

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; Suzanne L. Burnham, TX; Clarence L. Campbell, FL; Craig N. Carter, KY; Tony A. Caver, SC; Max E. Coats, Jr., TX; Leroy M. Coffman, FL; Tim R. Cordes, MD; Ed Corrigan, WI; Stephen K. Crawford, NH; Leonard E. Eldridge, WA; Dee 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; Gregg 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, DC; 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, GA; Jewell G. Plumley, WV; Jeanne M. Rankin, MT; Keith Roehr, CO; Earl Rogers, UT; Michael A. Short, FL; 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 Woods, MO; Ernest W. Zirkle, NJ.

The Committee met from 12:30 p.m. to 5:45 p.m. on Sunday, October 21, 2007 at John Ascuaga's Nugget Hotel, Reno, Nevada. In attendance were 31 Committee members and 46 visitors for a total of 77 persons. The meeting was Chaired by Peter Timoney with the assistance of Vice Chair, James Watson. In his introductory remarks, the Chair made reference to three Resolutions approved in 2006, responses to which had been circulated among the Committee membership in advance of the meeting. Any discussion of the responses would be deferred to the Business Session. The Agenda for this year's meeting addressed a number of diseases and health-related issues, some of which were considered of special economic significance to the equine industry at this time. By limiting the number of presentation topics, greater opportunity was provided for discussion of each agenda item.

Peter Timoney, standing in for George Allen, Gluck Equine Research Center, University of Kentucky, presented the Time-Specific Paper entitled Recent Advances in Our Knowledge of Equine Herpesvirus-1 (EHV-1) Myeloencephalopathy. The paper provided an overview of the latest information on the emerging significance of the disease, unique characteristics of the mutant neuropathogenic strains of equine herpesvirus-1, the pathophysiologic basis for their enhanced pathogenicity, advances in diagnostic testing for the disease, the immunologic basis of protective immunity, and finally, prospects for disease prevention through vaccination. The full text of this paper is included in these proceedings.

In addition, Dr. Timoney deputized for Charles Issel, Gluck Equine Research Center, University of Kentucky and gave his presentation entitled, Control of Equine Infectious Anemia Should Take New Directions at the Joint United States Animal Health Association (USAHA) American Association of Veterinary Laboratory Diagnosticians (AAVLD) Scientific Session, Monday, October 22. The paper proposed a series of new approaches to the control of equine infectious anemia embracing more targeted testing to identify carriers of the causal virus in the currently untested reservoir equid population, greater efficiency and accuracy in diagnostic testing, and regionalization of states with equivalent infection status so as to minimize the frequency of testing required of horses moving between those states. The text of this paper will be included in the USAHA/AAVLD Scientific Session section of these proceedings.

Josie Traub-Dargatz, Colorado State University and Centers for Epidemiology and Animal Health (CEAH), Veterinary Services (VS), Animal and Plant Health Inspection Service

(APHIS), United States Department of Agriculture (USDA) reported on a project being conducted by the Center for Emerging Issues (CEI) at CEAH on equine herpesvirus-1 neurological disease. On review of the increased frequency of occurrences of this disease in recent years, the CEI concluded that equine herpesvirus myeloencephalopathy met the criteria for a potentially emerging infectious disease. A disease is considered emerging when it meets at least one of three criteria: (1) disease is identified for the first time; (2) disease changes in virulence, host range or other pathogen behavior, or (3) disease changes in geographic range or incidence within a range. The current situation with equine herpesvirus myeloencephalopathy outbreaks reflects a change in virulence and behavior of the causal virus. In 2007, the disease was the focus of presentations at both the American College of Veterinary Internal Medicine and meetings of the American Horse Council. CEAH developed a project to identify what could be learned from outbreaks of equine herpesvirus myeloencephalopathy that would enhance current understanding of the disease as well as improve strategies for handling future outbreaks of other emerging diseases. Equine herpesvirus specialists and animal health officials involved in mitigation of these outbreaks were interviewed to gather information about methods used in outbreak management and the need for educational materials and research studies. A report summarizing lessons learned and common themes in the mitigation of outbreaks will be produced. The project will provide the equine industry, equine practitioners and animal health officials with a concise summary of approaches used to manage such outbreaks which hopefully would enhance the response of USDA-APHIS-VS when confronted with new or emerging diseases in the future. Considerable discussion followed both presentations on equine herpesvirus myeloencephalopathy. There was a widespread interest in how best to manage outbreaks of equine herpesvirus myeloencephalopathy especially at racetracks or other venues where horses are congregated in close proximity to one another. Concerns were expressed over interpretation of positive diagnostic test findings in the absence of clinical evidence of disease. Exclusion of such animals was questioned under the current case definition of the disease used in the National Animal Health Reporting System (NAHRS).

Two presentations followed on the USDA Review of the U.S. Contagious Equine Metritis (CEM) Import Program that was carried out in 2007. The goal of the review was to identify program improvements that would mitigate the risk of release of CEM carrier stallions or mares into the United States. Edward Arza, USDA-APHIS-VS, provided the background that prompted the review, identified the ten areas involving import activities which served as the focus of the report, and gave an executive summary of the major recommendations for each of the program activity areas that were evaluated. The risks involved in reintroduction of CEM into the United States are very real, especially for those states that import the greatest number of stallions and mares.

Ellen Buck, National Import Center, VS-APHIS-USDA, provided a detailed response by way of an action plan to the recommendations provided in the CEM Import Program Review report. Among the areas highlighted in the Action Plan were required changes to the Code of Federal Regulations, review and updates of policy documents and memoranda, inclusion of a CEM program review in Station Reviews, enhancement of existing systems of record-keeping and communication, improvement in the reliability of diagnostic testing for this disease by CEM-approved laboratories, provision for specific training and accreditation of veterinarians who perform sampling for the disease, and finally, a risk assessment of Spanish Purebreds and Racing Thoroughbreds which are currently exempt from CEM quarantine and testing in the United States.

