REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

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The Committee met on October 21, 2013 at the Town and Country Hotel, San Diego, California, from 1:00 to 5:50 p.m. There were 33 members and 28 guests present. The meeting was chaired by Dr. Kent Fowler. The mission statement was reviewed and the Committee decided no changes were necessary at this time. The monthly National Equine Conference Call was discussed and reported by Dr. Fowler to have an average of 54 call-ins on each monthly call. There are three proposed resolutions to be discussed in the business session. Dr. Fowler also mentioned a retrospective evaluation of the Committee's resolutions from 2009-12 would be presented by himself and Dr. Andy Schwartz in today's session.

NAHMS Equine 2015 Needs Assessment

Josie Traub-Dargatz, USDA-APHIS-VS, Center for Epidemiology and Animal Health (CEAH) National Animal Health Monitoring System (NAHMS) has conducted two previous equine studies (1998 and 2005). A third study is planned for 2015. Objectives for the NAHMS 2015 Equine study will be determined through a needs assessment process. Input on priorities for the study will be obtained through discussions and presentations at veterinary conferences, through contact with leadership on several groups including the American Association of Equine Practitioners (AAEP), members of the American Horse Council Health and Regulatory committee, members of the USAHA Infectious Diseases of Horses committee, leadership of the State Horse Council coalition, National Assembly of State Animal Health Officials (NASAHO), participants on the National Equine Industry Monthly conference calls and Veterinary Services (VS) Equine Group members. Once input from these groups is summarized, a broader survey of the industry regarding priorities for the NAHMS Equine 2015 study will be collected this fall. A summary of the group needs assessment survey was presented to the Committee facility further discussion by this committee related to their recommendations for focus areas for the upcoming study.

Equine Herpes Virus-1 Workshop Summary

Katie Flynn, California Department of Food and Agriculture

The American Association of Equine Practitioners Foundation (AAEP) and the USAHA Committee on Infectious Diseases of Horses sponsored the Equine Herpesvirus-1 Workshop held on October 19, 2013. Dr. Flynn presented a summary of the EHV-1 Workshop and that summary is presented in its' entirety at the end of this report.

Interlaboratory Comparison of Equine Herpesvirus type 1 Polymerase Chain Reaction Techniques Utilized in North American Diagnostic Facilities

Tim Baszler, Director of the Washington Animal Disease Diagnostic Workshop Laboratory in the College of Veterinary Medicine, Washington State University

In 2013, the USDA-APHIS National Veterinary Services Laboratory (NVSL) and American Association of Veterinary Laboratory Diagnosticians (AAVLD) conducted a joint interlaboratory comparison (ring trial) of neuropathogenic equine herpesvirus type 1 (nEHV-1) polymerase chain reaction (PCR) techniques in an effort to standardize testing methodology for equine herpesvirus myeloencephalopathy (EHM) carried out at state/university/provincial diagnostic facilities in North America.

A total of 28 state diagnostic facilities from the USA and Canada evaluated a ring test "panel" of field EHV isolates. Reference materials for all test panels consisted of EHV-2, EHV-4, EHV-5, wild-type EHV-1, and three strains of nEHV-1. The 28 participating laboratories used 38 different procedures (some laboratories tested multiple procedures) based upon modifications of ten peer-reviewed published methods for EHV-1 PCR. Two genes were utilized as PCR targets, the EHV-1 glycoprotein B gene, and the EHV-1 ORF 30, viral DNA polymerase gene which also is the gene including the neuropathogenic marker that has been associated with large outbreaks of EHM.

Glycoprotein B gene-based PCR assays, which are fundamentally designed as screening assays that detect wt-EHV-1 and nEHV-1, were used by 15 participating laboratories and had excellent diagnostic sensitivity for both wt-EHV-1 (100%; 30/30 samples identified correctly), and nEHV-1 (98.8%; 89/90 samples identified correctly), as well as excellent diagnostic specificity (98.3%; 59/60 non-EHV-1 samples identified correctly). As predicted, none of the glycoprotein B gene-based assays differentiated wt-EHV-1 from nEHV-1 and as such serve as excellent diagnostic tools to identify EHV-1 infected horses from non-EHV-1 infected horses but do not identify nEHV-1 specifically.

ORF 30 (viral DNA polymerase) gene-based PCR assay had more variable results from testing of the ring trial samples. Three published ORF 30 A/G $_{2254}$ assays: 1) Allen et al, 2007, 2) Pusterla et al, 2009, and 3) Smith et al, 2012), which differentiate wt-EHV-1 from nEHV-1 by detecting the A_{2254} (wt-EHV-1) or G_{2254} (nEHV-1) polymorphism, were used by 21 participating laboratories. The three assays had diagnostic sensitivity (based upon correct identification of nEHV-1 samples) of 93.1% (67/72 samples, Allen 2007), 100% (36/36 samples, Pusterla 2009) and 94.4% (17/18 samples, Smith 2013). The diagnostic specificity (based upon correct identification of non-nEHV-1 samples) was 88.9% (64/72 samples, Allen 2007), 72.2% (26/36 samples, Pusterla 2009), and 100% (18/18 samples, Smith 2013). Nearly all (17/18) of the "false positive" results for ORF 30 A/G assays 1 & 2 resulted from the nEHV-1 specific ORF 30G assay identifying wt-EHV-1 as nEHV-1.

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Genesis of the Equine Disease Communication Center

Nathaniel A. White, Professor Emeritus of Equine Surgery at Virginia Tech's Marion duPont Scott Equine Medical Center

In late April 2011, horses attending an equine event in Ogden, Utah, were exposed to equine herpsevirus-1. Three months later, when USDA, Animal and Plant Health Inspection Service (APHIS) declared the outbreak contained, more than 2,000 horses had been exposed. Of those, 90 tested positive for its neurologic form, equine herpesvirus myeloencephalopathy (EHM). A total of 242 exposed premises in 19 states, stretched from Oklahoma to California.

