REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

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The Annual Meeting of the Committee took place from 12:30pm to 6:15pm Sunday October 15, 2006 at the Minneapolis Hilton Hotel, Minneapolis, Minnesota.. Some 38 committee members and 41 visitors were in attendance. The meeting was Chaired by Peter Timoney and assisted by Vice Chair James Watson. The greater share of the agenda was given over to consideration of several diseases or equine health related issues of current importance. This was followed by a panel of timely updates on a range of topics of less major significance. Provision was made for adequate discussion time of each of the presentations.

Dr. Helen Aceto, University of Pennsylvania School of Veterinary Medicine, New Bolton Center, presented a Time Specific Committee Paper entitled *Salmonella* Infection in the University Veterinary Medical Teaching Hospital, University of Pennsylvania.

An additional Time Specific Paper was presented by Dr. Peter Timoney, M.H. Gluck Equine Research Center, University of Kentucky. The subject was 2006 Multi-State Occurrence of Equine Viral Arteritis. The full text of both of these Time Specific Papers are included in these proceedings.

Dr. Josie Traub-Dargatz, Colorado State University and Centers for Epidemiology and Animal Health (CEAH), Veterinary Services (VS), Animal Plant Inspection Service (APHIS), United States Department of Agriculture (USDA) reported on the National Animal Health Monitoring System (NAHMS) Equine 2005 Study. The highlights of Part 1 of the study, “Baseline Reference of Equine Health and Management 2005”, dealing with population estimates, on-farm health management and vaccination practices, biosecurity and equine movements are included in these proceedings.

Dr. Steve Halstead, Michigan Department of Agriculture, and Chair of Subcommittee on Equine Infectious Anemia (EIA) gave the annual report of the activities of the Subcommittee. The report was approved by the Committee and is included in these proceedings.

Dr. Kent Fowler, California Department of Food and Agriculture (CDFA) Chair of the Subcommittee on Equine Piroplasmosis presented the Subcommittee report. The report was approved by the Committee and is included in these proceedings.

Two presentations followed on the availability of electronic programs from the private sector and USDA with respect to certification of animal movements, individual animal status for specific diseases Equine Infectious Anemia (EIA), and transmission of diagnostic laboratory reports to the appropriate parties in real time. Mr. Kevin Maher, Global Vet Link Inc. (GVL) reviewed the range of programs GVL currently offers to practicing veterinarians, animal disease diagnostic laboratories, State Animal Health Officials and the animal industry. Special reference was made to electronic Certificates of Veterinary Inspection (eCVI) and test certification for EIA (eEIA) and the growing appeal to veterinary practitioners of moving from paper to electronic certification. Amellita Facchiano, VS-APHIS-USDA, provided an update on "Veterinary Services Process Streamlining", that encompasses an expanding range of electronic programs which have been developed by the agency at the urging of the United States Animal Health Association (USAHA) several years ago to evolve towards a more paperless system of recording animal identification, eCVI, laboratory test results relative to diseases required for health certification (eLAB) and Veterinary Accreditation. Ms. Facchiano pointed to the ease of access and value of the programs currently available to practicing veterinarians, animal health officials and state veterinary diagnostic laboratories. Both presentations underscored the considerable progress that has been achieved over the past several years in developing the electronic programs needed to transition away from paper in communicating information on the identification and health status of horses.

Dr. Larry White, CEAH-VS-APHIS-USDA presented a paper on "Vesicular Stomatitis: 2006 Developments." First confirmed August 17, 2006 in a 10 year old horse, the disease has been restricted this year to one state, Wyoming, in contrast to much more extensive occurrences in immediate past years, 9 states in 2005, and 3 states in 2004. Infection with the New Jersey serotype of vesicular stomatitis virus was confirmed in the clinically affected horse based on virus isolation and serological grounds. A total of 9 premises have been involved so far and there is every indication that the outbreak is winding down. Sequencing and phylogenic analysis of the 2006 isolate demonstrated a close relationship with animal isolates made in Montana and Wyoming in 2005. Based on these findings it is concluded that the virus likely over-wintered in the region from 2005. Dr. White commented on an on-going study at the National Veterinary Services Laboratories into the persistence of antibodies in horses following natural infection with vesicular stomatitis virus. Using virus neutralization and cELISA tests, antibodies have been found to persist at detectable levels for at least 24 months. Attention was drawn to the implications of this finding on shipping horses internationally.

Dr. Josie Traub-Dargatz concluded the formal program by providing an update on the activities of a Task Force established by the American Association of Equine Practitioners (AAEP) to develop guidelines for the diagnosis and containment of infectious contagious disease outbreaks at racetracks and like equine events. The goal of the guidelines which are written for veterinarians, is to promote an effective first response. Respiratory disease, diarrheal disease, neurological disease and vesicular disease have been addressed in the guidelines. The guidelines will be available to AAEP members on the AAEP website and will be updated as needed. Dr. Traub-Dargatz also provided an update on the recent workshop conducted by the Dorothy Havemeyer Foundation on infection control in equine hospitals and at equine events. Among the topics addressed at the workshop were disease surveillance systems, antimicrobial drug use and bacterial resistance to several drugs, lessons learned from recent disease outbreaks at racetracks, programs to control salmonellosis at equine hospitals and clinics and investigational methods for on-farm occurrences of equine infectious diseases.

Following the scientific program, the Committee considered and affirmed three resolutions that were forwarded to the Committee on Nominations and Resolutions for approval by the general membership.

The Committee unanimously approved two recommendations. The first recommendation requests that USDA-APHIS-VS immediately pursue regulation changes to address the movement into and within the United States of stallions and semen with regard to their status for equine arteritis virus. The second recommendation recommends that the National Assembly of State Animal Health Officials adopt a range of measures with respect to dealing with equines found positive for antibodies to *B. equi* and or *B. caballi*

Salmonella Infection at a Veterinary Teaching Hospital: The University of Pennsylvania Experience

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Introduction

Subclinical and clinical infections with *Salmonella* are reasonably common in large animal patients [Dunowska *et al.,* 2004; Smith *et al.,* 2004; Morley *et al.,* 2004]. Major outbreaks of nosocomial *Salmonella* have been documented at large animal veterinary teaching hospitals over the past several decades [Castor *et al.,* 1989; Dargatz and Traub-Dargatz, 2004; Hartmann, 1996; Paré *et al.,* 1996; Schott *et al.,* 2001; Tillotson *et al.,* 1997] and have been responsible for a number of veterinary teaching hospital closures. Among equine patients, horses presenting with gastrointestinal disturbances such as diarrhea and colic may be at highest risk of shedding *Salmonella* [Ernst *et al.,* 2004; House *et al.,* 1999; Kim *et al.,* 2001; Morley *et al.,* 2004; Palmer *et al.,* 1985].

The George D. Widener Large Animal Hospital, located within the University of Pennsylvania's New Bolton Center in Kennett Square, Pennsylvania, is one of the busiest large animal hospitals in the United States with approximately 6000 patient visits annually and a predominantly (82%) equine caseload. As an integral part of the University of Pennsylvania's School of Veterinary Medicine, in addition to clinical service, the Widener Hospital is crucial to the other primary missions of the School, namely the teaching of veterinary students and delivery of advanced training for interns and residents and in the conduct of research. As a tertiary care referral center, the hospital admits a large number of critically ill and emergency cases and it is one of the few facilities in the Mid-Atlantic region that provides 24/7 coverage for serious large animal health emergencies. The largest proportion of emergency admissions (approximately 50% of around 1,200 emergency admissions per year) present with the complaint of colic or diarrhea as their primary problem. As a consequence, the Widener Hospital, like other large animal veterinary teaching hospitals, is at greater risk of having animals that are actively shedding *Salmonella* within its environs than are many other animal housing facilities. Moreover, as the Widener Hospital invariably houses a high number of compromised animals that are at greater risk for infection with *Salmonella,* it is at increased risk for both cross-infection among patients with subsequent dissemination to the large animal (primarily equine) population and zoonotic infection.

