REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

Chair: Peter J. Timoney, Lexington, KY
Vice Chair: James A. Watson, Jackson, MS

Helen M. Acland, PA; Debbie Barr, CAN; Derek J. Belton, NZ; Carter Black, GA; Shane A. Brookshire, GA; Jones W. Bryan, SC; Clarence L. Campbell, FL; Craig N. Carter, KY; Tony A. Caver, SC; Max E. Coats, Jr., TX; Timothy R. Cordes, MD; Ed Corrigan, WI; Stephen K. Crawford, NH; Leonard E. Eldridge, WA; Dee B. Ellis, TX; J Amelita Facchiano, TX; Dave E. Fly, NM; W. Kent Fowler, CA; Tony G. Frazier, AL; Paul Gibbs, FL; Keith N. Haffer, SD; Nancy E. Halpern, NJ; Steven L. Halstead, MI; Jeffrey J. Hamer, NJ; Greg N. Hawkins, TX; Burke L. Healey, NC; Carl Heckendorf, CO; Steven G. Hennager, IA; Michael E. Herrin, OK; Robert B. Hillman, NY; Don P. Knowles, WA; Ralph C. Knowles, FL; Maxwell A. Lea, Jr., LA; Donald H. Lein, NY; Mary J. Lis, CT; Martha A. Littlefield, LA; Amy W. Mann, VA; MaryAnn T. McBride, NC; Patrick L. McDonough, NY; Richard D. Mitchell, CT; Donald S. Munro, PA; Lee M. Myers, GA; Sandra K. Norman, IN; Don L. Notter, KY; Eileen N. Ostlund, IA; Robert E. Pitts, WV; Jewell G. Plumley, WV; Jeanne M. Rankin, MT; Keith Roehr, CO; Earl Rogers, UT; Michael A. Short, FL; Shari C. Silverman, NJ; Robert C. Stout, KY; David Thain, NV; Belinda S. Thompson, NY; Kerry Thompson, DC; H. Wesley Towers, DE; Susan C. Trock, NY; Charles D. Vail, CO; Taylor H. Woods, MO; Ernest W. Zirkle, NJ.

The Committee convened at 1:00 pm Monday, October 27, 2008 at the Sheraton Greensboro Hotel Greensboro, North Carolina. The meeting adjourned at 6:15 pm. There were 29 members and 55 guests present. The meeting was Chaired by Peter Timoney with the assistance of the Vice Chair, James Watson.

In drawing up the agenda for this year's meeting, emphasis was placed on a limited number of diseases and health-related issues of topical interest and concern to the equine industry. As in recent years, the number of topics was restricted in order to provide ample time for discussion of each agenda item.

The opening presentation, a Time-Specific Paper entitled, Potential Threat of African Horse Sickness to the United States was given by William White, Foreign Animal Disease Diagnostic Laboratory (FADDL), Veterinary Services (VS), Animal and Plant Health Inspection Service (APHIS). The following areas were covered by the speaker in his presentation: the salient clinical features of the disease in different equid species; a historical perspective of major occurrences of African horsesickness (AHS) outside of the African continent; a summarized account of current knowledge on the species of *Culicoides* responsible for the transmission of this disease with special reference to the potential vector competency and geographic distribution of particular *Culicoides* species, e.g. *C. sonorensis*, found in the United States (U.S.), and an overview of the potential modes AHS could be introduced into the United States or Europe with possible strategies for mitigation of the risk of such an event. The full text of Dr. White's paper is included in these proceedings.

Josie Traub-Dargatz, Colorado State University and Centers for Epidemiology and Animal Health (CEAH), VS-APHIS provided a final report on a project on equine herpesvirus myeloencephalopathy that was undertaken by the Center for Emerging Diseases (CED) at CEAH. The primary aim of the project was to identify what could be learned from past outbreaks of this disease that could improve our understanding of the circumstances under which it occurs and hopefully, formulate more effective strategies for handling future outbreaks of this and other equine infectious diseases. The report entitled Equine Herpesvirus Myeloencephalopathy: Mitigation Experiences, Lessons Learned, and Future Needs is a highly informative source of information on the subject. It provides a basis for future discussion by veterinary officials and those in the equine industry regarding prevention and control of this disease and the research that that is needed to develop the best management practices for dealing with future occurrences including defining the regulatory framework needed to respond efficiently in such circumstances. The report contains a comprehensive description of research, education and other needs

related to equine herpesvirus myeloencephalopathy. Special mention is made of the importance of establishing validation criteria for polymerase chain reaction (PCR) testing for the disease and of being able to differentiate neuropathogenic from non-neuropathogenic strains of equine herpesvirus 1. Previous occurrences of equine herpesvirus myeloencephalopathy highlight the lack of efficacy of current commercial vaccines in preventing the disease and the need to develop more effective immunogens if the industry is to be successful in significantly reducing the risk of such occurrence in the future. A copy of the full report is available at no cost from USDA-APHIS-VS-CEAH.