When this type of disease outbreaks occur in the horse industry rumors and misinformation create panic and can cause further spread of disease. Significant losses including horse loss and loss due to restricted horses movement and use affect many segments of the economy. Because horse owners are not aware of the disease risks or needed biosecurity to prevent or contain disease outbreaks, there is a high risk of disease spread.

The EHM outbreak in Utah stimulated the American Association of Equine Practitioners (AAEP) and the American Horse Council (AHC) to move forward with creating a National Equine Health Plan (NEHP), which was conceived at a combined USDA and AHC workshop, held in June 2010. One of NEHP's goals is to establish the roles of the horse industry for infectious disease containment and prevention. Specifically how the horse industry needs to respond to a disease outbreak in concert with the state animal health officials (SAHO) and USDA. Using the Utah outbreak as an example an AAEP task force recommended creating an Equine Disease Communication Center (EDCC) organized and supported by the horse industry.

The goal of the EDCC is to provide a call center, which can provide real time information about disease outbreaks; an alert system for industry organizations about the current status of disease outbreaks; and a website with updates about disease outbreaks, biosecurity recommendations and information about equine diseases. Working with a group of state animal health officials, a plan for flow and exchange of information for the EDCC has been developed. Currently the horse industry is working to set up a call center and website. After it's functioning the decision tree for evaluation and distribution of information will be created to enable the EDCC to be the chief source of information flowing to and from SAHO and USDA during a disease outbreak. The goal is to make the EDCC "the source" of equine disease information for the horse industry.

Contagious Equine Metritis 2013 Incident Findings

Katie Flynn, California Department of Food and Agriculture

California CEM Incident Summary

In February 2013, a 17-year-old Lusitano mare in California was confirmed positive at the National Veterinary Services Laboratories (NVSL) for *Taylorella equigenitalis,* the bacterium that causes contagious equine metritis (CEM). She was detected positive on samples collected as part of a pre-breeding infertility examination. The positive mare was born in Brazil in 1996 and entered the United States as a foal.

In addition to the index mare, three other horses in California were subsequently found positive for *T. equigenitalis* at the NVSL; their isolates match the index mare's isolate. Using pulsed-field gel electrophoresis (PFGE), the NVSL determined that the isolate does not match any other isolates from previous CEM detections in the United States, and does not match any *T. equigenitalis* isolates ever found on post-entry CEM quarantine testing of horses entering the United States from CEM-affected countries. All four positive horses, two mares and two stallions, have now been treated, re-tested with negative results, treated again, and released from quarantine. Additionally, a filly foal born in June 2013 to one of the positive mares has been tested with negative results, treated, and released from quarantine along with her dam.

One positive stallion was a 20-year-old Lusitano imported from Brazil in 2003 that bred the index mare in 2012 both by artificial insemination (AI) and live cover. The other positive stallion was a domestic 25-year-old Lipizzaner that had semen collected at the same facility as the positive Lusitano stallion in 2012. The second positive mare was a domestic 13-year-old Andalusian-cross that was bred to the positive Lusitano by AI in 2012 and became pregnant. None of the positive horses have yet been identified as the source of the outbreak. The epidemiologic investigation is ongoing.

Along with the positive horses, a total of 18 exposed horses have been identified and quarantined, including the foal of the positive mare. Twelve exposed stallions, including one in Texas and eleven (including one gelding exposed as a stallion) in California, have been tested with negative results, treated, and released from quarantine. All exposed stallions had semen collected between 2008 and 2012 at one of two facilities in California where the imported positive stallion was also collected. Finally, there are five exposed mares in quarantine for testing and treatment in California, Illinois, and New Mexico. Four of the exposed mares received semen from the imported positive stallion by AI between 2007 and 2012. The fifth mare is required, by established CEM protocols, to be tested because she received semen by AI from an exposed stallion that is now a gelding and cannot be test bred.

The lessons learned by regulatory officials during the incident included:

- Variability in viability with CEM strains. The original sample from the index mare was not stored in appropriate CEM culture media however, was easily cultured. Additionally, the exposed mare samples were shipped with ice and still managed to culture positive.
- CEM organism can be detected in frozen semen. All thirty straws of frozen semen collected and processed in 2009 were heavily infected and positive on culture and PCR.

- Pregnant positive mare can have negative foal offspring.
- PCR tests on semen and swabs would be a beneficial tool in future investigations to shorten the time under quarantine.

The detection of CEM in California impacted the equine industry with increased testing requirements for exported horses and the loss of breeding revenue by stallion collection station and stallion owners. Exposed horses owners were significantly impacted by cost of quarantine specifically the costs associated with veterinary sampling and treatments and loss of breeding seasons. In California, veterinary costs for exposed horses ranged from $900 to $3,200 for exposed stallions and $500 to $800 for exposed mares.

Puerto Rico Incident Summary

In May 2013, a 2-year-old Thoroughbred filly in Puerto Rico was confirmed positive at the National Veterinary Services Laboratories (NVSL) for *Taylorella equigenitalis*, the bacterium that causes contagious equine metritis (CEM). The filly was born in central Florida in 2011 and moved to Puerto Rico in late April 2013. She was tested for *T. equigenitalis* relative to that move; direct swabs were cultured positive after an initial complement fixation test and a follow-up were both found positive.

A thorough epidemiologic investigation of the positive horse has now been completed. More than 30 potentially exposed horses were tested in Puerto Rico, Florida, Kentucky, and New York. No additional positive horses were detected and no relationship was found between the positive filly and any horses associated with previous U.S. cases of CEM. The positive filly was treated, retested with negative results, and released from quarantine in July.

Discussion on 2009-2012 Resolutions

Kent Fowler, California Department of Food and Agriculture

2009 - Resolution #5 (eight USAHA committees)

Background: United States (U.S.) livestock exporters are facing an escalation of animal health requirements by importing countries that make it difficult or impossible to export U.S. genetic material.

Resolution: The United States Animal Health Association (USAHA) urges the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) to initiate all trade negotiations on import and export protocols with reference to compliance with World Organization for Animal Health (OIE) guidelines and Sanitary and Phytosanitary (SPS) rules.

