Ceva-Biomune; Dr. Louise Dufour-Zavala, Georgia Poultry Laboratory; Dr. Maricarmen Garcia, University of Georgia; Mr. Ruud Hein, Intervet-Schering-Plough; Dr. Julie Helm, Clemson University Livestock-Poultry Health; Mr. Ray Hilburn, Alabama Department of Agriculture; Dr. Sarah Mason, North Carolina Department of Agriculture; and Dr. John Smith, Fieldale Farms Corporation.

Introduction: Vaccinal laryngotracheitis (VLT) is an acute viral respiratory disease primarily of chickens. Economic losses attributable to VLT have been important in many poultry producing areas throughout the United States and the world. Despite efforts to control the disease through vaccination and implementation of biosecurity measures, outbreaks of VLT are still a threat to the poultry industry. In 2007 the subcommittee suggested the following action items:

- Studies of currently available vector vaccines by the *in ovo* route should be continued.
- Research should be conducted to develop newer molecular vaccines to control and prevent VLT.
- States should adopt the Model State Program –VLT (USAHA – 2005).
- Procedures of proper administration of Chick-embryo-origin (CEO) and Tissue-culture-origin (TCO) vaccines must be reviewed.
- Field evaluations must be conducted in conjunction with laboratory research to evaluate the efficacy of control procedures.

Update – 2008 Observations on VLT outbreaks: One outbreak was unusual in that it started in the summertime and continued unabated through the following summer, despite very hot temperatures. Although biosecurity and vaccination zones were used according to the plan, the outbreak expanded and lasted longer than anticipated and included cases in TCO, CEO, and Recombinant-vaccinated broilers and adult bird flocks. The litter management strategies were repeatedly reviewed during this outbreak and involved many players with which consistent communications were critical for compliance.

Experience has demonstrated that cooperative programs in which industry, government, academia, laboratories, and allied industries work together to control the disease are the most successful approach currently available to minimize cases and stop outbreaks. These programs should consider the known epizootiology of the disease and should contain provisions for rapid diagnosis; the use of Geographical Information Systems (GIS) to design appropriate zones for biosecurity, vaccination, and movement routes to processing plants; and open communication and cooperation among all players. Rapid implementation of a vaccination program and inclusion of a larger area to gain more protection are important control measures.

Vaccination Strategies:

Vaccines: In addition to the commercially available CEO ILT vaccines and the TCO vaccine, there are two vectored ILT vaccines: Ceva-Biomune's recombinant Fowl Pox –LT and the Intervet/Schering Plough Animal Health recombinant HVT/ILTV.

Biomune's recombinant FP-LT vaccine (VECTORMUNE-FP-LT) was first developed for long-lived commercial layers and breeders. The product has been licensed for commercial layer pullets and breeders since September 2002. *In ovo* use by the broiler and broiler breeder industry prompted Biomune to acquire registration and labeling claims for use of the product at the hatchery. USDA approval of the efficacy data was granted on 12/11/07 and the final field safety trial is currently underway. The role and possible interference of maternal antibody is currently being investigated.

The recombinant HVT/ILTV (INNOVAX-ILT), officially licensed for use subcutaneously at one day of age, was introduced in the field in September 2007. The vaccine was intended to be used in long-lived birds. However, due to the vaccinal ILT situation in broilers in several states, the vaccine has been used extensively in broilers. More than 700 million broilers and approximately 50 million layers have been vaccinated. In broilers, the vaccine has been applied *in ovo* at mainly ½ dose and in several cases in combination with SB1. Layers have been vaccinated by the subcutaneous route and in most cases it has been combined with the Rispens MDV vaccine. Recently, several breeder flocks have been vaccinated at day of age subcutaneously in combination with SB1 or the Rispens MDV vaccine. Several vaccine administration issues have shown to play an important role in the outcome of the vaccination with the INNOVAX-ILT. These include application of the vaccine, onset of complete protection and compatibility with other vaccines or antibiotics.

Field evaluation: CEO vaccination affords excellent immunity but with it there is an economic downside of reactions and weight reduction. The use of CEO vaccine over an extended period in a dense production area was

very detrimental to broiler growth and performance and led many to reevaluate vaccination methods. The use of recombinant vaccines was preferable to the use of CEO in terms of zootechnical performance, but it did not stop the cases or the outbreak.

Individual companies would choose the best vaccination strategy (CEO and/or vectored) for their particular situation. This method grew from the diversity of broiler sizes and growing methods of the various industry partners. Some companies chose to use only vectored vaccine. Others used either entirely CEO or a combination of vectored and CEO in broilers. In general, both methods were used in most areas that have broiler operations. Vectored vaccines did not protect the broilers from ILT infection in some cases, but resulted in mild cases that recovered quickly and regained weight and livability by market age. CEO alone worked well in birds being raised to heavy market weights but was detrimental to birds marketed at a lighter weight. Combinations of CEO and vectored vaccines were used by other companies depending upon the prevalence of ILT field cases near their farms. A novel exit strategy that combines the use of CEO vaccines, the use of recombinant vaccines, and a gradual abandonment of CEO vaccination in a ring pattern towards a small zone considered most persistent was used and is still being evaluated.

Research Update: Decreased use of the CEO vaccines and the eventual substitution of live-attenuated vaccines by recombinant-LT vector vaccines have been considered the strategy towards a more efficient control of the disease. Fowl poxvirus (FPV) and herpesvirus of turkey (HVT) viral vector vaccines carrying ILTV genes have been developed and are commercially available. Recently the broiler industry has used FPV-ILT and HVT-ILT recombinant vaccines for off-label *in ovo* vaccinations against ILT achieving variable results. The objective of this study was to evaluate the efficacy of these vaccines to protect birds against currently circulating viruses and the standard USDA challenge strain under controlled experimental conditions. The first two experiments were performed to evaluate the protection elicited by the recombinant HVT-LT vaccine. Briefly, broilers vaccinated *in ovo* with half a dose of the vaccine and non-vaccinated broilers were challenged at 5 weeks of age. Protection was evaluated by scoring clinical signs, body weight gain before and after challenge, and the presence of the challenge virus by real-time PCR. Overall, vaccinated chickens showed a reduction in clinical signs, had a moderate reduction in body weight after challenge, but showed no reduction of the challenge virus replication in the trachea and conjunctiva, as compared to the non-vaccinated group. Therefore, based on this challenge model the HVT-LT vaccinated chickens were considered partially protected against clinical signs.

In a second group of experiments, broilers were vaccinated *in ovo* (half a dose) at 17, 18, and 19 days with the FPV-LT and HVT-LT vaccines, and challenged at 5 weeks of age. Protection was evaluated by scoring clinical signs. Chickens vaccinated with the FPV-LT vaccine at 17, 18 and 19 days showed 45 percent, 61 percent, and 55 percent protection, respectively. Chickens vaccinated with the HVT-LT vaccine at 17, 18, 19 days post vaccination showed 48 percent, 78 percent, 84 percent, protection respectively.

Conclusion: Extensive use of recombinant ILT vaccines by mass *in ovo* application of reduced doses to commercial broilers suggests that such uses do provide partial protection against clinical signs. Such birds may become infected and shed virus. The role of these extra-label uses in the control of outbreaks remains to be elucidated and further study is needed. In particular, the viral load in recombinant-vaccinated birds after challenge and the establishment of latency in recombinant-vaccinated birds deserves further investigation. An economical ILT vaccine suitable for mass application that provides good protection against infection and shed without harsh vaccine reactions is sorely needed.

Current suggested action items – 2008

- Evaluations (field and laboratory) of currently available vector vaccines by the *in ovo* route should be continued.
- Research should be conducted to develop newer molecular vaccines to control and prevent VLT.
- Future research with the vectored products should include quantitative evaluation of viral shed and evaluation of the potential development of a carrier state after challenge.
- Economics must be considered with the development of newer vectored products.
- In the future, an effort to collect detailed data on mortality, duration of clinical signs, weight gain, vaccine usage and other epidemiological parameters is essential to have a more comprehensive evaluation of the currently available vaccines and control measures.
- Further research studies on innate immunity to ILT should be conducted.
- States should adopt the Model State Program –VLT (USAHA – 2005).

REPORT OF THE SUBCOMMITTEE ON AVIAN INFLUENZA AND NEWCASTLE DISEASE

David E. Swayne
Southeastern Poultry Research Laboratory
Agricultural Research Service

There have been several major developments over the past year with avian influenza (AI) and Newcastle disease (ND). Since July 2007 to June 2008, 30 countries have reported outbreaks of H5N1 high pathogenicity avian influenza (HPAI) in poultry and/or wild birds including Afghanistan, Bangladesh, Benin, Canada, China, Egypt, Germany, Hong Kong, India, Indonesia, Iran, Israel, Japan, Korea, Laos, Myanmar, Nigeria, Pakistan, Palestinian Territories, Poland, Romania, Russia, Saudi Arabia, Switzerland, Thailand, Togo, Turkey, Ukraine, United Kingdom and Vietnam (Source: OIE). There have been only a few reported cases of H5N1 HPAI in wild birds in Asia and Europe, but not a repeat of the large number of wild bird cases in European Union as occurred in the winter 2006. The United Kingdom experienced an outbreak of H7N7 HPAI on a single farm of 25,000 chickens in Oxfordshire during May 2008 that was resolved by stamping-out before spread to other facilities. Limited outbreaks of low pathogenicity avian influenza (LPAI) were reported in Korea (H7N8 in meat ducks), Dominican Republic (H5N2 in fighting cocks and backyard chickens), Portugal (H5N3 in partridges and other poultry), Denmark (H7N1 in geese, ducks and mallards), Haiti (H5N2 in backyard poultry and fighting cocks), Korea (H5N2 in ducks) and Germany (H5N3 in zoo and backyard poultry).

The major exotic poultry disease around the world is Newcastle disease (ND). For the period of July 2006 to June 2007, 73 countries reported ND cases. Many countries in the developing world have endemic ND and do not report occurrences of ND.

The 7th International Symposium on Avian Influenza (AI) will be held at the University of Georgia, Athens, Georgia, USA on April 5-8, 2009. Currently, the conference has sponsorship from U.S. Department of Agriculture (Agricultural Research Service; Cooperative State Research, Education and Extension Service; and Animal and Plant Health Inspection Service), U.S. Animal Health Association, U.S. Geological Survey, American Association of Avian Pathologists, Merial, Schering-Plough/Intervet, ABI, and Fort Dodge. There will be one day of split scientific sessions for poultry and wild birds, and one full day and two half days of joint sessions. The sessions will include: Global Reports on Avian Influenza; Pathogenesis and Pathobiology; Intervention and Control Strategies; 21st Century Diagnostics for Centuries Old Problems; Host and Environmental Factors that Impact Transmission and Mechanisms of Spread; Zoonotic Aspects of Avian Influenza; Vaccinology; Trade, Regulatory Control and Economics; Modeling of Avian Influenza Spread in Developing Control Strategies; Epidemiology and Ecology of AIV in the Natural Reservoir; HPAI H5N1 in Wild Birds; and Late Breaking Issues.

2008 United States Broiler Industry Update

Scott Westall
Pilgrim's Pride, Inc

Based on yearly Agristats data for field condemnations, 7-day mortality, and total mortality, US broiler flock health has seen a slight decline over the past year. The decline was seen across all three parameters and is most likely due to continued issues with Infectious Laryngotracheitis (ILT), Runting Stunting Syndrome (RSS), and newly identified Infectious Bronchitis Virus (IBV) variants. A poll of broiler production veterinarians ranks ILT, RSS, and IBV as the top three challenges facing the poultry industry.