In March 2004 an increase in cases of clinical salmonellosis resulted in an investigation that identified of a protracted outbreak of salmonellosis. The outbreak was not responsive to vigorous control efforts and ultimately resulted in closure of the hospital for 85 days in May 2004. Vigorous decontamination and remediation efforts were made during the closure and after reopening a strict infection control and biosecurity program was implemented.

Descriptive Epidemiology

In March 2004, in response to increased cases of clinical salmonellosis, an intensive epidemiological investigation was initiated. Retrospective evaluation of medical records revealed that the proportion of inpatient admissions confirmed as culture positive for *Salmonella* had increased from 0% in 1998, when no positive animals were identified, to 3.8% of all patients admitted to the hospital between January 1st and May 10th of 2004 when the hospital was temporarily closed to all admissions. The increase in culture positive individuals in a subgroup of inpatients identified as high risk (i.e. those animals with the presenting complaint of colic, diarrhea or fever) was even more dramatic, going from 0% in 1998 to 19.8% of all high risk admissions that occurred between January 1st and May 10th of 2004. These increases could not be accounted for by changes in sampling frequency of patients or by changes in the make-up of the patient population over time; such as emergency and high risk admissions accounting for a larger proportion of the total case load.

Phenotypic and genotypic characterization of isolates from positive patients revealed the majority to be *Salmonella enterica* serotype Newport MDR-AmpC. Since first being isolated in 1998, *Salmonella* Newport MDR-Amp C has undergone epidemic spread in

animals and humans in the United States (Dunne *et al.,* 2000, Fey *et al.,* 2000, Winokur *et al.,* 2000, Rankin *et al.,* 2002, Zansky *et al.,* 2002, Gupta *et al.,* 2003, Zhao *et al.,* 2003). This strain is characterized by a plasmid mediated *amp*C gene (bla_{CMY-2}) that encodes resistance to extended-spectrum cephalosporins (Fey *et al.,* 2000, Winokur *et al.,* 2000, Carattoli *et al.,* 2002,). Newport-MDRAmpC strains are commonly resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline, amoxicillin-clavulanate, cefoxitin, ceftiofur and show reduced susceptibility to ceftriaxone (Zansky *et al.,* 2002). In Pennsylvania, the first sample positive for this organism was submitted to the Pennsylvania Animal Diagnostic Laboratory System (PADLS) in November 1999. In the interim, more than 1700 *Salmonella* Newport MDR-Amp C isolates have been identified by PADLS and had their identity confirmed by the Salmonella Reference Center (SRC) at NBC. The isolates were obtained from a variety of species but the vast majority of positive samples came from clinically ill cattle. During the initial work to determine how closely related isolates obtained from different patients at the Widener Hospital actually were, isolates from 29 animals were available for review. Pulsed-field gel electrophoresis (PFGE) was performed with two restriction enzymes (*Xba*I and *Bln*I). A dendrogram indicated that 25/29 isolates formed 6 small clusters of identical profiles with a high degree of genetic similarity (Dice ≥ 0.96). Compared to the predominant *S*. Newport MDR-AmpC strain in a database of 990 isolates obtained from Pennsylvania animals and submitted to PADLS in the preceding 18 months, the outbreak-related strains showed ≤ 88% similarity.

The animal retrospectively identified as the index case was a three year old thoroughbred racehorse admitted to the hospital as an emergency on July 1st 2003 with the primary complaint of colic. Between July 2003 and May 2004, *Salmonella* serogroup C2 isolates, all subsequently identified as MDR-AmpC Newports, were obtained from 60 patients during the course of what was ultimately recognized as a protracted outbreak. These 60 patients represent 40% of all *Salmonella* positive animals identified at the Widener Hospital between January 1st of 2000 and May 10th of 2004. The vast majority (52/60, 87%) of the 60 positive animals identified between July 2003 and May 2004 were horses, but five cattle (8%), two alpacas (3%) and a lamb were also affected.

Strain Characterization

All *Salmonella* isolates from the NBC clinical microbiology laboratory from July 2003 until May 2004 were referred to the SRC, for serotype confirmation, and molecular characterization. Antimicrobial susceptibility profiles of *S*. Newport were determined using the Sensititre® CMV2ECOF Companion/Equine MIC Veterinary Specific plate (Trek Diagnostics, Cleveland, Ohio) and the interpretation of breakpoints was as determined by the manufacturer according to NCCLS guidelines, when available.

Extraction of DNA, and pulsed-field gel electrophoresis (PFGE) analyses were performed as described by the Centers for Disease Control and Prevention PulseNet System (Anon 1998). Briefly, *Xba*I or *Bln*I (Invitrogen) was used to digest the genomic DNA, and PFGE was conducted with a CHEF-DRII apparatus (Bio-Rad Laboratories,

Richmond, Va.) with the following running conditions: an initial switch time of 2.2 s and a final switch time of 63.8 s at 6 V/cm and an angle of 120 degrees for 19 h. After electrophoresis the gel was stained with ethidium bromide (0.2 _g/ml). DNA fragments were visualized and photographed using an EDAS 290 digital camera system and BioNumerics software (Applied Maths, Kortrijk, Belgium) was used to compare the PFGE profiles. PFGE profile numbers were assigned to each isolate with at least a one band difference in the PFGE pattern.

Plasmids were transferred by conjugation from *S*. Newport strain SRC0307-213 to a nalidixic acid resistant *E. coli* recipient strain (ATCC 27662) using nalidixic acid (50 μg/ml) and ampicillin (50 μg/ml) as the selective agents and β-Lactamase genes were sequenced as described previously (Rankin *et al.,* 2005). Isoelectric focusing (IEF) for β-lactamases was performed on all strains at the Centers for Disease Control and Prevention using a small-scale freeze-thaw method (Miriagou *et al.,* 2003).

Detection of bla_{CMY} and bla_{TEM} genes was performed by PCR at NBC as described previously (Rankin *et al.,* 2002). Detection of bla_{SHV} was performed at the Centers for Disease Control and Prevention (Atlanta, GA) using a modification of the method described by Rasheed and colleagues (1997). Total genomic DNA was extracted from SRC0307-213 and *E. coli* transconjugants using a Wizard Genomic DNA Purification kit (Promega Corp., Madison, WI). β-Lactamase genes were amplified with Promega 2X PCR master mix using primers described by Rankin *et al.,* 2005. PCR amplicons were cleaned up using a QiaQuick® PCR purification kit (Qiagen, Valencia, CA). Clean amplicons were used to obtain 2X DNA sequence coverage in both directions using a CEQ2000 capillary sequencer (Beckman Coulter, Fullerton, CA), and sequence alignments were performed using SeqManII version 5.06 (DNAStar Inc., Madison, WI).

Newport strain SRC0307-213, isolated in July 2003 was identified as the index case. All 60 isolates were shown to be resistant to ampicillin, chloramphenicol, tetracycline, cephalothin, ceftiofur, amoxicillin-clavulanic acid, gentamicin, and trimethoprim-sulfamethoxazole. Isolates from all 60 *S*. Newport cases were susceptible to amikacin, imipenem and enrofloxacin.

Fifty of the sixty isolates were available for molecular characterization by PFGE. The restriction enzyme, *Xba*I was chosen for the primary analysis. A second enzyme, *Bln*I, was chosen to confirm the results obtained by *Xba*I. Twenty one *Xba*I profiles were obtained from the 50 isolates.

PFGE profile NP102 (18 strains) and NP109 (8 strains) predominated. There were three additional small clusters of 2, 4 and 2 strains that belonged to profiles NP103, NP105, and NP108 respectively. Sixteen strains were represented by 16 individual PFGE profiles. BioNumerics software was used to create a dendrogram and showed that NP102 and NP109 were highly related (Dice coefficient of similarity, 96%). All other PFGE profiles were also highly related and in many cases differed from NP102 by one or two bands. Overall, the Dice coefficient of similarity did not drop below 80%.

