

## REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

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The Committee met on Sunday, October 24, 2004 from 12:30 pm to 6:15 pm. A total of 33 committee members and 37 visitors were recorded on the roll. Chair Peter Timoney presided assisted by Vice Chair James Watson. Committee members were recognized and given the opportunity to introduce themselves. Papers on a variety of diseases or disease related issues of topical importance were presented. The program included two time-specific papers the first of which was entitled "A Better Understanding of Non-Immune Approaches to the Prevention and Control of *Streptococcus equi* Infections" by Dr. John Timoney, Gluck Equine Research Center, University of Kentucky. The second time-specific paper was entitled "Equine Infectious Anemia and Control of The Disease: How Much is Enough?" by Dr. Charles Issell, Gluck Equine Research Center, University of Kentucky. The complete text of both papers are included in these proceedings.

Dr. Sabrina Swenson, United States Department of Agriculture (USDA), Animal Plant Health Inspection Service, (APHIS), Veterinary Services (VS), National Veterinary Service Laboratory (NVSL) discussed

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a paper entitled "Vesicular Stomatitis: 2004 Experience". This year's occurrence, which has been restricted to Texas, New Mexico and Colorado, was caused by the New Jersey serotype of the virus. Characterized by a variable clinical attack rate on affected premises, the disease has been reported primarily in horses, with significantly fewer cases confirmed in cattle and an isolated case each in a llama and an alpaca. Diagnosis was based primarily on serological findings, the competitive Enzyme-Linked Immunosorbent Assay (cELISA) being used as a screening test on suspect cases of infection and any positive samples re-tested by the complement fixation test to establish recentness of exposure to the virus. Virus detection was attempted by virus isolation, polymerase chain reaction (PCR) and antigen-capture Enzyme-Linked Immunosorbent Assay (ELISA).

West Nile Virus (WNV) was the subject of two presentations. Dr. Katie Wetherall, California Department of Food and Agriculture, presented an overview of WNV in California for 2004. The state has recorded the highest number of equine cases of the disease nationally. Of particular concern was the higher than usual case-fatality rate in affected horses (over 40%) and under-reporting of cases of the disease from the field. The California Department of Food and Agriculture role in the outbreak included programs on awareness and education, disease prevention and control in horses and equine surveillance. Case findings to date clearly underscore the importance of vaccination as the best available means of preventing neurological disease caused by WNV. Dr. Stephanie Thompson, Merial, presented information on the development of a recombinant canary pox vectored WNV vaccine (Recombinek) and its safety and efficacy for use in horses. Due to host specificity, productive replication of the canary pox vector does not occur in mammalian cells; therefore stimulation antibody production directed at the virus vector does not occur. The recombinant equine WNV vaccine has been proven effective in the face of a live WNV-infected mosquito challenge. In a year-long, two dose duration of immunity study, horses were fully protected from viremia following a virulent WNV infected mosquito challenge. In a further study, 100 percent efficacy against viremia was demonstrated as early as 14 days after completion of the initial two dose vaccination series. Immunization has been shown to illicit better humoral and cell mediated immune responses in vaccinates.

Drs. Timothy Cordes and Freeda Isaac, USDA-APHIS-VS, Riverdale, MD, made a presentation entitled "Uniform Methods and Rules (UM&R) for Equine Viral Arteritis (EVA): What's Next?" The UM&R, published in 2004 after significant input from members of the horse industry, provide a framework for states to use in developing their respective control programs against this disease. The UM&R should not be regarded as a final definitive document, it can be modified as new

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scientific information and procedures become available. It was established that the UM&R for EVA was not binding on states, implementation of what it contained could not be enforced by the USDA without implementation of interstate regulations. Individual states should consider adoption of the UM&R standards. Under terms of World Trade Organization's Sanitary Phytosanitary Agreement, USDA can only enforce entry-testing requirements for EVA on stallions and imported semen or embryos after a domestic control program has been established. Implementation of a testing requirement on interstate movement was suggested as a possible first step in moving forward with a domestic control program. It was felt that the endorsement of the horse industry should be sought before any further action is taken.

Dr. Isaac then presented "Contagious Equine Metritis (CEM): Quarantine Facilities Guidelines for States and Related Issues". At the request of a number of State Veterinarians and veterinary practitioners, the USDA Working Group on CEM significantly revised guidelines used to approve premises and facilities for quarantine and testing infected stallions and mares for CEM. As a result, specific criteria were developed for the approval of premises and facilities, management, sampling and inspections of mares and stallions while under quarantine. The CEM Working Group has also been working on updating the current CEM Regulations. As various issues are still under consideration with respect to the diagnostic tests used to detect the carrier state in this infection, these recommendations have not been finalized. Reference was made to the importance of monitoring horses entering the United States under 90 day temporary import permits. The compliance agreement that is signed between USDA and the State Veterinarian during such events was considered sufficient to address this concern.

Dr. Isaac also made a presentation on "Equine Health Issues and the European Union". In the spirit of facilitating trade between the United States and Member States of the European Union (EU), a number of animal health working groups had been established to address specific animal health trade issues. Earlier this year, the USDA formed an Equine Technical Working Group which had an inaugural meeting on July 6, 2004 via teleconference with a group of EU Commission officials to discuss a range of issues of concern to both parties. Topics addressed were; "Concerns of potential equine health issues as a result of having 10 new member states"; "Pre and post-entry testing requirements for CEM and EVA"; "Standardization of diagnostic laboratory tests with respect to Dourine, Glanders and Piroplasmosis"; "Pre-embarkation Veterinary Inspections and Certification"; "Implementation of CELISA for Piroplasmosis on horses entering the United States" and "Collection of equine embryos for exportation to the United States". Dr. Isaac reported that this meeting was very productive and hoped that these meetings could occur on an annual basis.

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“Reclassification of Diseases by the Office of International des Epizooties (OIE)” was the subject of a presentation by Dr. Cordes. OIE disease classification and reporting of those diseases, has traditionally been based on criteria that delegates the disease to OIE List A or B. The new disease classification system, which will begin on January 2005, will be based on four criteria. These criteria are: ability of disease to spread internationally; zoonotic potential; significant spread in naive populations; and if it is an emerging disease. The new notification requirements will be as follows: emergency reports- within 24 hours; all endemic diseases-every 6 months; and annual reports will provide comprehensive disease outbreak and related information.

Dr. Cynda Crawford, University of Florida presented a paper on “Equine Influenza Virus Infection in Greyhounds”. What was described as a newly emergent disease of greyhounds was associated with respiratory disease of variable severity with a case fatality rate of over 30 percent in some recorded outbreaks. Epidemics of “kennel cough” had been recorded in track greyhounds in 1992, 1999, 2003, and 2004. The majority of the affected animals coughed for up to two weeks, with many dying from a peracute pneumonia. A strain of equine influenza virus was isolated from the lungs of a fatal case during an outbreak in Jacksonville, Florida earlier this year. The virus was sequenced and phylogenetically compared with other mammalian and avian influenza viruses. It was found to be genetically related to strains of equine influenza virus (H3N8) in circulation in the United States in 2002 and 2003. The canine prototype virus has been designated (Influenza A/Canine/Florida/43/04/H3N8). This year’s outbreaks of influenza virus related respiratory disease have resulted in nationwide restrictions of greyhound movements and significant loss of income from those involved in the greyhound racing industry.

Dr. Eileen Ostlund, USDA-APHIS-VS-NVSL, presented a paper entitled “Three Tiered Laboratory System for the Serologic Diagnosis of Equine Infectious Anemia (EIA)”. A Pilot Program was conducted to examine the impact of the proposed tiered laboratory system for EIA. Four states, Georgia, Iowa, Oklahoma and Oregon participated in the Pilot Program and required Tier 1 laboratories to conduct ELISA tests for EIA. Positive ELISA samples were referred to Tier 2 (State/university) laboratories for further testing. Discrepant samples were forwarded to the National Veterinary Services Laboratories (NVSL) for resolution. During the 6 months duration of the Pilot Program, approximately 60,000 samples were tested in Tier 1 laboratories. A total of 62 ELISA positive samples were referred from Tier 1 laboratories. Of these, 43 were resolved at Tier 2 laboratories and 19 were referred to NVSL (Tier 3). Twenty-four EIA positive horses were identified in participating states during the Pilot Program; of these, 4 required confirmation at NVSL.

It was estimated that the Pilot Program encompassed 6% of na-

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tional testing for the time period. Feedback from participant laboratories indicated general acceptance of the ELISA method at Tier 1 laboratories. Concerns about limiting Tier 1 laboratory testing options and prevention of Tier 1 laboratories from conducting tests for international movement were expressed. Several states declined participation in the Pilot Program for the same reasons. Tier 2 laboratories noticed an increase in workload with referred samples and additional EIA testing for international movement. Additional testing of ELISA positive samples, for horses that were eventually resolved as negative, inconvenienced laboratory clients. "False positive" ELISA results were possible with all licensed brands of EIA tests. Confirmatory testing at Tier 2 and Tier 3 laboratories was required for final determination of EIA status.