A second Time-Specific Paper entitled, Development of the Use of Tick-borne Transmission Models to Test the Efficacy of Imidocarb Diproprionate and Ponazuril in the Clearance of *Babesia caballi* and *Babesia (Theileria) equi* from Persistently Infected Horses was presented by Donald Knowles, Agricultural Research Service (ARS) and Washington State University. The basis for conducting this research was to minimize restrictions on the international movement of horses and furthermore, to prevent establishment of an area or areas of endemicity of *B. caballi* and/or *B. equi* in the U.S. Under the conditions of a controlled study of a limited number of horses persistently infected with a field strain of *B. caballi*, a single course of treatment with imidocarb diproprionate used at maximum therapeutic dosage level, eliminated the infection from the treated animals. The test horses continue to be monitored many months after they went negative for evidence of persistent infection based on various parasite detection tests, antibody determination assays, and a failure to successfully infect *Dermacentor nitens* ticks fed upon them. The author urged caution in extrapolation from this finding to cases of natural *B. caballi* infection in horses which are infected with drug resistant strains of the parasite as a possible result of prior treatment with imidocarb diproprionate used at sub-optimal levels necessary to clear the infection. Also, the results of this study are very promising, the number of test animals involved was very limited. The parallel study on the possible efficacy of imidocarb diproprionate for eliminating persistent *B. equi* infection in horses is currently in progress at the USDA's National Veterinary Services Laboratories (NVSL). Initial indications of drug efficacy do not appear promising. Dr. Knowles alluded to an additional study that is being carried out on the possible effectiveness of ponazuril (Marquis®, Bayer) for the treatment of *B. equi* infection in horses. In vitro studies with the drug reduced levels of *B. equi* in erythrocyte cultures but measurable parasite levels returned upon cessation of treatment. Results of the studies conducted to date confirm the need for further in-depth evaluation of these and other possible drugs with respect to their efficacy in eliminating persistent infection with either *B. caballi* in horses. The full text of this Time Specific Paper is included in these Proceedings.

Kent Fowler, California Department of Food and Agriculture (CDFA) and Chair of the Subcommittee on Equine Piroplasmosis, gave the Subcommittee Report. The Subcommittee was very active over the past year devoting itself to three major issues:

1. a seroprevalence survey of a representative sampling of horses throughout the U.S. for evidence of *B. caballi* or *B. equi* infection.
2. promotion of the need for additional federal funding for research on finding therapeutic means of clearing persistent *B. caballi* and *B. equi* infection in the horse.
3. organized the Third conference of Eqine Piroplamosis which took place on October 24, 2008.

The seroprevalence survey is to be based on equine infectious anemia residual sera submitted by the National Animal Health Laboratory Network (NAHLN). Response to the request for samples exceeded expectations with over 43,000 samples submitted to NVSL for testing.

There is an urgent need to expand the piroplasmosis studies that have been conducted during the past year. Expansion of the studies will require the availability of additional funding.

Dr. Fowler overviewed the Third Conference for Experts on Piroplasmosis program and highlighted the more significant issues that emerged, from current knowledge about equine piroplasmosis infection in the U.S., to a recommendation for establishment of a working group to evaluate the results of the national serosurveillance study, to discussion on a draft policy document on management of seropositive piroplasmosis infected horses. A report of the complete proceedings of the Third Conference for Experts on Equine Piroplasmosis will be available in the near future. The Subcommittee Report was approved by the committee and is included in these proceedings.

Peter Kirkland, Elizabeth MacArthur Agriculture Institute, Menangle, Australia, presented an overview of the widespread occurrence of equine influenza in New South Wales and Southeastern

Queensland in 2007. The introduction of the disease into Australia for the first time resulted in extensive spread of the virus in the non-vaccinated, naïve population in the two affected states with transmission rates and clinical disease approaching 100 percent on many premises. The disease was more severe in horses kept in close contact. Virus spread was believed to occur by aerosol transmission and also by indirect contact with contaminated fomites. Equine influenza viral nucleic acid was detectable on nasal swabs prior to the onset of clinical signs in acutely infected animals. Diagnosis of the disease was accomplished using a real-time reverse transcriptase-polymerase chain reaction assay and a blocking EWSA. A commercial Canary pox vectored vaccine which was approved for use on a restricted basis, induced a rapid immune response and provided the ability to distinguish between naturally infected and vaccinated horses. Overall, vaccination together with stringent restrictions on animal movement proved to be effective in curtailing further spread of the disease and limiting it to the two states in which it was originally introduced. Based on very extensive field surveillance and laboratory testing, there has been no evidence of residual equine influenza infection in the domestic equid population in Australia since early 2008. The disease is therefore considered to have been eradicated.

Ellen Buck, National Import Center (NIC), VS-APHIS, provided updates on a range of issues of current concern to the horse industry. She confirmed that the Non-Competition Entertainment Horse Rule came into effect July 2009. Legal review of the permanent private quarantine proposed rule is proceeding quickly and guidelines are being developed to approve such premises. Preparations for the World Equestrian Games in 2010 which will be held in Lexington, Kentucky, are in an advanced stage with Northern Kentucky International Airport, Cincinnati, Ohio the site for post-entry holding and testing of the majority of horses approved for three-day quarantine. A plan has been developed on how equine piroplasmosis seropositive horses will be managed at the Kentucky Horse Park, the site of the 2010 Games, as well as measures to mitigate the possible risk of transmission of either *Babesia* infection in the course of piroplasmosis seropositive horses competing in non-arena events. There has been continued progress in implementation of the recommendations provided in the 2007 Contagious Equine Metritis (CEM) Review Report. A training program was initiated in early 2008 dealing with aspects of the disease, how to sample the sites of persistence of the bacterium in the carrier mare and stallion, and how to submit such specimens for laboratory examination to optimize the chances of detection of the bacterium. A training program for laboratory personnel in states carrying out the diagnosis of this disease has also been implemented by NVSL. Efforts are also underway to address some of the other recommendations of the CEM Review Report. Dr. Buck overviewed the issue of the optimal time to hold horses on post-entry into the U.S. during which they are serologically tested for equine infections, anemia, dourine, glanders and equine piroplasmosis. Following a meeting with representatives of the equine industry, the American Horse Council and the American Association of Equine Practitioners, and realizing the significant careens of the industry over keeping performance horses in confinement for three days, USDA came to the decision that 42 hours was the minimum period such horses must be held under federal control following post-entry into the U.S.