ILT and IBV are the two highest-ranking respiratory diseases. New vaccines and vaccination techniques are currently being implemented to control ILT. Newly identified IBV variant GA 08 causes airsacculitis and increased salvage and condemnations. This virus has been isolated in Georgia and South Carolina and has a higher incidence in the winter. Work is underway to develop a vaccine for GA 08 virus.

RSS and Gangrenous Dermatitis are the two top-ranking immunosuppressive diseases. A consensus on the causative agent or agents of RSS has not been reached but Astroviruses and Rota-like or reo-like viruses are suspected. There is no doubt that RSS related immunosuppression has impacted flock uniformity and processability. It appears the incidence of Gangrenous Dermatitis has decreased and Infectious Bursal Disease (IBD) did not make the rankings this year.

Coccidiosis and Necrotic Enteritis are the top ranking enteric diseases. These issues are probably related and may take a more prominent role as feed costs increase.

Bacterial infections in pullets and breeders are of increasing concern. Spinal Abscesses in hens are being diagnosed more frequently as are *Staphylococcus spp.* infections in pullets and breeders.

Poultry exports have hit record levels in 2008. The weak U.S. Dollar and strong foreign currencies have created strong demand for poultry exports. The top three export markets are Russia, China, and Mexico. Russia has recently banned exports from several companies, which has created concern industry wide.

Input costs this year have been tremendous. High gas cost coming into winter will challenge producers and growers to look for ways to conserve. High grain costs due to ethanol mandates and subsidies along with weak market pricing have led to industry-wide cutbacks. Nutritional strategies are also changing due to high input costs. Nutritionists and Veterinarians will be challenged to make sure the nutritional needs of the birds are met. Failure to do so could result in classical deficiency diseases and immunosuppression.

Table 1. Ranking of Disease Concerns among 16 Broiler Production Veterinarians

Infectious Laryngotracheitis	11
Runting Stunting Syndrome	6
Infectious Bronchitis Virus	6
Mycoplasmosis	5
Gangrenous Dermatitis	3
Spinal Abscesses in Hens	2
Avian Influenza	1
Chick Quality	1
Coccidiosis	1
Femoral Head Necrosis	1
Legs-Skeletal Issues	1
Necrotic Enteritis	1
Salmonella	1
Staph in Pullets/Breeders	1

Table 2. Ranking of Non-Disease Concerns among 16 Broiler Production Veterinarians

Exports	5
High Gas	4
Feed/Nutrition	3
Welfare	2
Antibiotic Issues	1
Food Safety	1
Labeling Standards	1
Market Cutbacks	1

Mycoplasma Testing	1

United States Table Egg Industry Update October 2007 to October 2008

Eric Gingerich
University of Pennsylvania

Overall health of the national table egg layer flock is very good. This is due to the continued availability of high quality vaccines, flock supervision from professional, well-trained flock supervisors, readily available veterinary technical assistance from primary breeder, vaccine company, diagnostic laboratory, and consulting veterinarians, high quality nutrition provided by professional nutritionists, housing of a majority of layers in environmentally controlled facilities in cages off litter, and the use of sound biosecurity practices. An increase in the finding of diseases thought once to be eradicated has been seen on the rise in cage-free production.

A recent poll of the Association of Veterinarians in Egg Production (AVEP) was conducted and 11 of 68 members responded. The survey revealed the following diseases of concern occurring in US caged layer flocks - #1 – *E. coli*/peritonitis, #2 - *Mycoplasma gallisepticum* (Mg), #3 – Cannibalism, #4 – Starve outs of baby chicks, and #5 - Calcium depletion/tetany. In cage-free layer flocks (6 respondents), the diseases in order of concern were #1 (tie) Cannibalism and Colibacillosis, #3 – Mites, #4 – Coccidiosis, and #5 – Roundworms. Other issues and diseases of concern (11 respondents) were welfare, avian influenza (AI), *Salmonella enteritidis* (SE), and the lack of approved, effective treatments for most of our infectious or parasitic diseases.

Colibacillosis is a problem mainly of young flocks with mortality rates of 0.5 to 4 percent per week starting shortly after housing. It is felt that this condition is most often secondary to upper respiratory challenges with Mg, *Mycoplasma synoviae* (Ms), ammonia, infectious bronchitis (IB), etc. It also may be a primary problem if water lines are contaminated with *E. coli*. The overall incidence of early onset colibacillosis is down from recent years. A post-molt colibacillosis syndrome is also seen in some flocks due to declining immune system function, an ascending infection of the reproductive tract, upper respiratory infections, etc. A new tool to use against *E. coli*, a live *E. coli* vaccine, was introduced in mid to late 2006 and has been increasingly used successfully as both a preventative and as a treatment in the face of an outbreak.

Mg continues as an issue in multi-aged facilities and is successfully controlled in most cases through vaccination. Each complex must customize its vaccination program to control the strain on the farm. Ts-11 and 6/85 live vaccines are used for controlling mild strains of Mg while F-strain live vaccine is being used to control more pathogenic strains. The live pox-vectored recombinant Mg vaccine is being used in a variety of situations and appears to be useful in low challenge situations but still continues to be evaluated in high challenge facilities. Vaccine failures with all vaccines are somewhat common and the unit must resort to medication programs using tylosin or tetracycline antibiotics. Some operators are now applying the F-strain vaccine by eye drop in an effort to increase its efficacy.

Calcium depletion is normally associated with low intake of calcium, phosphorus, and/or vitamin D3 especially early in production with low feed intakes. Calcium tetany is seen when young flocks that are slow to mature are placed on calcium-rich feeds too early. A post-molt problem with calcium tetany is also being found due to excessive calcium intake during the molt resulting in a shutdown on normal hormonal action to pull calcium from the medullary bone.

Cannibalism continues to be seen especially in high light intensity situations in both caged and cage-free birds. In these cases, the 10-day rule for beak trimming results in longer beaks than desired compared to a beak trim at 4 to 8 weeks and results in an increase in incidence and severity of cannibalism.

AI continues to be a very high concern across the country. Active and passive surveillance programs are increasing across the U.S. in response to the threat of highly pathogenic H5N1 AI (HPAI) from Asia. There is great concern in the layer industry in regard to the amount of time before egg movement can take place once a quarantine is placed on a premise in a control zone. Egg storage on large farms is not capable of storing more than 72 hours of production. The United Egg Producers and the US Egg Association have proposed a “Movement Control Model Plan” for the table and breaking egg industries to allow movement of product within 48 hours after quarantine by assuring that a farm is negative for AI by 1) testing five dead birds per house per day by AI real time PCR and 2) reporting daily mortality and egg production to the authorities. Discussion and research as to the best ways of bird euthanasia and disposal from large cage layer houses and complexes continues. The threat of H5 or H7 low pathogenic AI (LPAI) for layer flocks on the East coast is much reduced due to the efforts by New York and New Jersey Departments of Agriculture and USDA to reduce the positivity of the live bird markets from 60 percent positive markets in 2004 to near 0 since. No significant AI isolations have been made in layer flocks in the US in the last year. A majority of egg operations are complying with the National Poultry Improvement Plan (NPIP) low pathogenic AI (LPAI) program for commercial layers.

SE was felt to be an issue that was being addressed adequately by state and industry egg quality assurance programs until the announcement on September 22, 2004 that FDA was proposing a program for "Prevention of SE in Shell Eggs During Production". FDA received over 200 written comments. Issues discussed were 1) laboratory procedures and laboratory availability for testing, 2) funding for testing, costs incurred if eggs are diverted, and administration of the program, 3) lack of egg pasteurization facilities in many egg producing areas to be able to effectively divert eggs from high risk flocks, 4) wet washing houses required between flocks where SE positive manure samples were found in the previous flock whereas dry cleaning, fumigation, vaccination of in-coming pullets, plus good rodent control has been found to be effective, 5) the excessively low requirement for 45 F egg storage prior to processing, etc. Comments were reopened in May of 2005 to ask questions about pullets. The initiation of this program continues to be in doubt as it is stalled in the Office of Management and Budget (OMB), which has been studying it for over 2 years. The incidence of egg-related SE outbreaks continues steady apparently due to areas of egg production where SE risk reduction programs are either not effective or totally embraced.

Coccidiosis and necrotic enteritis has been increasing in incidence in caged layers especially on the east coast and in one strain of layer. Vaccination of pullets is being used successfully as control.

Diseases under control and of low incidence are as follows: infectious laryngotracheitis (ILT), Infectious Bronchitis, fowl coryza, and urolithiasis/gout. These diseases tend to be localized to a region or a farm. The pox-vectored recombinant ILT vaccine has been determined not to be a suitable replacement for chick embryo origin (CEO) vaccines in high challenge areas. The relatively new HVT-vectored ILT vaccine is showing great promise in high challenge regions and should reduce the amount of CEO vaccine used in layer flocks that may spread to broilers.

Diseases that are very rarely a problem for table egg layers are pox, Marek's, Newcastle, infectious bursal disease, chick anemia virus, erysipelas, and fowl cholera.

Poultry welfare concerns are increasing as activist groups increase their activities in portraying the caged egg industry as not humane and promoting laws against caged egg production in several states including a major egg producing state, California. The ballot initiative sponsored by the Humane Society of the United States (HSUS) in California, Prop 2, is to take place this November and would essentially ban the use of cages for layers, veal calf stalls, and gestation stalls for sows. The group Californians for Safe Food (www.SafeCaliforniaFood.org) are leading an attack to vote down this proposal and numerous veterinary and industry groups are supporting this opposition.

The egg industry has experienced record egg prices and profits since early 2007 and throughout 2008 in spite of increased corn and feed prices. Reduced numbers of layers due the UEP required reduction in layers per cage, fewer layer houses being built due to uncertainty about the future of caged layer production, and increased exports to Europe and Asia are felt to be the reasons.

Current Health and Industry Issues Facing the Turkey Industry

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In preparation for this report to the USAHA Committee on Transmissible Diseases of Poultry & Other Avian Species, Drs. Clark, Tilley, and Mills polled a majority of the US turkey industry professionals and veterinarians involved in turkey production to inquire about the health status of turkeys produced in August 2007 through August 2008. The turkey industry reports several disease challenges for this 12 months varying by geographical regions within a state and across the United States. This report lists (Table 1) the challenges by disease and issues.

The lack of approved efficacious drugs continues to be the top disease issue. The withdrawal of the NADA for enrofloxacin in 2005 for use in poultry leaves the industry with no adequate therapeutic response to colibacillosis (ranked #4), or fowl cholera (ranked #8). The controversy over the use of antibiotics in animal agriculture remains a major concern for the turkey industry and for all of animal agriculture. A recent example is the proposal by FDA to eliminate extra label use of cephalosporins in animal agriculture without sufficient examination of the risks and benefits involved. This loss is of particular concern to the turkey industry because cephalosporin treatment of individual breeder toms is one of only a few tools available for poultry veterinarians to use in outbreaks of fowl cholera. We urge the American Veterinary Medical Association (AVMA) to continue to call for and support the scientific examination of the evidence in the cases against the use of antibiotics in agriculture and to support the judicious use of antibiotics in animal agriculture as long as the benefits outweigh the risk.

Late mortality increased to the second-ranked health issue, as Colibacillosis slipped to fourth place. Late Mortality may be defined as mortality, in excess of 1.5 percent per week, in toms (males) 17-weeks and older; mortality is not diagnosed to a specific disease or cause. Excess cumulative mortality of 5 – 10 percent in toms prior to slaughter has been reported. Late mortality may be associated with physiologic or biomechanical deficiencies following early rapid growth in heavy toms achieving genetic potential; aggressive behavior noted in mature toms; cannibalism; leg problems; and/or hypertension.