Ellen Buck then gave a presentation on the Proposed Rule for Importation of Non-Competitive Entertainment Horses into the United State from CEM affected countries. The comment period on the proposed rule was now closed, with a very limited number of comments being received. A primary concern that had been expressed was the risk of losing track of such horses following importation into the country. Adequate safeguards would be required to ensure that horses imported under the proposed rule were effectively tracked and monitored throughout

their period of residency in the United States, regardless of duration. A complete review will be conducted of the primary requirements that need to be met under the proposed rule.

Steve Halsted, Michigan Department of Agriculture, and Chair of the Subcommittee on Equine Infectious Anemia, gave the report of the activities of the Subcommittee. It had been an active year for the Subcommittee, highlighted by a National Direction Meeting on Equine Infectious Anemia that was held in Denver, Colorado, May 21-22, 2007. In concluding his report, Halsted called on Stanley Bruntz, CEAH-VS-APHIS-USDA, to comment on current progress in improving data compilation and analysis under the National Animal Health Reporting System (NAHRS) with specific reference to equine infectious anemia. It is anticipated that improvements to the existing system will be implemented before the end of 2007, enabling the provision of summarized national data on this disease on a monthly basis. The Subcommittee Report was approved by the full Committee the Report of the National Equine Infectious Anemia Direction Meeting is included in these proceedings.

Josie Traub-Dargatz gave a brief overview of the National Animal Health Monitoring System (NAHMS) Equine 2005 Equine Trends and Equine Events Reports. The NAHMS Report Equine 2005 Part II Report: Changes in the U.S. Equine Industry, Section I, presents demographic changes in the US equine population from a historical perspective based on data provided by the National Agricultural Statistics Service, Census of Agricultural and US Bureau of Census. Section II, includes historical data on equine infectious anemia, West Nile Virus and vesicular stomatitis. In Section III, results of the Equine 1998 and Equine 2005 studies are compared to provide an overview of changes in U.S. equine management and health from 1998 through 2005. Another NAHMS Report, Equine 2005: Baseline Reference of Equine Health and Management Strategies at Equine Events in Six States, 2005, contains information from a study carried out in California, Colorado, Florida, Kentucky, New York and Texas, that focused on identifying health-management factors at equine events that could potentially impact the occurrence and spread of equine infectious diseases. Copies of each report are available by contacting CEAH-VS-APHIS-USDA.

Thomas Chambers, Gluck Equine Research Center, University of Kentucky, presented an update on the international equine influenza situation with specific reference to major occurrences of the disease in Japan and Australia since mid-August, 2007. In both countries, the disease was caused by a strain of equine influenza A/equine 2 virus that is reported to be very closely related to one originally isolated during an outbreak of influenza in Wisconsin in 2003. In the case of Japan, this has been the second major occurrence of equine influenza since the first known incursion of the disease into that country's equine population in 1972. The number of infected horses in the current series of outbreaks has been estimated at a little over 600, significantly less than the figure of 7,000 involved in 1972. The reduced incidence is believed to be the result of a twice-yearly vaccination program against influenza mandated since the previous occurrence of the disease in the country. Vaccination is believed to have decreased the morbidity rate, mitigated the clinical severity of the disease and limited dissemination of the virus through reduced nasal shedding by infected horses. The recent occurrence resulted in cancellation of numerous race meetings and a ban on horse movements. Current field data would indicate that the 2007 occurrence has essentially run its course and racing is being resumed on a select basis and under specific conditions.

The occurrence of equine influenza in Australia was the first known incursion of the disease into that country's equine population. Vaccination against equine influenza was not permitted in Australia since the disease had hitherto been exotic to the country. Equine influenza is believed to have been introduced in a shipment of horses imported through the post-arrival quarantine station in Sydney. Since the second to third week in August, the virus spread widely in the racing and breeding horse populations in New South Wales and Queensland, the two states in which the disease has been confined to this point. Race meetings have been cancelled and there has been major disruption of the countries' thoroughbred and standardbred breeding industries. The equine industry in affected states is continuing to experience huge economic losses from the epidemic of equine influenza. As of October 17, 2007, 4,619 infected premises

had been identified in New South Wales and a further 1,291 in Queensland, and additional new outbreaks are still being reported. The total number of infected horses is currently estimated at over 40,000. There is very recent evidence of infection with equine influenza virus in dogs on a very limited number of affected premises. A strictly controlled program of vaccination with a commercial canary-pox vectored vaccine has been implemented in selected populations of racehorses and performance horses in the two affected states as well as in the state of Victoria. Federal and state animal health officials still maintain that eradication of equine influenza is an achievable goal, notwithstanding the continue spread of the virus in New South Wales and Queensland. It remains to be seen whether eradication can be accomplished or whether equine influenza will become endemic in Australia.

Kent Fowler, California Department of Food and Agriculture (CDFA) and Chair of the Subcommittee on Equine Piroplasmiasis presented the Subcommittee report. In spite of the best efforts of the Subcommittee, no progress had been achieved since 2006 in being able to carry out a serosurveillance study for equine piroplasmiasis in the U.S. slaughterhouse population due to closure of the three remaining horse slaughter plants in Texas and Illinois before the survey could get underway. A couple of alternative options were being pursued in an effort to accomplish the goal of establishing what the status of the US equine population was with respect to this disease. The Subcommittee Report was approved by the Committee and is included as part of this Committee Report.

Donald Knowles, Washington State University and USDA Agricultural Research Service (ARS), Animal Disease Research Unit (ADRU) gave a progress report on treatment studies of *Babesia caballi* infection in horses. The objective of the study is to analyze with current parasite detection methodologies, the ability of imidocarb dipropionate to clear horses of persistent *B. caballi* infection. The source of the parasite was *B. caballi* infected *Dermacentor nitens* ticks obtained from Puerto Rico. Following successful establishment of persistent infection in four horses, two were treated with the drug and two were left as controls. While infection in the untreated controls remained detectable by real time polymerase chain reaction (PCR) assay, parasite DNA remains undetectable by PCR in the treated horses. Anti-*B. caballi* antibody continues to be detectable by competitive enzyme linked immunosorbent assay (cELISA) and indirect fluorescent antibody (IFA) assay. Three months' post-treatment transfusion of 100 ml of blood from each treated and untreated horse to four naïve horses resulted in transmission from the untreated controls but not the imidocarb treated horses. Both treated and untreated horses are currently being tested for their ability to transmit *B. caballi* by *D. nitens*. It remains to be established whether treatment with imidocarb renders horses nontransmissible for *B. caballi*.