Salmonella Newport SRC0307-213 was phenotypically negative for ESBL production by double disk diffusion testing with ceftazidime and ceftazidime-clavulanate and also cefotaxime and cefotaxime-clavulanate (BD BBL™ Sensi-Disc™, Becton Dickinson, Franklin Lakes, NJ) but was positive for *bla*$_{CMY}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ genes by PCR. It has been noted previously that the presence of an ESBL can be masked by the expression of an AmpC-like enzyme such as *bla*$_{CMY}$ (Bradford *et al.,* 1997, Bush 2001, Livermore *et al.,* 2001). IEF showed enzymes with a pI of ≥8.4, 8.0 and 5.4 consistent with the CMY, SHV and TEM enzymes, respectively. Two *E. coli* transconjugants (SRC0307-213-1 and SRC0307-213-2) were tested by double disk diffusion as described above, and both exhibited an ESBL phenotype. Both transconjugant strains were *bla*$_{TEM}$ and *bla*$_{SHV}$ positive, and negative for the *bla*$_{CMY}$ gene by PCR. IEF of the transconjugants showed that only 2 β-lactamase genes had transferred, pIs 5.4 and 8.0, and this was consistent with the observation that the plasmid mediated *amp*C gene in Newport strains has previously been shown to be difficult to transfer by conjugation (Rankin 2002). DNA sequencing of *S*. Newport strain SRC0307-213 showed that it contained a *bla*$_{CMY-2}$ gene. *E. coli* transconjugant EC0307-213-2 was positive for *bla*$_{TEM-1b}$ and extended-spectrum β-lactamase gene *bla*$_{SHV-12}$.

PCR analysis showed that 35/50 strains were positive for *bla*$_{CMY}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ genes. In addition, 6/50 were *bla*$_{TEM}$ and *bla*$_{SHV}$ positive, 4/50 were *bla*$_{CMY}$ and *bla*$_{TEM}$ positive, 2/50 were *bla*$_{CMY}$, and *bla*$_{SHV}$ positive, one isolate was *bla*$_{CMY}$ positive and 2 isolates were negative for all three β-lactamase genes tested.

Characterization studies demonstrated that two PFGE profiles predominated, although all of the profiles observed were highly related and were determined to be the result of minor genetic events such as plasmid loss or acquisition.

ESBL-producing salmonellae are rare in the United States and this was the first report of an ESBL-producing *S.* Newport-MDRAmpC from animals (Rankin *et al.,* 2005). *bla*$_{TEM-1b}$ has frequently been found in the *Enterobacteriaceae,* including *Salmonella* (Liebana *et al.,* 2004) and the *bla*$_{SHV-12}$ gene, first described in *E. coli* and *Klebsiella pneumoniae* from Switzerland in 1997, is now becoming increasingly common in *Salmonella* serotypes from Europe and the United Kingdom but has not yet been described in *S.* Newport (Nuesch-Inderbinen *et al.,* 1997, Villa *et al.,* 2002, Munday *et al.,* 2004, Weill *et al.,* 2004, Hasman *et al.,* 2005). Isoelectric focusing of β-lactamases characterized so far from human *S*. Newport MDR-AmpC strains submitted to NARMS indicate that many express a putative TEM enzyme, but none show enzymes in the range expected for SHV β-lactamases. The identification of ESBL genes in *S.* Newport-MDRAmpC has considerable implications for veterinary and public health. Carriage of multiple β-lactamase genes is disconcerting because certain combinations of genes could effectively limit all β-lactam therapeutic options.

Response to the Outbreak

In March 2004 when an increase in clinical cases of salmonellosis was suspected, but in advance of the full recognition of the scope of the problem that was eventually

revealed by retrospective records analysis and isolate characterization, environmental surveillance in the hospital (which had been in place for many years prior to the outbreak) was increased. In addition, active surveillance of patients housed in high-risk areas was initiated. Prior to this, only those animals exhibiting clinical signs consistent with salmonellosis or those intimately associated with clinically ill animals (e.g. mares with foals) were generally subject to culture. As a result, some sub-clinical cases (i.e., animals with no clinical signs of salmonellosis that were nonetheless shedding *S*. Newport MDR-AmpC in their feces) were identified, although most of the 60 patients found to be positive between July 1st 2003 and May 10th 2004 did have clinical signs to one degree or another.

The Widener Hospital is a world-renowned equine clinic and one of the few tertiary care referral centers providing round-the clock coverage for large animal health emergencies in the Mid-Atlantic region where equine and other livestock industries are an important part of the local economy. The decision to close a facility of this kind even temporarily represents a serious loss to the equine and agricultural communities alike. Efforts were therefore made to clean and decontaminate high-risk areas while maintaining essential hospital services. Specific parts of the hospital were temporarily closed to patients; all disposables were discarded; the area was then cleaned, disinfected and restocked before reopening. For example, during this period, the intensive care units (ICU/NICU) were subject to two such rounds of cleaning and disinfection. Nevertheless, *S*. Newport culture positive animals continued to be identified. In April, the hospital was closed to elective in-patients; only emergency cases were admitted. Following expert consultation (Dr. Paul Morley, Colorado State University), improvements in collection procedures and sensitivity of detection for environmental samples revealed 37/140 sites throughout the hospital and animal housing areas positive for *Salmonella enterica* serotype Newport MDR-AmpC. Moreover, those areas that had been thoroughly cleaned and disinfected, culture negative and then reopened started returning to culture positive status. At this time all admissions were halted. In May it was apparent that the situation was not responsive to vigorous control efforts and it was deemed necessary to discharge all remaining patients and close the entire hospital until the adverse bacterial population could be brought under control.

Extensive decontamination and remediation began. An interim Director of Biosecurity was appointed to manage these efforts using the incident-command structure. All animal housing and clinical spaces and the routes connecting them were subject to rigorous, multistage cleaning and disinfection. Briefly, a three or four stage cleaning protocol was employed in all areas. Stage 1 utilized a plain anionic detergent applied with a small brush to ensure that all surfaces from the ceiling down to the floor were disrupted. After a contact time of 15 minutes the treated area was rinsed and left to dry. Next, a dilute bleach solution was applied, after a 20 minute contact time the area was again rinsed and allowed to dry. The bleach step was only used on non-porous surfaces. Stage 3 involved application of a solution of a quaternary ammonium disinfectant. This was left in place for 5 hours, or overnight, prior to rinsing. Once the surface was dry, the final disinfectant phase involved fogging of the area with a 4% solution of the peroxygen-based disinfectant Virkon-S® (Antec International, Sudbury,

UK; Dunowska *et al.,* 2005; Patterson *et al.,* 2005). Once dry, cleaned-areas were closed to all traffic except for the collection of environmental samples to assure their negative status. More than 220,000 square feet of animal housing and clinical spaces were cleaned in this manner.

Because of damage to painted-block wall surfaces in many stalls (from which two *Salmonella* positive environmental samples were obtained), many animal-housing areas were sandblasted and resurfaced. Cleaning and disinfection of sandblasted areas took place after blasting and re-pointing of walls but before application of the finish coats of urethane-based paints. All dirt stall-flooring bases were completely removed and replaced with concrete plus a polyurethane-based monolithic flooring system. Similarly, all rubber mats were removed from stalls and other animal areas and completely replaced with the monolithic flooring surface. In animal housing and clinical spaces, virtually all casework, sinks etc. were removed and cleaned separately prior to being replaced once the space itself was cleaned, disinfected and determined to be culture negative. Wood counter-tops present in some treatment areas and nursing stations were all replaced with stainless steel. Equipment and supplies in all areas were cleaned or discarded; an algorithm that that took into account the "cleanability", value and potential risk (i.e. the likelihood that a given piece of equipment had been used for positive patients and the intimacy of contact it would have with future patients) associated with each item was used to assist in making decisions about whether or not specific items should be discarded. In order to ensure the safety of all personnel involved in the cleaning process and the proper use of personal protective equipment, standard operating procedures were developed for all phases of the process in collaboration with the University of Pennsylvania's Office of Environmental Health and Radiation Safety. The multiphase liquid cleaning and disinfection procedure comprising detergent and disinfectant steps successfully eliminated the bacterial population at virtually all locations but failed to control *Salmonella* within the intensive care units. These were treated by professional contractors (Micro-Clean Inc., Bethlehem, PA; ChlorDiSys Solutions Inc., Lebanon, NJ) with a gas-phase space decontamination using chlorine dioxide [Luftman *et al.,* 2006].