Response: USDA will continue to promulgate a science-based approach and compliance with OIE guidance during all trade negotiations. However, not all countries have the same level of confidence in current science and OIE guidance, and some take a less scientific and more rigid approach to the risk associated with a certain commodity.

Results: National Center for Import and Export (NCIE) negotiates with foreign governments to the best of their ability on import and export issues. Politics may play a significant role in some of these decisions.

2009 - Resolution #8

Background: The 2009 United States contagious equine metritis (CEM) incident involving 48 states and 991 exposed equids initiated “The First Conference of Experts on CEM” at the United States Animal Health Association (USAHA) meeting in San Diego on October 9, 2009. The conference purpose and intent was to review recent developments concerning the national incident of CEM, discuss CEM protocols, review ongoing *Taylorella equigenitalis* research, and to discuss possible further CEM research and regulatory actions at the state and federal level. Concerns were addressed on the lack of consistent CEM testing and treatment protocols at both a state and federal level.

Resolution: The United States Animal Health Association (USAHA) urges the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) to immediately implement the recommendations of the 2007 Contagious Equine Metritis Working Group in a VS Memorandum.

Response: The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Service (VS) has undertaken and completed the majority of the

recommendations of the 2007 Contagious Equine Metritis (CEM) Working Group. Some recommendations cannot be addressed through a VS memorandum. For example, recommended changes in the testing protocols for mares and stallions require amending the existing regulations. APHIS anticipates publication of an interim rule incorporating those changes later this year. We have completed training for laboratory personnel and State CEM coordinators. We will be using the Emergency Management Response System (EMRS) as a database for tracking imported horses subject to CEM testing. We have drafted a new VS memorandum regarding testing and treatment of mares and stallions; however, the memorandum includes the revised testing protocols and will not be finalized until after the interim rule is published.
The recommendations included some elements that VS cannot implement. For example, the Review Group recommended hiring a full-time employee to oversee the CEM program, which the APHIS budget and workload could not justify. Recommendations that were best incorporated in a VS memorandum will be included in the revised testing and treatment memorandum, such as communication of horse movement and test results, revised testing protocols, and standards for CEM facilities.
Results: A CEM Subcommittee has been formed to identify unresolved issues and report next year at USAHA to the Committee membership. There are a number of CEM issues yet unresolved from the recommendations set forth by the 2007 CEM Working Group.

2010 - Resolution #28
Background: The equine industry incurs costs during disease outbreaks due to enhanced testing, movement restrictions, treatment required for sick animals, cancellation of equine events, and equine mortality. To optimize equine health through the control of equine infectious diseases, a framework document is required to develop a comprehensive United States Equine Health Plan.
Resolution: The United States Animal Health Association (USAHA) requests that the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) dedicate the necessary resources for continued collaboration with industry to develop a framework document for an Equine Health Program, with an initial emphasis on prevention and control of infectious diseases.
Response: VS also appreciates the interest of the United States Animal Health Association (USAHA) in developing a national framework to address equine health issues and is supportive of this concept. Such a framework could be a first step toward addressing potential vulnerabilities in a safeguarding system for the equine industry. However, immediate and long-term budget uncertainties prevent VS from making any substantial commitment to, or additional funding requests for, equine health at this time. VS will continue to work closely with USAHA's Committee on Infectious Diseases of Horses and other external stakeholder groups as we move forward on this framework.
Results: The Committee should continue to endorse this resolution and support this year's draft resolution for formation of an Equine Disease Communication Center in collaboration with equine stakeholder groups, including AAEP and AHC.

2011 - Resolution #4
Background: Diagnostic capacity is a critical asset in case of major animal disease events, and any reduction in the National Animal Health Laboratory Network (NAHLN) budget places our animal industries, the security of our food supply, and consequently our citizens' health and the United States (U.S.) economy at enormous risk.
Resolution: The United States Animal Health Association (USAHA) and American Association of Veterinary Laboratory Diagnosticians (AAVLD) request that the Secretary of Agriculture support and that Congress authorize $30 million in annual funding for the National Animal Health Laboratory Network (NAHLN). We further request that in order to adequately sustain the network to ensure food safety and security, animal and public health, and the United States economy, Congress fund the NAHLN through a stable funding mechanism.
Response: APHIS and National Institute of Food and Agriculture (NIFA) - We appreciate your support of NAHLN and will keep your suggestion in mind as we develop the FY 2013 budget.
Results: AAVLD has been working hard to try to get it included in the farm bill, but that process is still in the works. At this point, most of the efforts on NAHLN funding is trying to maintain what

they have (in the neighborhood of $9M) in the NIFA line item for diagnostics, which is shared with the plant side. It is still actively being pursued and that is an issue USAHA continues to monitor and support.

2012 - Resolution #7

Background: The National Assembly of State Animal Health Officials (National Assembly) requested in early 2012 that the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), National Veterinary Services Laboratory (NVSL) perform a brief survey of United States (U.S.) veterinary diagnostic laboratories across the country to determine the type of test methods in use for detection of neuropathic strains of Equine Herpes Virus-1 (nEHV-1). This survey highlights the National Assembly assumption that laboratories across the country were using different test methods to diagnose nEHV-1 infection.

Resolution: The United States Animal Health Association (USAHA) and the American Association of Veterinary Laboratory Diagnosticians (AAVLD) request that the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), National Veterinary Services Laboratory (NVSL) proceed with the neuropathic strains of Equine Herpes Virus-1 (nEHV-1) ring trial and make every effort to standardize testing methodology for nEHV-1 polymerase chain reaction testing at diagnostic facilities in the United States.

Response: The National Veterinary Services Laboratories (NVSL) has implemented a collaborative effort with the American Association of Veterinary Laboratory Diagnosticians (AAVLD) to establish a working group whose goal is to design and implement an inter-laboratory comparison test (ring test) that will allow laboratories to test existing polymerase chain reaction (PCR) assays used for the detection and typing of EHV isolates and neuropathogenic EHV-1, and to establish their performance limits.