Ms. Amelita Facchiano, Global VetLink, presented a paper entitled "Diagnostic Lab Connectivity and Electronic Health Certificates for Equids". Diagnostic Laboratory connectivity with electronic health certificates provide laboratories and private practitioners with real-time record keeping, accurate epidemiology data queries for the dissemination of information relating to the diagnosis of animal diseases, animal movement tracking and trace back reports necessary for regulatory surveillance, monitoring, and control of existing, emerging and/or foreign animal diseases.

The rationale for her presentation was built upon significant accomplishments in the development and implementation of electronic health certificates and diagnostic lab connectivity since 1999 and to present the epidemiology results with electronic health certificates with diagnostic laboratory connectivity for Equine Infectious Anemia (EIA) between September 2001 and September 2003.

In 1999, the Florida Department of Agriculture and Consumer Services (FDACS), contracted with GlobalVetLink, LC of Ames, Iowa, for a project encompassing Internet applications for all species and diagnostic lab connectivity for Equine Infectious Anemia (EIA) applications necessary for animal health regulatory management.

From a time period of September 2001 through September 2003, the State of Florida produced a total of 50,114 certificates for 19,701,679 total animals. Of the total queried, the numbers represent 19,437 EIA and 2,681 Official Certificates of Veterinary Inspection (OCVI) that include diagnostic lab test results. The system is used in the export of horses to more than 47 states and 3 U.S. territories.

Tests for the 20,200 EIA applications were submitted to one (1) state and two (2) private diagnostic labs. All tests were reported Negative. None were positive, suspect or needed retest. Ninety percent were run with Agar Gel Immunodiffusion test and 10% using ELISA. The reasons for testing were: 18,834 Annual, 9 Breeding, 49 Change of Ownership, 31 Export, 274 First Test, 87 Market, 123 Other, and 51 Show.

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Digital images, an additional method of identification, replace the hand drawings, are provided on the lab submittal form and are available in the lab applications should a Certified Copy be requested by a veterinarian and/or client. With diagnostic lab results and vaccination records readily available on OCVI's, the electronic health certificates provide immediate ability to verify tests results and vaccines requires for the movement of animals.

We conclude that electronic health certificates offer the practitioners and diagnostic labs the ability to create complete and legible documents, incorporate digital images and signatures of practitioners and lab technicians, compile real time data, allow for ease of data analysis, and disseminate documents to the appropriate animal health officials with the same ease as sending e-mail. Reduction of paper work and time/cost benefits to administrative staff accomplishes the goals supported by United States Animal Health Association, which are now in national implementation stages by USDA-APHIS-VS. This project compliments the goals of the National Animal Health Lab Network (NAHLN) and their partnership with state and federal agencies to safeguard animal health and fully coincides with the National ID Plan and U.S. Animal Identification Plan (USAIP).

The EIA Subcommittee Report was presented by chair Dr. Ernest Zirkle. The EIA Subcommittee met via teleconference May 5, 27, July 15, 29, August 19 and September 9. In addition there were several sub-subcommittee calls addressing specific issue assignments. During these calls, what a National EIA Control Plan should include and how it should be enforced was discussed. The subcommittee forwarded several documents to the full Committee for review and edification in preparation for today's Committee meeting. They included three resolutions and outcomes from the 2003 Annual Meeting:

- 1) Develop a proposal for National Control Program based on a nationwide census. USDA and the EIA Subcommittee have developed that proposal and are recommending a resolution to implement it.
- 2) Laboratory system for serologic diagnosis of EIA. A pilot study was implemented by USDA. Dr. Eileen Ostlund reported on that at this meeting.
- 3) Two year moratorium on training for new EIA laboratories. USDA implemented the moratorium November 17, 2003.

The EIA Subcommittee also distributed the following documents to the full Committee: Draft EIA National Control Program; EIA National Control Program, Cost/Benefit Analysis based upon 5 regions; Equine Ag. Census, 2002 as compared to EIA tests 2003; Draft Resolution requesting implementation of National EIA control program; and Draft Resolution requesting implementation of eEIA (electronic EIA) connectivity to those laboratories who desire it.

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Following the scientific program, the committee considered and approved the report of the activities of the EIA Subcommittee. In addition, three (3) resolutions were approved by the Committee and forwarded to the Committee on Nominations and Resolutions for approval by the general membership. They addressed:

1. Enhancement of USDA's data gathering program for outbreaks of infectious diseases of horses and sharing that information with stakeholders and state animal health officials;
2. Providing laboratory connectivity to states for the electronic Equine Infectious Anemia application; and
3. Development and implementation of a national State-Federal Cooperative EIA Control Program.

The following is the "Proposed Three Phase Plan for Implementation of a National State-Federal Cooperative Program for the Control of EIA," as reported by EIA Subcommittee Chair Zirkle.

### **PROPOSED THREE PHASE PLAN FOR IMPLEMENTATION OF A NATIONAL STATE-FEDERAL COOPERATIVE PRO- GRAM FOR THE CONTROL OF EIA**

E. Zirkle, Fairton, NJ

#### **Introduction:**

This document describes a proposed National State-Federal Cooperative Equine Infectious Anemia (EIA) Control Program. The goals of this program are to, without the burden of additional regulations, (a) reduce the overall national prevalence of EIA and (b) reduce the imposition of required EIA testing. Under this plan, EIA test requirements for equine movement will be standardized, simplified and, in some cases, eliminated; allowing greater freedom of movement while reducing the risk of being exposed to equidae of unknown EIA status. These proposed changes will reduce the overall cost of EIA control – a change that will be reflected in reduced expense across the equine industry. The Program proposal calls for a three-phase implementation with an open time frame. Phase One establishes EIA Risk Zones within the U.S. based on incidence levels derived from historical EIA testing records; Phase Two refines the Risk Zones and risk management as improved equine census and disease prevalence information becomes available; and Phase Three further develops the program, and its utility to the industry, through the development of a voluntary EIA Certification Program partially supported by Federal funding. This Program will reward equine owners who test and have historically tested their animals with reduced costs, increased ease of movement, and protection from punishment for the untested and non-commingled EIA reservoir equidae in their region.

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### **Assumptions:**

- There are two major goals of our efforts: to develop a proposal for a National EIA Control Program and to expedite testing according to risk
- Industry support for development of a National Control Program exists
- APHIS already considers EIA a Program Disease and can modify the program easily
- An adequate system of equine ID will be forthcoming under the auspices of the Equine Species Working Group (this will facilitate all control efforts)
- The National Control Program must be based on assisting states/regions to be successful

### **Perspective:**

- Certification for control of EIA virus transmission must be based on good science
- Movement from an EIA-free facility in a low risk region to a lower risk region should be seamless
- Knowledge of each equid is needed to accurately assess the risk
- The UM&R for EIA is a good starting point for development of certification schemes
- Cost benefit analyses indicate savings in excess of \$10,000,000 per year to owners if “test-by-risk” regional testing plans are implemented (see the attached COST-BENEFIT file)
- Testing the National Animal Health Management Services (NAHMS) Equine 98 serum bank might provide useful unbiased data about the national prevalence of EIA (please see the NAHMS serum file attached)
- To encourage cooperation between states and within regions where possible.

### **Points:**

- To support and participate in any meaningful control program, states would need:
  - 1) Authority to require testing of exposed equine. This would include those determined to be at significant risk epidemiologically
  - 2) Authority to control movement of test positive and suspect animals by use of ‘hold orders’ or ‘quarantines’
  - 3) Authority to conduct necessary epidemiologic investigations
- Case rate determinations must be based on accurate or reli-

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able numerators and denominators

- 1) Current numerators (numbers tested) reflect the numbers of tests conducted and do not directly measure the number of individual animals tested. A more accurate number will depend on the development and implementation of a unique Equine Animal ID system so that case rates represent the proportion of individual animals affected. Whatever figure(s) are offered for use must provide information about how they were derived and describe the strengths and weaknesses in the method used to generate the numbers.
  - 2) The denominators used to determine case rate estimates must likewise include descriptions of how they were generated and the strengths and weaknesses of the methods used. For this item there may be a variety of sources including USDA, National Agriculture Statistical Service data, state generated data or industry-developed figures used in arriving at the figure. Denominator estimates should also include a measure of error or confidence.
- Industry backing must exist to support the level of control desired
    - 1) This item considers the backing that the horse industry provides (philosophical, personnel, financial) to assist in monitoring compliance with regulations. Examples range from a group of lay people trained and authorized by the state to monitor testing requirements at congregation points to an EIA oversight committee formed by the state horse council to work with the state veterinarian.
    - 2) A determination of the level of participation/oversight as provided by the industry, state veterinarian and animal disease regulatory body is especially critical when test-positive equids are found, e.g., providing sufficient manpower to perform the epidemiologic analysis needed to identify the source and to follow contacts.
  - Epidemiology investigations must be complete for full participation in the program - How complete are the investigations when test-positive equids are found? This point is considered critical to the success of a program and is covered under "authority" above. Today, this may be inversely related to incidence
  - Movement of equids within and between areas is complex but manageable
    - 1) This is the most complex issue to consider, as the movement history of each individual must be tempered by the status of each equid encountered in the 60 days before

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the move. Thus, if the equid moving has only been in an “EIA-free facility” for the 60 days, movement to any area should be facilitated.