The final update provided was on the current status of the Proposed Rule for Equine Viral Arteritis. Dr. Timothy Cordes, VS-APHIS-USDA confirmed that the docket pertaining to the proposed rule had been finalized and that the latter should be published within the first half of 2009. The intent of the proposed rule is not to restrict the entry of carrier stallions or equine arteritis virus infective semen into the U.S., merely to identify virus positive animals and semen and share that information with the appropriate regulatory officials and the consignee of a particular shipment.

Dee Ellis, Texas Animal Health Commission and Subcommittee Chair on Equine Infectious Anemia (EIA) gave the Subcommittee Report. The primary focus of the Subcommittee's activities in 2007 and 2008 was to assess the feasibility of promoting a change in the current National EIA Control Program with the ultimate goal or achieving eradication of this disease. The outcome of the Subcommittee's deliberations over the past many months are summarized in a Resolution that was presented and approved with amendment by the Committee.

The main points proposed by the Subcommittee are:

1. request USDA-APHIS-VS to seek new money to fully fund an enhanced control program that eventually leads to disease eradication
2. focus funding of states with the highest seroprevalence of infection

3. incorporate needed changes into the DFR
4. establish a National EIA Prevalence Working Group
5. refine the existing EIA laboratory reporting system
6. revise existing EIA diagnostic protocols

An overriding concern expressed by the Subcommittee Chair was the apparent lack of encouragement and support from the equine industry for a change in the status quo of the current National EIA Control Program. The Subcommittee Report was approved and is included in these proceedings.

Committee Business:

Following conclusion of the scientific program, the Committee went into the Business Session. Three resolutions on equine piroplasmosis and equine infectious anemia were considered and approved and forwarded to the Committee on Recommendations and Resolutions for approval by the general membership. In addition, the Committee recommended that a letter be sent to USDA urging the finalization of the draft policy on handling of positive equine piroplasmosis horses. The Committee also recommended removal of the second option, which allows for the treatment and release from quarantine of positive horses.

REPORT OF THE SUBCOMMITTEE ON
EQUINE PIROPLASMOSIS (EP)

Kent Fowler, Chair
California Department of Food and Agriculture

The Subcommittee was formed March 2006 to better identify the risk of EP becoming an endemic disease within the U.S. Additional direction of the Subcommittee was based upon identified needs to estimate the prevalence of seropositive EP horses within the U.S. and to identify a more cohesive policy at both state and federal level for identification and disposition of EP seropositive imported horses. The Subcommittee has also strongly encouraged USDA to fund research to find an effective treatment for EP.

As a result of Subcommittee work and the preceding work of others, the following conclusions have been drawn:

1) The status of EP in the United States is in question. EP is classified as a foreign animal disease to the United States. Prior to February 1, 2004, the official test for piroplasmosis, on equidae presented for importation into the U.S., was the complement fixation (CF) test, a test that is known to occasionally yield false negative results. Unscrupulous owners, importers or agents compounded the problem by purposely treating EP infected horses with immunosuppressive medications to give rise to a false negative reaction in the CF test. The CF test was replaced by an upgraded c-ELISA test that was specified as the official test on August 22, 2005. The competitive enzyme linked immunsorbent assay (c-ELISA) is less likely to yield false negative results on adult horses. Because of the compromised reliability of the CF test to detect long-term carriers of *B. caballi* or *B. equi*. It is plausible that infection from either parasite exists at an undefined prevalence in horses that have been imported into the United States and perhaps in horses native to the United States.
2) Potential tick vectors exist, but the dynamics for transmission remain unclear. EP infected horses may exist in the U.S. at a sufficient prevalence level to infect various competent resident tick vectors and potentially result in the establishment of endemicity of *B. caballi* or *B. equi* in the resident equine population in the United States.
3) Treatment is not yet a validated viable option. There is no conclusive evidence that treatment of a carrier of either or the two causal agents of EP (*Babesia caballi* and *Babesia equi)* is a viable option in successfully eliminating the carrier state. Ongoing research by Dr. Don Knowles, ARS-USDA, and research at NVSL, has encouraging early results for successful treatment of *B. caballi*.
4) Validated research risk assessment is required. It is crucial to 1.) maintain stringent import restrictions that prevent the importation of seropositive horses into the United States, 2.) develop a cohesive and acceptable policy at both federal and state levels for identifying and dealing with resident EP seropositive horses, and 3.) request funding for research to devise effective treatment protocols for EP.