Leg problems (#6) are ranked among the top concerns of the turkey industry. Leg problems are a common complaint, including spiral fractures of the tibia or femur. Leg Problems may be defined as lameness, particularly in toms, several weeks prior to slaughter. Leg problems are attributed to various conditions (refer to Table 1), including, pododermatitis, fractured femurs, fractured tibia, osteomyelitis (OM), tibial dyschondroplasia (TDC), spondylolisthesis, “Shaky Leg”, etc.

Blackhead, also known as Histomoniasis, increased to position #16 in 2008, from #22 in 2007. It is one disease with no efficacious drug approved for use in turkeys. There were 63 reported cases of blackhead (Table 2). Losses to blackhead have been severe and sporadic cases are occurring in North America. The disease can be devastating in the individual flocks affected. Dimetridazole was extremely efficacious and previously approved for use in turkeys for the prevention and treatment of blackhead; it was banned in 1987. The lack of any legal treatment for histomoniasis is of concern, especially in the case of valuable turkey breeder candidate flocks. Losses to blackhead have been severe in several areas of Europe, and sporadic cases are occurring in North America. It seems unconscionable that we are unable to prevent the suffering and death in flocks affected by histomoniasis when effective treatments exist, but were taken away from the poultry industry due to misuse in another industry.

Cellulitis remains a major disease issue across all geographic regions, as the survey average increased to a score of 3.3 and ranked #3, from 3.1 and #5, respectively, the prior year. Analysis indicates a range of concern; 33 percent (26 percent, prior year) of respondents score cellulitis a 5 (severe), 8 percent (22 percent, prior year) score it a 1. Cellulitis is most commonly seen in, but not limited to, commercial male turkeys nearing market age. The prevalence and severity of cellulitis continues to increase. The disease is now confirmed at younger ages and in both toms and hens. *Clostridium septicum, C. perfringens* type A, or *C. sordelli* is isolated from fluid or affected tissue samples of affected or dead birds. Affected turkeys present with two or more of the following signs: subcutaneous emphysema (crepitus); serous or serosanguinous subcutaneous fluid; vesicles on the skin, especially on the breast/inguinal area; moist, dark, wrinkled skin, especially breast/inguinal area; cellular necrosis (microscopic); organ involvement (spleen/liver); vesicles on the skin, and/or moist, dark, wrinkled skin on the tail

area. The affected flock will have mortality greater than or equal to 0.5 dead per 1,000 birds, fitting the individual bird definition, for two consecutive 24-hour periods. Research on the pathogenesis and control is ongoing. Opinions vary as to risk factors and potential causes of the problem.

Poult enteritis of unknown etiologies has increased in importance, from position #7 to #5, although the score remained the same at 3.0. Some of the recent poult enteritis concerns have been characterized as Poult Immunosuppression Pancreatic Enteritis Syndrome (PIPES); controlled studies with astrovirus and rotavirus isolates have reproduced PIPES. The immunosuppression persists for the life of the bird. PIPES does not have excess mortality as associated with PEMS. Turkey Coronavirus (TCV), as a defined cause of enteritis, was ranked #34 (Table 1) with 10 reported cases (Table 2).

Heat stress decreased from #6 ranking to #18 following a milder summer. Poult Enteritis Mortality Syndrome (PEMS, ranked #33 versus #31 previously), and protozoal enteritis (#24 versus #22) all decreased in ranking on this year's survey. *Ornithobacterium rhinotracheale* (ORT, ranked #13 versus #17 previously), and Avian Metapneumovirus (AmPV, ranked #32 compared to #33) increased in importance in the latest survey.

Mycoplasma synoviae (MS, infectious synovitis) infections, ranked #27, are one cause of synovitis. It may be present in flocks 10-12 weeks of age with typically low mortality and low morbidity. There were 47 cases of MS reported (Table 2). The primary breeders have remained free of *M. gallisepticum* (MG), *M. mleagridis* (MM) and MS. Sporadic but increasingly frequent infections with Mycoplasma, both MG and MS, often in association with backyard poultry and broiler breeder flocks is an ongoing concern, having the greatest impact when a breeder flock is infected and has to be destroyed.

Unrealistic and uninformed demands on the turkey industry under the guise of animal welfare by activist groups opposed to any animal agriculture remain a major concern. The ultimate goal of these groups is the elimination of animal agriculture. AVMA has taken a leading role in the development of animal welfare principles and practices, affirming the ethical role of animal agriculture in our society. The industry continues to support the use of sound science in developing animal welfare principles and guidelines.

Current food-to-fuel mandates and subsidies have resulted in record high feed prices. Corn price has doubled, and price variability has more than doubled. The unprecedented increases in costs to produce turkeys and other food animals are proving to be very detrimental to both the livelihood of the livestock farmer and the consumer, with no relief in sight.

Highly pathogenic avian influenza (H5N1) continues to infect poultry in Southeast Asia, with sporadic introductions in Europe and Africa. Sporadic transmission to humans continues, and has world health authorities concerned about the possibility of further genetic mutation triggering a pandemic. Continued circulation of this virus through poultry allows for further genetic drift and/or shift that could result in a highly pathogenic and highly transmissible virus among humans. Eradicating this disease at its source continues to be the focus and burden of the international effort to eliminate this threat, but will demand more resources. The possibility of the spread of this virus to the United States through the illegal transport of infected birds or migration of infected wild birds remains a concern.

The use of invalid animal disease issues as non-tariff trade barriers, and the penalization of countries that have more open diagnostic systems remains a major concern for the industry. The most visible examples are the trade bans applied by Japan and others whenever a Low Pathogenic Avian Influenza (LPAI) subtype H5 or H7 is found in the U.S. Hopefully, importers of poultry and poultry products will align their policies with the new OIE chapter on avian influenza, if they have not done so. Industry professionals continue to support transparent and science-based standards in trade issues.

One industry concern is the recent FSIS focus on preharvest control of salmonella. While everyone desires safe food, public health officials and veterinarians must realize that the most effective interventions to prevent food-borne illness remain proper food preparation and handling. Proper food handling and appropriate processing technologies are the best way forward. Attempting to control food-borne disease by selectively eliminating what are normal intestinal inhabitants of domestic animals essentially represents a national certified raw meat program similar to the hazardous certified raw milk program. Such an effort is distracting to the main food preparation issues, and represents a major policy development failure. While significant progress has been made in *E. coli* 0157 control in beef, it must be pointed out that the improvements resulted from improved processing technology, not on-farm interventions. Pre-harvest interventions were not a factor.

Turkey Production in 2007 increased to 7869.22 from 7463.89 million pounds (live weight). Overall domestic per capita consumption for turkey products increased from 17.50 to 18.00 (lbs). Exports increased from 547 to 623 (million pounds) 2007 to 2008. Production increased to 271.685 million head slaughtered with an average live weight (lbs) of 28.96, compared to prior year of 262.460 and 28.44, respectively (reference: Turkey Sourcebook, National Turkey Federation).

Table 1. Turkey health survey (September) of U.S. veterinarians in turkey production ranking current disease issues (1= no issue to 5 = severe problem). Survey response (reply) is 100% (n=24).

Issue	Score Average (1-5)	Score Mode (1-5)
Lack of approved, efficacious drugs	4.5	5.0
Late Mortality	3.3	3.0
Cellulitis	3.3	2.0
Colibacillosis	3.1	3.0
Poult Enteritis of unknown etiologies	3.0	4.0
Leg Problems	2.9	2.0
Bordetella avium	2.8	4.0
Cholera	2.6	3.0
Salmonella	2.6	2.0
Breast Blisters and Breast Buttons	2.4	3.0
Cannibalism	2.4	2.0
Fractures	2.3	3.0
Ornithobacterium rhinotracheale (ORT)	2.3	2.0
Osteomyelitis (OM)	2.3	2.0
Tibial Dyschondroplasia (TDC, Osteochondrosis)	2.3	2.0
Blackhead (Histomoniasis)	2.3	1.0
H3N2 Swine influenza	2.3	1.0
Heat stress	2.1	2.0
Mycoplasma iowae (MI)	2.1	1.0
Shaky Leg Syndrome	2.0	2.0
Bleeders	2.0	1.0
Coccidiosis	1.9	2.0
Avian Influenza	1.9	1.0
Protozoal Enteritis	1.8	2.0
Round Worms (*Ascaridia dissimilis*)	1.8	2.0
Newcastle Disease Virus (NDV)	1.8	1.0
Mycoplasma synoviae (MS)	1.7	1.0
Mycoplasma gallisepticum (MG)	1.6	1.0
Necrotic enteritis	1.6	1.0
Erysipelas	1.5	1.0
Spondylolisthesis (Kinky-Back)	1.3	1.0
Avian Metapneumovirus	1.3	1.0
PEMS (Poult Enteritis Mortality Syndrome)	1.3	1.0
Turkey Coronavirus	1.2	1.0

Table 2. Turkey health survey (September) of US veterinarians in turkey production. Survey response (reply) is 100% (n=24).

	2008	2007
Cases (numbers) of Blackhead (Histomoniasis)	63	68
Cases (numbers) of *Mycoplasma synoviae* (MS)	47	52
Cases (numbers) of Turkey Coronavirus (TCV)	10	N/a

National Poultry Improvement Plan Status Report

Andrew R. Rhorer
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National Poultry Improvement Plan
USDA-APHIS-VS

Pullorum-Typhoid Status:

In calendar year 2007, there were no isolations /outbreaks of *Salmonella pullorum* reported to the Poultry Improvement Staff. There was one isolation/outbreak of *Salmonella pullorum* reported during calendar year 2008 from January to October 1, 2008. There have been no isolations of *Salmonella gallinarum* since 1988 in any type poultry.