Results: Dr. Baszler's presentation highlights the results of the ring test trial. "Glycoprotein B gene-based PCR assays, which are fundamentally designed as screening assays that detect wt-EHV-1 and nEHV-1, were used by 15 participating laboratories and had excellent diagnostic sensitivity for both wt-EHV-1 (100%; 30/30 samples identified correctly) and nEHV-1 (98.8%; 89/90 samples identified correctly), as well as excellent diagnostic specificity (98.3%; 59/60 non-EHV-1 samples identified correctly). ORF 30 (viral DNA polymerase) gene-based PCR assay had more variable results from testing of the ring trial samples. Nearly all (17/18) of the “false positive” results for ORF 30 A/G assays 1 and 2 resulted from the nEHV-1 specific ORF 30G assay identifying wt-EHV-1 as nEHV-1."

2012 - Resolution #19

Background: In April 2012, a USDA Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), National Center for Import and Export (NCIE) policy change was instituted dictating that the USDA-APHIS-VS, National Veterinary Services Laboratory (NVSL) would no longer test horses residing in the U.S. for dourine or glanders, unless they were suspected of having the disease or were required to be tested by law (e.g., plasma donor horses). USDA-APHIS-VS-NVSL, the only U.S. laboratory that performs these tests, is now prohibited from doing so on healthy horses residing in the US. So, despite the USDA recommendation that U.S. horses be tested for these diseases prior to shipping out of the country, there is no longer a way to test them and the passive surveillance for these diseases is lost. This USDA-APHIS-VS-NCIE testing policy change was not communicated to diagnostic laboratories or equine exporters.

Resolution: The United States Animal Health Association urges the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) to re-evaluate the dourine and glanders testing policy change for United States domestic equids and allow this testing recommended by USDA-APHIS-VS, National Center for Import and Export upon request (NCIE), at the owner’s expense. This testing provides United States (U.S.) owners exporting horses the opportunity to pre-test domestic horses and possibly avoid a domestic horse returning home from being denied entry into the U.S. due to a false positive test. Reinstitution of the USDA-APHIS-VS, National Veterinary Services Laboratory testing of domestic

equids for these diseases is necessary and valuable for the passive surveillance of our national equine herd.
Results: VS will allow horses originating in the U.S. to be pretested for dourine and glanders before export. This policy will apply only to horses exported from the U.S. with the intention of future re-import.

2010 Resolution # 29
Background: Recently, there has been increased concern over the differences in the United States and Canadian import test requirements for equine piroplasmosis (EP). In testing of EP-positive horses in the United States, the cELISA has been more sensitive than the IFA in detecting sero-positive animals.
Resolution: The United States Animal Health Association strongly urges the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), National Center for Import and Export (NCIE) to meet with the Canadian Food Inspection Agency (CFIA) to discuss equine piroplasmosis (EP) import testing and the maintenance of EP freedom in North America. This meeting should be dedicated exclusively to the topic of EP and, if necessary, be facilitated by USDA traveling to Canada.
Interim Response: The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) recognizes that standardizing equine piroplasmosis testing methods between the United States and Canada could be beneficial to both countries. VS is requesting a technical meeting with the Canadian Food Inspection Service to discuss the sensitivity of assays used in each country for detecting seropositive horses and how import testing might be further harmonized.
Final Response: The U.S. Department of Agriculture (USDA), 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.
USDA-APHIS-VS recognizes that standardizing equine piroplasmosis (EP) testing methods between the United States and Canada could be beneficial to both countries. VS has scheduled a technical meeting with the Canadian Food Inspection Agency on September 15, 2011. Meeting participants will discuss the sensitivity of assays used in each country for detecting seropositive horses and how import testing might be further harmonized to achieve the maintenance of EP freedom in North America.
Results: The technical meeting between NCIE and the Canadian Food Inspection Agency did occur. Canada requires immunofluorescence assay (IFA) test for import.

2010 Resolution #30
Background: In November 2009, the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) established an Equine Piroplasmosis Working Group (EPWG) to study the occurrence of equine piroplasmosis (EP) in the United States and to make recommendations for its management.
Resolution: The United States Animal Health Association (USAHA) urges the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) to consider submitted public comments on the April 2010 Equine Piroplasmosis Working Group Long-Term Recommendations and promptly accept and implement those recommendations.
Interim Response: The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) appreciates the interest of the United States Animal Health Association (USAHA) in addressing equine piroplasmosis (EP) in the United States. VS has reviewed the long-term recommendations for managing EP submitted by the Equine Piroplasmosis Working Group (EPWG). VS is also identifying what resources are available to implement those recommendations in light of immediate and long-term budget uncertainties.
VS will continue to work closely with the EPWG, the membership of the USAHA Infectious Diseases of Horses Committee, and other external stakeholder groups as we determine how best to implement the EPWG's recommendations.

Final Response: The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) appreciates the interest of the United States Animal Health Association (USAHA) in addressing equine piroplasmosis (EP) in the United States.
VS has reviewed the long-term recommendations for managing EP submitted by the Equine Piroplasmosis Working Group (EPWG). VS is also identifying what resources are available to implement those recommendations in light of immediate and long-term budget uncertainties. VS will continue to work closely with the EPWG, the membership of the USAHA Committee on Infectious Diseases of Horses, and other external stakeholder groups as we determine how best to implement the EPWG's recommendations.
Results: Most of the recommendations have been implemented. A Program Standards document is being written to pull all the implemented practices and procedures together. Reorganization of VS currently underway should allow key staff more time to complete the standards document.

2010 Resolution #31
Background Information: The identification of EP-positive imported equids and the recent large-scale EP incident in a domestic population of horses have increased the need and interest for an effective treatment in the management of EP-positive equids identified in the United States.
Resolution: The United States Animal Health Association (USAHA) requests the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) to prioritize and fund the research for a safe and effective treatment for elimination of the carrier state for *Babesia caballi* and *Babesia equi* and for the development and validation of a post-treatment clearance assay for establishing and monitoring the status of equids following approved equine piroplasmosis treatment protocols.
Response: As you know, ARS has an active research program at our Pullman, Washington location to solve problems related to equine piroplasmosis. We agree that this work is critical to ensuring the protection of the U.S. horse population. Although immediate and long-term budget uncertainties prevent us from making any commitments regarding funding requests, we will consider your input as we formulate future budget initiatives for Congress.
Results: ARS produced and validated a Western Blot clearance test, which has been included in the policy for release of test negative treated horses. There is a dire need to validate and establish this test at NVSL for greater accessibility.