The Equine Piroplasmosis Subcommittee introduced two resolutions at the 2007 USAHA Annual Meeting. The two resolutions were approved:

Resolution 19

REQUEST FOR SERUM FROM THE NATIONAL ANIMAL HEALTH LABORATORY NETWORK (NAHLN) FOR AN EQUINE PIROPLASMOSIS SEROLOGICAL SURVEY

Resolution:

The United States Animal Health Association (USAHA) requests that NAHLN laboratories make available and submit residual banked equine serum samples to the National Veterinary Services Laboratory (NVSL) for testing by competitive enzyme linked immunosorbent assay (C-ELISA) for the presence of antibodies to equine piroplasmosis (EP). The absolute requirement is that all samples submitted for evaluation carry no identification (ID) whatsoever as to animal name/numerical ID, date of collection, premises of origin or the laboratory or state from which they originated.

USAHA also requests the United States Department of Agriculture (USDA) to determine what constitutes a representative number of samples from the above NAHLN submissions to provide meaningful estimates of the current prevalence of EP in the United States resident horse population

or accept the previously statistically recommended number of 15,000 samples and use previously identified funding which was obtained through the slaughter surveillance initiative.

RESPONSE:

USDA-APHIS-Veterinary Services

The U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) recognizes the concerns of the United States Animal Health Association (USAHA) and appreciates the opportunity to respond. APHIS and the Agricultural Research Service (ARS) support this project and are in the process of planning the survey design, development, and implementation.

Resolution 20

SUBJECT MATTER: REQUEST FOR SERUM FROM THE NATIONAL ANIMAL HEALTH MONITORING SYSTEM (NAHMS) FOR AN EQUINE PIROPLASMOSIS SEROLOGICAL SURVEY

The United States Animal Health Association (USAHA) requests that the Centers for Epidemiology and Animal Health (CEAH) provide residues of sera collected during the 1998 NAHMS survey to be tested by competitive enzyme linked immunosorbent assay (C-ELISA) for the presence of antibodies to Equine Piroplasmosis (EP). The sera would carry no identification (ID) whatsoever as to animal name/numerical ID, premises of origin or state from which they originated.

RESPONSE:

USDA, APHIS, Veterinary Services

The U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services recognizes the United States Animal Health Association's concerns and appreciates the opportunity to respond. The decision was made by the EP Subcommittee to use the residual serum submitted to diagnostic laboratories (Resolution 19) as the basis of the survey rather than the 1998 NAHMS samples.

This past year four Subcommittee meetings took place via telephone conference call. The following decisions and discussions were a result of those meetings:

1) A decision to move forward with the possibility of testing the 1998 National Animal Health Monitoring System (NAHMS) Equine sera for the presence of antibodies to *Babesia caballi* and *Babesia equi* was delayed for several months as the request moved forward within USDA. In addition, only 8,000 equine NAHMS samples were collected in 1998, and testing serum gathered ten years previously would not have been very helpful to determine current national prevalence of EP. Therefore, it was decided to drop this survey proposal. In the meantime, plans to move ahead using National Animal Health Laboratory Network (NAHLN) laboratory Equine Infectious Anemia (EIA) banked sera samples were progressing nicely.
2) The response to date from the NAHLN laboratories to the EP survey project was tremendous. The participation of the laboratory was a critical element in constituting the critical mass for this very important survey. It was re-emphasized that all EIA residual sera samples that were submitted as part of this project shall remain unidentifiable as to source and remain strictly anonymous. Each participating laboratory was assigned a code number, and submitted samples were not to be accompanied by any information whatsoever with respect to horse name or identification (ID), premises name, or name of state that would identify the source specimen. Sample shipping boxes were identified with the assigned unique laboratory code. This laboratory code will not appear in the data set comprising the survey results. This shall ensure that there is no link whatsoever between any laboratory and any positive test result. By design, this purposely blinds all participating laboratories from the study's findings. Reporting of results will be at the national level and the prevalence estimate will have no links at a regional, state or laboratory level.
3) Center for Epidemiology and Animal Health (CEAH) developed a sample allocation plan for the EIA sera sent to NVSL. The plan allocated 17,000 samples with the expectation of completing 15,000 tests. The number of samples per laboratory will be proportional to the number of EIA tests performed by that laboratory. Samples will be selected at a specified interval for each lab. If a particular sample is untestable (eg, broken tube, hemolysis, etc.) then the next tube will be selected. The initial sampling timeframe proposed was October 2007 through April 2008. This was extended to June 2008.

4) Thirty-nine (39) NAHLN labs, in 35 states, were asked to participate in the EP national survey using EIA banked sera. CEAH assigned a unique code number to each lab to assist in assuring that no laboratory, state or region was identified with a positive sample. 35/39 laboratories submitted over 43,000 serum samples to NVSL.
5) Don Knowles agreed to direct the laboratory component of this project through collaboration with his laboratory and NVSL as follows:
    a. NVSL to receive samples and provide refrigerator space.
    b. NVSL to provide labor by utilizing additional wells in their routine testing process.
    c. Agricultural Research Service (ARS) (Pullman laboratory) to do Western Blot confirmatory testing on all c-ELISA positive NVSL samples.
    d. ARS (Pullman laboratory) to procure bulk-rate Veterinary Medical Research and Development (VMRD) kits.
6) Don Knowles estimated cELISA cost for 1 well per horse at $1.65. Therefore, with 15,000 horses the total cost would be $24,750 and then doubled to test for both B. equi and B. caballi.
7) Don Knowles offered for ARS to match APHIS funding for this project, which means $49,500 divided, or $24,750 per agency.
8) A working group would then be formed to develop recommendations for dealing with EP in the U.S. based on their evaluation of the survey results.
9) A need for a Third Conference for Experts on EP was identified and discussed on Subcommittee conference calls. Initially, this conference was proposed for June 2008 in Riverdale, Maryland, but the inability to select a date for the majority of the Subcommittee dictated it be rescheduled in Greensboro, North Carolina to coordinate with participants' travel to USAHA. The Third Conference for Experts on Equine Piroplasmosis is scheduled for October 24, 2008, in Greensboro, North Carolina.
10) Appreciation was noted for the hard work and productive efforts of Dr. Tim Boone, California Department of Food and Agriculture (CDFA), as a member of the Equine Piroplasmosis Subcommittee. Dr. Boone, Equine Program Lead, retired this past year from CDFA after 24 years of service in the Animal Health Branch.