To assure negative status, environmental samples were collected from designated areas of the hospital using commercially available electrostatic dust collection wipes (Swiffer®; Procter and Gamble, Cincinnati, OH). The charge on the wipes attracts debris and bacteria and they can be used to sample large surface areas. Samples were collected using the procedures described by Burgess and colleagues [2004].

A multistage process involving pre-enrichment and enrichment steps followed by differential plating was used for the detection of *Salmonella*. Briefly, the samples collected on Swiffer® disposable cloths were placed in a Whirl-Pak® bag to which 100 ml of buffered peptone water (BPW) was added. Samples in BPW were then incubated at 37°C for 24 hours. The pre-enriched samples were subsequently inoculated into Rappoport-Vassiliadis broth and incubated for a further 24 hours at 42°C, followed by subculture onto DCA (deoxycholate-citrate-agar), MacConkey and XLD (xylose-lysine-deoxycholate) plates. The Rappoport-Vassiliadis broth was then reincubated and a second subculture performed after 48 hours. In all cases, plates were incubated

overnight at 37°C. All organisms isolated by the above techniques and presumptively identified as *Salmonellae* were subject to serogrouping. Antimicrobial susceptibility profiles (Sensititre® CMV2ECOF Companion/Equine MIC Veterinary Specific plate; Trek Diagnostics, Cleveland, OH) and serotyping (Reilly *et al.,* 1991) were performed on selected samples using standard WHO approved techniques.

During the course of cleaning and disinfection, and the conduct of other steps to improve the biosecurity of the facility, all areas of the hospital were subject to rigorous and repeated environmental sampling to ensure that every part of the hospital moved to culture negative status. During this period 960 environmental samples were processed. Ultimately, samples from all parts of the hospital complex were shown to be negative on three separate occasions and, after 85 days of intense activity, the hospital began the process of reopening and returning to full function on August 2nd 2004.

Tertiary care veterinary referral centers that treat large animal patients, particularly those with heavy case loads, are at risk for the introduction of infectious disease, notably *Salmonella* [Castor *et al.,* 1989; Dargatz and Traub-Dargatz, 2004; Hartmann, 1996; Paré *et al.,* 1996; Schott *et al.,* 2001; Tillotson *et al.,* 1997]. Where significant problems do develop, they may not be amenable to even the most vigorous remedial efforts if such efforts are limited to specific areas at a time and patients that could potentially be exposed to the inciting organism and/or add to the environmental load of the pathogen are still being admitted to other parts of the facility. A proactive biosecurity program that includes a significant surveillance component can help to rapidly identify and limit infectious disease problems [Dunowska *et al.,* 2004, Morley *et al.,* 2004, Smith *et al.,* 2004]. In busy hospitals especially, implementation of effective monitoring and surveillance and development of biosecurity protocols should be considered. A demonstrably effective biosecurity program improves the quality of the facility by optimizing patient care, reducing nosocomial infection, and protecting personnel and clients from zoonotic agents. In teaching institutions a biosecurity program also provides educational opportunities. The ability of such programs to limit financial losses and liability, and restore confidence to staff and clients are also important considerations.

Development and implementation of a biosecurity program

After decontamination, the hospital partially opened in August 2004, and returned to full operation in January 2005. Implementation of effective monitoring and surveillance and development of biosecurity protocols were critical to reopening. A full commitment to biosecurity was made at the highest level of the University. A Director of Biosecurity was charged with developing a long term biosecurity plan with the assistance of a Biosecurity Advisory Committee that includes representatives from all clinical (medicine, surgery, emergency critical care, sports medicine, reproduction and field service) critical support (nursing, housekeeping, animal attendants, facilities) and diagnostic services (microbiology, pathology).

The biosecurity program at the Widener Hospital has several major components:

1. Risk Stratification – patients, people and traffic

Patients are divided into risk categories and housed accordingly. Barrier precautions are applied based on risk and there is strict attention to animal and human traffic flow. Strict hand hygiene and rigorous routine cleaning and disinfection are stressed.

2. Monitoring and surveillance – patients, environment and clinical status

Surveillance of the hospital's environment and patients is the critical sensory input into the program. Although routine monitoring is focused on *Salmonella*, data are collected and collated on other agents of concern and on the antimicrobial sensitivity profiles of selected organisms including *S. aureus*, *E. coli*, *Enterococcus* and *Enterobacter* species. It is the responsibility of the Director of Biosecurity to adjust the focus of surveillance testing based on developments in the hospital, literature, etc. Clinical status of patients is monitored by a Biosecurity Assistant in liaison with attending clinicians. Based on specific algorithms, changes in patient status trigger additional testing and other actions designed to contain potential infection problems. The algorithms mandate certain actions e.g. initiation of clinical sample submission, implementation of barrier precautions etc other actions are made at the discretion of the Director of Biosecurity. A decision was taken to make the position of the Director of Biosecurity one without patient-related clinical duties. In this way the Director of Biosecurity has no specific relationship to cases of concern in terms of infection control and can make difficult or unpopular decisions about the disposition of specific patients without bias. Nevertheless, all decisions must balance the needs of the hospital with those of the individual patient and must also take into account any impact on client relations.

For patient surveillance, 10 g fecal ball (or 10 ml of gastrointestinal reflux or a rectal swab if those are the only samples available) is placed into a Whirl-Pak® bag with 100 ml of selenite broth. The mixture is then incubated overnight at 35ºC after which an aliquot of the resulting broth is streaked to an XLD and a MacConkey plate. The plates are incubated overnight at 35ºC and observed for typical colony types the following day. Organisms presumptively identified as *Salmonellae* are serogrouped. Antimicrobial susceptibility profiles (Sensititre® CMV2ECOF Companion/Equine MIC Veterinary Specific plate; Trek Diagnostics, Cleveland, OH) and serotyping (Reilly *et al.*, 1991) are performed on selected samples using standard WHO approved techniques.

Samples collected at admission and during hospitalization from over 6,500 inpatients revealed that 1.3% of elective and non-GI patients were positive for *Salmonella*. In equine colic patients and those admitted with fever or diarrhea rates were 9.8% and 20.7%, respectively. Among bovine patients 11.8% were positive are for *Salmonella*, 72.5% of which were detected at admission (compared to only 16.0% in equine colic patients). Based on these data surveillance protocols were changed so that an admission sample is collected from low-medium risk patients but they are no longer subject to in-hospital surveillance. High-risk patients continue to be sampled at admission and twice weekly during hospitalization.

Environmental samples are collected weekly from designated areas of the hospital using commercially available electrostatic dust collection wipes (Swiffer®; Procter and Gamble, Cincinnati, OH). The charge on the wipes attracts debris and bacteria and they can be used to sample large surface areas. Samples are collected using the procedures described by Burgess and colleagues (2004). In addition to sampling large common areas in specific animal housing and clinical spaces, electrostatic wipes are also used to collect samples from all stalls located in high risk areas (colic and isolation facilities) and stalls in other areas that were occupied by animals positive for *Salmonella* on fecal culture. After stalls are vacated by the patient they are subject to cleaning and disinfection. Once the stall is dry, composite samples are collected from each stall by using an electrostatic dust wipe on equipment, hand, wall, and floor surfaces; in that order.