Jeffrey Nelson, National Veterinary Services Laboratory (NVSL), VS-APHIS-USDA, presented a brief report on the treatment of *B. equi* experimentally infected horses with imidocarb dipropionate. The source of the parasite used in the study originated from a horse imported into the United States several years ago from Peru. Species of *Boophilus* ticks will be used in later tick transmission studies with the parasite. As the study is still at a preliminary stage, few experimental findings were available for review.

Following conclusions of the scientific program, the Committee went into Business Session and The Committee considered and approved three resolutions on equine piroplasmiasis that were forwarded to the Committee on Nominations and Resolutions for approval by the general membership.

Report of the Equine Infectious Anemia Subcommittee to the USAHA Committee on Infectious Diseases of Horses

Steven L. Halstead, Chair

The Subcommittee met by conference call through 2007. The primary activity of the Committee in 2007 was to convene a second EIA National Direction meeting (the first being held in March, 2006) of a small group of State animal health officials to further consider the most appropriate and acceptable direction in which to guide the nation's EIA management efforts. The recommendations and conclusions of the participants are detailed in the attached report, and can be summarized as follows:

- Areas where the EIA program works well are those of uniformity, compliance, prevalence modeling, and available regulatory tools.
- Areas of concern include low interest in pushing for further prevalence reduction; passive attitude about EIA, low enthusiasm for program change, and educational gaps within some segments of the industry; resource shortages, and disproportionate testing across all segments of the national equine herd.

The attendees recommended the following next steps to address the above concerns and to capitalize on the program successes:

1. Develop National dialogue about refining and standardizing testing requirements nationally— standardize the testing requirements and base the minimum testing requirement on the general (testing or estimated) prevalence of the disease in the state.

- Require change-of-ownership testing nationally
- Set the minimum testing for states with lower prevalence at 2 years
- Set the minimum testing for states with higher prevalence at 1 year
- Movement among lower prevalence states or from a lower prevalence state to a higher prevalence state would be allowed with a current test (within 2 years)
- Movement among higher prevalence states or from a higher prevalence area to a lower prevalence state would be allowed with a current test (within 1 year)

The group suggested that the chair of the EIA Subcommittee discuss these proposals with and among the members of the National Assembly and the APHIS Senior Staff Veterinarian for Equine programs discuss the proposals with the APHIS VS AVICs. After those meetings, the National Assembly members and/or AVICs would discuss the proposals with pertinent stakeholders in their state:

- State veterinarian
- State horse councils
- Farm bureau as appropriate
- State veterinary medical associations
- State equine practitioners
- State breed associations

At the same time, the group suggested that members of the EIA Subcommittee discuss these proposals with the following:

- American Horse Council at their June meeting in Washington DC.
- State Horse Councils—there will be an opportunity to address the state horse councils the first day of the AHC meeting in June 2007
- American Association of Equine Practitioners (AAEP) at their national meeting on December 1 through 3 in Orlando FL as part of the AAEP Infectious Diseases Committee meeting
- National breed associations

2. Suggestions for further EIA Subcommittee consideration and action—the group suggested the EIA subcommittee pursue a number of other areas as well, some of which support the change in the testing requirements described above and some are meant as national program improvements. These suggestions are summarized from the meeting report below:

Ensure accuracy of prevalence model—the group would like more discussion about the CEAH prevalence model, especially with states that would be considered to be higher prevalence.

Additional testing requirements— explore the feasibility of pursuing state specific legislation to require uniformly a current EIA test for gatherings in state. A current test would be one that has been done in the last 1 year or 2 years depending if the state is a low or high prevalence state.

Laboratory moratorium—there is a perception that NVSL will not allow any more labs to do EIA work. NVSL however, has a set of criteria for approving new labs when they are needed. The group encourages NVSL to clearly communicate that it is a moratorium and not a ban and what the criteria are for getting a new lab approved. This would be especially helpful to those in states where prevalence is higher.

Reporting and compiling of test results—as part of the approval process labs need to be required to report the results in a way that enhances accurate compilations, including having labs report test results to both the state where the horse resides and the state where the lab is located to help ensure accurate compilations and avoid double counting.

Revision of the VS 10-11—poll lab personnel, field veterinarians and others who use the form to make sure the new form meets their needs before making official revisions. Also, talk with those states who do not use the APHIS form to learn why and to make the form more useful.

Use of VS 10-11 at the borders—make use of VS 10-11 for imported horses from Mexico and Canada. In addition, explore the use of the VS 10-11 for BLM horses. In both cases, the use of the electronic form would be ideal.

EIA Summit—consider holding a summit to discuss the direction, iron out problems and improve the national effort to control EIA. This should include the range of key stakeholders including state, federal and industry personnel and practitioners including accredited veterinarians

EIA free zones—explore the idea of having EIA free zones in higher prevalence states so horses could move with fewer restrictions. While the group was not opposed to the idea there was concern that the regulatory work involved in making sure the zone stayed free might out-weigh the benefits of having the zone in the first place

Guidance/regulation—follow through on plans to update the UM&R for the program and incorporate the UM&R into the CFR

Pursue Federal funding—the group encourages APHIS to pursue funding for a national EIA program and to use a portion to provide cooperative agreement money for states.

Subcommittee on Equine Infectious Anemia (EIA) National Direction Meeting Results

Stanley Bruntz
Veterinary Services

Background

As a result of a recent meeting of the Equine Infectious Anemia (EIA) Subcommittee of the United States Animal Health Association (USAHA) Infectious Diseases of Horses Committee (IDOHC) the chair of the subcommittee invited a number of people to join him for a national direction setting meeting in Denver on May 21 and 22, 2007. See Appendix 1 for a list of those who attended.

The agenda for the meeting included several updates on work being done on EIA and several discussions guided by a series of questions. The updates include the following:

- National Direction Meeting in 2006
- Recap of 2007 EIA Subcommittee work
- Follow up from IDOHC – Resolution 8
- Progress on Uniform Methods and Rules (UM&R) and the development of an EIA rule in the Code of Federal Regulations (CFR)
- Prevalence Modeling
- Work related to EIA by the National Surveillance Unit (NSU)
- Changes to National Animal Health Reporting System (NAHRS) related to EIA
- Results of the Equine NAHMS study related to EIA
- EIA / VS 10-11 Revision

The key questions used to guide the discussion included the following.