Upon majority consensus of the Subcommittee and industry interaction, the following resolutions for progressively dealing with the current status of EP in the United States are as follows:

1) Resolve that USAHA request USDA-APHIS-VS to form an EP Working Group to evaluate the results of the National EP Survey and to provide recommendations for distribution of those results. It is also requested that the EP Working Group provide Committee with the survey results.
2) That USAHA request USDA-APHIS-VS to implement, recommendations from the EP Working Group and the committee, a national Policy for EP reactor equids.
3) That USAHA urge USDA-APHIS-VS to expand the funding for research to find an effective and safe treatment that eliminates the carrier state for *B. caballi* and *B. equi*.
4) That USAHA urge USDA-APHIS-VS to implement provisions in the 2007 Resolution 40 that requires all horses imported into, or returning to, the U.S. be identified with radio frequency identification (RFID) microchips that comply with the International Organization for Standardization (ISO) 11784 and 11785 standards (134.2 kHz). Universal RFID readers should be present at all import centers and border stations to read both 125 and 134.2 kHz microchips.

Challenges of the Subcommittee includes gathering continued feedback from the equine industry and developing science-based recommendations for dealing with all existing and evolving issues pertaining to the impact of EP on the U.S. The vision of the Subcommittee should be to do what it takes to ensure that EP does not become endemic in the resident horse population of the U.S.

REPORT OF THE EQUINE INFECTIOUS ANEMIA SUBCOMMITTEE

Dee Ellis, Chair

The focus of the equine infectious anemia (EIA) Subcommittee activities in 2007-2008 was to determine the feasibility and necessity of encouraging/facilitating change from a national EIA control program to an eradication program.

The Subcommittee had a number of conference calls over the year. First to discuss the pros and cons of a national eradication program, then how to go about making it happen.

As the new Subcommittee Chair, I traveled to Kentucky in February to discuss EIA program issues with Dr.'s Issel, Timoney, and Cordes. I also visited with a number of other participants in the existing EIA program activities to be fully aware of the many concerns. I would like to thank the Gluck Center and the University of Kentucky for their hospitality.

Members of the Subcommittee initiated dialogue in a number of outside venues on this subject to garner input, including Regional USAHA meetings, the National Assembly of Animal Health Officials, and the National Institute of Animal Agriculture.

The state veterinarians from the 4 at-risk states met in Dallas, Texas in August to discuss the viability of an eradication program. They agreed that they could support an eradication effort if properly funded and written.

The Subcommittee recommends that a resolution be approved that encourages and supports a national eradication program which includes the following necessary actions by the USDA-APHIS-VS:

- fully fund eradication efforts with new money
- focus the funding in a small number of at-risk states
- incorporate needed changes into the Code of Federal Regulations(CFR)
- create a national Prevalence EIA Working Group
- refine the existing EIA Lab reporting system
- revise the existing EIA diagnostic protocols

The most important unfinished business of the Subcommittee is to continue to support outreach efforts with stakeholders, and continue to garner support for a national eradication program from the many industry partners.

I would like to especially thank Amelita Facchiano for her help in supporting the group.

POTENTIAL THREAT OF AFRICAN HORSE SICKNESS TO THE UNITED STATES

William R. White
Foreign Animal Disease Diagnostic Laboratory

Timothy R. Cordes
Veterinary Services

Introduction

African horse sickness virus (AHSV) and bluetongue virus (BTV) are both members of the genus *Orbivirus* of the family Reoviridae. Both cause serious, non-contagious but infectious, arthropod-borne diseases in equids and ruminants respectively. AHSV infects all equids, causing asymptomatic infection in zebra and African donkeys, but it causes the most lethal infectious disease of horses known, with mortality as high as 95 percent (1). BTV is thought to infect all known species of ruminant, however, severe disease usually occurs only in certain breeds of sheep, particularly the fine-wool and mutton breeds and some species of deer, most notably the North American white-tailed deer (2). Zebra are thought to be the reservoir host of AHSV and cattle of BTV. Both diseases are OIE listed diseases that disrupt international trade in live animals and animal products from countries or regions with enzootic or epizootic occurrence.

The distribution of both diseases is a reflection of the distribution of their infected arthropod vectors, which are certain species of *Culicoides* biting midges, the temperature required for viral replication in these vectors, and transmission by these vectors. AHSV is confined to Sub-Saharan Africa and possibly Yemen and the Arabian Peninsula, but it has made brief excursions into Spain and Portugal in the west and into India and Pakistan in the east (3). BTV occurs much more widely, traditionally stretching in a band around the world from latitude 40^{o} N to 35^{o} S (4), but in certain areas like western North America it may extend up to 50^{o} N. Those midges that transmit AHSV also transmit BTV, and the reverse is likely true. *Culicoides imicola* is the major vector of AHSV and the major Old World vector of BTV.

