Second International Workshop on Equine Viral Arteritis

convened by

The Dorothy Russell Havemeyer Foundation, Inc.

Monday, October 13th – Wednesday, October 15th 2008

Maxwell H. Gluck Equine Research Center
University of Kentucky
College of Agriculture
Department of Veterinary Science
Lexington, KY 40546-0099
USA

Telephone: (859) 257-4757
Fax: (859) 257-8542
Web Site: http://www.ca.uky.edu/gluck/index.htm
Greetings to each of you on the occasion of the Second International Workshop on Equine Viral Arteritis to be held at the University of Kentucky. A warm welcome to those who also attended the first workshop that took place at the University’s Maxwell H. Gluck Equine Research Center in 2004. A notable absentee from this year’s workshop is Dr. William McCollum, international authority on this disease and friend and colleague to many, who, regrettably passed away a little over a year ago.

The workshop, which has been endorsed by the World Organization for Animal Health (O.I.E.), was convened by The Dorothy Russell Havemeyer Foundation, Inc. Especial thanks and appreciation to Mr. Gene Pranzo, President, The Havemeyer Foundation, for the interest and support he has provided in enabling this meeting to take place.

The intent of the workshop is to bring together members of the scientific community from around the world to further our understanding of a disease that has significantly impacted international trade in horses and equine germplasm since 1984. Through sharing our knowledge and experience on equine viral arteritis, it is hoped we can arrive at solutions to some of the problems that continue to confound the laboratory diagnosis of this infection, and also, consider strategies for achieving greater control of the disease at a national and international level.

I am confident the workshop will be scientifically rewarding and a socially enjoyable occasion. Of added importance is the opportunity it will provide each participant with to forge new relationships with colleagues from other countries as well as renew and enhance old friendships.

Peter Timoney
Frederick Van Lennep Chair in Equine Veterinary Science
OIE Designated Specialist on Equine Viral Arteritis
Contact Information

If you have any specific needs or concerns during your stay, please feel free to contact any of the following:

Dr. Peter Timoney  
(859) 257-4757, ext. 8-1094  
E-mail: ptimoney@uky.edu

Ms. Debbie Mollett  
Administrative Associate  
(859) 257-4757, ext. 8-1085  
E-mail: dmoll01@uky.edu

Ms. Patsy Garrett  
Administrative Assistant  
(859) 257-4757, ext. 8-1112  
E-mail: patsy.garrett@uky.edu

Mr. Roy Leach  
Business Manager  
(859) 257-4757, ext. 8-1132  
E-mail: royleach@uky.edu

Hotel

Hyatt Regency  
401 W. High Street  
Lexington, KY  
(859) 253-1234  
Fax: (859) 254-7430

Airlines

American Airlines  1-800-433-7300  
Continental  1-800-523-3273  
Delta  1-800-221-1212  
Northwest  1-800-225-2525  
United  1-800-864-8331  
US Airways  1-800-428-4322
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preface</td>
<td>1</td>
</tr>
<tr>
<td>Contact Information</td>
<td>2</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>3</td>
</tr>
<tr>
<td>List of Participants</td>
<td>4</td>
</tr>
<tr>
<td>Program Overview</td>
<td>7</td>
</tr>
<tr>
<td>Workshop Goals</td>
<td>9</td>
</tr>
<tr>
<td>Scientific Program</td>
<td>10</td>
</tr>
<tr>
<td>Abstracts (in order of presentation)</td>
<td>17</td>
</tr>
<tr>
<td>Contact Information for Participants</td>
<td>57</td>
</tr>
<tr>
<td>Pages for Note-Taking</td>
<td>62</td>
</tr>
</tbody>
</table>
### List of Participants