Hatchery Participation in the National Poultry Improvement Pla: Testing Year 2007	
Egg and Meat-Type Chicken Hatcheries Participating	283
Egg and Meat-Type Chicken Hatcheries Capacity	718,723,839
Turkey Hatcheries Participating	48
Turkey Hatcheries Capacity	35,224,523
Waterfowl, Exhibition Poultry and Game Birds Hatcheries (WEGBY) Participating	775
WEGBY Hatcheries Capacity	25,592,182

Egg-Type Chicken Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary:Testing Year 2007	
U.S. Pullorum-Typhoid Clean: Participating- Number	187
Birds in Flocks-Number	3,205,906
Average per Flock	17,144
Primary Breeding Flocks – Flocks Proportion of Total	27.8%
Primary Breeding Flocks—Birds Proportion of Total	14.3%

Meat-Type Chicken Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary: Testing Year 2007	
U.S. Pullorum-Typhoid Clean: Participating- Number	5,140
Birds in Flocks-Number	75,820,652
Average per Flock	14,751
Primary Breeding Flocks – Flocks Proportion of Total	9.7%
Primary Breeding Flocks – Birds Proportion of Total	6.5%

Turkey Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary: Testing Year 2007	
U.S. Pullorum-Typhoid Clean: Participating –Number	518
Birds in Flocks-Number	4,603,212
Average per Flock	8,886
Primary Breeding Flocks – Flocks Proportion of Total	20.6%
Primary Breeding Flocks – Birds Proportion of Total	7.1%

Waterfowl, Exhibition Poultry, and Game Birds Breeding Flocks In the National Poultry Improvement Plan: Participation and Testing Summary: Testing Year 2007	
U. S. Pullorum-Typhoid Clean Participating	3,648
Birds in Flocks	1,475,373
Primary Breeding Flocks – Flocks Proportion of Total	32.6%
Primary Breeding Flocks – Birds Proportion of Total	48.2%

Mycoplasma gallisepticum, Mycoplasma synoviae, and *Mycoplasma meleagridis* positive breeding flocks

National Poultry Improvement Plan 2007/8

	WEGBY	Egg-type Chickens	Meat-Type Chickens	Turkeys
Mycoplasma gallisepticum	17	0	25	2
Mycoplasma synoviae	17	4	86	5
Mycoplasma meleagridis		0		2

Mycoplasma status:

Number of flocks and birds in flocks by State with *Salmonella enteritidis* isolates, 1990-2008

	Environmental	Dead Germ	Bird
Arkansas			
Flocks	1		2
Birds in Flocks	6000		15000
Georgia			
Flocks	1	2	
Birds in Flocks	400	46000	
Illinois			
Flocks	3	2	1
Birds in Flocks	3900	3700	1200
Indiana			
Flocks	15	2	1
Birds in Flocks	158345	27479	15092
Kentucky			
Flocks	1		
Birds in Flocks	6625		
Ohio			
Flocks	16		9
Birds in Flocks	183700		91600
Oregon			
Flocks	2		
Birds in Flocks	19516		
Pennsylvania			
Flocks	16		6
Birds in Flocks	166385		78450
Texas			
Flocks	1		
Birds in Flocks	10000		

Phage types of *Salmonella enteritidis* isolates

	Environmental	Dead Germ
Phage Type 13		
Flocks	10	2
Birds in Flocks	143000	3700
Phage Type 13A		
Flocks	5	2
Birds in Flocks	54321	27479
Phage Type 2		
Flocks	2	
Birds in Flocks	28900	

Phage Type 23		
Flocks	21	
Birds in Flocks	16000	
Phage Type 28		
Flocks	2	2
Birds in Flocks	15000	46000
Phage Type 34		
Flocks	2	
Birds in Flocks	12500	
Phage Type RNDC		
Flocks	1	
Birds in Flocks	7000	
Phage Type Untypable		
Flocks	2	
Birds in Flocks	24000	
Phage type 8		
Flocks	19	
Birds in Flocks	207701	

Egg-type Chicken Breeding Flocks with Isolates of *Salmonella enteriditis* by Phage Type and Year 1989-2008

Year	Number of Flocks	Phage Type
1989	1	13A
1990	11	13A, 13, 8, 28
1991	12	13A, 13, 8
1992	10	13A, 8, 28, 34, Untypable
1993	5	8, 2, Untypable
1994	3	13A, 8
1995	2	13A, 28
1996	5	13A, 8, 2, RNDC, Untypable
1997	2	8
1998	2	8
1999	1	13
2000	4	13, 8
2001	1	13
2002	0	
2003	0	
2004	0	
2005	1	13
2006	1	34
2007	4	13, 8
2008	3	8

U.S. *salmonella enteritidis* clean Egg-type Chickens: Number of flocks and birds in the flocks with *Salmonella enteriditis* isolates, 1990-2008

	Environmental	Dead Germ	Bird
Flocks	68	6	19
Birds in Flocks	679,871	77,179	201,342

National Veterinary Services Laboratories Summary of Poultry *Pasteurella-Salmonella-Mycoplasma* Activities 2008

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***Pasteurella*:** During the period of August 15, 2007 through August 14, 2008, the National Veterinary Services Laboratories (NVSL) received 282 *Pasteurella multocida* isolates for characterization. Of these, 57 percent were submitted for somatic type analysis, 15 percent were submitted for DNA fingerprint analysis, and 53 percent of isolates were submitted for both tests.

***Salmonella*:** During the period of July 1, 2007 through June 30, 2008, the NVSL serotyped 18,267 *Salmonella* isolates recovered from animals, their environment, or feed. Of the 4830 poultry isolates (26 percent of total isolates), 3417 were recovered from chickens or their environment and 1413 were recovered from turkeys or their environment. The most common serotypes found in poultry this year are listed in Tables 1 and 2.

Table 1: Most Frequently Identified Serotypes from Chickens

Clinical	Monitor
Enteritidis	Heidelberg
Kentucky	Kentucky
Typhimurium	Enteritidis
Heidelberg	Typhimurium
	Senftenberg

Table 2: Most Frequently Identified Serotypes from Turkeys

Clinical	Monitor
Senftenberg	Senftenberg
Hadar	London
Montevideo	Hadar
Agona	Muenster
	Saintpaul

Mycoplasma: During the period of October 1, 2007 through September 30, 2008, the NVSL performed 187 avian *Mycoplasma* hemagglutination inhibition tests; a 35 percent decrease in testing from last year. During this same period, 1050 ml of hemagglutination antigen and 1058 ml of control sera were provided to other diagnostic laboratories.

Avian Import Activities Fiscal Year 2008

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Poultry and Hatching Eggs: During fiscal year (FY) 2008, 10,879,134 poultry including day old chicks, and 20,557,574 poultry hatching eggs imported into the United States.

Commercial Birds: The imports of commercial birds are limited to those that are exempt for the Wild Bird Conservation Act, serviced by the U.S. Fish and Wildlife Service. During FY 2008, 172,128 commercial birds were released from USDA-supervised private bird quarantine facilities.

Pet Bird Program: There were 531 pet birds imported into the United States through the home quarantine program during FY 2008.

National Veterinary Services Laboratory Avian Influenza and Newcastle Disease Activities Report FY 2008

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Avian Influenza

Live Bird Marketing System (LBMS). As part of the ongoing LBMS surveillance for presence of avian influenza virus (AIV) and avian paramyxovirus type-1 (APMV-1), the National Veterinary Services Laboratories (NVSL) tested 4,129 specimens in 673 submissions from 9 states (Connecticut, Delaware, Florida, Massachusetts, Maine, New Jersey, New York, Pennsylvania, Rhode Island) by virus isolation in embryonating chicken eggs. The surveillance is a collaborative effort between individual States and the United States Department of Agriculture. However, only specimens submitted to the NVSL, which includes all presumptive positive specimens detected at the State level, are reported here.

In FY 2008, AIV or APMV was isolated from 12 percent (82 of 673) of submissions and 4 percent (167 of 4129) of specimens tested. AIV subtype H5N2 was the most common subtype found in the LBMS this year; it was isolated from 51 specimens in 19 submissions. The H5N2 virus was isolated from 30 specimens from NJ, 19 from NY, and 2 from PA. AIV subtype H7 was isolated from 9 specimens; an H7N3 was isolated from one specimen from NJ and H7N7 from seven specimens from NJ and one specimen from PA. The H5 and H7 AIVs were shown to be low pathogenicity avian influenza (LPAI) virus by the chicken pathogenicity test and/or deduced amino acid profile at the hemagglutinin (H) cleavage site. Genetic studies showed the H5 and H7 viruses to be most closely related to North American H5 and H7 viruses circulating in wild ducks. Other subtypes of AIV isolated, the states from which the specimens originated, and the numbers of isolations were: H1N1 (NY, n=8), H1N1,4 (NJ, n=1), H3N6 (NJ, n=5), H3N8 (PA, n=1). Twelve AIVs were isolated that were believed to be mixed infections where the N subtype was shown to be N1 (NJ, n=1; NY, n=2; PA, n=4) N1,4 (PA, n=2; NJ, n=1) or N2 (NJ, n=1; NY, n=1) but the H subtype could not be identified by conventional subtyping assays. The remaining 82 viruses isolated were identified as APMV; 80 were APMV-1 from 7 states (CT, FL, MA, NJ, NY, PA, and RI) and 2 were identified as pigeon paramyxovirus type-1 (PPMV-1) from NJ and PA, respectively. Pathogenicity of representative APMV-1 isolates was determined by the intracerebral pathogenicity index (ICPI, n=9) test and/or by analysis of the deduced amino acid profile at the fusion protein cleavage site (n=55). All but 2 isolates were characterized as low virulent (lentogenic pathotype) strains; the 2 isolates were characterized as pigeon paramyxovirus type-1 (PPMV-1), a pigeon-adapted variant of Newcastle disease virus.

Low Pathogenicity Avian Influenza (LPAI) in Commercial Poultry and Backyard Birds. Surveillance for AIV in commercial poultry is conducted under provisions of the National H5 and H7 Low Pathogenicity Avian Influenza Control Program implemented in September 2006. Although most of the testing is performed locally, the NVSL provides reagents for the agar gel immunodiffusion (AGID) test and confirmation testing of positive specimens. During FY 08, two detections of LP notifiable AI (LPNAI) involving commercial poultry were reported to the World Organization for Animal Health (OIE). The first detection occurred in June 2008 in a single flock (two houses) of 16,000 65-week-old broiler breeders in Arkansas that tested positive for antibodies to H7N3 during routine pre-slaughter testing. No clinical signs were noted in the flock at the time of testing. However, about three weeks prior to testing, the flock experienced a mild increase in mortality and drop in egg production and wild geese were observed to be present on ponds near the poultry houses. An H7N3 virus was isolated from the flock and shown to be LPAI and most closely related to North American H7 viruses circulating in wild waterfowl. The premises was depopulated. The second LPNAI detection occurred in August 2008 in a breeding and raise-for-release upland game bird facility in Idaho. The facility housed approximately 30,300 birds (pheasants, ducks, quail, chukars, and pigeons) and was involved in interstate sales. The flock was first detected when three pheasant carcasses, submitted to the Pennsylvania State University diagnostic laboratory, were found positive for H5 AIV, Pasteurella, and Mycoplasma. Additional specimens collected from the flock yielded AIV H5N8 and antibodies specific to H5N8 in the pheasants and mallard ducks and an H4N7 AIV in the ducks. The H5N8 and H4N7 virus were both shown to be LPAI. Active and passive surveillance in the surveillance zone surrounding the infected premises and trace backs have been negative for additional AIV infections. The flock was depopulated.

In FY 08, infections with LPNAI (H5 or H7 subtypes) were detected in five backyard flocks. Detection of H5 or H7 infection in backyard flocks, by virus isolation or PCR, is included in the semiannual reports to the OIE. Isolated detections of antibodies (alone) in a flock in the absence of clinical disease or epidemiologic link to an outbreak are not notifiable. The first detection in a backyard flock occurred in November 2007 when antibodies to H5N2 (and H3) were detected in turkeys reared in a multi-age, mixed species (turkeys, chickens, and ducks) operation in South Dakota. No virus was isolated and the premises was depopulated by on-site slaughter and

controlled marketing of virus negative birds. The second case involved a mixed-species operation in Massachusetts in January 2008. Premovement testing of pheasants in the facility showed presence of antibodies to H5N2. Swab specimens collected from the birds were H5 positive by real-time RT-PCR but no virus was isolated. The flock was released from quarantine following two negative virologic tests. The third case was detected in February 2008 when H7N7 virus and specific antibodies were detected in a backyard flock of chickens, game fowl, and ducks (170 birds) in North Carolina. The virus was shown to be LPAI by sequencing and chicken pathogenicity test. The flock was depopulated. The forth case occurred in July, 2008 when a backyard flock of about 1,000 birds (multiple species) in New Hampshire was found to be positive for antibodies to H7N7 through routine serologic surveillance. Swab specimens were negative for AIV by rRT-PCR and virus isolation. The flock was released from quarantine following two negative virologic tests. The fifth and final detection of AIV in a backyard flock occurred in September 2008 when a mixed species operation of about 200 birds in Massachusetts was found to be positive for antibodies to H5N2. The flock was under quarantine at the time of this report.