- What excites you about the progress made so far?
- What concerns you about the progress made so far?
- What crossroads are we facing?
- What are the possibilities for the future of dealing with EIA?
- What future do we want to create?
- What are the next steps (short and long term)?

Areas that seem to be working well

The group identified areas of EIA program that are going well. Some of the ideas that came up included:

- **Uniformity**—even though there are areas that need more standardization, what has been accomplished has been good
- **Compliance**—some of the state veterinarians say that there is good compliance in their states on change of ownership requirements. In fact this regulation may be the best educational tool they have. People begin to do the policing themselves by not accepting a sale without a current Coggins test.
- **Low prevalence**—the testing that occurs shows that the tested horses have a low prevalence
- **UM&R**—the UM&R is pretty good
- **Potential for changes**—there is the possibility of making some changes that would make sense and move the program forward

Areas of concern

The group identified areas of concern about the EIA program and efforts:

- **Reduction in prevalence**—because the infection has gone down, some thought the program needs to do some things differently to take it to the next level
- **Industry/Owner support and incentives**—there is little incentive for pleasure horse owners to participate. The industry is generally not enthusiastic about the program. Some thought the national program is the result of top-down pressure rather than a groundswell of support. There is no public health threat, so zoonotic diseases get more attention. Because of the low tested prevalence, it is hard for people to get excited about controlling or eradicating the disease. The perceived risk to owners and the industry at large is low.
- **Education is needed**—there is concern that people don't know enough about the disease. According to two NAHMS studies some progress has been made between 1998 and 2005 in making people more aware of the disease but little change in those that were knowledgeable. See the table below. Participants thought more progress could be made if people were more knowledgeable.

Level of Familiarity	% ops in 1998 (Std error)	% ops in 2005 (Std error)
Had not heard of it	16.7 (1.9)	9.8 (0.6)
Recognized name but not much else	14.5 (1.6)	18.7 (0.8)
Knew some basics	23.9 (2.1)	25.9 (0.9)
Knowledgeable	44.9 (2.7)	45.6 (1.0)

- **Direction**—it is not clear what direction the national program is taking. The possibilities include 1) the status quo, enhanced control and eradication. While many supported working for eradication, participants recognized that even the word eradication would cause concern among certain horse owners and the industry in general.
- **Resources**—there is little slaughter capacity for horses in the United States. As a result there are fewer options for dealing with animals that test positive.
- **Written guidance and regulations**—the biggest concern is the lack of standards for the EIA efforts nationally. Also, while generally good, some thought the UM&R needs some work. There is no regulation for EIA in the Code of Federal Regulations, though regulations are expected to be completed and published within a year.

- **Testing**—some are concerned that the horses being tested are just the same ones over and over. The amount of money spent on EIA testing is out of kilter compared to the risk of the disease (the horse industry is spending too much). There is concern about the lack of standards about the intervals between testing. There was concern that even though the change of ownership requirements for a current Coggins test is effective the requirement is not standardized nationally. Some thought the focus on time intervals was wrong and the testing should focus on testing based on movements and risk.
- **Laboratories and Reporting**—there was concern that the current moratorium on new EIA testing laboratories is resulting in not having labs available where and when they are needed. A participant from NVSL pointed out that there are guidelines for getting new laboratories approved and that the moratorium is not a total ban. There was also concern about how results get reported to states and nationally to APHIS, VS.

Crossroads

There are a number of key areas of the program that are at crossroads

- **Overall direction**—the control of EIA could go in one of three general directions 1) Continue with the status quo 2) enhance/streamline the control program 3) Work for eradicating EIA .
- **Frequency of testing**—the variation that exists nationally needs to be resolved in a rational, science-based way
- **Basis of testing**—most of the basic testing guidance is based on time rather than movement and risk.
- **Possible regionalization**—with prevalence differences nationally, there is an opportunity to regionalize, that is regulate differently based on the prevalence and the risk in various states or groups of states

Next Steps

The group decided there are two major categories of next steps for managing EIA. The first involves a national dialogue about refining and standardizing testing requirements nationally that would begin soon after this meeting ends. The second involves a number of improvements the group would assign to the EIA subcommittee for further consideration and action:

1. National dialogue about refining and standardizing testing requirements nationally—the group suggested that the program standardize the testing requirements and base the minimum testing requirement on the general (testing or estimated) prevalence of the disease in the state.

- Require change-of-ownership testing nationally
- Set the minimum testing for states with lower prevalence at 2 years
- Set the minimum testing for states with higher prevalence at 1 year
- Movement among lower prevalence states or from a lower prevalence state to a higher prevalence state would be allowed with a current test (within 2 years)
- Movement among higher prevalence states or from a higher prevalence area to a lower prevalence state would be allowed with a current test (within 1 year)

The group suggested that the chair of the EIA Subcommittee discuss these proposals with and among the members of the National Assembly and the APHIS Senior Staff Veterinarian for Equine programs discuss the proposals with the APHIS VS AVICs. After those meetings, the National Assembly members and/or AVICs would discuss the proposals with pertinent stakeholders in their state:

- State veterinarian
- State horse councils
- Farm bureau as appropriate
- State veterinary medical associations
- State equine practitioners
- State breed associations

At the same time, the group suggested that members of the EIA Subcommittee discuss these proposals with the following:

- American Horse Council at their June meeting in Washington DC.
- State Horse Councils—there will be an opportunity to address the state horse councils the first day of the AHC meeting in June 2007

- American Association of Equine Practitioners (AAEP) at their national meeting on December 1 through 3 in Orlando FL as part of the AAEP Infectious Diseases Committee meeting
- National breed associations

2. Suggestions for further EIA Subcommittee consideration and action—there are a number of other issues the group suggested the EIA subcommittee pursue. Some support the change in the testing requirements described above and some are meant as national program improvements. The EIA Subcommittee would help ensure follow up:

Ensure accuracy of prevalence model—the group would like more discussion about the CEAH prevalence model especially with states that would be considered to be higher prevalence. Getting national agreement on a way to tell what states should be considered higher prevalence and what states should be considered lower prevalence is key. Also, there needs to be a way of determining when a state moves from one category to the other.