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gian Luca Autorino</td>
<td>National Reference Centre for Equine Diseases (CeRME) – Istituto Zooprofilattico Sperimentale del Lazio e della Toscana - Rome, Italy</td>
</tr>
<tr>
<td>Udeni Balasuriya</td>
<td>Gluck Equine Research Center - Lexington, KY, USA</td>
</tr>
<tr>
<td>Maria Barrandeguy</td>
<td>Instituto de Virología CICV, INTA – Buenos Aires, Argentina</td>
</tr>
<tr>
<td>Reinhard Böse</td>
<td>Labor Dr. Böse GmbH – Harsum, Germany</td>
</tr>
<tr>
<td>Ivaylo Chenchev</td>
<td>National Diagnostic and Research Veterinary Medical Institute (NRRVMI) – Sofia, Bulgaria</td>
</tr>
<tr>
<td>Ann Cullinane</td>
<td>Irish Equine Centre – Johnstown, Ireland</td>
</tr>
<tr>
<td><strong>Trevor Drew</strong></td>
<td><strong>OIE Designated Specialist on Equine Viral Arteritis</strong>, Veterinary Laboratories Agency – Weybridge, United Kingdom</td>
</tr>
<tr>
<td>Guillaume Fortier</td>
<td>Frank Duncombe Laboratory – Caen, France</td>
</tr>
<tr>
<td>Yun Young Go</td>
<td>Gluck Equine Research Center - Lexington, KY, USA</td>
</tr>
<tr>
<td>Aymeric Hans</td>
<td>French Food Safety Agency (AFSSA) – Laboratory for Studies and Research on Equine Diseases – Goustranville, France</td>
</tr>
<tr>
<td>Jessica Hennig</td>
<td>Gluck Equine Research Center - Lexington, KY, USA</td>
</tr>
<tr>
<td>Ákos Hornyák</td>
<td>Szent Istvan University - Budapest, Hungary</td>
</tr>
<tr>
<td>Name</td>
<td>Affiliation</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Peter Kirkland</td>
<td>Elizabeth MacArthur Agricultural Institute – Camden NSW, Australia</td>
</tr>
<tr>
<td>Takashi Kondo</td>
<td><strong>OIE Designated Specialist on Equine Viral Arteritis</strong>, Epizootic Research Center, Equine Research Institute, JRA – Tochigi, Japan</td>
</tr>
<tr>
<td>Magdalena Larska</td>
<td>National Veterinary Research Institute – Pulawy, Poland</td>
</tr>
<tr>
<td>Pat Lenihan</td>
<td>Central Veterinary Research Laboratory, Backweston Campus – Celbridge, Ireland</td>
</tr>
<tr>
<td>Chengzhu Liang</td>
<td>Equine Disease Research Laboratory, Technique Center of Shandong Inspection and Quarantine Bureau of China – Shandong, P.R. China</td>
</tr>
<tr>
<td>Zhengchun Lu</td>
<td>Gluck Equine Research Center - Lexington, KY, USA</td>
</tr>
<tr>
<td>N. James MacLachlan</td>
<td>University of California – Davis, CA, USA</td>
</tr>
<tr>
<td>Sara Mankoč</td>
<td>University of Ljubljana – Ljubljana, Slovenia</td>
</tr>
<tr>
<td>Barry Meade</td>
<td>Gluck Equine Research Center - Lexington, KY, USA</td>
</tr>
<tr>
<td>Elizabeth Medcalf</td>
<td>Animal Health Trust – Newmarket, United Kingdom</td>
</tr>
<tr>
<td>Zvia Mildenberg</td>
<td>Kimron Veterinary Institute – Bet Dagan, Israel</td>
</tr>
<tr>
<td>Richard Newton</td>
<td>Animal Health Trust – Newmarket, United Kingdom</td>
</tr>
<tr>
<td>Name</td>
<td>Affiliation</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Gene Pranzo</td>
<td>The Dorothy Russell Havemeyer Foundation, Inc. - New York, NY, USA</td>
</tr>
<tr>
<td>Stéphane Pronost</td>
<td>Frank Duncombe Laboratory – Caen, France</td>
</tr>
<tr>
<td>Jena Richards</td>
<td>Gluck Equine Research Center - Lexington, KY, USA</td>
</tr>
<tr>
<td>Marcello Sala</td>
<td>National Reference Centre for Equine Diseases (CeRME) – Istituto Zooprofilattico Sperimentale del Lazio e della Toscana - Rome, Italy</td>
</tr>
<tr>
<td>David Schaengold</td>
<td>The Dorothy Russell Havemeyer Foundation, Inc. – New York, NY, USA</td>
</tr>
<tr>
<td><strong>Beverly Schmitt</strong></td>
<td><strong>Vice-President, OIE Standards Commission</strong>, National Veterinary Services Laboratory – Ames, IA, USA</td>
</tr>
<tr>
<td>Maria Teresa Scicluna</td>
<td>National Reference Centre for Equine Diseases (CeRME) – Istituto Zooprofilattico Sperimentale del Lazio e della Toscana - Rome, Italy</td>
</tr>
<tr>
<td>Kathy Shuck</td>
<td>Gluck Equine Research Center – Lexington, KY, USA</td>
</tr>
<tr>
<td><strong>Peter Timoney</strong></td>
<td><strong>OIE Designated Specialist on Equine Viral Arteritis</strong>, Gluck Equine Research Center – Lexington, KY, USA</td>
</tr>
<tr>
<td>Claudia Valdivieso</td>
<td>Livestock Disease Diagnostic Center – Lexington, KY, USA</td>
</tr>
<tr>
<td>David Westcott</td>
<td>Veterinary Laboratories Agency – Weybridge, United Kingdom</td>
</tr>
<tr>
<td>Jianqiang Zhang</td>
<td>Gluck Equine Research Center – Lexington, KY, USA</td>
</tr>
</tbody>
</table>
## Program Overview