- 2) In the absence of complete testing data, movement should be constrained according to the regional estimates (testing required when moving to an area of lower risk). Several scenarios are presented below as examples of movement within and between Risk Areas under several levels of testing.
- 3) The movement column requires additional individual definition, not just region-wide restraints. We must develop a means for rewarding those owners who have tested and not punish them for the incidence in the untested and non-commingled reservoir in their region. Thus, the designation and definition of EIA-free equid, facility, community etc. becomes important.
- 4) Once such entities are agreed to, then movement from low to high-risk areas and back again becomes refined according to the contacts encountered while in the high-risk area. For example, a horse moving from New York to an EIA-free facility in Louisiana (a closed, controlled race-track) can move back to New York without testing. By contrast, the New York horse moving to Louisiana and encountering horses on trail rides where testing is not required or not monitored, requires a retest and a quarantine period of up to 60 days is recommended. We will need to develop practical ways to deal with the multiple combinations to accommodate movement between situations according to risk.

An example of movement within zones with “A” being the lowest risk and C the highest follows:

From Proposed Area Proposed Movement

- A Free within A, B and C
- B Free within B and C [Free within A, B and C (if certified free)]
- C Free within C [Free within A, B and C (if certified free)]

Number of Risk Designations:

This proposal recommends that the U.S. be separated into 5 regions or Risk Areas based, in part, on the previous ten year testing data. The relatively overtested Northeast U.S. nine states plus Alaska and Hawaii comprised Risk Area A. The states with historical data (ten-year averages) showing the highest rates, namely Louisiana, Texas, Oklahoma and Arkansas, comprised Risk Area C. The other 35 states have similar historic rates and we suggest they form 3 regions already extant within the USAHA organization. Thus B1, B2 and B3, are South-

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eastern, Central, and West, respectively. These Risk Status assignments are the first step in establishing a National Control Program for EIA. We wish to foster viable cooperative programs between states and with the USDA. Thus, we prefer to wait until census data are compiled and real prevalence estimates can be made with accuracy before defined borders for the Risk Areas are established in cooperative programs between states and the USDA. Therefore, we consider it prudent to establish 5 zones initially.

Risk Areas (presented in the following map): (A) North East (plus Hawaii and Alaska), (B1) South East, (B2) Central, (B3) Western, and (C) South Central

Once the population estimates (census) are available, we can further define the parameters of risk within each of the areas, a point where "industry support" and "epidemiology" become important considerations.

### **Other considerations:**

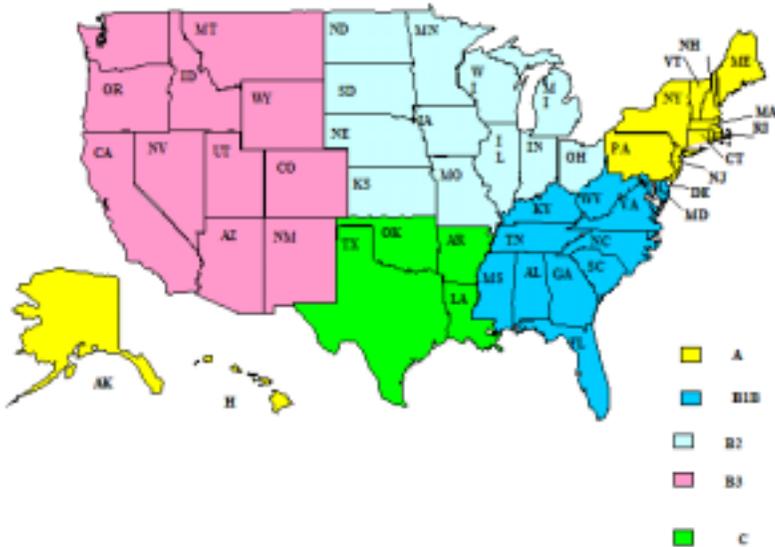
A future voluntary certification program (see Phase 3) would be a subordinate part of a comprehensive National Control Program as opposed to preceding the development of a control program. Currently, if supported by the states' equine owners, individual states may choose to participate in "multi-state regional EIA arrangements" based on state assessments of EIA risks. The future certification effort should be based on devising a means of industry and state approval for a practical way to designate an "EIA free equid", an "EIA-free facility" and so forth on which to base a rational plan for EIA control. This type of planning is consistent with the "Health Assurance Program" certification schemes in New York State.

An overriding consideration for our efforts should be the convincing evidence that owners in many areas of the country are "overtesting" for EIA according to the risk. It is agreed that testing at a lower frequency today (maybe every 2<sup>nd</sup> or 3<sup>rd</sup> year vs annual) would not increase the risk of acquiring EIA in many areas of the country. Therefore, encouraging regional efforts for control of EIA should be in the best interest of all involved parties.

Based on the above points, the EIA Subcommittee recommends the following Three Phase EIA Control Program with immediate implementation of Risk Areas A-C to facilitate early discussions between states within each Risk Area to take advantage of the projected savings to their industry.

The EIA Subcommittee recommends prompt review of population estimates and participation in a nationwide census to obtain population numbers with sufficient confidence to permit accurate estimation of the percentage of resident equids tested in each state over the last 3 years. These data will then be used to obtain accurate estimates of

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the expected prevalence of EIA in contiguous states within Risk Areas. These data will be used to design tailored control efforts within states and to refine risk estimates between states. Once those data are accumulated and analyzed, refinements based on industry support and epidemiologic investigations will have meaning and relevance.

### Phase One Implementation:

Phase One would utilize the cost-benefit analysis for a proposed National Control Program for EIA based on risk status assessments from prior years testing. These risk status assignments would divide the nation into 5 Risk Areas with no separation according to quantifiable information derived from a census (enumeration) or from “industry support” and “epidemiology”, as they do not seem to be appropriate. If this plan is adopted by states within the Risk Areas and “test-by-risk” plans are implemented, it is estimated to result in savings in excess of \$10,000,000 per year to horse owners, while not increasing risks of EIA. We feel strongly that dividing the 35 mid-risk states into 3 sub-regions along the USAHA regional zones (Western, Central, South eastern) will facilitate discussions and tend to build better interstate regulations and/or cooperation.

### Phase Two Implementation:

Phase Two would be developed once quantitative information (census, enumeration estimates) becomes available to calculate, within defined confidence limits, the ability of the state to accurately assess the true prevalence of EIAV infections within their jurisdiction. This sound knowledge can then be used to refine/define appropriate levels of EIA

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Control and activate different levels of support from the USDA in a Certification Program (see Phase 3 below).

### **Phase Three Implementation:**

Phase Three would involve the development of a voluntary EIA Certification Program. Should individual states elect to participate, two outcomes of such a program could logically follow:

- **Equivalency**—It would provide for states of equal certification status to implement the appropriate legislative/regulatory measures to move horses without EIA testing across borders to states and/or regions of equivalent status (regionalization).
- **Funding**—It would provide USDA funding for states/regions wishing to improve their certification status. Foremost, funding would be for a state or federal veterinary medical officer (VMO) assigned full-time to specific state/regional EIA programs in the form of salary, benefits, and travel expenditures. Such designated EIA Program VMO's would define and develop testing, epidemiology and industry support for EIA programs in individual states/regions specifically to the needs of that area. Additionally, money would be available for educational materials and research. A proposed budget has already been designed and is ready for presentation to the VSMT once the industry supports the endeavor.

### **EIA Regionalization Program: Benefit-Cost Analysis**

A regionalization scenario for EIA in the United States was proposed by the USAHA-EIA Subcommittee (Figure 1). The Subcommittee distinguished between three classifications of risk for the disease in the United States. The area of highest risk is comprised of the states of Texas, Oklahoma, Arkansas, and Louisiana. The area of lowest risk consists of a collection of the northeastern states, Hawaii, and Alaska. The remaining mid level risk states are located in the West, Central, and Southeast of the United States. For purposes of the regionalization program, these latter mid level risk states are divided into three subregions.

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### EIA - 3 risk classifications, 5 regions

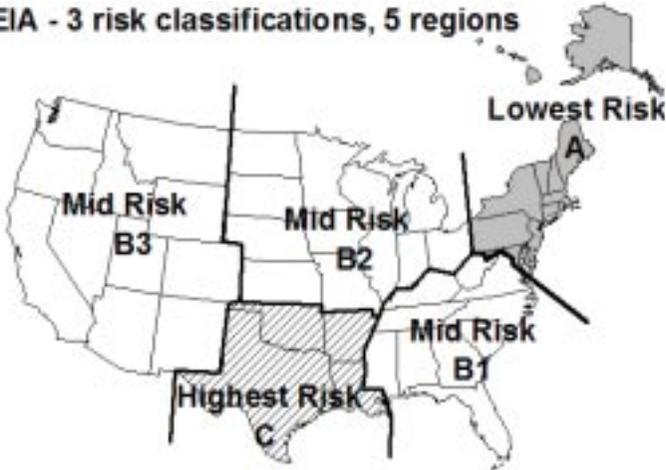


Figure 1.