2010 Resolution #32
Background Information: In August 2005, the official test for equine piroplasmosis (EP) on equids entering the United States was changed from Complement Fixation (CF) to the competitive Enzyme-Linked lmmunosorbent Assay (cELISA). This change was a result of disclosure that the rate of false negative CF test results was unacceptably high.
Resolution: The United States Animal Health Association requests the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), National Center for Import and Export (NCIE) to provide, upon request, individual states with owner and animal information for all equids imported into the United States since 1995. USDA-APHIS-VS-NCIE should provide owner and imported horse information to the respective chief animal health official of the state of destination of the imported horse at the time of release of the equid from the United States equine import facilities.
Interim Response: The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), National Center for Import and Export (NCIE) agrees with the United States Animal Health Association's request, NCIE will provide the information to States that request it.
Final Response: The U.S. Department of Agriculture (USDA), 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.
USDA-APHIS-VS, National Center for Import and Export (NCIE) agrees with the United States Animal Health Association's request. To date, VS has received one request from one State for information about horses previously imported. The requested information has been provided to that State. VS has agreed to provide information upon request from the appropriate State officials.

Results: In the one state requesting the information, owners were contacted and offered a test at no cost if they still had the imported horse(s). No positive horses were disclosed through this effort, but the process raised awareness of the potential disease threat and provided an opportunity to educate equine owners.

2011 Resolution #21

Background Information: Over the past two years, approximately 170 Equine Piroplasmosis (EP) affected horses in the United States were enrolled in an approved treatment plan for Theileria equi (T.equi) designed by the United States Department of Agriculture *(USDA), Agriculture Research Service (ARS). Preliminary reports on the treatment outcome are very encouraging.

Resolution: The United States Animal Health Association (USAHA) requests that the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) develop and publish guidelines for Equine Piroplasmosis (EP) Theileria equi test negative horses after completion of an approved EP treatment plan and that have met the following conditions to be considered for state quarantine release:

- Enrolled in the USDA-APHIS-VS/ USDA, Agriculture Research Service (ARS) treatment research program as per VS Memo 555.20; and
- Treated using the USDA-ARS published imidocarb treatment protocol under state or federal supervision; and
- Be identified with ISO-compliant microchip and that the identification number be held in a repository accessible by states; and
- Nested real-time reverse transcriptase polymerase chain reaction and complement fixation test negative on post-treatment testing; and
- Negative by transfusion to a splenectomized horse OR negative by the USDA-ARS Western Blot clearance test; and
- Competitive Enzyme-Linked lmmunosorbent Assay (cELJSA) negative at USDA-APHIS-VS National Veterinary Services Laboratory.

Additionally, annual cELISA tests should be conducted for the first three years after release as added assurance of disease freedom.

Interim Response: The U.S. Department of Agriculture (USDA), 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.

VS is reviewing the data on equine piroplasmosis (EP) affected horses that test negative after being treated according to the program that the Agricultural Research Service (ARS) designed for *Theileria equi*. We will report on this data review at the 2012 annual USAHA meeting and will develop a policy on the disposition of EP-affected horses that test negative after treatment.

Results: A Concurrence Memorandum was signed by Dr. Clifford, USDA in February 2013, with two additional nested polymerase chain reaction (PCR) tests added to the protocol. A letter from Dr. Clifford to State Veterinarians and area veterinarian in charge (AVICs) making the Concurrence Memo public is with the federal technical writers. The letter will likely state that either nested PCR or real-time PCR could be used for the series of three negative PCRs taken at least 30 days apart.

2011 Resolution #22

Background Information: An upgraded competitive enzyme linked immunosorbent assay (cELISA) test was specified as the "official test" on August 22, 2005, and is highly unlikely to yield "false negative" results on chronically equine piroplasmosis (EP) infected adult horses. While the cELISA has a significantly higher sensitivity in detecting the chronically infected EP horse, the sensitivity to detection of the acutely infected horse is much lower when compared to the complement fixation (CF) test.

Resolution: The United States Animal Health Association (USAHA) urges the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Services (APHIS), Veterinary Services (VS), National Center for Import and Export (NCIE), a negative Complement Fixation test and a negative competitive enzyme linked immunosorbent assay test for Equine Piroplasmosis (*Theileria equi* and *Babesia caballi*) prior to importation of equids into the United States.

Interim Response: The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) concurs with this recommendation. In the second quarter of calendar year 2012, the VS, National Center for Import and Export (NCIE) plans to formally incorporate complement-fixation testing into the standard equine import testing protocol, which already includes the competitive enzyme-linked immunosorbent assay. Before implementing this change, VS must notify brokers and importers about the new requirement and associated costs. Further, the National Veterinary Services Laboratories (NVSL) must prepare additional reagents to supply foreign laboratories that conduct pre-export screening tests.
Results: Final response is needed. The cELISA is required for import, but National Center for Import and Export (NCIE) has not officially added the complement fixation (CF) test requirement. The CF is being conducted at U.S. expense post entry, and multiple cases of equine piroplasmosis (EP) have been confirmed by this test that would have been missed by cELISA alone.