### Monday, October 13th

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00 – 9:00 pm</td>
<td>Opening Reception&lt;br&gt;Kentucky Room, Hyatt Regency Hotel&lt;br&gt;Open Bar 7:00 – 9:00 pm</td>
</tr>
</tbody>
</table>

### Tuesday, October 14th

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:40 am</td>
<td>Shuttle departs Hyatt Regency Hotel for Culton Suite, Good Barn</td>
</tr>
<tr>
<td>8:00 – 8:30 am</td>
<td>Continental breakfast at Culton Suite</td>
</tr>
<tr>
<td>8:30 – 8:40 am</td>
<td>Introductory Remarks&lt;br&gt;Dr. Nancy Cox, Associate Dean for Research, College of Agriculture&lt;br&gt;Dr. Mats Troedsson, Chair, Department of Veterinary Science and Director, M.H. Gluck Equine Research Center</td>
</tr>
<tr>
<td>8:40 am – 8:50 am</td>
<td>Program Overview &amp; Workshop Goals&lt;br&gt;Dr. Peter Timoney</td>
</tr>
<tr>
<td>8:50 am – 12:00 pm</td>
<td>Scientific Program</td>
</tr>
<tr>
<td>12:00 – 1:00 pm</td>
<td>Catered lunch at the Culton Suite; Group Photo</td>
</tr>
<tr>
<td>1:00 – 4:00 pm</td>
<td>Scientific Program</td>
</tr>
<tr>
<td>4:00 pm</td>
<td>Shuttle departs for Boone Faculty Center/Hyatt Regency Hotel</td>
</tr>
<tr>
<td>4:30 – 5:10 pm</td>
<td>Reception hosted by the University of Kentucky Gluck Equine Research Foundation Board, Boone Faculty Center [Optional]</td>
</tr>
<tr>
<td>5:10 pm</td>
<td>Shuttle departs for Hyatt Regency Hotel</td>
</tr>
<tr>
<td>5:50 pm</td>
<td>Shuttle departs Hyatt Regency Hotel for Shaker Village</td>
</tr>
<tr>
<td>7:00 pm</td>
<td>Dinner at Shaker Village</td>
</tr>
<tr>
<td>9:00 pm</td>
<td>Shuttle departs for Hyatt Regency Hotel</td>
</tr>
</tbody>
</table>
**Wednesday, October 15th**

7:40 am  Shuttle departs Hyatt Regency Hotel for Culton Suite

8:00 – 8:30 am  Continental breakfast at Culton Suite

8:30 am – 12:00 pm  Scientific Program

12:00 – 1:00 pm  Catered lunch at Culton Suite

1:00 – 4:00 pm  Scientific Program

4:00 – 5:00 pm  Tour of the Gluck Center [Optional]

5:00 pm  Shuttle departs for Hyatt Regency Hotel

6:00 pm  Shuttle departs Hyatt Regency Hotel for Keeneland Racetrack

6:30 pm  Reception and dinner in the Lafayette Room, Keeneland
Open Bar 6:30 – 9:00 pm

9:00 pm  Shuttle departs Keeneland Racetrack for the Hyatt Regency Hotel

**Thursday, October 16th**

Opportunity to visit:

- Horse Farms
- Local Equine Veterinary Clinics
- Kentucky Horse Park
- Attend Races at Keeneland
- Visit Other Local Sites of Interest