The following assumptions are made in this benefit-cost analysis of the proposed regionalization scheme.

- Reducing Federal and State requirements for EIA testing will cause horse shows and/or gatherings in the lowest and mid risk areas to reduce their EIA testing requirements without increasing the risk of EIA spread.
- In contrast to the current pattern of testing which requires horses be tested in their home states on a periodic basis, the certification program as proposed here would direct testing toward a pattern of testing horses as they move out of the highest risk areas into the mid and lowest risk areas. It is assumed that this testing will take place recognizing appropriate waiting periods for serological detection from time of exposure without the need for regulatory imposition of quarantine.
- Any unusual periodic increase in EIA positive tests in the mid risk area will be addressed by temporary reclassification as an area of highest risk and the reinstatement of locality based testing for within state gatherings.
- EIA testing at all changes of ownership will continue to be standard practice in all areas of the United States. The results of this testing would be employed in a continuing surveillance program for detection of EIA. A sample of 3,000 tests would be required for each area in order to detect 0.1 percent prevalence of EIA infection with 95.0 percent confidence. A sample of 4,600 tests would be needed in order to detect infection at the same prevalence but with 99.0 percent confidence.
- The National Animal Health Monitoring System (NAHEMS)

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Equine98 study estimates for management practices are frequently in terms of percent of operations rather than in terms of percent of horses. For purposes of this analysis, the NAHMS Equine98 database was used to generate new horse-level estimates of reasons for EIA testing and reasons for equine movement.

- States already joining together with other states to form areas for recognition of EIA testing continue to operate as areas. An example of such an arrangement is the cooperative agreement between the states of Oregon and Washington. Expansion of these areas is also considered desirable under the scenario examined here.

<b>Table 1    Lowest Risk, Mid Risk, Highest Risk Scenario with 5 regions</b>					
Regions	South	West, Central, Southeast	Northeast	Total	Value Cal culated at \$24.65/test
Risk	High	Medium	Low		
Number positive, 2003	159	123	3	285	
Number of tests, 2003	432,753	1,297,337	229,811	1,959,901	
Number of horses, 1999	905,000	3,870,000	542,400	5,317,400	
% show testing within state		31.9%	60.8%		
% interstate test			12.7%		
% moving >500mi			17.1%		
Tests Avoided		413,851	168,934	582,785	\$14,365,640
Tests Added		38,280	92,588	130,868	\$3,225,888
Net Savings					\$11,139,751
Tests Remaining	432,753	921,766	153,465	1,507,984	
South - Arkansas, Texas, Louisiana, Oklahoma Northeast – Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New Jersey, New York, Pennsylvania, Delaware, Maryland, Alaska, Hawaii  West, Central, and Southeast - all other states NAHMS Equine 98 estimates found here use horse weightings rather than operation weightings.					

Table 1 contains 1999 equine population estimates from NASS and 2003 EIA testing data for the lowest, mid, and highest risk classifications as shown in Figure 1. Using these classifications, horses in the highest risk area would continue to be tested as is currently the prac-

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tice for horse shows and gatherings, sale, surveillance, and interstate movement. Horses in states with the mid-risk classification would be required to test for sale purposes and for interstate movement. Horses in states with the lowest risk classification would only be required to test for sale purposes. Any horses which enter states classified as highest risk from states in the lowest risk area would need to be tested in order to return to states of lowest and mid risk.

For the West and the Northeast regions of the United States as defined by the NAHMS Equine98 study, analysis of the NAHMS Equine98 survey data indicates that operations representing 31.9 and 60.8 percent of horses, respectively, gave within state show requirements as the primary motivation for EIA testing. For the Northeast, interstate testing requirements accounted for 12.7 percent of the responses. The remainder of the operations cited within state change of ownership, international movement, for personal knowledge, and veterinary recommendation due to equine illness, and other as their primary motivations. If EIA regulatory testing requirements for movement of horses to shows within lowest and mid risk states and requirements for testing for movement out of states considered to be of lowest risk are reduced, the percentages of horses testing is assumed to decline. Using the NAHMS Equine '98 estimates on primary motivation of operations for EIA testing, it is assumed that 31.9 percent of testing in the mid risk area and 73.5 percent of testing in the lowest risk area would no longer take place. This would mean a reduction of an estimated 582,785 EIA tests at a cost savings of approximately \$14.4 million annually, if valued at \$24.65 per test.

However, EIA testing in the highest risk area would be expected to increase above current levels due to horses having entered the highest risk area from the lowest risk area, desiring to return to the lowest risk area as well as for any flare up of EIA in the mid risk area. Again from the NAHMS Equine98 study, estimates provided that 17.1 percent of horses in the NAHMS Equine98 Northeast region were on operations that moved their horses a distance of 500 miles or more. If this percentage is applied to the equine population in this region, an additional 92,588 tests would need to be performed assuming these equine movements beyond 500 miles are into the highest risk area with the intention of returning to lowest or mid risk areas. These additional tests would offset the earlier reductions in costs by \$2.3 million annually. In order to account for the costs of additional testing during flare ups of EIA in mid risk areas, the average equine population for one state of 120,000 is multiplied by the percentage of horses on operations which reported testing for shows within state of 31.9 percent and the per test cost to obtain an additional testing cost increase of \$0.9 million annually. This assumes one anomaly outbreak of EIA in one state per year in the mid risk area. Taken together, the net benefits

## INFECTIOUS DISEASES OF HORSES

of the regionalization scheme then would be \$11.1 million, or \$14.4 million in decreased testing costs minus \$3.2 million in increased testing costs.

If states in the mid risk zone are able to be further regionalized such that requirements for interstate testing can be reduced within mid risk subregions, there would be additional savings from decreased EIA testing.

Continued testing of samples within the lowest risk area due to change of ownership and continued testing of samples within the mid risk areas due to change of ownership and interstate movement would be more than sufficient to continue surveillance for detection of EIA in these areas at 0.1 percent prevalence of EIA infection with 99.0 percent confidence.

Administrative expenses of this option have been estimated at \$240,000 per year in the form of one full time Federal staff position plus expenses. These administrative costs as well as costs to the horse owner would be expected to increase substantially should testing upon exit from the highest risk area require regulatory enforcement of quarantines. Should such enforcement and owner costs reach only \$120 per horse for the 92,750 horses involved, the net savings of \$11.1 million from regionalization would be entirely offset by quarantine expenses.

In terms of the distribution of the benefits and costs of the proposed testing changes for EIA, horse owners in the lowest and medium risk areas, particularly those who do not move their horses into the highest risk zone, would enjoy an overall reduction in testing costs. Those who move their horses into the highest risk zone and then return to lowest or medium risk areas would incur testing costs similar to those of the past. The Federal government would be increasing its outlays by \$240,000 annually in order to administer a new program. Because of the large benefits to horse owners of this option, it might be possible to consider channeling some of the savings from testing toward indemnifying or paying sanctuary costs for animals testing positive for EIA in the high risk zone, thus also achieving a reduction of risk of disease to the entire industry.

### **Summary:**

Implementation of the proposed regionalization scenario for EIA indicates savings of \$11.1 million to the horse industry from an overall reduction in testing. It is clear from this initial analysis, however, that the choice of regionalization scheme and its administrative costs have a marked impact on the estimation of costs and benefits of moving away from the current program.

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CEAH contributors to this report include Ann Hillberg Seitzinger, Josie Traub-Dargatz, Al Kane, Lindsey Garber, George Hill, Bruce Wagner, John Green, and Ziad Malaeb.

### **EIA AND EQUINE INVENTORIES - 2/18/04**

J. Trabu-Dargatz, L. Garber and G. Hill  
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This summary was prepared at the request of Drs. Tim Cordes and Chuck Isel for use by members of the Committee on Infectious Diseases of Horses.

The attached spreadsheet includes demographic data from the 1997 Agriculture Census and the January 1, 1998 and January 1, 1999 NASS inventories as well as historical EIA test data. The spreadsheet calculations use the NASS January 1, 1998 equine inventories to measure the coverage of the number of official EIA tests performed. Assuming the equine inventory has not changed over time, the number of EIA tests corresponds to 30.6 percent of the equine population in 1999, 33.7 percent in 2000, and 37.4 percent in 2003. If inventories have increased, the 2003 figure is estimated to be high by 2 to 3 percent. The conclusion from these data is that the US consistently tested about one third of its equine population between 1999 and 2003. This estimate of percent of horses tested for EIA in the U.S. is similar to NAHMS Equine '98 study estimate of 35.6 percent.

At the State level the number of tests performed relative to the equine population vary widely. Some States test a small percentage while others do a lot of testing. Examples include:

<10 percent tested (6 States) AZ, CT, HI, ID, OR, WA  
=10 and <20 percent tested (6 States) CA, IA, KS, MT, NE, SD  
=70 percent tested (9 States) AR, DE, FL, GA, KY, MD, MO,  
NH, RI

While currently available inventory numbers of equids are adequate for a general comparison to number of EIA tests as was done in the NAHMS Equine '98 study, they are not adequate to enable Veterinary Services to perform up-to-date equine health monitoring activities. If the EIA program moves forward with a certification program, USDA needs to estimate the number of equids and the number of operations with equids at least every 5 years. Extrapolations from Census of Agriculture data which exclude horses located off of farms, or an estimated 39.1 percent of the equine population, will not accurately reflect equine population demographics. Because this estimation effort will require NASS list building efforts in the ag-urban sector, it will be expensive. Therefore, it may be advisable to request NASS and CEAH to look at the most cost effective scheme for equine estimation/enumeration.