Environmental samples are processed using that The International Standards Organization (ISO) culture procedure for *Salmonella* detection. This is a multistage process involving pre-enrichment and enrichment steps followed by differential plating; all of which are designed to select for *Salmonella*. All environmental surveillance samples, including samples gathered from vacated stalls, are subject to this procedure. Briefly, the samples collected on Swiffer® disposable cloths are placed in a Whirl-Pak® bag to which 100 ml of buffered peptone water (BPW) is added. Samples in BPW are then incubated at 37°C for 24 hours. The pre-enriched samples are subsequently inoculated into Rappoport-Vassiliadis broth and incubated for a further 24 hours at 42°C, followed by subculture onto DCA (deoxycholate-citrate-agar), MacConkey and XLD (xylose-lysine-deoxycholate) plates. The Rappoport-Vassiliadis broth is then reincubated and a second subculture performed after 48 hours. In all cases, plates are incubated overnight at 37°C. All organisms isolated by the above techniques and presumptively identified as *Salmonellae* are subject to serogrouping. Antimicrobial susceptibility profiles (Sensititre® CMV2ECOF Companion/Equine MIC Veterinary Specific plate; Trek Diagnostics, Cleveland, OH) and serotyping (Reilly *et al.*, 1991) are performed on selected samples using standard WHO approved techniques.

Results from over 5,000 environmental samples for *Salmonella* collected over the last two years suggest that appropriate environmental surveillance is an effective indicator of containment.

3. Test Development and Review – patients, environment, nosocomial pathogens
Surveillance tests and strategies, notably implementation of real-time PCR for *Salmonella,* are under development. Briefly, *Salmonella* DNA is extracted from a 1 ml aliquot using a commercial kit as described by the manufacturer (MoBio, Carlsbad, CA). Real-time PCR is performed as described by Hoorfar *et al.* (2000). The *inv*A probe 5' FAM - TCTGGTTGATTTCCTGATCGCA BHQ5 3' was modified and was labeled at the 3' end with a black hole quencher rather than TAMRA. Reactions are amplified on a SmartCycler System (Cepheid, Sunnyvale, CA). Cycle threshold (CT) values are recorded for all samples tested to determine which samples were positive.

PCR data obtained so far demonstrate that across-the-board implementation of *Salmonella* PCR is not viable, but the test performs better in high risk colic patients where the prevalence of Salmonella positives is higher; primarily manifest as improvement in sensitivity from 30% in non-colic, non-fever, non-diarrhea equine patients to 55% in horses presenting with colic. The utility of real-time PCR for **targeted surveillance** in horses presenting with colic is currently under investigation.

The management of multidrug resistant *Salmonella* serotypes in a veterinary hospital requires an environmental sampling technique that can rapidly identify as many of the areas that need to be cleaned as possible (true positives) without necessarily increasing the burden of cleaning or stalls held closed by also identifying large numbers of locations that do not need cleaning (false positives). Although currently our only option, the standard culture techniques for *Salmonella* have a turn-around time is too long for our purposes. The real-time PCR test has also been examined for evaluation of environmental samples. It has described has a maximum turn-around time (including overnight pre-enrichment of the environmental sample in BPW) of between 26-30 h, which is a large improvement over 3-5 days for culture. However, compared to the ISO culture technique, the test characteristics (with a cycle time < 35; sensitivity = 86.5%, specificity = 92.3%). of a single real-time PCR test were such that the balance between the number of sites correctly and incorrectly identified as needing cleaning (we should have cleaned 74 areas; 32 of them correctly, 42 incorrectly) would place an onerous burden on hospital staff and impact stall availability. It is possible that these burdens could be lessened considerably (with only a small decrease in the number of sites correctly identified as needing cleaning) if a second PCR test were to be carried out on environmental samples that were positive first time around (based on the calculated sensitivity of the single PCR test, when successive PCR tests are used it could reduce the number of areas needlessly cleaned from 42 to 3 and we would miss only 9 of the 37 areas that should be cleaned; overall test characteristics for the serial procedure: sensitivity 75.7%, specificity 99.8%). Further work is underway to test this hypothesis.

The need to initiate testing strategies for other potential agents of hospital-acquired infection is under constant review.

4. Evidence-Based Decision Making – patients, people, protocols, monitoring and surveillance

In the wake of an outbreak, the level of risk aversion is high. It may not be possible or practical to sustain the rigor and cost of initial biosecurity procedures. It is essential to appreciate that evidence-based modification of biosecurity protocols is crucial to program success. The data gathered from monitoring and surveillance are used to make evidence-based decisions on the effectiveness of the biosecurity protocols, define the level of risk that different types of case represent and optimize the benefit to risk ratio of the program.

5. Education and Awareness – people

Education of all stakeholders (faculty, staff, house officers, students, clients, referring veterinarians, etc.) is critical to success. It is not possible sustain a long-term

biosecurity program or shift the perception of the need for biosecurity from something that is **"bad"** to being a **truly positive** attribute without a persistent commitment to education among all concerned.

Concluding Remarks

The appearance of increasingly resistant organisms in community as well as hospital settings combined with the mobility of our animal populations make it likely that the risk of introduction of infectious agents capable of causing outbreaks of disease will increase over succeeding years. All facilities, but particularly veterinary hospitals must consider the development of infection control procedures and a biosecurity program to protect them against such events. A demonstrably effective biosecurity program improves the quality of the facility by optimizing patient care, reducing hospital-acquired infection, protecting personnel and clients from zoonotic agents, providing educational opportunities, limiting financial losses and liability, and restoring confidence to staff and clients. Having operated a strict infection control and biosecurity program for two years we have found that written plans, careful data management, attention to detail, good communications and a persistent message are imperative to success.

Implementation and, more importantly, long-term maintenance of a biosecurity program is not without problems. Acceptance of biosecurity requires commitment to dealing with a variety of fundamental issues across many constituencies including, but not limited to: education, communication, cost, data management, impact on patient care, legal liability. Nevertheless, even in a relative short period, with proper commitment it is possible to effect major changes in attitude and behavior with respect to biosecurity.

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2006 MULTI-STATE OCCURRENCE OF EVA

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Introduction

The 2006 occurrence of equine viral arteritis (EVA) in NM and several other states has increased awareness among horse owners and breeders of a disease that can have significant financial repercussions, especially for the breeding sector of the nation's equine industry. Economic losses directly or indirectly attributable to this infection include abortion, illness and death in very young foals, the carrier state in stallions, and denied export markets for certain categories of horses with prior exposure to this infection, as well as virus infective semen or embryos. [12,14]

Ever since 1984, when EVA occurred on a widespread scale in Kentucky, involving an estimated 41 Thoroughbred breeding farms, the disease has gained considerable international notoriety.[10] There is a growing awareness of the heightened risk of global spread of the causal agent, equine arteritis virus (EAV), inherent in the ever-increasing volume of trade in horses, semen and embryos.[11] Ironically, notwithstanding the huge economic impact of the horse industry on the national economy, to this day, the United States stands alone as the only country with zero import testing requirements or controls for EVA. Over the years, there have been numerous proven introductions of EAV into the resident US breeding population either from the importation of carrier stallions or shipped virus infective semen. Regretfully, on occasion these have resulted in economically damaging outbreaks of EVA and the multi-state dissemination of strains of the virus of considerable pathogenic potential.[1]

Notwithstanding the widespread global distribution of EAV, from a historical perspective, relatively few confirmed outbreaks of EVA have been reported. This situation has been changing, however, in more recent years. The number of recorded occurrences of the disease has increased in the United States and Canada, due in part to greater awareness of EVA among veterinarians and members of the horse industry, as well as improved laboratory capability to diagnose the infection.[14] Two of these occurrences in particular which took place in 1988 and 1993, led to dissemination of the virus among a significant number of states, resulting from the movement of horses

either incubating the infection or subclinically infected with EAV. The most recent was a major occurrence of EVA at Arlington Park Racetrack, Chicago, Illinois in 1993.[9]

Prevalence of Infection

It has been known for many years that based upon the results of a range of serological surveys carried out in the United States, the prevalence of EAV infection varies widely among different horse breeds.[2,5,6,14] Highest rates of infection have been found in Standardbreds and Warmbloods and much lower rates in Thoroughbreds (Timoney & McCollum, unpublished data).[2] At the time of the NAHMS Equine 1998 study, there was very little evidence of circulation of EAV in a significant and representative sampling of the Quarter Horse population, with a seroprevalence of only 0.6 percent.[7] This indicated that the single most numerous horse breed in the country was essentially totally naïve with respect to prior contact with EAV and, therefore, fully susceptible should future exposure to infection occur.