Departure date for most participants
Workshop Goals

A major goal of this workshop will be to share information on new and existing laboratory tests for the diagnosis of equine arteritis virus infection. Emphasis will be placed on established test procedures described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (6th edition). Notwithstanding the availability of highly reliable time-tested laboratory procedures for virus detection and antibody determination, problems continue to be encountered by some laboratories in the diagnosis of this infection. It is important to identify where differences in opinion, methodology and approach exist if there is to be progress in achieving greater consistency and reliability among laboratories involved in the testing of horses and equine germplasm for international trade. I would strongly encourage participants to share information and ideas freely and to work towards advancing the principal goal of the workshop, namely, greater standardization among laboratories that test for this infection. It is especially appropriate that the workshop is being attended by Dr. Beverly Schmitt, Vice President, O.I.E. Biological Standards Commission, as well as the three O.I.E. designated specialists on equine viral arteritis, Dr. Trevor Drew, Dr. Takashi Kondo and Dr. Peter Timoney.

An additional goal of the workshop is to share the latest information on the epidemiology of equine arteritis virus. With continued escalation in the volume of horse movements and shipment of equine semen around the world, the attendant risk of dissemination of the virus has never been greater. The potential for economically significant outbreaks of equine viral arteritis in unprotected equine populations is of continuing concern. Full advantage should be taken at this workshop to apply current knowledge of the biology and epidemiology of equine arteritis virus towards development of prevention and control strategies that would mitigate the risk of virus dissemination at a national and international level while not proving overly restrictive and economically burdensome to equine industries worldwide.
Second International Workshop on Equine Arteritis Virus

Tuesday, October 14th, 2008

8:00 – 8:30 am  Continental Breakfast at the Culton Suite

8:30 – 8:40 am  Welcome
                   Nancy Cox  
                   (Assoc. Dean for Research, College of Agriculture)  
                   Mats Troedsson  
                   (Chair, Dept. of Veterinary Science & Director, Gluck Equine Research Center)

8:40 – 8:50 am  Program Overview and Workshop Goals  
                   Peter Timoney

8:50 – 9:00 am  The Role of the O.I.E. in Assay Standardization  
                   Beverly Schmitt

9:00 – 9:10 am  Discussion

Session 1

Detection and Identification of Equine Arteritis Virus

Moderators: Udeni Balasuriya & Takashi Kondo

9:10 – 9:20 am  Study of a Diagnostic Technique for Equine Viral Arteritis and its Application  
                   Chengzhu Liang

9:20 – 9:30 am  Discussion

9:30 – 9:40 am  First Detection and Characterization of Equine Viral Arteritis in France during the 2007 Outbreak Illustrating the Importance of Molecular Tools for the Diagnosis of this Infection  
                   Stéphane Pronost

9:40 – 9:50 am  Discussion
9:50 – 10:00 am Rapid Detection of Equine Arteritis Virus by a Reverse Transcription Loop-Mediated Isothermal Amplification Assay

Takashi Kondo

10:00 – 10:10 am Discussion

10:10 – 10:20 am Comparison of Two Real-time Reverse Transcription Polymerase Chain Reaction Assays for the Detection of Equine Arteritis Virus Nucleic Acid in Equine Semen and Tissue Culture Fluid

Zhengchun Lu

10:20 – 10:30 am Discussion

10:30 – 10:45 am Break

10:45 – 10:55 am Detection of a Novel European Genotype of Equine Arteritis Virus in Slovenian Lipizzan horses by One-Step MGB Real-Time RT-PCR Assay

Sara Mankoč

10:55 – 11:05 am Equine Arteritis Virus – Virus Isolation (VI) and PCRs

David Westcott

11:05 – 11:15 am General Discussion

Session 2

Assays for Antibody Determination to Equine Viral Arteritis

Moderators: Ann Cullinane & Guillaume Fortier

11:15 – 11:25 am Evidence that Use of an Inactivated EHV Vaccine Induces Serum Cytotoxicity Affecting the Equine Arteritis Virus Neutralisation Test

J. Richard Newton

11:25 – 11:35 am Discussion

11:35 – 11:45 am Serological Testing for Equine Arteritis Virus in Ireland 2003 to 2008

Ann Cullinane

11:45 – 11:55 am Discussion

12:00 – 1:00 pm Lunch at Culton Suite and Group Photo
1:00 – 1:10 pm  Evaluation of a Method to Reduce the Problem of Cytotoxicity in the Virus Neutralization Test during the 2008 Breeding Season and an Update on Use of an ELISA in France

Guillaume Fortier

1:10 – 1:20 pm  Discussion

1:20 – 1:30 pm  Observations on Equine Sera Giving Borderline Positive Results in the Virus Neutralisation Test for Equine Arteritis Virus (EAV)

Reinhard Böse

1:30 – 1:40 pm  Discussion

1:40 – 1:50 pm  Comparative Evaluation of the EVA VN-Test with a Commercial ELISA Test to Detect Antibodies Against the Major Envelope Protein (G_L) of Equine Arteritis Virus.