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### **EIA AND USE OF NAHMS EQUINE '98 SERUM BANK - 2/6/04**

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Fort Collins, CO

This summary was prepared at the request of Drs. Tim Cordes and Chuck Issel for use by the members of the Committee on Infectious Diseases of Horses.

The following issues relate to use of the NAHMS Equine '98 serum bank for estimation of prevalence of EIA infections regionally and nationally.

There are approximately 8,000 sera from just over 900 operations in 28 States banked at NVSL from the NAHMS Equine '98 study. The operations include those with three or more resident horses as of spring 1998.

Since the national prevalence of test-positive equids is quite low, enough positive animals may not be found in the serum bank to make reliable regional estimates. For example, if the prevalence of EIA-positive horses in the serum bank is equivalent to the national prevalence in 2003 (approximately 0.01 percent), there would be only 1 positive sample in the serum bank of just over 8,000 samples. In addition, the samples represent about 900 premises which, given an expected low herd-level prevalence, makes it likely that no or very few positive premises will be in the sample. If the prevalence of test positives is higher in the serum bank samples, then using the weighting system for the biological samples as was applied to all of the NAHMS Equine '98 data might allow creation of regional estimates. No State-by-State estimates of the prevalence of EIA would be possible.

To consider using this serum bank for the purpose of looking back in time regarding the prevalence of EIA-positive animals, the following would need to be kept in mind:

- NAHMS study data does not include a life-long history of individual horse movement, so for a given horse we could not say where it was exposed to EIA, only where it was when it was tested as part of the NAHMS Equine '98 study.
- Support must be gained for testing, e.g. from the horse industry, from State Veterinarians and AVICs from the 28 States in the study, and from a national representative of the equine industry.
- The horses included in the NAHMS Equine '98 study are more likely to represent the general equine population than do those now tested routinely for EIA, e.g. those that show or move

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interstate. Thus, the prevalence of EIA among the horses sampled as part of NAHMS Equine'98 may be higher than that officially reported. Decisions need to be made in advance as to how to deal with this outcome if it occurs. A higher prevalence may be due to several factors. As the same horses year after year are in the EIA testing program, the prevalence of test-positive animals would go down, as positive animals are generally not retested in subsequent years.

- An agreement would have to be in place so that there would be no trace back of positive animals.
- There would have to be adequate funding for the serologic testing and a laboratory identified to do the testing. The test to be used would have to be agreed upon by all stakeholders.
- This serum bank represents a valuable asset that we must use judiciously.

This retrospective look could give us a bench mark for any future studies of EIA prevalence studies. There is a large amount of information available based on the NAHMS Equine '98 study that could be matched to these sera including but not limited to information on individual horses, such as signalment (age, sex, and breed). There is also information available for the premises or operations on which the horses resided, including location and management aspects, such as information about observation of insects, methods of insect control, proximity to surface water, and movement of horses on and off the operation.

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### NON-IMMUNE APPROACHES TO THE PREVENTION AND CONTROL OF *STREPTOCOCCUS EQUI* INFECTIONS

Dr. John F. Timoney, Gluck Equine Research Center,  
University of Kentucky, Lexington, KY

Strangles is among the 3 most significant respiratory diseases of the horse throughout the world. Although the causative organism, *Streptococcus equi* of Lancefield Group C, is highly host adapted, rarely shows antigenic or other variation, survives only briefly in the environment, and is susceptible to most commonly used antibiotics, it nevertheless maintains its place as an ubiquitous and much feared equine pathogen. This reflects its ability to efficiently transfer, infect, and subsequently establish a carrier state in a small proportion of recovered horses. Perhaps of greater importance, however, is the inherent mobility of its host. In an era of rapid transportation, a *S. equi* infected horse may travel between hemispheres in less than a day and then initiate an outbreak of strangles thousands of miles from its farm of origin.

A high level of immunity to reinfection is generated in 70 to 80% of recently infected horses, a level much superior to that following vaccination with heat inactivated *S. equi* or with adjuvanted protein-rich extracts. The disappointing efficacy of these vaccines has forced a reliance on identification and separation of infected animals and interruption or reduction of direct or indirect transmission during outbreaks as a means of reducing the occurrence and impact of infection.

*Environmental Survival.* Older reviews, e.g. Stableforth and Galloway (1959), cite earlier studies that indicated that *S. equi* remained viable in pus for weeks. More recently, Jorm (1992) noted survival for up to 2 months on previously disinfected wood and glass at 2°C and 20°C. In our studies of *S. equi* CF32 in local soil (Lexington, KY), horse feces and in water, we have observed survival in soil and feces for less than 3 days. In sterilized feces, viable *S. equi* were detectable for 14 days suggesting a potentially hostile effect of the fecal flora. Survival in water was detected for up to 40 days when a total die-off occurred similar to that noted by Jorm. Thus, drinking water and its container is potentially an important source of *S. equi* during an outbreak since nasal discharges from affected horses are certain to enter as they drink. Daily disinfection of the water trough is essential to minimize transmission during an outbreak.

*Shedding of S. equi and its detection.* Most horse farms enjoy extended periods of freedom from strangles, a situation that would not be possible were *S. equi* to survive for long periods in the environment or to be shed persistently by carrier animals. Nasal shedding begins 2 to 3 days after onset of fever and persists for 2 to 3 weeks for some but not all animals. Shedding from ruptured mandibular abscesses is very brief as the abscess cavity is quickly invaded by *S. zooepidemicus*.

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Horses which develop empyema of the guttural pouch may continue to harbour viable *S. equi* for a year or longer. Shedding is intermittent and some shedder animals may have a unilateral nasal discharge or soft cough. Although transmission between guttural pouch carriers and susceptible horses has not been demonstrated experimentally, herds of horses in which these carriers have been identified and treated have become disease-free.

Culture on Columbia CNA blood agar remains the 'Gold Standard' for detection of *S. equi*. Nasal swabs are more convenient but less sensitive than nasal washes in detection of nasal shedding. Since shedding is usually not detectable until a day or two after onset of fever, daily monitoring of rectal temperature facilitates recognition and isolation of new cases to limit further transmission. A polymerase chain reaction (PCR) based on the *SeM* gene, a sequence specific to *S. equi* is about three times more sensitive than culture (Timoney and Artiushin, 1997). However, it does not distinguish between dead and live organisms. Culture accompanying PCR on a nasal swab/wash is used in control programs to select animals for guttural pouch endoscopy (Newton, *et al.* 2000). Since PCR is capable of detecting *SeM* DNA in guttural pouch lavages for weeks following disappearance of live organisms, culture should always be performed on samples positive by PCR. The cost of guttural pouch endoscopy precludes its widespread use as a tool in routine detection of chronically infected horses in herds experiencing a protracted outbreak, or with unexplained periodic recurrences. It is usually selected for animals identified by initial screening of nasal swabs by culture or PCR or that have an unexplained unilateral nasal discharge.

Detection and segregation of shedding animals during an outbreak is also of value in reducing transmission. Experimental data have shown that the greater the number of challenge organisms administered intranasally the shorter the incubation period and the more severe the disease that results. Observations during outbreaks indicate that the clinical attack rate, mortality, number of lymph node abscesses and complications such as purpura, guttural pouch empyema, and lower respiratory tract involvement are more frequent in outbreaks where there is overcrowding and lack of space to quarantine sick horses (Sweeney, *et al.* 1987).

*S. equi*-free status is eventually attained by most herds following a strangles outbreak, and is a consequence of clearance by a competent host immune response, poor environmental survival of *S. equi*, a low frequency of carriers and intermittent shedding by these carriers. Thus, large geographic areas, even countries, e.g. Argentina, Japan, Ireland, have been strangles-free for long periods during the past century.

*Future Research.* Besides a need for safer, more effective vaccines

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to aid in prevention, control and management of strangles outbreaks would greatly benefit from the availability of a rapid, inexpensive horse-side test for detection of nasopharyngeal shedding of *S. equi*. Progress in the identification of its unique antigens suggest that such a test is feasible.

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### **EQUINE INFECTIOUS ANEMIA AND CONTROL OF THE DISEASE: HOW MUCH IS ENOUGH?**

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#### **Summary**

The accuracy of serologic tests for equine infectious anemia (EIA) is the foundation upon which control strategies against this persistent lentivirus infection of equids have been built. The agar gel immunodiffusion (AGID) test for EIA developed in 1970 has been regarded internationally as the gold-standard serologic test for EIA. Statistics gathered by the United States Department of Agriculture (USDA) since 1972 document over 100,000 positive tests/equids and clearly document the progress made in reducing the numbers of positive equids found annually in the United States. Today, using test kits in AGID and enzyme linked immunosorbent assay (ELISA) formats, we expect that less than 0.02% of the 2,000,000 tests performed in a year will be positive. Testing for EIA and subsequent regulatory actions on test-positive equids has reduced significantly the threat of encountering EIA virus. With the huge successes in reducing the incidence of EIA in the mobile and tested population, what are the prospects for further improvements and at what costs? This presentation will focus on two areas where modest changes could assist in delivery of more accurate testing at a lower cost to the industry.