Culicoides imicola is an Afro-Asiatic species that extended its distribution into the Mediterranean Basin of Europe from North Africa and the Middle East, causing the emergence of BTV into parts of Europe never before affected and causing the largest bluetongue disease epizootic on record (5, 6, and 7) with over one million sheep deaths. The reasons for this unexpected change in BTV epidemiology are complex but involve recent geographic extension of the distribution of *C. imicola*, involvement of novel and locally residing *Culicoides sp.* vector(s), and on-going climate change (7). *Culicoides imicola* is now found as far north as France and Switzerland, and overlapping in distribution with local *C. obsoletus* and *C. pulicaris* which are able to transmit BTV and extend BTV further north.

In addition, BTV unexpectedly entered northern Europe in August of 2006, creating a rapidly spreading bluetongue epizootic in The Netherlands, Belgium, Germany, France and Luxembourg with over 2,000 cases (6, 8 and 9). Northern Europe was experiencing a very hot summer with daily average 6^{o} C higher than normal (P. Mertens, personal communication). The virus overwintered by an unknown mechanism, although the 2006-7 winter was the second mildest winter in northern Europe on record. The epizootic continued into 2007 causing 45,000 cases and expanding to include the United Kingdom (U.K.), Denmark, Switzerland and the Czech Republic. Models had predicted the disease by wind-borne vectors to jump the English Channel into the UK which occurred in September 2007 following the wettest May to July period in 250 years (10, 11). In September 2008 Sweden also reported its first BTV case. The outbreaks were caused by BTV serotype 8, which had never been identified in the European Union before, and the exact origin and route of introduction still remains unknown. No importation of semen or embryos occurred during the period of interest, and importation of possible infected ruminants could not be identified. In addition, BTV serotype 8 is absent from southern Europe, and significant geographical barriers exist (Alps and Pyrenees Mountain chains) to prevent wind-borne spread from the south.

Since discovered in the Cape Colony of South Africa in the early eighteenth century, South Africa has had major epizootics of AHS every 10 to 15 years on average. The cause of this pattern was uncertain until Baylis et al (12) found a very strong association between the timing of these epizootics and the warm (El Nino) phase of the El Nino/Southern Oscillation (ENSO). When the ENSO caused heavy rain followed by drought, as occurred in 42 ENSOs since 1803, no AHS outbreak occurred. However, when the reverse occurred as a subset ENSOs, i.e. drought was followed by heavy rain, 13 of 14 major epizootics occurred. It was suggested by the authors that drought causes congregation of zebra near remaining

water holes leading to more contact and infection of vectors, which then disperse rapidly once rain provides more breeding sites. *Culicoides* populations can increase by over 200-fold in this scenario. Monitoring this pattern during ENSOs will help predict future AHS epizootics in South Africa.

Because of the recent dramatic change in epidemiologic status of BTV and its *Culicoides sp.* vectors in Europe, both the United States equine and ruminant industries have become concerned about their potential for entry into the U.S. Potential pathways for the entry of AHSV will be briefly described, as well as possible AHS disease scenarios in the U.S. if entry were successful.

Pathways Analysis for Release of AHSV into the U.S.

The ultimate purpose of a pathways analysis is to provide information to decision makers about the feasible route(s) that a disease agent (e.g. AHSV) can use to enter a geographic region so that a surveillance plan can be developed for rapid detection of the organism (13). A pathways analysis may then lead to a qualitative or quantitative risk assessment that measures the likelihood of a disease outbreak occurring from an identified pathway(s) and the consequence of such an outbreak. The following discussion is meant to identify feasible routes for entry of AHSV into the U.S. that could be evaluated during a formal pathways analysis for AHSV.

1. Importation of AHSV-infected animals

Federal regulations exist for the legal importation of domestic and wild equidae from countries the USDA-APHIS considers to be affected with AHS. Specifically, in the Code of Federal Regulations, Title 9, Part 93, paragraph 93.308a2:

Horses intended for importation from regions APHIS considers to be affected with African horse sickness may enter the United States only at the port of New York, and must be quarantined at the New York Animal Import Center (NYAIC) in Newburgh, New York, for at least 60 days. This restriction also applies to horses that have stopped in or transited a region considered affected with African horse sickness. APHIS considers the following regions to be affected with African horse sickness: Oman, Saudi Arabia, the Yemen Arab Republic, and all the regions on the continent of Africa except Morocco.

Further regulations are available on the USDA-APHIS-VS-NCIE website regarding the minimum 60 day quarantine for all equines originating in AHS-affected countries: http://www.aphis.usda.gov/import_export/animals/animal_import/equine/equine_import60day.shtml. Quarantine charges are currently $210 for day 1-3, $195 for day 4-7, and $166 for all remaining days.

Regarding equine traffic through the NYAIC during the last three years, only 16 horses and no zebra entered from AHS-affected countries. There were 15 horses in one shipment in 2006 and one horse in 2008 (K. Davis, NYAIC, personal communication). Zebras are not generally imported into the U.S. because of the expense and presence of successful breeding programs in the U.S. In contrast several thousand horses from non-affected countries were quarantined at NYAIC. There were about 3800 in FY 2007 and 2600 in FY 2008.