In FY 2007, 392 submissions were received from 25 states for AIV antibody detection and antibody subtyping. The majority of the submissions (320) were from commercial turkeys in 13 states (Arkansas, Iowa, Illinois, Indiana, Michigan, Minnesota, Missouri, North Carolina, North Dakota, Ohio, South Dakota, Virginia and Wisconsin) that were positive for antibodies to subtypes H1 and/or H3 in combination with N1 and/or N2. Vaccination for H1 and H3 is commonly practiced in turkey breeder flocks that are raised in close proximity to swine. Therefore, the total number of positive flocks may represent multiple testing of the same breeder flocks to fulfill the quarterly testing requirements under the National Poultry Improvement Plan. Detection of non-H5 or H7 AIV or AIV-specific antibodies to AIV in poultry/birds is shown in Table 1.

AI Diagnostic Reagents Supplied by the NVSL. A total of 17,423 units of AGID reagents (antigen and enhancement serum) were produced and shipped to 88 state, university, and private laboratories in 37 states during FY 2008. The quantity is sufficient for approximately 2,090,760 AGID tests. An additional 1,343 units (161,160 tests) were shipped to 18 foreign laboratories.

rRT-PCR Test Development and Proficiency Test Panels. A new version of the H7 rRT-PCR assay (2008 H7 rRT-PCR assay) was developed at the Southeast Poultry Research Laboratory (Spackman et al.) to replace the H7 assay that has been in use since 2002. The new assay was needed to detect recent H7 viruses that were missed by the 2002 H7 assay. The 2008 H7 assay has been validated and a new protocol developed and distributed to the National Animal Health Laboratory Network (NAHLN) laboratories. The NAHLN laboratories conducting surveillance testing for AI and/or ND are required to have one or more diagnosticians pass an annual proficiency test (PT) to perform official rRT-PCR tests. In FY 2008, PTs were distributed to 257 diagnosticians in 55 laboratories for AI rRT-PCR and 254 diagnosticians in 54 laboratories for APMV-1 (Newcastle disease) rRT-PCR.

AIV Surveillance in Wild Waterfowl. In 2008, waterfowl surveillance for the highly pathogenic Asian strain of H5N1 in Alaska and the lower 48 states continued. The surveillance is a cooperative effort between USDA's Animal and Plant Health Inspection Service (APHIS, NVSL), Wildlife Services (WS, National Wildlife Research Center, Fort Collins, CO) and the Department of Interior's United States Geological Survey (USGS, National Wildlife Health Center, Madison, WI). Specimens collected from wild-caught and hunter-killed waterfowl, the environment and feces were screened by rRT-PCR for AIV specific RNA at WS, National Animal Health Laboratory Network (NAHLN) laboratories and at the USGS laboratory in Madison, WI. All presumptive H5 and H7 positive specimens were submitted to the NVSL for confirmation and virus isolation. Between October 2007 and September 2008, 814 presumptive positive specimens were received for confirmation testing. No HPAI H5N1 was detected; however, LPAI H5N1 virus was detected in specimens submitted from 2 states (MI, MT). A total of 58 H5 viruses (various N subtypes) from 22 states and 23 H7 viruses (various N subtypes) from 8 states were isolated. All H5 and H7 AIVs were characterized as LPAI viruses of North American lineage. Other AIV subtypes isolated included H1, H3, H4, H6, H10, and H11. Details of the wild bird surveillance will be reported separately.

Newcastle Disease

Isolations of Virulent Newcastle Disease Virus (vNDV). In FY 2008, no vNDV was isolated from domestic poultry or birds confiscated by U.S. Customs. However, vNDV was isolated from one lot of Passerine birds imported through a quarantine facility in California and pigeon paramyxovirus type-1 (PPMV-1) was isolated from 8 pigeons in two states (FL, and NY). In addition, vNDV was isolated from 15 wild cormorants specimens (10 submissions) from three states (CT, n=1; MN, n=5; and WI, n=9).

Isolations of Low Virulent Newcastle Disease Virus (LoNDV). During FY 2008, 52 isolates of APMV-1 were received for characterization at the NVSL or were isolated at the NVSL from diagnostic submissions from 9

states. All of the isolates were characterized as low virulent NDV by the intracerebral pathogenicity index (ICPI) and/or by deduced amino acid motif at the cleavage site of the fusion protein.

ND Diagnostic Reagents Supplied by the NVSL. A total of 262 vials (2ml each) of inactivated LaSota antigen were shipped to 10 domestic laboratories in 7 states and to 5 foreign laboratories. In addition, 45 vials (2ml) of ND antiserum were shipped to 9 domestic laboratories in 5 states and 5 foreign laboratories.

Table 1. Subtypes of non-H5 or H7 low pathogenicity avian influenza virus (AIV) or specific antibodies detected in poultry/birds, FY 2008.

State	Species	Subtype of AIV* (number)	Antibody Subtypes (number)
Florida	Ostrich[a] Black Swan Emu Chicken	 H6N2 (7)	H11N2, H?N2 H6 Multiple H6 (3)
Idaho	Mallard Duck	H4N7	H1N2, H4N7
Maryland	Wild Turkey		H10N3
Massachusetts	Chicken Pheasant		H6 H2,4,6N3
Minnesota	Chicken Turkey	 H6N5	H6N1(2) H6
New York	Ostrich		H8N2 (2)
Pennsylvania	Duck Ostrich	H3N8, H4N8	H1,6N2
South Dakota	Turkey		H1N1 (3), H3N2,(8), H6N5 (7), H1,10N7 (2), H1,6N5 (2), H10N7
Wisconsin	Turkey	H3N2	H3N2 (8)

*Low pathogenicity AIV by the chicken pathogenicity test.

[a]Zoological garden

North American Animal Health Laboratory Network (NAAHLN)

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The NAAHLN is one of several initiatives resulting from the Security and Prosperity Partnership (SPP) of North America, committed to in 2005 by leaders of United States, Canada, and Mexico. The SPP is a trilateral effort to increase security and enhance prosperity among the three nations through greater cooperation and information sharing. The purpose of the NAAHLN is to harmonize laboratory testing procedures to facilitate early detection of targeted diseases that could pose a threat to the animal/poultry industries of North America. In 2007 three Working Groups, comprised of subject matter experts from the National Veterinary Laboratories in each country, were formed to develop harmonization strategies for avian influenza, vesicular diseases, and tuberculosis, respectively. Diagnostic tests targeted for avian influenza include the agar gel immunodiffusion test, hemagglutination-inhibition and neuraminidase-inhibition tests, virus isolation, and real time RT-PCR. To date, efforts have focused on sharing of diagnostic test protocols, exchange of diagnostic reagents, training of laboratory staff, and inter-laboratory testing of harmonization panels. It is anticipated that harmonization for avian influenza will be completed in 2009.

National Animal Health Monitoring System Poultry Studies Update and 2010 Needs Assessment

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The National Animal Health Monitoring System (NAHMS) is a nonregulatory division of the United States Department of Agriculture (USDA) designed to help meet the Nation's animal-health information needs. The Small Enterprise Chicken study was NAHMS' third study of the poultry industry, focusing on biosecurity and bird movement on operations with 1,000 to 19,999 chickens. A questionnaire was mailed in August 2007 to a sample of operations identified as having 1,000 to 19,999 chickens on the National Agricultural Statistics Service (NASS) 2002 Census of Agriculture, with a follow-up reminder sent to non-respondents 2 weeks later. In September 2007, non-respondents were contacted by telephone and surveys were completed via telephone interview. About two-thirds of these operations had any chickens at some time between October 2006 and September 2007 and provided information regarding their biosecurity and bird movement practices.

Over half of operations were contract operations with breeding birds, and one-fourth were contract operations without breeding birds. Only 17 percent of operations were independent (non-contract) operations. Contract operations generally had stricter biosecurity requirements compared to independent operations. A higher percentage of independent operations had outdoor bird access. Additionally, a higher percentage of operations with birds other than chickens had outdoor bird access compared to operations with chickens only. Movement of birds to locations in which other birds were present was rare. Employee contact with birds off the operation was also rare.

Results from this study will be used to parameterize the avian disease model to better prepare for potential disease outbreaks.

NAHMS is currently preparing for a poultry study to take place in 2010. In order to identify the information needs, an information needs assessment was conducted. A questionnaire was distributed to broiler, layer, turkey, and primary breeder veterinarians via their respective professional organizations. The questionnaire was also distributed to federal and state veterinarians and university research/extension personnel. Additionally, discussions were held with each poultry veterinary group to further clarify their information needs.

Research and government stakeholders placed a high priority on avian influenza and biosecurity. Primary breeder and layer veterinarians were mostly concerned with compartmentalization, while broiler and turkey veterinarians were interested in cellulitis/gangrenous dermatitis. Based on the input from stakeholders, we are considering objectives for a NAHMS 2010 poultry study as follows:

Objective 1: Estimate the prevalence and identify risk factors associated with cellulitis and gangrenous dermatitis on broiler and turkey farms.

Objective 2: We are having on-going discussions with industry and APHIS regarding a potential role for NAHMS in the compartmentalization efforts.

Perspectives on FSIS Salmonella Initiatives

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In 2000, the U.S. Department of Health and Human Services (HHS) developed a project called Healthy People 2010 that set a number of ambitious public health goals for the nation including goals addressing illness caused by food borne pathogens such as *Salmonella* and *Campylobacter*. The human salmonellosis goal established by Healthy People 2010 was set at 6.8 cases per 100,000 persons by 2010. In 2000, the case rate was 14.08. In 2005, it was 14.47, higher than the 2000 case rate and over twice the 2010 goal.

In response, the U.S. Department of Agriculture's Food Safety Inspection Service (FSIS) embarked on an ambitious program of actions designed to reduce the contribution of the food products under their jurisdiction (red meat and poultry) to the human salmonellosis case rate. This program was detailed in a Federal Register Notice in February 2006 and consisted of a specific list of actions that the Agency intended to take, one of which was classifying establishments into process control categories (Category I, II, or III) based on their *Salmonella* sample set results. The Agency then established a goal of having 90 percent of all meat and poultry establishments in Category I by October 1, 2010. By June 30, 2007, however, neither turkey nor young chicken establishments had reached the desired 90 percent compliance rate. At that point, FSIS determined that more robust *incentives*, including web-based publication of individual establishment verification results, was necessary to *encourage* the industry to improve its performance in controlling *Salmonella*.

At the same time, it became increasingly apparent that several serotypes of *Salmonella* were contributing disproportionately to the salmonellosis burden. These were deemed to be "serotypes of public health concern." CDC reported that *Salmonella* serotypes accounted for 38.6 percent of all human food borne infections in 2006. A CDC study determined that poultry *meat* is an effective vector for *S. enteriditis* (SE). FSIS data from 2006 showed that the proportion of SE isolates found among all poultry isolates of *Salmonella* jumped from 7.71 percent in 2005 to 13.66 percent in 2006. In addition, a *Salmonella* serotype having the antigenic formula I4,[5],12:i:- has been increasingly recognized by CDC as a leading cause of human illness. This same serotype was also becoming more common in young chicken isolates rising to 5th place in 2006 according to FSIS.