Additional testing requirements—the EIA subcommittee would discuss the feasibility of pursuing state specific legislation to require uniformly a current EIA test for gatherings in state. A current test would be one that has been done in the last year or two years depending if the state is a low or high prevalence state.

Laboratory moratorium—there is a perception that NVSL will not allow any more labs to do EIA work. NVSL however, has a set of criteria for approving new labs when they are needed. The group encourages NVSL to clearly communicate that it is a moratorium and not a ban and what the criteria are for getting a new lab approved. This would be especially helpful to those in states where prevalence is higher.

Reporting and compiling of test results—as part of the approval process labs need to be required to report the results in a way that enhances accurate compilations. One suggestion is to have the labs report test results to both the state where the horse resides and the state where the lab is located to help ensure accurate compilations and avoid double counting.

Revision of the VS 10-11—poll the lab personnel, field veterinarians and others who use the form to make sure the new form meets their needs before making the official revision. Also, talk with those states who do not use the APHIS form to learn why and to make the form more useful.

Use of VS 10-11 at the borders—make use of VS 10-11 for imported horses from Mexico and Canada. In addition, explore the use of the VS 10-11 for BLM horses. In both cases, the use of the electronic form would be ideal.

EIA Summit—consider holding a summit to discuss the direction, iron out problems and improve the national effort to control EIA. This should include the range of key stakeholders including state, federal and industry personnel and practitioners including accredited veterinarians

EIA free zones—explore the idea of having EIA free zones in higher prevalence states so horses could move with fewer restrictions. While the group was not opposed to the idea there was concern that the regulatory work involved in making sure the zone stayed free might out-weigh the benefits of having the zone in the first place

Guidance/regulation—follow through on plans to update the UM&R for the program and incorporate the UM&R into the CFR

Pursue Federal funding—the group encourages APHIS to pursue funding for a national EIA program and to use a portion to provide cooperative agreement money for states.

Appendix 1—List of Attendees

Name	Organization	E-mail
Amelita Facchiano	USDA APHIS CEAH	Amelita.Facchiano@aphis.usda.gov
Henry Moreau	State Veterinarian – Louisiana	hmoreau@ldaf.state.la.us
Taylor Woods	Missouri Department of Agriculture	taylor.woods@mda.mo.gov
Dee Ellis	Texas Animal Health Commission	deee@tahc.state.tx.us
Carl Heckendorf	Colorado Department of Agriculture	carl.heckendorf@ag.state.co.us
Kerry Rood	Vermont Agency of Agriculture	Kerry.rood@state.vt.us

Warren Hess	Utah Department of Ag and Food	wjhess@utah.gov
Valerie French	University of Liverpool/ USDA- NCAHCM	Valerie.french@md.aphis.usda.gov vkfrench@yahoo.com
Kent Fowler	California Department of Food and Ag	KFowler@cdfa.ca.gov
Don Hoenig	Maine Department of Agriculture	donald.e.hoenig@maine.gov
Bev Schmidt	USDA, APHIS, NVSL	Beverly.J.Schmitt@usda.gov
Leonard Eldridge	Washington Department of Agriculture	leldridge@agri.wa.gov
Ernie Zirkle	Retired State Veterinarian and consultant	ezirkle@msn.com
Steven Halstead	Michigan Department of Agriculture	halsteads@michigan.gov
Peter Timoney	University of Kentucky	ptimoney@uky.edu
Chuck Issel	University of Kentucky	cissel@uky.edu
Tim Cordes	USDA, APHIS, VS	Timothy.R.Cordes@aphis.usda.gov
Josie Traub-Dargatz	Colorado State University/USDA APHIS CEAH	jtraub1@lamar.colostate.edu
Aaron Scott	USDA APHIS CEAH	Aaron.E.Scott@aphis.usda.gov
Stan Bruntz	USDA APHIS CEAH	Stanley.D.Bruntz@aphis.usda.gov

Report of the Subcommittee on Equine Piroplasmiasis

Kent Fowler, Chair
California Department of Food and Agriculture

Subcommittee members include:

Kent Fowler, Peter Timoney, Lee Coffman, Tim Cordes, Leonard Eldridge, Steve Hennager, Bob Hillman, Ralph Knowles, Amy Mann, Richard Mitchell, Don Knowles, Mike Short, Robert Stout, Tim Boone, Kerry Thompson,

The Equine Piroplasmiasis (EP) Subcommittee was formed in 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.

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

- 1) The current status of EP in the United States is in question. For many years, EP has been classified as a Foreign Animal Disease to the United States. Prior to February 1, 2004, the "official test" for Piroplasmiasis, conducted on all equines presented for temporary or permanent importation into the United States was the complement fixation (CF) test, a test that is known to occasionally provide "false negative" results. Unscrupulous owners, importers or agents compounded the problem by purposely treating EP infected horses with immunosuppressive medications which would cause them to give a false negative reaction in the CF test. To improve diagnostic efficacy the CF test was replaced by an upgraded c-ELISA test that was specified as the "official test" on August 22, 2005. The 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 level in horses that have been imported into the United States over a significant number of years and perhaps also in horses native to the United States.
- 2) Potential tick vectors of both causal agents exist, but the dynamics for transmission remain unclear. EP infected horses may exist in the United States 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 a validated viable option. There is no conclusive evidence currently available 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.

4) Validated research risk assessment is required. It is crucial to: a) maintain stringent import restrictions that prevent the importation of seropositive and carrier horses into the United States, b) develop a cohesive and acceptable policy at both federal and state levels for identifying and dealing with resident EP seropositive horses, and c) request funding for research to devise effective treatment protocols for EP.

The Equine Piroplasmosis Subcommittee introduced five proposed resolutions at the 2006 USAHA Annual Meeting. The following two resolutions were accepted by USAHA:

1) USAHA Resolution 9 urges USDA to expand the funding for research to find an effective and safe treatment for elimination of the carrier state for *Babesia caballi* and *Babesia equi*.