The first change would move testing toward a three-tier laboratory system advocated by the Committee on Infectious Diseases of Horses of the United States Animal Health Association (USAHA) where ELISA tests would be the preferred primary test. This system is advocated because ELISA tests are more sensitive than AGID tests in detecting antibody against EIA virus (EIAV), and ELISA test results are more objective than AGID test results, i.e., less affected by interpretation. If our analyses are correct and if the new system is adopted, accuracy of negative EIA test reports would be higher than today as the majority of errors appear to be false-negative AGID reports. These are most likely associated with samples with less intense AGID test-reactions, i.e., where a line of identity with the reference positive serum does not form, and where accurate interpretation is critical. In the vast majority of these cases ELISA test results are unequivocally positive.

The tradeoff for adopting the ELISA test formats as the primary test for EIA is the complication introduced when the initial positive ELISA test results are not confirmed by AGID testing. In the majority of these cases we would expect that additional testing, e.g., immunoblot, would fail to show recognition of viral proteins or would show recognition of no more than one viral protein, indicative of a false-positive ELISA test result. In other cases, multiple viral proteins would be recognized and

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would indicate a false-negative AGID test result. We argue that at this stage in the control of EIA, it is preferable to have resolvable laboratory problems associated with occasional false-positive ELISA results than to release false-negative AGID horses to move and mingle freely. It is probable that such equids have helped perpetuate EIAV in the past.

In reviewing the data, it is clear that the power of a negative ELISA test result for EIA is greater than that of the negative AGID test. This fact should be recognized as such by international groups, e.g., placed in the "prescribed" diagnostic test group rather than "alternative test" group by the OIE (World Organisation for Animal Health) standards commission, and negative ELISA tests for EIA accepted for import by all nations. For the proposed three-tier laboratory system to function, equids with negative ELISA tests for EIA must be allowed to move freely: intrastate, interstate, and internationally. Today, only AGID tests have universal acceptance.

Testing has been required by many jurisdictions for movement on public roads, for congregations, for interstate travel, for change of ownership, *et al.* In two states, Louisiana and Arkansas, annual testing is required of all equids. The costs for EIA testing have been borne by owners and annually are estimated at greater than \$50,000,000. Thus for the year 2003 when 273 positive equids were reported nationally, over \$180,000 was spent to find each. In areas where EIA test-positive horses are rare, the average testing costs to find each positive is even higher; e.g., in the northeastern states over the last 3 years owners expended about \$1,000,000 to find each positive. When such expenditures are reviewed critically, it is evident that testing should be applied more in line with risk, especially to deliver testing to those who have eluded surveillance testing to date, i.e., the so-called untested reservoir.

The second change utilizes projections by the USDA that indicated regionalization and reduced testing in low risk areas could dramatically reduce testing costs to the industry without increasing the risk of acquiring EIA. When coupled with increased accuracy of testing, the risk of acquiring EIA could be further reduced. An improved control program for EIA at a lower cost to the industry seems intuitive and overdue. We urge adoption of a national program utilizing the three tier laboratory system.

### **Historical aspects**

Prior to 1970, EIA was a disease feared by veterinarians and horse owners because of its capacity to spread between horses without control because no practical and accurate diagnostic test was available. Today, the accuracy of serologic tests for EIA forms the basis for effective control strategies against this persistent lentivirus infection of equids. The AGID test for EIA developed in 1970 by Coggins and Norcross<sup>1</sup>

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has been regarded as the gold-standard serologic test for EIA. The wide international acceptance of the AGID test was garnered because of the excellent correlation of AGID test results and horse inoculation tests for detection of EIAV using 250ml transfusions of whole blood. Guidelines for the control of EIA were drawn up by an inclusive industry and veterinary group led by the Committee on Infectious Diseases of Horses of the U.S. Livestock Sanitary Association (now the U.S. Animal Health Association) in 1966, adopted in 1967, and increased in scope in 1974 with an outline for an EIA state control program once the AGID test proved effective in detecting EIAV-infected horses.<sup>2</sup> The guidelines have stood the test of time and were recently expanded as the Uniform Methods and Rules for the control of EIA promulgated by the USDA (issued first in 1998 and revised in 2002). These can be accessed from the USDA website <http://www.aphis.usda.gov/vs/nahps/equine/eia> as "UMR(PDF)".

An appreciation of the changing role of EIA to the horse industry can be gleaned by review of testing statistics since 1972. The database of statistics on EIA testing compiled by USDA-Animal and Plant Health Inspection Service (APHIS) can be accessed through the following web site: <http://www.aphis.usda.gov/vs/nahps/equine/eia/web-mapping/Main.htm>. When testing was first available, veterinarians often focused their testing on facilities where known or suspect cases had resided. The result was often the discovery of a high rate of reactors, many of which were inapparent carriers of EIAV. Even though the testing was biased toward positive initially, and today is biased toward negative because the same equids are tested each year, review of the numbers of positive tests (1972-1995), corrected in 1996 to be numbers of positive equids (1996-2003), and the numbers of total tests (1972-2003) reveals numerous points worth discussion.

In the early stages of testing for EIA, once the suspected/known foci were identified and removed the moniker "swamp fever" fit well, i.e., higher rates of infection were noted in the Gulf Coast states. Official test statistics from Florida and Louisiana show the historical perspective well and document the progress in control of EIA (see the web site for details.) Peak numbers of positives were noted in 1974 and 1976 for Florida and Louisiana respectively and nationally in 1975 when 10,381 positives were reported.

Nationally, over 100,000 positive tests/equids have been detected since 1972 and the compiled data show clearly the progress made in reducing the numbers of positive equids found annually in the United States. Today, we expect that less than 0.02% of the roughly 2,000,000 tests performed in a year will be positive (a rate of about 1 in 5000). Thirty years of intense testing for EIA has reduced significantly the threat of encountering EIAV-infected equids. With the huge successes in reducing EIA in the mobile and tested population, what are the pros-

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pects for further improvements? At what cost to the industry? This presentation will focus on two modest changes that could assist in delivery of more accurate testing at a lower cost to the industry, with an emphasis on explaining the differential strengths of available test kits for the serologic diagnosis of EIA.

### **Diagnostics for EIA**

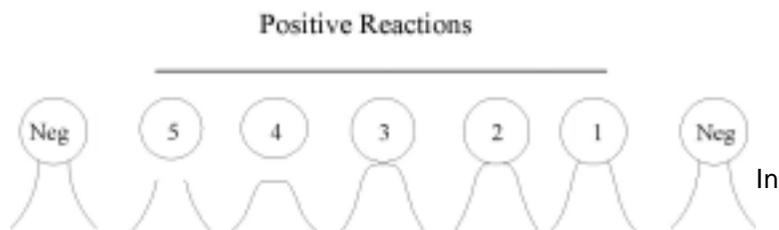
Today in the United States, in addition to three licensed AGID test kits, there are three licensed ELISA-based test kits marketed for detection of anti-EIAV antibodies. The AGID test and the ELISA-based kits all detect antibodies against the major core protein of EIAV, the p26 antigen. One ELISA test kit also includes determinants of the transmembrane protein of EIAV (gp45) but the final reaction does not discriminate between the two (the SA-ELISA II test kit from Centaur, Inc.) The power of the positive AGID test is higher than that of the ELISA tests because a positive AGID test is proven to be correlated with EIAV presence; all positive ELISA tests, therefore, must be confirmed by AGID. Results for AGID testing, however, are more subjective than for ELISA testing, and in AGID tests reagents are dispensed by eye not by volume. Although ELISA tests are currently labeled for visual reading, ELISA results can be easily made more objective by reading test plates with a spectrophotometer, with a permanent result in the form of a print-out.

Personnel who conduct laboratory tests for EIA must be initially trained and certified by the USDA, and successfully complete annual proficiency tests to maintain their certification. Today that entails reporting satisfactory results from 20 equid check-test serum samples. Because of statistical considerations, NVSL is forced to bias the testing in favor of clearly positive or negative tests, to the detriment of testing critically the technicians' ability to accurately interpret results of samples with low levels of antibody.

We have monitored published results of proficiency testing of approved laboratories in the United States that have been conducted by the USDA over the past 20 years. The samples used in these exercises are carefully selected as representative field samples where no "abnormal" reactions are expected, i.e., they have proven to be clearly positive or negative in all official test formats. Although not possible to document with numbers because of purported confidentiality issues, it is clear that the error rate from EIA testing is higher when AGID tests are used than when ELISA tests are used. The majority of errors are false-negative AGID reports, especially common in samples with less intense AGID test-reactions, i.e., in samples where a line of identity with the reference positive serum does not form (see reaction intensity 1 in Figure 1).