In January 2008, FSIS published a Federal Register Notice[1] detailing the *Salmonella* Initiative Program (SIP). The Agency developed the SIP to offer regulatory waivers to Category I establishments as an incentive for *volunteer* meat and poultry slaughter and processing establishments to increase process control efforts for *Salmonella* and *Campylobacter*. The SIP is also intended to benefit the Agency by providing key microbial data from sampling and analysis conducted *by the establishments* that volunteer for SIP.

Unfortunately, this voluntary program became a de facto *mandatory* program for the broiler industry when the Agency required establishments currently operating with On Line Reprocessing (OLR) and HAACP-based Inspection Models Project (HIMP) waivers to participate in SIP. Over 90 percent of broiler slaughter establishments operate under one or both of these waivers. The program is essentially an unfunded federal mandate requiring extensive microbial testing that is both laborious and expensive. The data collected will increase the exposure of participating establishments to adverse regulatory and activist group actions that could threaten business continuity. The resultant data is neither confidential nor protected from Freedom of Information Act (FOIA) requests and FSIS has stated that it will be linked to public health databases in order to improve attribution efforts.

FSIS is also pursuing several additional initiatives that seek to reduce the acceptable level of *Salmonella* organisms in raw poultry products to essentially zero and to improve the ability of public health officials to trace back the source of salmonellosis cases to specific meat and poultry processing facilities. These include significantly reducing the *Salmonella* performance standard and corresponding categorization levels when the recent broiler baseline study is completed; including comminuted meat in the ground poultry performance standard; and considering an adulterant determination for *Salmonella* in microwaveable convenience foods containing raw poultry. The Agency has also forewarned the Industry that pathogen testing of raw finished poultry products is coming.

While we all agree that human cases of salmonellosis are a legitimate public health issue, the U.S. poultry industry should be rightfully concerned that this drive to de facto zero tolerance for a large group of organisms that are part of the normal microflora of healthy birds may ultimately be unachievable. The pathogen management tools available to the industry today will not result in compliance with the ever-diminishing expectations of the Agency and new interventions or strategies do not appear to be on the near horizon. The result of this constellation of FSIS efforts may not result in the intended outcome of safer meat and poultry, but instead have a marked and potentially economically devastating impact on U.S. poultry processors and, eventually, on the supply of domestically produced poultry meat.

[1]U.S. Federal Register Volume 73, Number 18, January 28, 2008

Research Update on Exotic and Emerging Poultry Diseases

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Research on the understanding and control of viral diseases remains the goal at the Southeast Poultry Research Laboratory (SEPRL), Agriculture Research Service (ARS), USDA. The laboratory is administratively divided into two research units, the exotic and the endemic viral disease research groups. The following is a brief description of research accomplishments over the past year.

Avian Influenza Virus diagnostics

Support of the real-time RT-PCR tests used by the National Animal Health Laboratory Network (NAHLN) for both the H5 and H7 subtype tests were performed during the year. The H5 subtyping test, although originally designed to detect North American viruses, could detect with a previous modification to the forward primer, H5 viruses from Europe and Asia including the H5N1 HPAI viruses. However, diagnosticians at the veterinary diagnostic laboratory in Hong Kong identified several H5 viruses from Hong Kong that were missed by the current PCR test. These viruses were forwarded to SEPRL and sequence analysis showed the viruses were missed because of 3 nucleotide mismatches in the probe region. This was confirmed through further testing. The viruses were a variant of the Fujian H5N1 lineage (Clade 2) that is widespread in China, Vietnam, and likely other countries in the region. Several alternative probes were designed and shown to improve the specificity so that all the H5 viruses in the panel were detected. These changes have not been validated for use in the NAHLN, but the information is being made available to national and international laboratories. A similar story was seen with the H7 subtype test. The H7 test was targeted to North American viruses, and because of the large sequence differences, it did not detect Eurasian viruses. In recent wild bird surveys in the U.S. a large number of wild bird H7 viruses were not detected by the H7 test. Sequence analysis showed multiple changes in the primers and probes that likely accounted for these differences. Because of the large number of differences, a new primer and probe were designed to develop a broader reacting test. The new test was able to identify all the North and South American viruses tested, and it had slightly improved sensitivity over the original test. The National Veterinary Services Laboratories (NVSL) performed a validation study on experimentally infected animals and field samples, and the test performed as expected. With this validation information the test replaced the original H7 test as the official USDA test for the NAHLN program.

Avian Influenza Virus epidemiology

The USDA full coding sequencing genome project is starting to provide large amounts of data that is being annotated and released into GenBank. Currently about 175 viruses have been released in GenBank. As part of this project, a large number of recent H5N2 viruses from Live Bird Markets (LBMs) from the Northeast U.S. and H5 wild bird viruses were sequence and compared. Since the eradication of H7N2 viruses from the LBMs in the U.S., a small number of H5N2 viruses have been isolated. Over 30 viruses isolated over the course of the year were compared with sequence from all eight gene segment to see if the viruses were from a single or multiple introductions into the market. It appeared that at least 6 different introductions of virus occurred in the market system, but evidence of 3 lineages persisting for at least a few months was present. One lineage was predominant, and was present at the end of the study in 2007. Most of these viruses were still in ducks, so the virus had not appeared to have jumped into chickens at this time. Wild bird viruses were also compared from the Eastern U.S. at similar time points as the LBM isolates. The interest was not only wild bird ecology of H5N1 viruses, but to see if wild bird surveillance could be used predicatively to see what would be isolated from the LBM system. Over 30 viruses were examined, but all the viruses appeared to have unique combinations of genes from each other and from the LBM isolates. The surveillance, although designed to detect HPAI viruses in wild birds, did not appear to be useful for predicting poultry outbreaks.

Avian Influenza vaccination

Considerable effort is being made to evaluate new vaccine technology and provide advice on how to most effectively use the vaccines that are currently available. One area of work was to evaluate and develop different DIVA (Differentiate Infected from Vaccinated Animals) vaccine strategies. Two of these strategies are the heterologous neuraminidase (hNA) DIVA strategy and the NS1 DIVA strategy. The hNA strategy is a vaccination in which the hemagglutinin of the vaccine is matched to the field strain, i.e. H5 vaccine for H5 field strain, but the neuraminidase subtype is purposefully mismatched, i.e. N1 vaccine for N2 circulating strain. In experimental studies, this approach appeared to provide a clear differentiation of vaccinated and vaccinated and then infected birds. This DIVA still suffers from several issues including a lack of a simple companion diagnostic test, availability of appropriate vaccines, and adequate validation information.

An alternative strategy is the NS1 (non-structural protein 1) approach. The NS1 protein is a non-structural protein that is not found in the influenza virion. Since killed influenza viruses are primarily whole virion preparations, vaccinated birds should not develop an antibody response to this protein. However, the NS1 protein is produced at high levels in infected cells, and infected birds can develop an NS1 antibody response. Several reports suggested this would be a useful strategy, and we developed a baculovirus expressed NS1 protein to produce an ELISA test that had low background with avian samples. With this ELISA test, the antibody response was evaluated in infected and vaccinated and infected chickens. Unfortunately, using a H6N2 virus as challenge, only 2 of 10 birds were documented to have a serologic response after challenge. Similar results were seen with vaccinated and then challenged birds, in which only a few birds seroconverted after challenge. The lack of uniform response makes the NS1 strategy more difficult to apply since larger numbers of birds would need to be sampled to show freedom of disease with a high confidence interval.

A difference in response to AIV vaccination between Pekin-like and Muscovy ducks has been reported in Vietnam. A vaccination study was conducted in which ducks were vaccinated with the Chinese RE-1 vaccine using three different schedules (at 1 and 14 days of age; only at 14 days of age; and at 7 and 21 days of age) and then challenged with Dk/VN/88/07 HPAI H5N1 virus at 30 days of age. Although the best vaccination strategy for both Pekin and Muscovy ducks was to vaccinate at 7 and boost at 21 days of age, clear differences in response to vaccination was observed between them. Vaccinated and challenged Muscovy ducks presented higher mortality and more neurological signs than Pekin ducks. Pekin ducks had at least 2 $\log_2$ higher HI titers in serum at the moment of vaccination compared to the vaccinated Muscovy ducks, regardless of the vaccination schedule. This study underlines the importance of tailoring AIV vaccination programs to different bird species.

Avian Influenza Virus pathogenesis

The mean infectious doses of selected avian influenza virus (AIV) isolates, determined in domestic poultry under experimental conditions, were shown to be both host and virus dependent and could be considered one measure of the infectivity and adaptation to a specific host. As such, the mean infectious dose could serve as a quantitative predictor for which strains of AIV, given the right conditions, would more likely be transmitted to and maintained in a given species and/or subsequently cause an AI outbreak in the given species. The intranasal (IN) mean bird infectious doses (BID50) for HPAIV isolates for White Leghorn (WL) chickens ranged from 1.2-4.7log10 mean embryo infectious dose (EID50). Although the upper limit for BID50 to predict infectivity and sustainable transmissibility for a specific species is unknown, a BID50 <4.7log10 was suggestive of such transmissibility. For the LPAIVs, there was a trend for domestic ducks and geese and Japanese quail to have the greatest susceptibility and WL chickens to be most resistant, but turkeys were susceptible to all three LPAIV tested when used at moderate challenge doses. This suggests domestic ducks and geese, turkeys, and Japanese quail could serve as bridging species for LPAIVs from wild waterfowl to chickens and other gallinaceous poultry. These data provides support for the commonly held and intuitive belief that mixing of poultry species during rearing and in outdoor production systems is a major risk factor for interspecies transmission of AIVs and the emergence of new AIV stains capable of causing AI outbreaks as these situations present a more diverse host population to circumvent the natural host dependency or host range of naturally circulating viruses.

Better understanding of the molecular basis of AIV pathogenesis contributes to the development of improved prophylactic, therapeutic, and diagnostic reagents to control AI virus infections. With the use of a whole genome chicken 60-mer oligonucleotide 44K microarray we analyzed the transcriptional profiles of ducks infected with different strains of H5N1 HPAI viruses. This permitted the identification of genes differentially regulated after AIV infection. Differences observed in the innate immune response indicate different mechanisms potentially induced by AIV's to modulate the host response in ducks. The differentially expressed genes identified are candidates for further hypothesis-driven investigation of genes determining resistance to AI viruses in ducks and other bird species.

The contribution of the *Mx* gene in resistance of chickens against AIV infection was also investigated. The Mx protein is an IFN-induced protein that confers resistance to influenza virus infection in mammalian species. A single *Mx* gene has been identified in chickens and is induced by interferons-α and β. Asn/Ser dimorphism at residue 631 in the Mx determines antiviral activity against H5N1 influenza virus in transfected cell lines. The *Mx* gene in chicken breeds is polymorphic; Mx 631Asn (Mx+) has antiviral activity; Mx 631Ser (Mx-) lacks antiviral activity. A higher frequency of the favorable allele +/+ is found in native breed lines of chickens, including breeding stock, and the -/- allele is more commonly found in highly selected lines of chickens (e.g. broiler lines). Mx +/+ or -/- chickens were infected with a highly pathogenic H5N2 virus and mortality was followed over 2-week period. A lower mean death time was observed in -/- Mx631 SNP lines of birds following HP challenge. Differences in cytokine responses, in particular IFNα, were also observed between Mx types. Increased IFNα expression correlated with increased MDT following high dose HPAI challenge. Future studies are planned to further examine the role and contribution of avian Mx to resistance to AI infection.