The incubation period of AHSV in horses is 5-7 days experimentally with a range of 2-10 days. Viremia in horses is 4-8 days and has not been detected beyond 21 days, while in donkeys and zebra viremia may last up to 28 days (1). The maximum infectivity period would therefore be 31 days in horses and 38 days in donkeys and zebras. Therefore, 60 day quarantine in a vector-proof stable ensures that no equidae will leave while still infectious. The OIE infectivity period for AHS is 40 days (2007 OIE Terrestrial Animal Health Code, online) which also indicates that a 60 day quarantine is more than adequate.

Despite early reports, there is little evidence of antibody to AHSV in domestic or wild ruminants, except possibly camels (1). Antibody was detected by ELISA in white and black rhinoceroses and neutralizing antibody was detected in elephants in Kenya. Clinical disease has never been described in camels, rhinoceroses and elephants, and no information is available on possible viremia. They are unlikely to play a role in the epidemiology of AHS. Dogs eating infected horse meat develop a peracute, highly fatal pulmonary form of AHS, but they also are unlikely to play a role in transmission since *Culicoides spp.* do not readily feed on them.

It is possible that equine semen collected from a viremic donor could contain AHSV and expose a mare during breeding by artificial insemination or live cover. Semen, urine and all secretions may contain virus, and semen may also be contaminated by red blood cells with adhering virus. It is extremely unlikely an imported stallion would be viremic during mating in view of the 60 day quarantine.

2. Introduction of infected vectors

Wind-borne: Dispersion of *Culicoides spp.* over distances up to 700 km over water and 150 km over land (8) has been postulated. However, the shortest distance from Africa to the U.S. is 4830 km. Global wind speed is typically 6.64 m/s (14.9 miles/h) near (10 m [33 ft]) the ocean surface but faster (8.6 m/s [19.3 miles/h]) when at a higher (80 m [262 ft]) altitude (10). Thus it would require 6 to 8 days for wind leaving Africa to reach the continental United States. Because the maximum adult life span is 10 to 14 days, AHSV-infected midges are unlikely to survive being transported from Africa to the continental United States on wind currents, even those of a hurricane. To cover such long distances transport would need to be at high altitude (6,000 m), at which air temperature is far below 0^{o} C, and *Culicoides spp.* would not survive (8).

Factors affecting the potential windborne spread of *Culicoides spp.* (9)

a. Distance. The successful transport of infected midges decreases with increasing distance.
b. Warm temperatures. Temperatures at 27-30°C are optimal for AHSV transmission in the laboratory, while temperatures below 15°C inhibit virus replication within the midge. As temperatures increase, midge infection rates increase and virogenesis quickens, but midge survival rates decrease. At cooler temperatures, AHSV within the *Culicoides spp.* vector becomes 'latent', but replication commences rapidly as temperatures warm. This may be a viral overwintering mechanism (3).
c. Light wind speeds around dusk and night when the midges are most active. Wind speeds of greater than 3 m/sec reduce midge activity.
d. Minimal/no precipitation. Midge activity is substantially reduced during rain.
e. A steady wind from the origin to the U.S. Steady winds reduces midge mortality.
f. Relative humidity (RH) of 75-85%. The midge can become desiccated at low RH, and oversaturated at high levels.
g. Susceptible equidae at the end of the transit. The minimum size of a zebra population to maintain an enzootic infection is unknown.

Plants: There are no references available describing *Culicoides spp.* in cargo, including imported flowers or plants (8, 13). If present, *Culicoides* would have to be infected adult females, since transovarial transmission of AHSV has not been demonstrated. In addition, *Culicoides spp.* associate much more closely with their mammalian hosts than with plant species normally sold in the export trade.

Airplanes and Ships: When originating from an AHS-affected country, they may theoretically contain infected *Culicoides spp*. Although mosquitoes have been commonly documented, there are almost no data recording the presence of *Culicoides spp.* on aircraft. Reye in 1964 (14) reported a probable spread of *Culicoides spp.* by aircraft from Fiji to the Society Islands. For more comprehensive data on the mechanical transport of insect vectors from Africa to the U.S., see Kasari 2008 (13).

3. Introduction of other infected materials

Contaminated biologicals, like equine serum and fetal equine serum, should also be considered since they are used for growth of hybridomas and cell cultures. For example, BTV was found in contaminated canine vaccine leading to abortion and death in pregnant bitches (8, 15), and epizootic hemorrhagic disease virus was found in contaminated bovine serum imported into Germany (8, 16).

Outbreak Scenario in U.S.

The U.S. has multiple components that would support at least a focal outbreak of AHS:

1. horse population estimated to be 9.2 million, concentrated in Texas (1 million),California (700,000) and Florida (500,000) (17),
2. warm temperate climate in several southern and western states that would encourage viability of an introduced vector, and
3. highly competent laboratory vector for AHSV, *Culicoides sonorensis*. This vector has a wide U.S. distribution (absent only from the northeastern states), and is the biological vector for BTV. If a foreign midge vector was to successfully invade *C. sonorensis* eco-niche and begin an AHSV epizootic, *C. sonorensis* would soon become infected and the likely primary vector.

The only component missing for establishment of enzootic areas in the U.S. is the occurrence of zebra or another yet unknown reservoir host. However, the number of zebra needed to establish an area enzootic for AHS is unknown, and safari and hunting lodges with zebra do occur in Texas and other states. In

addition, several enzootic countries in Africa do not have zebra. In these countries an unknown animal or 'biological mechanism' must serve as the reservoir host and allow overwintering. In parts of Africa, it has been suggested the African donkey may be the reservoir host.