In 2005, an outbreak of EVA was diagnosed retrospectively on a large Quarter Horse breeding farm in NM, which was characterized by a very high seroprevalence of infection, minimal clinical expression of disease but confirmed evidence that the virus strain involved was abortigenic. Such was the known background status of the Quarter Horse population in NM and various other western and some mid-western states prior to this year's major occurrence of EVA in the breed.

Primary Disease Outbreak

Based on extensive epidemiological investigation, the 2006 multi-state occurrence of EVA originated on a large Quarter Horse breeding farm in NM that stood 4 Quarter Horse breeding stallions.[8] The first indication of a disease problem on the index premises occurred on June 4th during a routine 60 day pregnancy examination of a group of mares previously confirmed in foal. A number of mares sharing the same pasture were found to have lost their pregnancies. Over the ensuing 1 to 2 weeks, additional mares in this group and in several other groups, some at pasture and others kept in dry lots were also confirmed to have aborted. By June 16th, the significant pregnancy losses (up to 50 percent) which had occurred to that point, prompted the owner to seek advice as to the cause of the problem. Immediate contact was made with the M.H. Gluck Equine Research Center, University of Kentucky, and EVA was suggested as a likely cause of the abortions. Upon request, a total of 26 sera, mostly from mares that had aborted, and semen samples from two of the breeding stallions were received for testing on June 20th. At the time, the recommendation was made to the farm owner to halt all shipments of semen from any of the breeding stallions to premises within NM or out of state, pending the outcome of the laboratory tests for EVA. By June 23rd, the serological results were available and these confirmed evidence of EAV infection in 24 of the 26 sera. This was followed on June 26th by detection of EAV in the semen of both stallions. These findings provided very strong circumstantial evidence of recent exposure to the virus; this was unequivocally confirmed upon subsequent examination of paired sera from individual horses. Upon notification of the NM State Veterinarian, the farm was placed under quarantine until further notice.

Disease Tracings

On subsequent investigation, it was determined that fresh-cooled semen from one of the infected stallions had been shipped to premises in a significant number of other states prior to June 16th, when such shipments were suspended. The assistance of USDA-APHIS-VS professional staff was sought and promptly provided to help carry out a complete epidemiological investigation of the extent of spread of the infection from the index premises to states besides NM. Trace information for both mares and semen shipments was obtained from the owner of the affected farm and from Certificates of Veterinary Inspection. Fresh-cooled semen collected from the breeding stallions on the index premises in the late spring and early summer of 2006 together with mares (both donor and recipients) that had visited the premises during the same time-frame were traced to premises in 6 eastern region states (AL, FL, IN, KY, MN, MS) and 12 states in the western region (CA, CO, ID, KS, LA, MO, MT, OK, SD, TX, UT, WY). The horses traced in States other than NM and UT were horses exposed on the index premises in NM or to potentially infective semen as described above and were considered direct exposures. A total of 69 direct exposures were identified, of which 48 (69.5 percent) were mares inseminated with shipped semen and 20 (29 percent) involved mares and foals that had visited the index premises for some period during the time-frame in question. One mare (1.5 percent) had been exposed through insemination with shipped semen and also as a result of visiting the index premises in NM. A summary of the outbreaks in NM and UT will be provided separately.

Diagnostic Criteria

In attempting to identify animals that became infected in the course of this occurrence of EVA, two important points need to be borne in mind. Firstly, no matter how suggestive of EVA the clinical signs exhibited by an affected horse may be, they cannot *per se* serve as the basis for establishing a diagnosis of the disease. Simply stated, EVA can clinically mimic a range of other infectious and non-infectious equine diseases.[13] Consequently, a provisional clinical diagnosis of the disease must always be corroborated by appropriate laboratory findings. Secondly, the presence of a neutralizing antibody titer ($\geq$1:4) to EAV in a single serum sample, no matter how high, is not of itself diagnostic confirmation of recent exposure to infection. It must be emphasized that neutralizing antibody titers which develop following natural infection with EAV can persist at high levels for a year or more. Serological confirmation of EVA or EAV infection is based upon demonstration of seroconversion or a significant (4-fold or greater) rise in antibody titer between paired (acute and convalescent) sera. A strict case definition was applied with respect to the epidemiological investigations surrounding the 2006 multi-state occurrence of EVA. A confirmed case of EAV infection was an animal that had had an epidemiological link to the index premises and met one or more of the following criteria:

- EAV detected in blood leukocytes, serum or semen,
- seroconversion or significant ($\geq$4-fold) rise in serum neutralizing antibody titer to EAV between paired sera,

- a positive serum neutralizing antibody titer (≥1:4) in a directly exposed animal with evidence of spread to other horses on the same premises based on virus detection, seroconversion, or a significant rise or decline in antibody titer.

Distribution of Infection

In accordance with this case definition, diagnostic confirmation of recent EAV infection was established for 6 states, 5 in the western region (KS, MT, NM, OK and UT) and 1 in the eastern region (AL). Strongly suggestive but not confirmatory proof of recent spread of EAV infection was found in horses in an additional 4 states (CA, CO, ID, TX), each of which had one or more animals with epidemiological links to the index premises in NM and high antibody titers to EAV. No evidence of EAV infection was found in any of the 9 remaining states (FL, IN, KY, LA, MN, MO, MS, SD, WY) that received shipped fresh-cooled semen and/or had mares visit the index premises in NM.

Several important issues arose in the course of undertaking the epidemiological tracings connected with the 2006 multi-state occurrence of EVA which limited the completeness of the investigations. The lack of a national program for the prevention and control of EVA together with the lack of uniformity among states in reporting the disease hampered efforts to define more accurately the extent of spread of the infection in certain states. The situation was further complicated by the fact that in some states, owners were not required to share test results for EAV infection on their animals with federal or state animal health authorities.

Characteristics of Disease Outbreaks

Based on detailed information provided by the index premises in NM, it was believed that EAV was initially introduced onto the farm at some point during the latter half of May 2006, most probably by means of an infected mare from a source as yet undetermined.[8] It is thought the virus circulated through various groups of mares, causing significant pregnancy losses before spreading to the 4 stallions on the farm, all of which became carriers and semen shedders of EAV. Serological examination of over 200 animals confirmed an extremely high seroprevalence of infection, with every mare, stallion and foal found positive. A third of the yearling colts were also seropositive. Notwithstanding the widespread dissemination of the virus on the farm, reported clinical evidence of infection was minimal. As already stated, the early pregnancy loss rate was very high. The principal mode of transmission of EAV on the index premises was almost certainly by the respiratory route; spread of the virus was undoubtedly facilitated by the large number of animals kept under conditions of close physical contact with one another. Once the stallions became infected, venereal transmission would also have played a role in the spread of EAV.

Aside from the outbreak of EVA on the index premises, the reported incidence of clinical disease apart from abortion on the other affected farms in NM was low. This contrasts with the corresponding situation in UT, the other most severely affected state. The clinical attack rate was reported to be moderate to high on a significant number of affected premises in that state, not all of which were Quarter Horse breeding farms. Infection occurred on 3 boarding stables, 12 private farms, 1 breeding facility, 4 training

stables and 1 veterinary clinic. While EVA was confirmed primarily in Quarter Horses, it also occurred in a range of other breeds, Warmbloods, Paint horses, Arabians and Thoroughbreds. Clinical signs observed in the majority of cases of the disease included fever, dependant edema of the hind limbs, mid-ventral edema of the sheath and scrotum in the stallion, and mammary glands in the mare, supra or peri-orbital edema and a variable degree of conjunctivitis. Less frequently encountered signs included a unilateral or bilateral serous nasal discharge, lacrimination, depression and anorexia and hives which was present in about 10 percent of affected animals. Clinical signs of EVA were more severe in older horses, greater than 20 years of age.