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**Figure 1. Range of reactions to expect with the AGID test** (adapted from USDA APHIS Program Aid #1738)



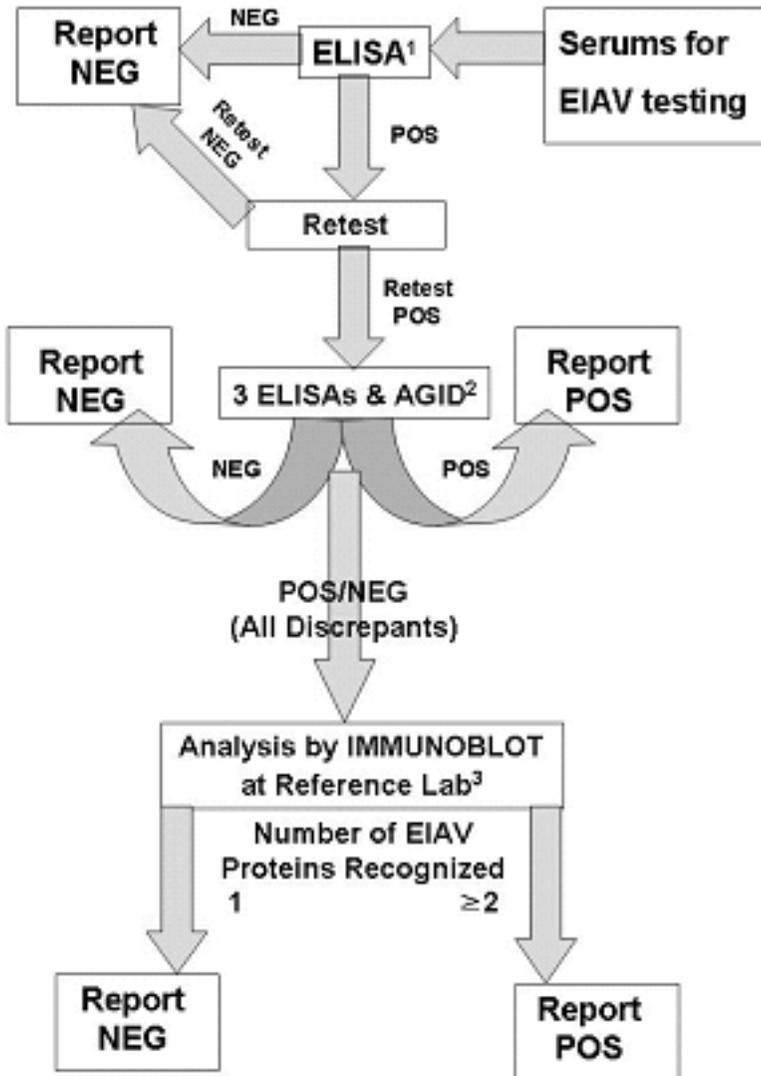
In 2004, 7% of approved laboratories reported as negative the only sample included in the proficiency test set with a reaction intensity of less than 2. (see Figure 1) These errors were made when the laboratory technicians knew their certification was at stake and arguably used their keenest sense and skill to perform at the highest level. It is logical and reasonable to conclude that such errors in AGID test reporting occur at an even higher rate during routine testing. It is interesting to note that sales of ELISA test kits are reported to increase each year just prior to submission of check test results to USDA, suggesting that laboratories that report they are using AGID tests for the proficiency exam might be verifying their results with ELISA tests before filing their report with the USDA. We speak with authority on this subject because we used this strategy when ELISA tests were first available. More recently, however, our technical staff insists on only using ELISA tests for these exercises because of the ease of interpretation and high degree of accuracy.

Review of these "check test" results indicate an excellent correlation between reporting of ELISA tests as measured in proficiency testing and EIA status (Dr. Eileen Ostlund, USDA-APHIS-VS-NVSL; personal communication).

To minimize the impact of inaccurate AGID test reporting, we agree with the USAHA resolution for adoption of a three-tier laboratory system. In the proposed three-tier system for EIA testing, repeatedly positive ELISA samples would be forwarded to referral laboratories where further testing, including AGID tests, would be performed by individuals whose expertise in interpreting AGID test results is optimal. An algorithm is presented below to outline our perspective on optimal testing for EIA based on methods available today (Figure 2.) Use of the proposed three-tier laboratory system would permit development of a central repository of samples that pose diagnostic challenges that could be used to perform systematic critical evaluations of the effectiveness of available test kits. Such information would be invaluable for continuing dialog with test kit manufacturers to improve the accuracy of licensed kits.

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Figure 2. Algorithm showing optimal testing procedures for EIA based on available tests – 2004



In the proposed 3 tier system

1. 1<sup>st</sup> tier labs (ELISA)
2. 2<sup>nd</sup> tier labs (Referral)
3. Reference labs

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If initial testing for EIA utilized ELISA tests, the rate of false-negative reports is expected to be lower than with AGID testing. The tradeoff for adopting the ELISA test formats as the primary test for EIA is the complication introduced when the initial positive ELISA test results are not confirmed by AGID testing. In the majority of these cases we would expect that additional testing, e.g., immunoblot, would fail to show recognition of viral proteins or would show recognition of no more than one viral protein, indicative of a false-positive ELISA test result. In other cases, multiple viral proteins would be recognized and would indicate a false-negative AGID test result. We argue that at this stage in the control of EIA, it is preferable to have resolvable laboratory problems associated with occasional false-positive ELISA results than to release false-negative AGID horses to move and mingle freely.

In an attempt to make testing more standardized, the three-tier system would require spectrophotometric reading of ELISA test results with a permanent record kept for at least 3 years. This minor change would further increase the objective nature of ELISA test reporting. For the proposed three-tier laboratory system to work, equids with negative ELISA tests for EIA must be allowed to move freely: intrastate, interstate, and internationally. Thus, international acceptance of negative ELISA results must be pursued vigorously to effectively utilize the greater accuracy of negative ELISA test results.

### **Sensitivity of current tests for EIA**

Because of the proven correlation of the AGID test to horse inoculation test results, any new test for detection of antibodies against EIAV is required to show results of equivalence to the AGID test. Thus, it is difficult if not impossible to develop procedures with higher sensitivity because producers would have to perform what many would refer to as unwarranted animal trials to prove EIAV presence, i.e., specificity. As a result of these constraints, manufacturers must make their new test kits as sensitive as the AGID, and in the process lose some of the inherent sensitivity of the new test format.

The differential sensitivity of the available test kits can be seen best in horses during the 20-45 days after exposure to EIAV and in foals with passive antibodies to EIAV. As the AGID test format has the capacity to detect both IgM and IgG antibodies, it may have an advantage in the early post-infection period over ELISA tests that use an IgG based detection system. Generally, antibodies against EIAV are detected first in immunoblot tests using an IgM detection system, then in immunoblot tests using an IgG detection system, then with available ELISA and AGID test kits with about equal results. The slight differences noted between accurate reporting of very weak AGID reactions that require interpretation (subjective) and ELISA results read by spectrophotometer (objective) are only worth mentioning because these

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very weak AGID reactions are often interpreted incorrectly under routine conditions in approved laboratories. Thus, with the available test kits, we would expect the ELISA tests would be reported as positive from 1-3 days earlier than AGID tests. As few of the new cases of EIA found each year appear to be from recent infections, the differences noted are probably of minor consequence.

More illuminating, however, are results of detection of antibodies against EIAV acquired as a result of passive transfer, i.e., in uninfected foals out of test-positive mares. We have participated in numerous prospective studies of inapparent carriers of EIAV and have done comparisons of available test-kits for detection of anti-EIAV antibodies with the immunoblot test. In the most recent published analysis<sup>3</sup>, results in the ELISA test kits for EIAV which are specific for the p26 antigen and still available today (CELISA from IDEXX Laboratories, Inc) were first reported negative a mean of 202 days, compared to 183 days for AGID (IDEXX Laboratories test kit), and beyond 210 days for immunoblot. We estimated that the first negative AGID reports for routine testing in the field would be about 30 days earlier, i.e., at 153 days of age. In that report, the first generation SA-ELISA test (Centaur, Inc.), specific for anti-gp45 antibodies, were first reported negative at a mean of about 82 days. The new generation SA-ELISA II incorporates both gp45 and p26 determinants and appears to have equivalent sensitivity to the other ELISA tests which are based on detection of anti-p26 antibodies only. Thus in our opinion, the available ELISA tests today would be expected to detect antibodies against EIAV in the serum of uninfected foals from 19-49 days longer than the AGID test. If we assume a half-life for IgG of 21 days, this suggests that the ELISA tests are about 2-4 times more sensitive than the AGID for detecting antibody against the p26 antigen of EIAV.