2) USAHA Resolution 10 calls for a national serosurvey of slaughter horses to determine the prevalence of equine piroplasmosis (EP) in the U.S.

This past year two Subcommittee meetings took place via telephone conference call. The following represent the outcome of discussions to result from those meetings:

1) The EP Subcommittee believed the slaughter horse survey to be the best methodology available to address the much-debated issue of establishing prevalence of EP in the U.S.

2) An estimated sample size of approximately 14,000 horses was suggested based on an anticipated low prevalence of EP.

3) Horse slaughter plant management in Illinois and Texas agreed to assist with the serosurvey and approved the collection protocol.

4) It was agreed that Dr. Don Knowles direct the laboratory component of this project through collaboration between his laboratory and NVSL as follows:

a) NVSL Ames would receive the serum samples and provide storage accommodation.

b) ARS Pullman would procure bulk-rate VMRD kits

c) NVSL Ames would provide labor and utilize available wells in the course of their routine testing program.

d) ARS Pullman would do confirmatory testing if needed.

5) Dr. Don Knowles estimated the cELISA cost for one well per horse to be \$1.65. Based on a total of 14,000 horses the total cost would be \$23,000 for testing for each parasite and then doubled to test for both *B. equi* and *B. caballi*.

6) Dr. Don Knowles offered to provide an ARS match for APHIS' funding of this project. Each agency would provide \$23,000.

7) 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.

Unfortunately, U.S. District Court rulings in Texas and Illinois shut down U.S. horse slaughter plants before a representative number of samples could be obtained to conduct the survey.

With the slaughter horse survey no longer a viable option, the Subcommittee consensus was to identify other meaningful sources of equids to survey. The first recommendation was to request CEAH that residues of sera collected during the 1998 NAHMS survey be tested by c-ELISA for the presence of antibodies to EP. These sera would contain no identification whatsoever. A letter outlining this request was sent to Dr. Nora Wineland at CEAH on July 19, 2007 and no response had been received as of October 17, 2007.

The second recommendation was to request regional NAHLN laboratories to make available and submit residual banked EIA serum samples to NVSL for testing by c-ELISA for the presence of antibodies to EP. Again, it was determined crucial that these samples contained no individual identification whatsoever, including the State or region from where they were obtained. A letter was sent to 33 NAHLN Directors on October 5, 2007, and as of October 17, 2007 seven supportive responses were received offering EIA residual serum samples to be sent to NVSL.

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 the Committee/USAHA initiate a request to CEAH that residues of sera collected during the 1998 NAHMS survey be tested by c-ELISA for the presence of antibodies to EP. The sera would carry no identification whatsoever as to animal name/numerical ID, premises of origin, or state from which they originated.
- 2) Resolve that the Committee/USAHA initiate a request to regional NAHLN laboratories to make available and submit residual banked EIA or other equine serum samples to NVSL for testing by C-ELISA for the presence of antibodies to EP. The absolute requirement is that all samples submitted for evaluation carry no identification whatsoever as to animal name/numerical ID, date of collection, premises of origin or the laboratory or state from which they originated.
- 3) Resolve that the Committee/USAHA requests USDA to determine what constitutes a representative number of samples to evaluate from the above NAHLN submissions to provide meaningful estimates of the current prevalence of EP in the U.S. resident horse population or accept the previously statistically recommended number of 15,000 samples and to use previously identified funding which was to have been obtained through the slaughter surveillance initiative.

Pending ratification of these proposals by the Committee and USAHA, challenges confronting the Subcommittee include 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 United States. The goal of the Committee should be to do what it takes to ensure that EP does not become endemic in the resident horse population of the United States.

RECENT ADVANCES IN OUR UNDERSTANDING OF EQUINE HERPESVIRUS-1 (EHV-1) MYELOENCEPHALOPATHY

George P. Allen, Peter J. Timoney
Gluck Equine Research Center,
University of Kentucky

Introduction

When an equine viral pathogen evolves and, in so doing, increases its virulence, and if there are no effective prevention or treatment options for use against the new virus strain, the potential for negative impact on equine welfare and for economic losses to the U.S. horse industry are great. The increasing incidence of EHV-1 neurological disease currently poses such a threat and is of national concern. Because vaccination and antiviral drugs offer little assistance in blunting the morbidity and mortality rates of EHV-1 myeloencephalopathy, the emerging disease presents a new and significant equine health problem to U.S. animal health officials. This report summarizes the recent, and largely unpublished, progress that has been made in our understanding of this emerging herpesviral disease of the horse.

Evidence for designation of equine herpesvirus-1 myeloencephalopathy as an emerging disease of the horse

The recent increase in reported occurrences of epizootics of EHV-1 myeloencephalopathy in assemblages of horses in the U.S. and the discovery that the majority of such recent neurologic disease outbreaks were caused by a mutant, hypervirulent strain of the herpesvirus have fueled concerns that we are witnessing the beginnings of an emerging viral disease of the horse (United States Department of Agriculture [U.S.D.A.], 2007). During the first 7 years of the current millennium (2000 – 2006), the disease has struck, in heretofore unprecedented numbers and with alarming clinical severity, groups of horses around the country congregated at venues for racing, eventing, boarding, pleasure riding, veterinary care, and university equitation training. Contributing to the uneasiness about this apparent upsurge of outbreaks of EHV-1 neurologic disease in the U.S. is the recent finding that the etiologic herpesvirus mutant has established a significant and well entrenched presence in the current biological reservoir of latently infected horses. Of 132 Thoroughbred broodmares in central Kentucky screened during necropsy examination for the presence of latent EHV-1, 8% of the animals harbored a neuropathogenic strain of the virus as a latent infection (Allen *et al.*, 2007). Eighteen percent of the total reservoir of mares latently infected with EHV-1 carried a mutant, neuropathogenic strain of the virus. Such a high prevalence of latency by the neuropathogenic mutant of EHV-1 rules out any hope for eradication of neurobiovar-carrier horse subpopulations from horse herds and provides a large and permanent pool of the mutant virus for initiating future outbreaks and perpetuating the neurologic disease. Analysis of virus isolates from recent U.S. epizootics of EHV-1 neurologic disease has demonstrated that the majority of the outbreaks were caused by a genetic substrain of EHV-1 unique to the North American continent (Nugent *et al.* 2006).