In contrast to the above, it is believed that the hunting and removing of zebras from most of South Africa has prevented the country from becoming enzootic (18). The only enzootic region in South Africa is in the northeast portion where continuous circulation of the virus occurs between *C. imicola* and herds of zebra in the Kruger National Park. Outbreaks occur each year in a southerly direction as climatic conditions become favorable for the infected midge to reach susceptible equine populations.

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DEVELOPMENT AND USE OF TICK-BORNE TRANSMISSION MODELS TO TEST THE EFFICACY OF IMIDOCARB DIPROPIONATE AND PONAZURIL IN THE CLEARANCE OF *BABESIA CABALLI* AND *BABESIA* (*THEILERIA*) *EQUI* FROM PERSISTENTLY INFECTED HORSES

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Equine piroplasmosis (babesiosis) caused by two distinct protozoan parasites presents significant challenges to the global veterinary profession. These challenges include protecting equine health while moderating the economic impact of restricted movement of infected horses within and between countries. Crucial to this effort is defining the ability of certain chemotherapeutics to clear persistently infected horses of these parasites and remove their transmission risk. Recent data have clearly established the tick-borne transmission risks of horses persistently infected with *Babesia (Theileria) equi* and *Babesia caballi*. Horses persistently infected with *B. equi* or *B. caballi* are efficient reservoirs for transmission by *Rhipicephalus* (*Boophilus*) *microplus* (13, 14) and *D. nitens* respectively (10). As previously reported *B. caballi* is transovarially transmitted, however without re-exposure of ticks to an infected horse, transmission is restricted to one generation in *Dermacentor nitens* (10). The experimental *D. nitens* transmission model was recently applied to testing the hypothesis that high dose imidocarb dipropionate treatment of horses persistently infected with *B. caballi* removes transmission risk. Collaborative efforts testing the efficacy of ponazuril in clearing persistent *B. equi* and/or *B. caballi* has been initiated.

Imidocarb is a carbanilide derivate usually administered as a dipropionate salt by intramuscular injection (1). A number of therapeutic protocols have been described in horses, however 2mg/kg administered in two doses at 24 hour interval is reported to be effective in eliminating *B. caballi* infection (5). For *B. equi* 4mg/kg administered for up to four doses at 72 hour interval is recommended, however the efficacy in eliminating *B. equi* infection in the horse is unclear. Previous reports (4, 5, 6, 7, & 12) indicated that imidocarb dipropionate cleared *B. caballi* but not *B. equi*. A recent study (2) reported that repeated high dose imidocarb dipropionate treatment did not eliminate *Babesia caballi* from naturally infected horses as determined by polymerase chain reaction (PCR)-reverse line blot hybridization. In this study five doses of imidocarb dipropionate (4.7 mg/kg, intramuscularly at 72 hour intervals) was tested. Further complicating interpretation of studies is the uncertain treatment histories of horses. This is an important issue due to the possibility of the emergence of treatment resistant strains due to the use of drug dosages below levels necessary to provide parasite clearance. Also, due to an inherent lack of sensitivity in the complement fixation test (CFT), previous use of the CFT to determine efficacy of certain chemotherapeutics in the clearance of persistent infections with *B. equi* or *B. caballi* precludes definitive conclusions.

Due to these collective data and the importance of knowledge concerning the ability of chemotherapeutics, including imidocarb dipropionate to eliminate *B*. *caballi* from infected horses a research plan was developed. The first hypothesis tested was that imidocarb dipropionate at 4mg/kg given three times at seventy two hour interval would eliminate *B. caballi* infection. The elements of this plan include (1) the acquisition of a tick transmittable *B. caballi* isolate with a history unlikely of exposure to imidocarb dipropionate; (2) derivation of a tick colony from the field tick transmitting the acquired *B. caballi* isolate; (3) use of currently validated competitive enzyme linked immunsorbent assay (cELISA) to measure equine anti- *B. caballi* responses; (4) experimental establishment of horses persistently infected with *B. caballi*, and (5) testing the ability of imidocarb dipropionate to eliminate *B. caballi* infection by tick transmission and needle transfer of whole blood. The derivation and characterization of the *Dermacentor nitens* tick colony and associated *B. caballi* isolated was recently reported (10). The outcome of a recent experiment (11) testing the above hypothesis and using with listed criteria will be reported.

Through collaboration with colleagues at the National Veterinary Services Laboratory, Ames Iowa the efficacy of imidocarb dipropionate in the elimination of *B. equi* is being tested. Additionally collaborations have been established to test the efficacy of ponazuril for efficacy in anti-*B. equi* activity. Ponazuril is currently used to treat equine protozoal myeloencephalitis (EPM) and has been shown to have efficacy in vitro against the apicomplexans *Neospora caninum*, *Sarcocystis neurona* and *Toxoplasma gondii* (8,9). The use of ponazuril within the oral paste preparation referred to as Marquis (Bayer) showed an ability to

reduce *B. equi* levels in vitro, however upon cessation of treatment, measurable parasite levels returned. The dosage of ponazuril necessary to eliminate *B. equi* from erythrocyte cultures is currently being determined and these data will be used to test ponazuril in horses persistently infected with *B. equi*. Similarly to the experimental protocol used for testing imidocarb dipropionate in elimination of *B. caballi,* the ability of ponazuril to eliminate *B. equi* infection will be tested using the established *B. equi* transmission model using *Rhipicephalus (Boophilus) microplus* (13,14).

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