Committee Business:

Following conclusion of the scientific program, the Committee went into Business Session. Three resolutions were considered, approved and forwarded to the Committee on Nominations and Resolutions for approval by the general membership. One of these resolutions requests USDA-APHIS-VS dedicate the necessary resources for continuing collaboration with the horse industry and state animal health officials (SAHOs) to develop the Equine Disease Communication Center (EDCC). The second resolution urges the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Services (APHIS), Veterinary Services (VS) and Agricultural Research Services (ARS) and the National Veterinary Services Laboratory (NVSL) to research, develop and validate genetic strain typing capabilities for both Equine Piroplasmosis (EP) organisms (*Theileria equi* and *Babesia caballi*). The third resolution urges the USDA-APHIS-VS to prioritize what research is required to validate and secure World Animal Health Organization (OIE) approval of a polymerase chain reaction (PCR) assay for the detection of *T. equigenitalis*. Dr. Fowler announced the newly formed EHV-1 and CEM Subcommittees would be chaired by Dr. Katie Flynn and Mr. Rusty Ford, respectively. Dr. Fowler also thanked the Committee on Infectious Diseases of Horses membership for their excellent work over the past five years and welcomed Dr. Andy Schwartz as the incoming Chair. The meeting was adjourned at 5:50 p.m.

REPORT OF THE SUBCOMMITEE ON EQUINE PIROPLASMOSIS

Mike Short, Chair
Florida Department of Agriculture and Consumer Services

Equine piroplasmosis (EP) continues to be a disease of concern in the United States with continued efforts in surveillance and research. EP testing of horses continues to be driven primarily by industry but some regulatory testing is occurring as well. Industry testing continues to occur thorough multiple routes including, sanctioned race tracks and breed sponsored events and sales. The majority of regulatory testing is being done though disease investigations and international export with some interstate testing occurring.

According to the September, 2013 National EP Situation Report, there have been more than 231,664 U.S. horses tested for EP since November 2009, with approximately 41,000 tested in the past year. Since 2009 there have been 215 horses determined to be positive for EP, with 26 detected in the past 12 months, (excludes the horses detected as positive during the investigation of the 2009 Texas ranch outbreak). All but one of the positive EP horses have been in one of two high risk categories; horses imported prior to August 2005 using the CF test and those involved in racing, primarily Quarter Horse racing.

During the past year the EP Subcommittee held one meeting which took place via conference call. The primary discussion points and continued areas of interest and concern of the subcommittee are:

- Recent USDA support for treatment of EP reactors in the U.S.
  - In February 2013, APHIS-VS established a policy to support the quarantine release of treated, cleared, test-negative horses that have been enrolled in the APHIS-VS, Agricultural Research Service (ARS) EP treatment research program and have met specific post-treatment testing criteria to prove organism clearance. These criteria were established based on treatment results from 163 treated horses in the 2009 Texas ranch outbreak and recommendations from the 2011 USAHA Resolution #21.
- EP Uniform Standards Document
  - The USDA-VS is working on an EP Uniform Standards document to include the current guidance in VS Memo 555.20, the Long-term recommendations from the EP Working Group and the laboratory EP testing approval notice, in one comprehensive document.
- Need for development of a method to strain type the EP organism
  - The identification of EP-positive imported equids and the recent large-scale EP incident in a domestic population of horses have increased the need to identify the genotypic strains of organisms in positive EP equids detected in the United States. While natural, endemic transmission of Equine Piroplasmosis is occurring at a very low level in the United States, a small number of EP positive horses continue to be detected. Many of these positive EP horses have direct ties to foreign countries endemic for EP, where the horse was believed to be infected. Currently, however, there is no validated method to determine different strains of each organism *(Theileria equi* and *Babesia caballi)* complicating the epidemiological and trace back investigations.
- South Texas EP Surveillance Program
  - In March of 2013, the Texas Animal Health Commission (TAHC) designated Kleberg County equine as high risk for exposure to Equine Piroplasmosis. As a result, the TAHC began mandatory testing of all equine in Kleberg County in April. Surveillance testing is continuing, however, at the end of May, 283 premises with a total of 747 horses had been tested. Of the 747 horses tested 19 tested positive on six premises.

EP positive horses continue to be detected at low levels in the U.S. horse population. Since November of 2009 more than 231,664 U.S horses have been tested for EP with a total of 215 positive horses identified that are unrelated to the 2009 Texas ranch outbreak. Of the 215 positive horses, no more than 73 are still alive with 32 of those horses enrolled in the EP treatment research program. All but one of the positive horses has been in one of two high-risk categories: horses imported prior to August 2005 using the complement fixation (CF) test and those involved in racing, primarily Quarter Horse racing.

Since January 1 of this year, 26 positive horses have been detected in the U.S. Twenty five were racing Quarter Horses and one was a Thoroughbred racehorse. Most were involved in unsanctioned racing. At least two of the EP positive horses were also positive for Equine Infectious Anemia (EIA).

Until recently, equine owners and state animal health officials were faced with one of three options for managing positive EP reactors as provided in USDA Veterinary Services (VS) Memorandum 555.20. Memorandum 555.20 states that the regulatory options available for management of these positive horses are permanent quarantine (which may include chemotherapy), exportation, or euthanasia. However, based on recent treatment research and published efficacy data concerning the effective clearance of *T. equi* infected horses (*Efficacy of imidocarb dipropionate in eliminating Theileria equi from experimentally infected horses, Grause et. al. 2012*), the USDA, VS has recently (February 2013) established a policy in support of quarantine release of treated, cleared, and test-negative horses. These horses must be enrolled in the APHIS-VS, ARS EP treatment research program and meet specific post-treatment testing criteria to prove organism clearance. These criteria were established based on treatment results from 163 treated horses in the 2009 Texas ranch outbreak and recommendations from the 2011 USAHA Resolution #21.

Thomas Lansford, Texas Animal Health Commission

Equine piroplasmosis was first diagnosed in south Texas in October 2009, as part of the diagnostic work-up on a clinically ill horse. Testing of equine on adjacent premises ensued during the following year and disclosed no additional cases. In January 2012, a positive horse, unrelated to the original premises, was disclosed in Kenedy County. Subsequent epidemiological investigation led to the testing of all of the equine in the county, disclosing 17 horses on three separate premises as positive for *Theileria equi*.

Based on the high level presence of competent tick vectors and common equine movement practices of equine in both counties, the Texas Animal Health Commission designated Kleberg County as a high risk county for equine piroplasmosis in March 2013. A county-wide test of all equine in Kleberg County was conducted during the spring and summer of 2013. A total of 987 equine on 358 premises were tested for both *Theileria equi* and *Babesia caballi*. The county-wide testing disclosed 19 horses (1.9% prevalence) on 6 premises as positive for *T. equi*.