Of the 10 states in which there were confirmed cases of EVA or strong circumstantial evidence of infection, NM and UT had the greatest estimated number of affected premises. A total of 8 premises were placed under official quarantine at the height of the occurrence in NM. The number of horses involved was 428. Additionally, 15 other premises in the state were placed under voluntary quarantine by the respective attending veterinarians and/or the farm owners. The total number of animals on these farms was 653. The last laboratory confirmed evidence of EAV infection on any premises in the state was July 29th. Restrictions have been lifted from all but one of these premises effective August 14th. There is no evidence of further circulation of or active infection with EAV since the end of the July, 2006.

In the case of UT, an estimated 591 horses on some 21 affected premises were placed under quarantine. Some 7 of the premises were involved through direct exposure either to shipped fresh-cooled semen from the index premises in NM or had mares (donor/recipient) visit that premises. A total of 14 (66 percent) of the known outbreaks of EVA were secondary/tertiary occurrences of the disease linked not directly to NM, but to one or other of the 7 affected premises in UT which had direct exposure to the index premises in NM. In the main, the morbidity rate on affected premises was very high, with clinical evidence of the disease observed in over 90 percent of at-risk horses. A quarantine was also imposed on an additional 350 horses on 6 premises, but restrictions were lifted once there was laboratory confirmation of absence of EAV infection in these animals. As of November 26th, the quarantine has been removed from the last remaining known EVA-affected premises on which there was evidence of virus circulation up to November 6th, 2006.

Laboratory Findings

A range of laboratories (USDA-APHIS-VS, National Veterinary Services Laboratory, M.H. Gluck Equine Research Center [OIE designated reference laboratory for EVA] and various state veterinary diagnostic laboratories) were involved in testing samples from horses involved in the 2006 occurrence of EVA.

Based on available results, there was a very high seroprevalence ($\geq$90 percent) of antibodies to EAV on many affected farms. Isolations of EAV were obtained from 10 of 14 aborted fetuses from 4 affected breeding farms in NM and 1 in UT. All of the abortions occurred in mares between the third and seventh month of pregnancy. The virus was also recovered from blood leukocytes of 24 horses located in 4 states (KS,

NM, OK, UT) and the serum of two additional animals, both in NM. It is worth noting that the dams of 4 of the virus positive fetuses were viremic at time of abortion. Persistent EAV infection (the carrier state) was confirmed in 8 stallions (6 Quarter Horses and 1 Warmblood). Five were located in NM and 2 in UT. Seroconversion or significant rises in antibody titers to EAV was demonstrated in 8 horses.

Salient Features of 2006 Occurrence

The 2006 multi-state occurrence of EVA presented a number of significant features, some not encountered in previous outbreaks of the disease. Of overriding importance was the ease with which infection was very effectively spread among an immunologically naïve population through the use of semen from a stallion acutely and later, persistently infected with EAV. From this and past experience, the virus has been proven to be readily transmitted using either fresh-cooled or frozen semen.[11,13,14]

This occurrence of EVA was the first in which there was widespread dissemination of EAV in Quarter Horses, a breed essentially not previously exposed to this virus. Aside from the major role shipped semen from one carrier stallion played in spread of the disease both within NM and to other states, movement of donor/recipient mares also contributed to transmission of the virus. The widespread practice of embryo transfer in the Quarter Horse breed and proliferation in the number of recipient mare farms in recent years, were significant industry-driven factors not previously recognized as playing a role in the epidemiology of EVA.

Another important factor that undoubtedly promoted spread of EAV during this occurrence of EVA was the very intensive "feed-lot" system of managing mares on many of the affected Quarter Horse breeding farms. The number of mares kept in close proximity to one another either in pasture or dry-lot situations, which was frequently significant, greatly facilitated transmission of the virus by the respiratory route.[8]

A final and very important point that, without question, has had a major influence on the continued circulation of EAV in states in which it was introduced was the lack of adequate supplies of the commercial MLV vaccine against EVA (Arvac®, Ft. Dodge Animal Health). From experience in dealing with previous large scale outbreaks of EVA both at racetracks and on breeding farms,[9,14] implementation of a widespread program of prophylactic vaccination of horses at risk of natural exposure to EAV would have rapidly curtailed further dissemination of the virus and brought this year's occurrence to a more timely conclusion. Significant supplies of the vaccine are once more available.

It should be emphasized that in spite of the extended duration (approximately 5-6 months) of the 2006 multi-state occurrence of EVA, no restrictions were imposed at any time on the interstate movement of horses or shipment of semen from affected states. Hopefully, the significance of what has taken place will galvanize the horse industry and animal regulatory authorities in non-affected as well as affected states to address the issue of EVA in a more progressive and realistic manner. The USDA-APHIS-VS has developed Uniform Methods & Rules (UM&R) for EVA that provides minimum standards for detecting, preventing and controlling the disease. These minimum standards and

requirements which were endorsed by the United States Animal Health Association, American Horse Council and the American Association of Equine Practitioners, represent a framework for states to develop their own control programs as well as serve as the basis for a national control program for EVA.[3,4] Only time will tell whether the 2006 occurrence of the disease has provided the necessary impetus for the parties concerned to address what has long been sorely needed, namely, a concerted effort at the level of the states to achieve greater prevention and control of EVA and lessen its economic impact on the nation's equine industry.

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Report of the Subcommittee on Equine Infectious Anemia
Steven L. Halstead, Chair

The Subcommittee met by monthly or more frequent conference calls through 2006. Activities and respective outcomes included:

1. Following the 2005 report of the Sub-Committee to the IDOHC and the resulting discussion, the Sub-Committee convened a meeting of a small group of State animal health officials to consider the most appropriate and acceptable direction in which to guide the nation's EIA management efforts. The report and conclusions of the participants are as follows:

   The following items represent the consensus of the attendees of the 2006 EIA National Direction Meeting in Dallas, TX, on March 21-22, 2006. The meeting's goal was to develop clear consensus to guide the USAHA IDOHC on the issue of EIA management. These ideas will be shared with other states and then submitted to the EIA Subcommittee for incorporation into its report to the full IDOHC committee and, as appropriate, for follow-up with the full USAHA.

   Working with USDA, all 50 states and the equine industry…

   Highest priority should be placed on accomplishing:

- EIA UMR should be revised in the immediate future to incorporate current science on testing and be reviewed and amended where appropriate.

- USDA should request enhanced analysis of EIA surveillance data from the National Surveillance Unit and request that they make an assessment and recommendations, including documenting and sharing smarter testing schemes as best practices. After the results of surveillance analysis, seek funding to support implementation.

- An organized effort to engage industry and stakeholders in examining EIA and its future management and potential eradication should be developed through an interactive process. Equine industry groups and AAEP should be enlisted as partners for both getting input and providing information. Also critical would be 4-H, CSREES programs, and others using EIA educational materials developed from this organized effort as part of their equine health programs. They would all be a good link to the end users.

- Procuring funding, to be directed to USDA and possibly managed through a cooperative agreement, to conduct a census of the U.S. horse population.

Other important recommendations for consideration and implementation:

- The revised EIA UMR should be used as a basis for all state programs and selective elements of the UMR should be considered for incorporation into the CFR when appropriate.

- States should be encouraged to explore agreements such as MOUs, etc. to enhance EIA programs and consider regionalization approaches.

- Encourage states to support the development of identification criteria and standards for premises and individual equids under the auspices of NAIS.

- Encourage states to document and share innovative enforcement techniques.