Subsequent molecular characterization of 450 archived isolates of EHV-1 recovered from Kentucky Thoroughbred fetuses that were aborted during the 50-year time span from 1956 through 2006 revealed a statistically significant, time-related increase in the proportion of such herpesviral isolates that possess the genetic marker (ORF30 A₂₂₅₄ → G mutation) which identifies neuropathogenic strains of EHV-1 (Smith, 2007). Phylogenetic analysis of a large, worldwide collection of EHV-1 isolates indicates that the neurologically associated mutation has arisen and been selected on numerous occasions and in multiple evolutionary lineages of the virus, suggesting that the mutant phenotype possesses a survival advantage over parental, wild-type strains of EHV-1 (Nugent *et al.*, 2006).

The recent, dramatic resurgence of large-scale epizootics of EHV-1 neurologic disease with a widespread geographic distribution and the evolving increase in virulence of the causative

herpesviral agent have led to the designation by U.S.D.A. of the hypervirulent, mutant strain of EHV-1 as a potentially emerging herpesvirus pathogen of the horse (U.S.D.A. 2007).

Enhanced neuropathogenicity of ORF30 A₂₂₅₄ → G mutant strains of EHV-1

A number of opinions have recently been expressed and published, in lay and online press articles and in agency-based guidelines for EHV-1 vaccination, stating that any strain of EHV-1 has the potential for causing neurological disease in horses and, further, that the only difference between myeloencephalopathic epizootics caused by mutant and wild-type EHV-1 strains is their frequency of occurrence. The results of comparison of both naturally occurring and experimentally generated outbreaks of EHV-1 neurologic disease by the two virus genotypes fail to support these stated views.

Recent experimental inoculations, with a wild-type, abortion-storm isolate of EHV-1, of 12 elderly horses possessing no detectable cellular immunity against the herpesvirus and demonstrated to be highly susceptible to neurologic disease following infection with a mutant strain of EHV-1 (67% neurologic attack rate and 75% neurologic mortality) failed to elicit clinically observable neurologic signs in any of the 12 horses (Allen, 2007a). Likewise, analysis of two recent, natural epizootics of EHV-1 myeloencephalopathy associated with infection by a wild-type strain revealed a neurologic attack rate of less than 2% with no instances of mortality or required euthanasia. Compared to the magnitude of the neurologic disease threat posed by neuropathogenic strains of EHV-1, that posed by wild-type strains is relatively innocuous. Clearly, epizootics of myeloencephalopathy caused by wild-type and mutant strains of EHV-1 exhibit significant differences in neurologic morbidity and mortality and in their potential for negative impact on the welfare of the horse and on the equine industry.

Pathophysiologic basis for increased neuropathogenicity of ORF30 mutant strains of EHV-1

The pathogenic signature of ORF30 mutant strains of EHV-1 is characterized by a greater potential for epidemics, an increased risk for neurologic morbidity, and a higher neurologic mortality. The mechanistic basis for such an increase in virulence of mutant EHV-1 strains, relative to that of wild-type strains, lies in the enhanced replicative capacity of the mutated variant of the virus (Nugent *et al.*, 2006). The point mutation – a single amino acid substitution within the DNA polymerase enzyme that replicates the viral genome – endows mutant strains of EHV-1 with exaggerated replicative powers. As a consequence, the hypervirulent mutants replicate more efficiently in the infected horse to achieve 10-fold higher levels of leukocyte-associated viremia, relative to those generated by wild-type EHV-1 infections (Allen and Breathnach, 2006; Allen, 2007a). The end pathologic result of such high levels of viremia is ischemic damage to the central nervous system (CNS) of the infected horse, ignited by a widespread and intense inflammation within virus-infected endothelial tissue of blood vessels that supply the CNS (Whitwell *et al.*, 1992).

As a result of their replication-facilitated increase in the level of nasal shedding of infectious virus, mutant EHV-1 strains have also acquired the ability to spread more efficiently from horse to horse and, as a consequence, to cause more extensive epizootics of infection.

Because vaccine-stimulated, immune effector mechanisms of the horse are overwhelmed by the massive viremic load that develops during infection with neuropathogenic strains of EHV-1, immunization of horses with current-generation, inactivated vaccines is unable to prevent the high level of post-infection viremia and thus offers little protection against development of the lesions of vasculitis that lead to ischemic neurologic disease (Henninger *et al.*, 2007).

Diagnostic testing of horses for infection by neuropathogenic strains of EHV-1

Rapid, laboratory-based diagnosis of horses infected with neuropathogenic strains of EHV-1 is essential for guiding the planning and implementation of strategies for controlling epizootics of the disease. The laboratory test that yields the most rapid and sensitive diagnostic results is the polymerase chain reaction (PCR). Equine samples of choice for testing are nasopharyngeal secretions and buffy coat leukocytes recovered from venous blood collected in anticoagulant. Since the post-exposure, temporal profiles of the presence of EHV-1 in nasal secretions and circulating leukocytes of the horse do not completely overlap, the collection and testing of both clinical specimens is necessary for achieving maximal diagnostic sensitivity.

Because of significant prognostic differences in the clinical outcome of horses infected with ORF30 A₂₂₅₄ wild-type and G₂₂₅₄ mutant strains of EHV-1, delineation between the two genetic biovars during diagnostic examination provides relevant and useful ancillary information to veterinary clinicians and regulatory officials. Facilitation of such EHV-1 genotype discrimination in the diagnostic laboratory has recently been achieved by a real-time PCR-based test procedure (Allen 2006b).