Another dataset that must be considered is that derived from a state where a representative (pseudonym JC, to maintain confidentiality) of the state veterinarian's office took unidentified selected samples, provided by our laboratory, to 28 approved laboratories for testing by their routine methods. In this voluntary program, JC returned to each laboratory the following day and reviewed the AGID test plates and/or results, and/or the ELISA results or spectrophotometer printouts. These results were discussed together with expected results and photographs of the AGID test reactions of the positive samples (from EIAV-infected equids from our laboratory with reactions of 5, 3 and =1 as shown in Figure 1.) In this program, laboratories with ELISA tests had results in agreement with expected. By contrast, more than half of the 47 laboratory personnel that used the AGID test format would have reported as negative serum from our reference weak positive horse (Flicker)<sup>4</sup>, serum with a reaction of =1 in the AGID test, if this had been a routine sample. In the majority of cases, with coaching, the AGID test reaction

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of this sample was interpreted as positive. In others the sample was negative, possibly because of operator error or antigen content issues (discussed below). One of the results of this exercise was that many of the laboratories voluntarily agreed to perform testing in only ELISA formats for the foreseeable future. Any sample positive by ELISA in those labs is forwarded to the state laboratory for confirmation by AGID.

The third major evidence for higher sensitivity of current ELISA tests over AGID reactions are derived from testing the USDA Reference weak positive serum and our reference weak positive serum (an EIAV-infected horse named Flicker<sup>4</sup>). Serum from both horses was tested at serial two-fold dilutions by AGID and ELISA with licensed and commercially available test kits (companion AGID and ELISA kits from Synbiotics Corp. were used.) The USDA reference weak positive serum had an endpoint titer (last positive AGID test-positive interpretation) of 1:2 by AGID and 1:8 by ELISA. Undiluted serum from Flicker was interpreted as a weak weak weak weak positive by AGID with the same AGID test-kit; by contrast, a 1:4 dilution of serum was positive by the Vira-CHEK ELISA. Analysis of these results indicate an approximate 4-fold difference in sensitivity of detection of antibody against EIAV between these ELISA and AGID test kits, consistent with the approximate 2-4 fold difference noted through the testing of serum from foals of test-positive mares (using companion AGID and ELISA test kits from IDEXX Labs.<sup>3</sup>) A definitive difference cannot be calculated because neither the ELISA test formats for EIA nor the AGID test is quantitative. We have listed approximate differences in sensitivity as a guide for comparison. We realize that the sensitivity of the AGID test cannot be improved substantially. We are confident, however, that manufacturers of the ELISA test kits for EIA could meter their reagents and be able to interpret both the USDA reference W+ serum and serum from Flicker at 1:16 dilutions, albeit at the risk of increasing the rate of false-positive reactions.

### **Current diagnostic problem areas and discrepant results:**

Some of the potential problems with AGID testing and interpretation can be ascribed to antigen content. When AGID testing was initiated for EIA in 1972, antigen for AGID testing was a relatively crude preparation extracted from splenic tissue of horses with acute EIA. Initially, it was difficult to obtain high enough antigen concentrations to get sharp lines of precipitation in agar, making interpretation of AGID test-reactions somewhat of an art form. This was remedied when EIAV production in vitro was made practical by Malmquist et al in 1973.<sup>5</sup> Today, antigen for use in AGID tests is often of recombinant origin. As recombinant antigen is relatively inexpensive to produce, there is no problem getting enough antigen for sharp lines of reaction with reference positive serum. In fact, it is relatively simple to get very dense and

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sharp lines of precipitation by increasing antigen and reference antibody concentrations. The problem now becomes one of metering antigen concentration so that samples with low levels of antibody against the p26 antigen of EIAV can still be interpreted as positive in all approved laboratories. Often the bias is toward dense sharp lines, because laboratory technicians appreciate the ability to discern the lines more clearly, i.e., it becomes a selling point. Over the years there have been several recalls of specific lots of AGID test kits because of this specific issue, sometimes, unfortunately, only noted after laboratory failures using the specific kits on proficiency tests.

We would suggest that USDA seriously consider adopting a quantitative standard reference weak positive antibody preparation against EIAV for use in qualifying/certifying AGID test kits, if not already in existence. The reference weak positive serum from Flicker or the international reference weak positive antibody standard developed by Professor Toma in France would seem to be excellent choices. One of the problems associated with attaining that level of sensitivity in the AGID test is that reactions would become more difficult to interpret, a known limitation of the AGID test format. Perhaps it is time to consider adopting more contemporary standards where test sensitivity is the major consideration, without abandoning the known strengths of the AGID test. At the very least, the reference labs should use AGID test reagents that are proven to have the highest sensitivity, while still retaining a sharp enough line for accurate interpretation. The proposed three-tier laboratory system would address this problem, as all ELISA positive – AGID negative samples would be tested further by immunoblot to resolve the discrepancy. We are fortunate to have options for EIA-testing today; we should capitalize on their strengths.

As alluded to above, false-positive ELISA test results occur with higher frequency than with AGID, in part because in the AGID test lines of identity can be distinguished from lines of non-identity. With today's more purified AGID test reagents, lines of non-identity are rarely encountered. ELISA tests results, on the other hand, are determined by a color change and anything that causes a color change in the right direction must be interpreted as positive. In some cases, repeat testing reveals operator error as the result is clearly negative.

In other cases, several explanations are tendered to help understand why samples would be positive by ELISA and negative by AGID. Samples that are repeatedly positive by ELISA, negative by AGID, and do not recognize EIAV proteins in research immunoblot tests are truly false-positive ELISA reactors. The rate and reason for these results appear to differ in the 3 ELISA test formats; thus, these samples, positive in one ELISA format, will often be negative in the other ELISA test formats. As the reason for the false-positive reactions appears to differ, if a field sample is repeatedly positive on all three ELISA test formats

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and a negative AGID test interpretation is tendered, it is highly probable that the sample will recognize the three major EIAV proteins in immunoblot tests.<sup>6</sup> To date, we have found no exceptions. The extra power afforded by using all 3 ELISA procedures is recognized by the Infectious Diseases of Horses Committee proposed three tier system (see Resolutions of the USAHA in 2002 and 2003).

Another class of discrepant reactions is from equids at no known risk for EIA whose serum is reactive only against the major core protein of EIAV (p26) in immunoblot tests, in one or more ELISA tests and occasionally in AGID tests. It is thought that this reactivity is due to equid exposure to related lentiviruses where interspecies determinants of the major core proteins stimulate cross-reactive antibodies. As these agents are not thought to multiply in equids, the low levels of antibody detected in immunoblot and ELISA tests (only rarely high enough to be detected by AGID) generally wane to lower, i.e., undetectable, levels in the AGID test in a relatively short time (30-60 days.) In our 30 years of experience with EIAV diagnostics, we have seen only 6 of these cases, hardly enough to mention except that one of them was presented one week before this meeting. In some cases, these types of reactions are noted in equids at risk for EIA; their p26 reactivity could potentially occur as a result of repeated exposure to low levels of inactivated EIAV on insect mouthparts.

A third class of discrepant results in official tests for EIA can occur in EIAV-exposed and EIAV-infected equids. Although there is no proven case of clearance of EIAV from a previously infected equid, there is a growing body of evidence that it may occur rarely. We have followed 3 such horses with field exposures and have monitored a gradual decline in antibody titer with time. We have been unable to demonstrate EIAV in blood, plasma or tissues from these horses in sensitive PCR tests for highly conserved genes of EIAV (unpublished results). In one case, the horse is now negative in AGID and 1 of 3 ELISA tests, although the serum still recognizes the 3 major proteins of EIAV in immunoblot tests. Additionally, we have evidence from experimental infections of horses and donkeys with a variety of strains of EIAV (fully virulent intact EIAV as well as specific gene-deleted mutants) that equids can be persistently infected with EIAV and escape immunosurveillance with some or all of the current licensed diagnostic tests. In all of these cases, antibody is detectable by research immunoblot tests (unpublished results). To date, such cases have not been reported in epidemiologic investigations of EIA, suggesting that such cases occur rarely in nature. It is probable that such cases would be discovered more readily if ELISA tests became the primary test of choice.

### **Testing costs, risks and planning**

Testing has been required by many jurisdictions for movements on

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public roadways, for congregations, for interstate movement, and for change of ownership. In two states, Louisiana and Arkansas, annual testing is required of all equids. The costs for testing for EIA have been borne by owners and annual testing costs are estimated at greater than \$50,000,000. For the year 2003 when 273 positive equids were reported, over \$180,000 was spent to find each positive equid. The average testing costs to find each positive equid in the northeastern states over the last 3 years was about \$1,000,000. A cursory examination of those numbers suggests that testing may be in greatly in excess of the expected risk in many areas. Perhaps it is time reassess our strategy for control of EIA and design programs which address risk estimates based on accurate census data and which adjust testing across state lines according to equivalency, i.e., regionalization.

Projections by the USDA have indicated that regionalization and reduced testing in low risk areas could dramatically reduce testing costs to the industry (Anne Seitzinger, USDA-APHIS-Veterinary Services, Center for Epidemiology and Animal Health, personal communication.) When coupled with increased accuracy of testing, the risk of acquiring EIA could be further reduced. An improved control program for EIA at a lower cost to the industry seems intuitive and overdue. We urge adoption of a national program utilizing the three tier laboratory system. Incidentally, a proposal for a National State-Federal Cooperative Program for the Control of EIA will be discussed at this meeting and a resolution may be approved by this Committee and forwarded for approval and ratification by USAHA.

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