- NAHMS sera study – Consideration of analysis of sera collected for the NAHMS equine study for EIA antibody was requested of each state department of agriculture. All states authorized this use of the stored sera. Analysis is ongoing through NVSL at this time, with approximately half of the ~8,000 specimens analyzed. No positive

testing sera have been found. Specimens collected through the NAHMS project are stripped of premises-specific information but might prove useful to indicate regions of the country where reservoirs of EIA exist within lightly tested equine populations.

2. As follow-up to the Dallas National EIA Direction meeting, the Sub-Committee requested that the USDA VS National Surveillance Unit (NSU) develop an EIA prevalence model for the United States. This work is on-going, with the concept and methodology presented to the IDOHC. Once completed, the model will be used to develop EIA prevalence estimates for states and/or regions to more accurately guide regionalization and other EIA management efforts.
3. Also following the Dallas meeting, the Sub-Committee made the recommended amendments to the existing EIA UMR. The Sub-Committee asked the IDOHC to support a resolution directing the USDA to incorporate specific components of the amended UMR into the CFR, with the clarification that comments on the proposed CFR wording will be requested through the proposed rulemaking process.
4. Finally, the Sub-Committee presented the IDOHC with a CEAH fact sheet report of the 1998 NAHMS sera study. In summary, these sera were found to have a reactor rate consistent with that extrapolated from annual movement testing reports.

Report of the Subcommittee on Equine Piroplasmosis

Kent Fowler, Chair

The committee members include:

- Kent Fowler, California
- Lee Coffman, Private Consultant
- Tim Cordes, USDA-VS
- Leonard Eldridge, Washington
- Steve Hennager, USDA-VS
- Bob Hillman, Texas
- Ralph Knowles, Private Consultant
- Amy Mann, Industry Expert
- Richard Mitchell, Private Practitioner
- Don Knowles, ARS
- Mike Short, Florida
- Robert Stout, Kentucky
- Tim Boone, California

It should be noted that prior to the formation of the IDOHC EP Subcommittee, there were two previous national meetings of EP experts at the USDA Center to discuss the options for addressing the EP situation in the United States. These meetings took place on August 21, 2003 and February 16, 2005 at the USDA Center. The purpose for the formation of the IDOHC EP subcommittee was based upon the clear and continuing

identified need for a more cohesive policy at both state and federal level for identifying and disposing of EP seropositive imported horses.

The first meeting of the Subcommittee took place on May 3, 2006 via telephone conference call. Subsequent meetings of the committee took place on June 14, July 18, and September 12, 2006. As a result of these meetings and the preceding work of others, the following conclusions were drawn:

- **The status of Equine Piroplasmosis in the U.S. is in question.** Equine Piroplasmosis (EP) is classified as a Foreign Animal Disease to the United States. In view of the deficiencies of the CF test in detecting the long-term carrier of B. caballi or B. equi, it is proposed that the infection with one or the other parasite exists at some undefined prevalence level in horses that have been imported into the United States and perhaps in horses native to the United States. Prior to February 1, 2004, the "official test" for Piroplasmosis, conducted on equine animals presented for importation into the United States was the Complement Fixation (CF) test, a test that is known to occasionally yield "false negative" results. Unscrupulous owners, importers or agents compounded the problem by purposely treating EP infected horses with immunosuppressive medications to give rise to a false negative reaction in the CF test. The CF test was replaced by an upgraded C-ELISA test, that was specified as the "official test" on August 22, 2005. The C-ELISA is highly unlikely to yield "false negative" results on adult horses.

- **Potential tick vectors 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 possibly result in establishment of endemicity of B. caballi or B. equi in the resident equine population in the United States.

- **Treatment is not a validated viable option.** There is no conclusive evidence that treatment of a carrier of either of the two causal agents of EP (Babesia caballi and Babesia equi) is a viable option in successfully eliminating the carrier state.

- **Research, risk assessment, and validation are required.** It is crucial to 1) maintain stringent import restrictions that prevent the importation of seropositive horses into the United States, 2) develop a cohesive and acceptable policy at both federal and state levels for identifying and dealing with resident EP seropositive horses, and 3) request funding for research on devising effective treatment protocols for EP.

Upon majority consensus of the committee and industry interaction inclusive of presentations at the National Institute of Animal Agriculture at Louisville, Kentucky in April, 2006 and the American Horse Council Equine Health Forum in Austin, Texas in

September, 2006, the following resolutions for progressively dealing with the current status of EP in the U.S. are as follows:

- **1** That IDOHC urges USDA to investigate the prevalence of EP infection in the United States utilizing accepted survey methodology. The IDOHC recommends that the first component of this incentive is to conduct a national survey of slaughter horses, which should provide an estimate of the prevalence of EP infection in the United States. It is further recommended that USDA establish a working group consisting of representatives from equine industry groups, state animal health officials, researchers and veterinarians knowledgeable about EP to evaluate the survey results, and if indicated, develop recommendations for control of EP positive horses in the United States and/or elimination of EP from the United States. If insufficient information is provided through the slaughter horse survey, consideration should be given to other ways to survey the resident equine population in the United States for the purpose of establishing its status with respect to infection with B. caballi and / or B. equi.

- **2** That all owners and/or custodial agents of known resident seropositive EP horses (C-ELISA positive) immediately report or re-confirm the existence of these horses to their resident state health and regulatory officials (State Veterinarian) and the USDA. These horses will be identified with a National Animal Identification System (NAIS) permanent identification chip, and a permanent resident address of the horse shall be declared. Any change of the permanent resident address of the horse shall be reported immediately to state regulatory officials.

- **3** That it will be the inherent responsibility of the owner or custodial agents of resident seropositive EP horses to maintain these horses in tick-free environments for the life of the horse or until such time as the USDA and the resident State Veterinarian are satisfied that the horse is free from EP. Regulatory officials shall inspect permanent resident premises as needed to ensure that the horse is being maintained in a tick free environment. If it is determined that the horse is not being maintained in a tick-free environment, all movements shall continue to be restricted and dependent on compliance with any action, to include quarantine, as determined necessary by the State Veterinarian to ensure the containment of EP.

- **4** That so long as a resident EP horse remains infected, any movements away and returning to the resident premises must be approved (reciprocal permit issued) in advance by the resident state as well as any state of destination. In addition to existing CVI requirements, prior to any approved movement away from and returning to the permanent residence, horses must be 1) inspected by an accredited or regulatory veterinarian and determined to be free of ticks, 2) treated with an approved acaricide, and 3) issued a VS 127.

- **5** That the IDOHC urges USDA to fund research into to finding an effective treatment for elimination of the carrier state either with B. caballi and / or B. equi. Additionally, the IDOHC encourages owners of EP carrier horses found in the United States to make their EP horses available for participation in this research.

Pending ratification of these concepts by the IDOHC and the USAHA, challenges of the Committee will 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 U.S. The vision of the Committee is to do what it takes to ensure that EP does not become endemic in the resident horse population of the United States.

NAHMS EQUINE 2005 STUDY

The NAHMS Equine 2005 study collected health and management information from 2,893 equine operations regarding health practices influencing equine infectious disease incidence and estimated the occurrence of selected equine health-related events. For details regarding study design and data analysis, and to view the full report, go to <http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/equine>.

Mortality Rate and Causes of Death for Equids – In the 12 months preceding the study interview, 4.9 percent of foals born alive died in the first 30 days of life. The largest percentage of foal deaths was attributed to injury or trauma followed by failure to get milk or colostrums.

The overall mortality rate for resident equids 30 days and older during the 12 months before the interview was 1.8 percent. Old age was the leading cause of death in equids older than 6 months, followed by injury, wounds, trauma, and colic.

Vaccination Practices for Equids – Overall, 75.9 percent of operations indicated that they had given at least some type of vaccines to resident equids during the 12 months preceding the interview.

Movement of Equids – Overall, 36.9 percent of operations had not moved resident equids off the operation and back onto it in the previous 12 months.