REPORT OF THE SUBCOMMITTEE ON EQUINE INFECTIOUS ANEMIA (EIA)

Andy Schwartz, Chair
Texas Animal Health Commission

Equine Infectious Anemia Proposed Rule

A decision memorandum on the issue of continuing progress and publication of a draft proposed rule on equine infectious anemia (EIA) was prepared by USDA, Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) staff and presented to the Deputy Administrator on June 7, 2012.

- Option 1 was to continue the rulemaking process and publish a proposed rule. This option would give all stakeholders the opportunity to comment on the proposed rule in a transparent manner. This option would satisfy the USAHA resolutions (2006) requesting codification of the Uniform Method and Rules. Additionally, this option would allow the USDA to move one step closer in addressing numerous issues with not having EIA regulations in the CFR.
- Option 2 was to discontinue the current EIA proposed activities. APHIS would continue to require that EIA reactors be officially documented on Veterinary Services Form 1-27 for interstate movement purposes, and would continue to approve individual laboratories. This option would satisfy stakeholders who prefer a State-based approach.

The Deputy Administrator made the decision to pursue Option 1, though as of this report the rule has not been published for comment.

Electronic EIA Test Documents

In 2004, a USAHA resolution was passed requesting USDA, Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) provide laboratory connectivity to all states wishing to utilize or develop the electronic equine infectious anemia (EIA) form with digital identification. The background information provided in the resolution indicated acceptance by practitioners in Florida, Wisconsin, Missouri, Iowa, and Texas, the first states to implement the GlobalVetLink electronic document on a test basis. Laboratory connectivity was established, facilitating the use of the GVL product and the electronic EIA form made available through USDA-APHIS-Veterinary Services Process Streamlining.

Over the past year there has been some interest among state animal health officials to develop and standardize an electronic EIA form for possible presentation to and acceptance by all states. The concept is to have a form that allows importation of digital images of horses in lieu of a description and hand drawn representation of unique markings. The concept does not include laboratory connectivity at this point. Some work has been done by Colorado, with input from Kansas and Texas.

Equine Passports

There was a brief presentation made at the Committee meeting in 2012 on Equine Passports. A number of states in the south have signed a Memorandum of Understanding (MOU) allowing the movement of equine between their states for a six month period if there is a current EIA test and veterinary inspection, and the owner keeps an itinerary detailing events attended. The MOU effectively takes the place of a veterinary inspection every 30 or 45 days. There are similar agreements in place between some western states. There seemed to be enough interest to warrant future consideration of a passport agreement between all states.

Equine Herpes Virus-1 Workshop Summary

Katie Flynn, Ellen Wilson, Kent Fowler
California Department of Food and Agriculture

The American Association of Equine Practitioners Foundation (AAEP) and USAHA Committee on Infectious Diseases of Horses sponsored the Equine Herpesvirus-1 Workshop held on October 19, 2013.

The recent outbreaks of Equine Herpes Virus-1 (EHV-1) in North America highlight regulatory infectious disease control issues and the importance of biosecurity. The outbreaks also identify regulatory disease control challenges for animal health officials across the country. The challenges include lack of national definition of a reportable EHV-1 case, lack of national case database, inconsistencies in regulatory mitigation (quarantines, monitoring and movement), lack of standardization of diagnostic tests, inconsistent and inaccurate dissemination of information and gaps in knowledge about disease agent. Increasing knowledge of the disease and biosecurity practices benefits all animal health officials

addressing the challenges of EHV-1. The morning workshop speakers provided state animal health officials an overview of the virus, the diagnostics, vaccination and mitigation measures. The presentation summaries are in the EHV-1 Workshop proceedings.

The afternoon panel discussion, facilitated by the Chair, Dr. Kent Fowler, provided state animal health officials and industry representatives an opportunity to discuss regulatory control of EHV-1 with experts. The panel discussion topics included EHV-1 as a reportable disease, strain variations of EHV-1, vaccination in the face of an EHV-1 outbreak, diagnostic testing, quarantine, communications and horse show biosecurity.

1. **Reportable Disease:** Why do we want to report EHV-1? Are we merely counting cases or are we doing something with the data? Panel members agree that there is a need for consistency across states with respect to what is or should be reportable. However, reportable does not necessarily mean actionable. States can evaluate each reported situation to determine what action, if any, is necessary to stop the spread of disease. The group consensus is that neurologic horses, regardless of EHV-1 strain type, should be reportable to state animal health officials.
2. **Strain Variation:** Science and field experience demonstrate the variation in transmission, clinical presentation and disease outcome for the neuropathogenic and non-neuropathogenic strains of EHV-1. Research and field EHV-1 incidents demonstrate a higher viral load and increased shedding with the mutated strain as compared to the wild type. The pre-workshop state veterinarian survey and published research data suggest a higher mortality rate with the neuropathogenic strain of EHV-1. Some variations in strain type, such as why some horses develop neurologic disease and others display milder respiratory signs, are unknown. From a clinical perspective, strain type does not impact treatment decisions, but does impact client education and the animal health official response, specifically the quarantine and recommended biosecurity parameters. The question of why some horses develop neurologic disease and others develop respiratory signs remains. Research is needed to identify specific risk factors for Equine Herpesvirus Myeloencephalopathy (EHM).
3. **Vaccination in the Face of an Outbreak:** Panel members shared varying opinions on vaccinating exposed horses in the face of an EHV-1 outbreak. The panel members recognized the need for research to demonstrate the effect of vaccination in an outbreak. Current vaccines are not labeled for prevention of EHM therefore; the use of vaccination in EHM incidents would not be supported. However, some panel members support vaccination, as a means of decreasing viral shedding and viremia, with the understanding that high antigenic mass vaccines may cause adverse reactions in the healthy horse. The panel agrees that vaccination is NOT a substitute for good biosecurity.
4. **Diagnostic Testing:** Knowing why you are conducting a test and what you are going to do with the results are essential for the appropriate use of any diagnostic tests. Sample collection and handling influence validity of the test result. Therefore, the panel recommends contacting the laboratory for guidance on sample handling, such as recommended type of swab to use (synthetic swab preferred) and the ideal storage (refrigeration) of sample. When evaluating test results, it is important to remember that sample results represent the animal status at the time of sample collection. During an investigation, the panel recommends testing both nasal swabs and whole blood from symptomatic exposed horses. Testing of asymptomatic exposed horses may be appropriate for determining quarantine release; for example, negative test results on an asymptomatic exposed horse sampled twice ten days apart with negative results may be the criteria for release from quarantine. Due to the lack of standardization and quality control of diagnostic PCR assays, state animal health officials are more comfortable taking action on results obtained from a NAHLN approved laboratory. Laboratories report qualitative (positive or negative) and/or quantitative results (viral load and/or CT values). According to the panel, the quantitative results would have no impact on treatment of clinical horses, but would impact guidance provided to the horse owner on exposure risks and necessary biosecurity measures.
5. **Quarantine:** Isolation is critical for the control of EHV-1 and should be a component specified with the quarantine. Effective isolation does not have to be expensive. Panel members emphasized the need for pre-planning to be able to address adequate isolation of