In the context of an ongoing epizootic of EHV-1 CNS disease, positive PCR test results on horses associated with the epizootic by physical proximity, a history of exposure, and/or clinical signs consistent with infection by EHV-1 can reliably be interpreted as evidence of active infection by the herpesvirus. The interpretation of positive PCR results on nasal secretions or blood leukocytes from horses not linked to an outbreak of EHV-1 infection and tested only for purposes of general herd screening, health certificate requirements, or pre-transport monitoring for virus shedding is more problematic and carries less certitude. A part of that interpretative uncertainty derives from lack of any information on the positive or negative predictive values of EHV-1 PCR tests. Adding to the confusion are recently expressed suppositions that PCR detection of EHV-1 DNA in blood leukocytes may result from the presence of non-viable virus particles, recent vaccinations, or latent virus DNA. However, strong evidence now exists that latent EHV-1 does not reside in circulating leukocytes of the horse at levels detectable by conventional, diagnostic methods of PCR (Allen 2006). In 24 horses demonstrated by nested PCR to harbor latent EHV-1 in their submandibular lymph node tissue, viral DNA was not detected in an equivalent mass of blood leukocytes from any of the same horses. Equally compelling is the recent report that of 590 blood samples collected from 40 mares on a farm on which EHV-1 was endemic, none of the blood samples tested positive for EHV-1 by PCR (Brown *et al* 2007). Therefore, horses whose blood leukocytes test positive for EHV-1 DNA by PCR are most likely to be actively infected by the virus.

Immunologic requirements for vaccine-induced protection from EHV-1 myeloencephalopathy

Studies have recently been carried out to clarify the immunological mechanisms that horses use for controlling the magnitude of EHV-1 cell-associated viremia and its vasculitis-mediated sequela of ischemic damage to the equine central nervous system. Such information has provided insight into the types of immunologic effector responses required to be elicited by vaccines for successful immunoprevention of EHV-1 paralytic disease and has identified reliable, *in vitro* immune correlates of vaccine protection against the neurologic disease. Results of the investigations revealed that horses possessing high levels of pre-existing, EHV-1 specific cytotoxic T lymphocyte precursors (CTLp), regardless of age, strain of virus, or SNA titer, were more likely to control the magnitude of post-infection, leukocyte-associated viremia and the subsequent development of EHV-1 neurologic disease (Allen, 2007a). Such results emphasize that a critical-mass reservoir of circulating memory CTL, in place at the moment and location of virus exposure and capable of being activated into functional CTL with specific cytolytic activity against EHV-1, is required for controlling EHV-1 neurological disease. These observations serve to identify CTLp as one of the critical immune requirements for protective immunity to EHV-1 induced myeloencephalopathy. It follows that, to achieve protective efficacy against EHV-1 myeloencephalopathy by vaccination, the vaccines must be able to drive the equine immune response toward the production of such cytolytically functional, effector CTL.

Prospects for eliciting protective immunity against EHV-1 myeloencephalopathy by vaccination

A particular concern of members of the equine establishment about the emergent nature of EHV-1 myeloencephalopathy is that the cornerstone for prevention of infectious diseases – prophylactic vaccination – offers little assistance for preventing epizootics of the paralytic herpesviral disease. Reports of EHV-1 neurological disease outbreaks in fully vaccinated horses are commonplace (Henninger *et al.*, 2007), and there is limited scientific evidence that any currently marketed vaccine for EHV-1 will provide significant protection against the neurological disease caused by neuropathogenic strains of the virus.

The highest priority of ongoing research activities on this emerging viral pathogen of the horse is development of an efficacious vaccine. Efforts to develop such an improved vaccine for controlling the neurologic manifestations of EHV-1 infection are underway and are focusing on (1) identification of the specific immune defenses of the horse that are most active against the mutant EHV-1 strain, and (2) construction of a safe and effective, live-virus vaccine able to stimulate such protective immune mechanisms. Recent, proof-of-concept studies on the efficacy of live-virus EHV-1 vaccines have demonstrated that experimental horses inoculated intranasally with a live, virulent, wild-type strain of EHV-1 develop an immunity that is fully protective against the development of central nervous system signs after subsequent challenge inoculation (3 months later) with a highly neuropathogenic, mutant strain of the herpesvirus (Allen, 2007c).

References

1. Allen GP. Quantifiable risk factors for post-exposure development of equine herpesvirus-1 neurological disease. *Am J Vet Res* 2007a, submitted.
2. Allen GP. Development of a real-time PCR assay for rapid diagnosis of neuropathogenic strains of equine herpesvirus-1. *J Vet Diagn Invest* 2007b; 19:69-72.
3. Allen GP. Antemortem detection of horses latently infected with neuropathogenic strains of EHV-1. *Am J Vet Res* 2006; 67:1401-1405
4. Allen GP. Equine experimental disease model for evaluation of the efficacy of vaccines in protection of horses against EHV-1 myeloencephalopathy. *Vaccine* 2007c, in preparation.
5. Allen GP, Bolin DC, Bryant U, et al. Prevalence of latent, neuropathogenic equine herpesvirus-1 in the Thoroughbred broodmare population of central Kentucky. *Equine Vet J* 2007, accepted.
6. Allen GP, Breathnach CC. Quantification by real-time PCR of the magnitude and duration of leukocyte-associated viraemia in horses infected with neuropathogenic versus non-neuropathogenic strains of equine herpesvirus-1. *Equine Vet J* 2006; 38:252-257.
7. Brown JA, Mapes S, Ball B, et al. Prevalence of equine herpesvirus-1 infection among Thoroughbreds residing on a farm on which the virus was endemic. *J Am Vet Med Assoc* 2007; 231:577-580.
8. Henninger RW, Reed SM, Saville, J, et al. Epidemic neurologic disease due to equine herpesvirus-1 at a university equestrian center. *J Vet Intern Med* 2007; 21:157-165.
9. Nugent J, Birch-Machin I, Smith KC, et al. Analysis of equine herpesvirus type 1 strain variation reveals a point mutation of the DNA polymerase strongly associated with neuropathogenic versus non-neuropathogenic disease outbreaks. *J Virol* 2006; 80:4047-4060.
10. Smith, K. Tracing the Origin of the Recent Rise in Neuropathogenic EHV-1. *Master of Science Thesis*, University of Kentucky, 2007.
11. United States Department of Agriculture. Equine herpes virus myeloencephalopathy: A potentially emerging disease. *USDA:APHIS:Veterinary Services:Centers for Epidemiology and Animal Health, Center for Emerging Issues Information Sheet*, 2007.
12. Whitwell KE, Blunden AS. Pathological findings in horses dying during an outbreak of the paralytic form of Equid herpesvirus type 1 (EHV-1) infection. *Equine vet J* 1992; 24:13-19.