Newcastle Disease Virus Epidemiology

Sequence analyses of both domestic and foreign NDV isolates were conducted in the last year. In a collaborative project, inactivated viral samples from Mexico were sequenced and evaluated. The recent viruses were still Class II, Genotype V viruses as were viruses evaluated from 1996-2002, but they clearly separated into a distinct group. This clear difference in viruses may be contributing to the perceived increase in clinical disease in layer flocks in Mexico because of the antigenic differences. These differences should remain a concern to the U.S. also, because of previous history of Mexican lineage viruses causing outbreaks in the U.S. In a separate study of wild pigeons and doves in the U.S. and number of virulent NDV viruses were isolated. The study was originally a West Nile Virus surveillance project, but a class II Genotype VI NDV virus was isolated from birds from two different states in the project. These viruses are the pigeon adapted variants that are seen worldwide, but this documents that they are relatively common in our wild pigeon and dove populations, at least in urban settings. The risk of spread to poultry remains unknown, but previous outbreaks in the U.K. have been traced to pigeon origins, and therefore this should remain a concern for U.S. producers.

Newcastle Disease Virus Vaccine Studies

Newcastle disease virus is recognized as having only a single serotype, so antibody to one virus will neutralize all NDVs. However, antigenic differences between different NDV viruses are also known to occur, and this antigenic variation does appear to affect protection. In previously published experiments with killed vaccines, it has been demonstrated that using a homologous vaccine provides better protection based on virus shedding than vaccinating with a less closely related virus vaccine based on differences in virus shedding. Recent work was conducted to see if homologous vaccination also provided improved protection with live vaccines. Using reverse genetics techniques, the HN and F genes of CK/CA/02 velogenic NDV was inserted into a less virulent virus that could be used as a live vaccine. A comparison of the recombinant vaccine with LaSota and using two different challenges, TXGB and CK/CA/02, it was demonstrated that using homologous vaccination provided better protection as measured by the number of birds shedding virus and the amount of virus being shed. This data provides support for consideration of updating our current vaccine viruses to more closely match the circulating strains to achieve the best protection possible.

Enteric Viruses in Chickens and Turkeys

Enteric diseases continue to cause substantial economic losses to the US poultry industry. Similar to surveys previously conducted at SEPRL, of 46 pooled samples from turkeys collected in the Midwest United States, 98 percent were positive for astrovirus, 65 percent were positive for rotavirus, and 22 percent were positive for reovirus. However, no specific association was found between the presence of a particular virus and enteric disease.

We continue to develop and improve diagnostic tests for enteric viruses in poultry. A reovirus S4-specific RT-PCR test was developed for use in chickens and turkeys, and the astroviruses-multiplex test to differentiate chicken and turkey viruses was improved. Characterization of rotaviruses continues by sequencing the NSP4 and VP6 genes**.** The use of the MA104 cell culture allowed the isolation of rotavirus from samples containing other enteric viruses, permitting the further study of these viruses.

Pathogenesis studies using genetically distinct turkey-origin reoviruses (TRVs) showed that poults infected with certain TRV isolates had moderate to severe bursal atrophy, suggesting virus-induced immune dysfunction. In order to characterize the effect of TRV infection on the turkey immune system, studies were conducted to quantify the humoral and cell-mediated immune responses in poults infected with the TRV isolate NC/SEP-R44/03. A marked effect on the cutaneous basophil hypersensitivity response and on the antibody response to Newcastle disease virus (NDV) exposure was noted in commercial and SPF poults inoculated with NC/SEP-R44/03 at three days of age. All inoculated poults had moderate to severe bursal atrophy. This immune dysfunction and bursal atrophy was not noted in commercial poults inoculated at three weeks of age.

The World Organization for Animal Health (OIE) Updates--Poultry

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The World Organization for Animal Health (OIE) has updated several animal disease Code chapters and appendices for 2008. At its May 2008 General Session Meeting, the International Committee adopted new text to several existing chapters. Of interest to the poultry industry, the following chapters were updated:

Avian Influenza (AI). For 2008, the Code chapter on AI received only minor updates, however, the United States has asked the Terrestrial Animal Health Standards Commission to consider revising a couple of the sections in the chapter as many Member countries continue to misinterpret the chapter as it pertains to the export of fresh poultry meat.

Zoning and Compartmentalization. Like the Code chapter on AI, the Code chapter on Zoning and Compartmentalization received only minor updates. Included in the chapter is the concept of "containment zones" which was first introduced last year. In addition, the Code now contains an appendix, which provides some general guidelines on the application of compartmentalization.

Newcastle disease (ND). The new Code chapter on ND was adopted at the May 2008 General Session. This chapter was further modified since it was first distributed for review and comment last year. The only ND virus that is reportable is that which either has an intra-cerebral pathogenicity index of 0.7 or greater in day-old chicks, or whose protein structure follows the basic amino acid sequence known to cause virulence. In addition, its definition of "poultry" has been modified to parallel that found in the AI Code chapter.

Animal Welfare. No new specific guidelines for animal welfare were adopted this past May. However, the definition of "animal welfare" was revised. This definition introduces a certain amount of subjectivity to the term, which has prompted the United States, as well as several other Member countries, to send in comments to the OIE asking that it be revised. In addition to the definition of animal welfare, the OIE will also be producing a discussion paper that will address guidelines on housing and husbandry of terrestrial animals. This document should be available by November or December of 2008.

HPAI Preparedness and Response
Quarantine and Movement Control: Continuity of Business in a Control Area

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Preparing for and responding to foreign animal diseases such as Highly Pathogenic Avian Influenza (HPAI) is a critical mission to safeguard our nation's animal health and food supply. Coordination and cooperation between multiple levels of local, State and Federal government, and coordination and cooperation with the food and agriculture industry sector, is necessary to achieve strong capabilities for the emergency management goals of prevent, prepare, respond and recover.

A specific challenge of foreign animal disease preparedness and response is the ability to rapidly incorporate veterinary functions and countermeasures into emergency management operations, and the ability to scale-up veterinary functions and countermeasures in a moderate to large-scale outbreak.

Another challenge of foreign animal disease and response is establishing priorities for goals and objectives, and identifying those goals and objectives that become (or remain) competing interests during an actual incident or outbreak.

For instance, the goal of containing and eradicating a foreign animal disease within a control zone may be in potential competition with the continuity of business planning for food and agriculture sector premises or facilities located within a control zone, that seek to maintain continuity of business, or that seek to re-establish continuity of business as rapidly as possible, by demonstrating non-infection and effective biosecurity practices.

While some competing priorities may be impossible to identify or resolve prior to a specific incident or outbreak, other competing priorities can be partially resolved or mitigated prior to the incident or outbreak, by elevating the awareness of those competing priorities, identifying the resources needed to accomplish those competing priorities, and establishing commonly accepted and understood response objectives.

As each State and industry sector develops their HPAI and FMD response plans, it is critical that incident goals, objectives, strategies, procedures and timelines are coordinated with Federal planning. This will enhance coordination and communication between all partners, produce less chance for unmet expectations or overlooked actions, and speed up a successful response. In short, the coordination objective is to integrate, synchronize, and de-conflict all levels of preparedness and planning, as much as possible, prior to an incident.

Assessments of the current capability for veterinary functions and countermeasures will help identify gaps or shortcomings in current response preparedness and planning, and help provide a framework to local, State, Tribal, Federal Tribal and Industry officials in assessing their individual response capabilities for HPAI and FMD, and identify those capabilities that need to be further addressed or elevated.

Preparedness goals for continuity of business in a control zone are the following:

- Identify the FAD agents that may cause potential quarantine or movement control restrictions.
- Establish biosecurity programs that are demonstrable and measurable, prior to the incident or outbreak of the FAD. Identify gaps or critical control point in biosecurity process and functions and provide correction prior to the incident or outbreak.
- Prioritize disruptions to business continuity by specific animal movements or animal commodity movements.
- Perform risk analysis or risk assessments for the animal movements or animal commodities that are potentially disrupted.
- Establish capability to perform diagnostic testing, as part of the infected zone or buffer zone surveillance plan, prior to the incident or outbreak. Surge capacity requirements for materials and personnel requirements need to be addressed.
- Establish capability to record and track herd health or flock health production parameters, to demonstrate herd or flock health at the start of an incident or outbreak. Identify information management systems or capability for storing and transferring information.
- Perform epidemiology assessment or questionnaire at the start of an incident or outbreak, to document the status of any contacts or traces to infected premises, contaminated personnel, or contaminated conveyances.
- Establish the relationships necessary to develop and implement continuity of business planning, with all associated local, State, and Federal stakeholders and agencies. Identify the resources needed to implement continuity of business planning. Recognize, discuss and analyze the economic consequences for the potential competing goal of containment and control of foreign animal diseases in control zones.

- -Develop movement control model plans and protocols that can be implemented during an incident or outbreak. These model control movement plans need to take into account the emergency management and NIMS requirements for command, administration, logistics, planning, and operations.

The Egg Sector Working Group (comprised of egg industry officials and the United Egg Producers (UEP), the University of Minnesota Center for Animal Health and Food Safety (CAHFS), the Iowa State University Center for Food Security and Public Health (CFSPH), APHIS CEAH staff, and APHIS NCAHEM staff have participated in a private-public-academic partnership to develop effective science based solutions for the continuity of business in a Control Area (Infected Zone and Buffer Zone) during a HPAI incident or outbreak.

The FY 2008 products of this private-public-academic partnership are the following:

- Draft Egg Movement Control Model Plan: Commercial Layer Industry Operations: Protocol for the Movement of Liquid Egg Product, Further Processed Egg Products, Inedible Egg, Table Eggs and Broken Egg Shells, Egg-Type Hatching Eggs, and Day-Old Chicks Within, Out of, and Into a Defined "Control Area".
- Rapid Decision Making Tool (FAST Eggs); Cooperative Agreement between ISU and APHIS.
- FAST Eggs Rapid Approval Program Biosecurity Checklist for Egg Production Premises and Auditors.
- FAST Eggs Active and Passive Surveillance Program using RRT-PCR and Flock Performance Indicators.
- FAST Eggs Spatial Risk Analysis Algorithm.
- CEAH, Egg Sector Working Group, and UMN CAHFS, An Assessment of the Risk Associated with the Movement of Pasteurized Liquid Egg and Its Products Into, Within, and Outside of a Control Area during a Highly Pathogenic Avian Influenza Outbreak for Pasteurized Liquid Eggs (PLE) Risk Assessment.

The FY 2009 goals are to complete the FAST Eggs cooperative agreement and deliverables, produce more commodity based risk assessments, and most importantly, obtain additional private-public-academic policy and science support, and identify the emergency management and incident command capabilities and resources needed to implement such planning in terms of command, administration, planning, operations, and logistics.

National Veterinary Stockpile Report

Dr. Lee M. Myers
National Veterinary Stockpile
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The National Veterinary Stockpile (NVS) is the nation's repository of supplies, vaccines, equipment, and other critical veterinary resources. Established by Homeland Security Presidential Directive 9 and operational in 2006, we are able to deploy large quantities of veterinary resources anywhere in the continental U.S. within 24 hours. We exist because of the nation's concern after 9/11 that terrorists could release animal diseases of catastrophic proportions that would deplete State and local response inventories, generate surge material requirements that would overwhelm traditional commercial sources, and prevent unaffected States from providing significant help for fear of the threat crossing their borders.

Our goal is to deploy countermeasures against the 17 most damaging animal diseases that affect human health and the economy, including foreign poultry diseases such as highly pathogenic avian influenza and exotic Newcastle disease. We also help state, tribe and U.S. territory animal health officials before an event, plan for and exercise the rapid request, receipt, staging, storage, and distribution of NVS resources during an event.