sick horses or movement to emergency veterinary clinics. The session highlighted the lack of science-supported quarantine biosecurity recommendations and release parameters. Panel members agreed that asymptomatic exposed horses on a quarantined premise could be released from quarantine without testing after 21 days. In general, panel members agree that quarantine release parameters should be cased-based. The panel suggests the collation and analysis of outbreak data to develop science-based guidance for quarantine protocols and quarantine release parameters.

6. **Communications:** With the advance of social media, communication is a challenge during any EHV-1 incident. Stakeholders recognize the need for accurate, clear, consistent messaging. For successful communications during an incident, panel members urge media training for individuals involved in EHV- 1 message communication. To encourage cooperation of all parties, the panel strongly recommends face-to-face meetings with horse owners, veterinarians and the state animal health official. Inclusion of the regulatory officials helps to give the perspective of "we are all in" for the welfare of the horse and the industry. Communication should target those impacted by the detection, such as the individual horse owners, whose concern is how the detection affects their horse.
7. **Horseshow Biosecurity:** Since 2011, there has been an increase in awareness of biosecurity at equine events; however, panel members agree that there is not a recognizable change in practices at these events. Recently, the United States Equestrian Federation recognized the need for biosecurity and is developing good practices for implementation at equine events. Some panel members believe that buy in and support of the private practitioner, specifically the horse show veterinarian, is necessary for successful implementation of horse show biosecurity measures. Veterinarians need to set the example with routine use of good biosecurity practices. In the United Kingdom, farms which comply with the Codes of Practice receive reduced insurance rates. This concept could be applied to the equine event biosecurity with the potential for reduction in insurance rates for events implementing biosecurity measures.

Moving Forward

The *USDA Equine Herpesvirus Myeloencephalopathy (EHM) Mitigation Experiences, Lessons Learned and Future Needs* document published in 2008 identified knowledge gaps, which were similar to those presented at this workshop. Those areas of future focus are:

1. **Regulatory official consensus on case definitions, outbreak definition, quarantine parameters, diagnostic testing and biosecurity practices for Equine Herpes Myeloencephalopathy (EHM) incidents.** As the recent state veterinarian survey results indicate, there are numerous inconsistencies and variations in EHV-1 regulatory mitigation. Some states indicate lack of understanding and guidance related to EHV-1 as part of the challenges of EHV-1 control. As the USAHA Committee on Infectious Diseases of Horses contains a cross section of regulatory officials interested in equine infectious diseases, it would be most appropriate to appoint a subcommittee to develop consensus documents related to EHV-1 regulatory mitigation.
2. **Comprehensive validation and standardization of real-time PCR assay for the detection and differentiation of EHV-1 strains from nasal swabs and blood.** The NVSL initiated the ring trial study with results to be presented at the 2013 USAHA Committee on Infectious Diseases of Horses Meeting. However, there is a need for continued discussion to ensure a validated, standardized PCR assay can be used to provide consistent equivocal results from laboratories across the states. State animal health officials should be provided guidance for interpretation of qualitative and quantitative results to enable the most appropriate and effective EHV-1 mitigation.
3. **Development of new vaccine for EHV-1 and evaluation of efficacy of current vaccines against EHM.** There is limited evidence that vaccination is effective against the neurologic form of EHV-1. Currently, there is no commercial EHV-1 vaccine labeled for the prevention of neurologic disease. Development of a vaccine that could prevent latency and the neurological form of EHV should be a top research priority.
4. **Encourage EHV-1 research to address the knowledge gaps specifically related to prevalence, risk factors, disease outcomes, latency and treatment.** Historically, EHV-1 has been considered a ubiquitous organism in the environment. However, research has not demonstrated the prevalence of

the various strains in the equine population. It would be interesting to know if two EHV-1 strains can coexist in the same horse. If they can, is there an impact of one on the other for reactivation and recrudescence? Is EHM truly an emerging condition? Can an increase in prevalence be documented? Unfortunately, the pathogenesis of EHM is still not well understood. As has been described, not all horses that develop viremia develop EHM, but viremia is required for EHM to develop. Latency is an aspect of all herpes virus infections, but there has not been research on the role of latency in the development of EHM. Several incidents involving the neuropathogenic strain of EHV-1 have animal health officials wondering why some horses develop EHM and others with significant viral loads don't develop EHM. It would help animal health officials to know what factors, favorably or unfavorably, influence the development of EHM. Additional, studies designed to investigate the role of antivirals in the prevention of EHM and overall decrease of viral load would be of value to regulatory officials. As there are a small number of EHM cases associated with individual outbreaks, it is essential that all pertinent data is gathered during these outbreaks for future analysis. Panel members encourage states to collect data on all EHM cases, as this would be beneficial for future analysis.