We provide the United States Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) new, significantly improved support for responding to animal diseases. Our logistics experts manage critical delivery of the following emergency response resources in support of a damaging animal disease outbreak.

- Personal protective equipment for responders
- Depopulation equipment and supplies
- Decontamination supplies
- Field diagnostics
- Vaccines and vaccine delivery equipment/supplies
- Satellite communications equipment for reliable voice and data capabilities
- Depopulation, disposal, and decontamination (3D) commercial services

Using 3D commercial contractors to support an animal disease response is a novel approach. We qualify and manage these companies to assure they can provide large numbers of trained, medically qualified responders starting within 24 hours. The 3D personnel are experienced in responding to all-hazards, and working within the incident command system. They have expertise in cleaning and decontamination, transporting biological hazards, and other specialized skills. We value the poultry industry's emergency response capabilities as local assets prior to and during deployment of NVS resources for a damaging poultry disease outbreak (i.e. highly pathogenic avian influenza and exotic Newcastle disease).

Our strategy is to secure countermeasures for all 17 animal disease threats. Future capabilities will include the following.

- Contractors fully trained in livestock and poultry animal disease response
- Additional animal handling & depopulation equipment
- Supply chain management system to coordinate inventory and deployments
- Vaccines and test kits
- Additional distribution centers to reduce deployment time

As a component of the APHIS National Center for Animal Health Emergency Management, we respond at the direction of APHIS management. Federal government emergency funding pays the costs for deployed NVS resources, including 3D commercial services, in response to a damaging animal disease outbreak. Payment is dependent upon concurrence of the state veterinarian, the area veterinarian in charge, and the regional director, and approval by APHIS executives.

We focus our outreach efforts on state/tribe/U.S. territory animal health partners, and appreciate the following groups providing information and recommendations through their representatives on the NVS Outreach Working Group during FY2008.

- APHIS VS National Incident Management Team
- APHIS VS Area Emergency Management Coordinators
- Multistate Partnership for Security in Agriculture
- National Assembly of State Animal Health Officials
- National Animal Health Laboratory Network
- National Association of State Departments of Agriculture
- National Emergency Management Association
- National Livestock Commodity Groups (National Pork Board, USA Poultry & Egg Export Council)

- Strategic National Stockpile
- State Strategic National Stockpile Coordinators
- Southern Animal & Agriculture Disaster Response Alliance
- U.S. Department of Homeland Security Office of Health Affairs

In collaboration with the NVS Outreach Working Group, we are developing NVS planning tools to assist state/tribe/U.S. territory NVS preparedness and response plans. The tools will provide guidance, suggested processes and mechanisms for how state/tribe/US territory animal health officials will plan to request, receive, stage, store, distribute and recover NVS resources. The toolkit will be posted on a secure portal of the NVS website http://nvs.aphis.usda.gov for state NVS planners.

2008 Live Bird Marketing System (LBMS) H5/H7 Low Pathogenic Avian Influenza (LPAI) Program Working Group Report

Patrice Klein, Fidelis Hegngi, and Angela Pelzel
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In October 2004, Veterinary Services (VS) published Uniform Standards for H5 and H7 LPAI Prevention and Control in the LBMS to establish a more consistent approach by participating States in the control of LPAI in the LBMS. A revised and updated edition of the Uniform Standards was published in August 2008, which includes a new section on General Criteria for Indemnification of H5/H7 LPAI in the LBMS.

State participation is voluntary; participating States will enact regulations necessary for compliance of their live bird markets (LBMs), producers, and distributors. All LBMs, producers, and distributors that supply the markets must be registered or licensed with the State and must allow Federal and State inspectors access to their facilities, birds, and records. These facilities must also have written biosecurity protocols in place. USDA-APHIS coordinates and administers the program. USDA-APHIS provides personnel and resources to assist States with implementation and compliance with program requirements.

Surveillance in the LBMS remains a high priority. USDA APHIS initiated cooperative agreements with 32 States and Territories in fiscal year (FY) 2008. In the Western Region, 12 States were awarded LBMS - LPAI cooperative agreements (Alaska, California, Colorado, Idaho, Iowa, Kansas, Missouri, Nebraska, Oklahoma, Oregon, Texas and Washington) to conduct LBMS surveillance. In the Eastern Region, 20 States and Territories have been awarded LBMS cooperative agreements (Alabama, Connecticut, Delaware, Florida, Georgia, Indiana, Kentucky, Massachusetts, Marlyand, Michigan, Minnesota, New Jersey, New York, North Carolina, Ohio, Pennsylvania, South Carolina, Vermont, Virginia, and Puerto Rico) to conduct LBMS surveillance. Many of these States also were awarded separate LBMS – HPAI cooperative agreements to conduct AI surveillance in higher risk areas. Additional States not previously listed that were awarded LBMS – HPAI cooperative agreements were Arizona, Illinois, Louisiana, New Hampshire, North Dakota, Rhode Island, South Dakota and West Virginia.

In February 2008, the annual LBM Working Group business meeting was held in Miami, Florida to address H5/H7 LPAI Prevention and Control program issues. More than 87 participating members of the industry and States attended the meeting. Even though the Northeast region remains a focal area of LBMS – AI disease control concern, the program has expanded to a national scope with the addition of new states in the Midwest and the Western regions. In addition, the LBMS-WG discussed the program's progress, shared ideas, and agreed on further implementation of the program.

As part of USDA's continued initiative to combat Notifiable AI (NAI), APHIS/VS has conducted annual LBMS Continuing Education training workshops since 2004. The 2008 LBMS-CE course was held at the University of Connecticut, Storrs, CT on Aug 21-23, 2008. The purpose of the course was to inform and familiarize State and Federal employees working in the LBMS NAI surveillance activities throughout the United States with various aspects of the LBMS. These aspects included respiratory diseases that affect poultry, laboratory testing and sample collection, biosecurity and records auditing, personal protective equipment, demonstration of correct euthanasia techniques, geographic information system, State and Federal regulations, the role of USDA's Investigation and Enforcement Services, Smuggling Interdiction and Trade Compliance, risk communication, an update on HPAI H5N1 events worldwide, and cultural sensitivity in the LBMS setting. Activities included the lectures, discussion groups, hands-on practical wet-labs, and a field trip to a LBMS Distributor. A total of 83 registrants attended to include 70 State and Federal personnel from 25 States and territories and 13 international participants from 9 countries representing Mexico, Dominican Republic, Egypt, Guadeloupe, St. Vincent and the Grenadines, Barbados, St. Lucia, Haiti, and Jordan,

From July 2007 to June 2008, 75,456 tests were conducted for H5/H7 LPAI surveillance in the LBMS. Virus isolation and real-time reverse-transcriptase polymerase chain reaction (RRT-PCR) tests commonly were done on pooled samples of 5 swabs per tube. Therefore the actual number of samples collected is reflected as follows:

- 24,999 birds were tested for AI antibodies on agar gel immuno-diffusion (AGID).
- 44,105 birds and environmental samples were tested for AI virus-by-virus isolation (VI) represented by.
    - 6713 environmental tests conducted on pooled samples of 5 swabs per tube)
    - 2108 bird tests conducted on pooled samples of 5 swabs /tube)
- 141,112 tracheal/oral pharyngeal swab samples were tested for AI antigen RRT-PCR (pooled samples 5/tube).

Testing at the National Veterinary Services Laboratories (NVSL) is not included in this report, but all presumptive positive samples were submitted to NVSL for confirmation.

In FY08, 20 LBMS premises were found positive for NAIV (all H5N2 with an exception of 1 H5N9): 3 production flocks; 2 auctions; 15 retail LBMs. Also in FY08, 5 backyard premises were positive for NAIV.

As a result of the H5/H7 LPAI LBMS program and the surveillance and response efforts by VS and the States, the incidence of LPAI in LBMS, especially in the Northeastern United States, has decreased steadily. In comparison of the same time periods of July 06 – June 07 to July 07 – June 08, the percent of LBMS positive premises decreased 41 percent (from 34 to 20) and the number of positive live bird markets decreased 48 percent (from 29 to 15). None of the registered distributors tested were positive during July 07 – June 08 although 2 auction markets did test positive. Overall, since the initiation of the H5/H7 LPAI –LBMS program, the total number of LBMS positive premises has decreased tremendously especially in the Northeast region of New York, New Jersey, PA, and the New England States.

Description of and Implementation Considerations for Compartmentalization

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The World Organization for Animal Health (OIE) has fairly recently developed guidelines for different approaches that a country may consider when affected by animal diseases which could allow them to continue international trade. These approaches of zoning and compartmentalization have several basic similarities but are operationally different. Zoning creates subpopulations of susceptible animal species within a country on a geographic basis whereas compartmentalization creates subpopulations of susceptible animal species within related establishments having a common biosecurity management system.

Both approaches involve the establishment of biosecurity measures, which protect the sub-population of interest and continuous oversight to assure that the biosecurity measures are effective. The involved industry in a compartment has the primary responsibility to assure that the approved biosecurity measures are enforced and sufficient information is available to the certifying authority, the official Veterinary Services to initially approve the compartment and to assure that the biosecurity measures remain effective as disease prevalence changes in the country. Disease pathway analysis and a Hazard Analysis Critical Control Point approach are beneficial tools for identifying and mitigating disease risk when addressing biosecurity.

Proposed trading partners will also need to evaluate and approve compartments prior to initiating or continuing trade in the event of disease outbreaks of concern in an exporting country. Because of costs of biosecurity, the involved industry should also evaluate the benefit/cost of establishing and maintaining compartments as a factor in considering compartmentalization as a method to achieve business continuity.

The poultry industry in the U.S. is interested in the concept of compartmentalization and may propose the development of compartments.

Simultaneous Detection and Identification of Multiple Pathogens for Differential Diagnosis of Avian Influenza

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A prototype application is presented in which a relatively comprehensive differential diagnosis of avian influenza is performed in a single assay – directly and explicitly distinguishing all possible A/HN subtypes. The assay simultaneously tests for strains and variants of other avian viral and bacterial pathogens that can confound diagnosis of avian influenza, or compound the morbidity and mortality of influenza infection. The technology can detect and differentiate known and unknown, emergent strains and variants of targeted pathogens. Since the specimen must provide a template if diagnostic sequencing is to succeed, the likelihood of a false positive detection event is nil. Extensive sequence-based genotyping from assay results supports forensic epidemiology, critical for detection and tracking of naturally emergent infectious diseases, and attribution in possibly hostile encounters with a particular biothreat agent.

This new technology will enhance the role of regulatory agencies and practitioners in assuring the security of agriculture and the food supply. Diagnostic methods to date have employed classical culture or biochemical tests based on labeled markers, focused upon serial testing of single or few agents. Today's evaluative methods are based on the limitations of such tests, and need to estimate likely risk of assay failure (false positive/false negative) compared to benchmark gold standard assay methods. If modern and unequivocal genomic methods of detection are ever to replace classical but slower and less precise "gold standards", an entirely new paradigm for validation and official certification is essential. A cost-benefit assessment supports the upgrading of validation protocols and resources, as an investment with expectation of returns as superior information for critical agricultural, veterinary and public health decisions.

The threat of bioterrorism requires multiple pathogen detections within hours, not weeks, and test results must be specific enough to warrant appropriately measured response. The test must provide authorities with proof positive as to the strain, subtype and perhaps the source of the agent, and it should provide law enforcement agencies with forensic evidence leading to attribution. This set of capabilities and requirements are met by highly multiplexed pathogen gene sequencing-based diagnostic methods available today.