David Westcott

1:50 – 2:00 pm  Discussion

2:00 – 2:10 pm  Development of a Fluorescent Microsphere Immunoassay for Detection of Antibodies Specific to Equine Arteritis Virus and Comparison with the Virus Neutralization Test

Yun Young Go

2:10 – 2:30 pm  General Discussion

2:30 – 2:40 pm  Break

Session 3

Epidemiology of Equine Arteritis Virus Infection

Moderators: J. Richard Newton & Peter Timoney

2:40 – 2:50 pm  Studies on the Equine Viral Arteritis Situation in Bulgaria

Ivaylo Chenchev

2:50 – 3:00 pm  Discussion
3:00 – 3:10 pm  Epizootiology of Equine Arteritis Virus (EAV) Infection in the Horse Population in Poland
   
   Magdalena Larska

3:10 – 3:20 pm  Discussion

3:20 – 3:30 pm  Prevalence of Equine Arteritis Virus and other Infectious Microorganisms Causing Abortion among Mares in Hungary
   
   Ákos Hornyák

3:30 – 3:40 pm  Discussion

3:40 – 3:50 pm  French 2007 Equine Viral Arteritis Outbreak: Epidemiological and Pathological Features
   
   Aymeric Hans

3:50 – 4:00 pm  Discussion

3:50 – 4:00 pm  Adjourn

4:30 – 5:10 pm  Reception at Boone Faculty Center

5:10 pm  Return to the Hyatt Regency Hotel
Wednesday, October 15th, 2008

Session 3 (Cont.)

Epidemiology of Equine Arteritis Virus Infection

Moderators: J. Richard Newton & Peter Timoney

8:30 – 8:40 am  UK Breed Seroprevalence Figures: Proxies for Underlying Differences in Attitudes to EVA?
J. Richard Newton

8:40 – 8:50 am  Discussion

8:50 – 9:00 am  The Situation over the Diagnosis of Equine Viral Arteritis in Japan
Takashi Kondo

9:00 – 9:10 am  Discussion

9:10 – 9:20 am  Epidemiological Features of the 2006/2007 Multi-state occurrence of Equine Viral Arteritis in the USA
Peter Timoney

9:20 – 9:30 am  Discussion

9:30 – 9:40 am  Molecular Characterization of Equine Arteritis Virus Isolates Associated with the 2006/2007 Multi-State Disease Occurrence in the USA
Jianqiang Zhang

9:40 – 9:50 am  Discussion

9:50 – 10:00 am  Isolation of Equine Arteritis Virus (EAV) From Stallions Whose Semen was Imported into the United States, 1996-2007
Barry Meade

10:00 – 10:10 am  Discussion

10:10 – 10:20 am  Break

10:20 – 10:30 am  Serosurveillance of the US Camelid Population for Evidence of Equine Arteritis Virus Infection
Jessica Hennig
Session 4

National/International Control of Equine Arteritis Virus Infection

Moderators: Trevor Drew & Gian Luca Autorino

10:50 – 11:00 am  Equine Viral Arteritis: Current Status in Argentina
                  Maria Barrandeguy

11:00 – 11:10 am  Discussion

11:10 – 11:20 am  Equine Viral Arteritis Screening Methods to Ensure Disease-free Status of the Horse Population in Israel
                  Zvia Mildenberg

11:20 – 11:30 am  Discussion

11:30 – 11:40 am  Equine Viral Arteritis – an Australian Perspective, September 2008
                  Peter Kirkland

11:40 – 11:50 am  Discussion

11:50 – 12:00 pm  Approaches to prevent and control EVA in the United Kingdom in 2008
                  J. Richard Newton

12:00 – 12:10 pm  Discussion

12:10 – 1:00 pm  Lunch at Culton Suite

1:00 – 1:10 pm  Vaccination against Equine Viral Arteritis in Ireland
                Pat Lenihan

1:10 – 1:20 pm  Discussion

1:20 – 1:30 pm  Use of an Inactivated EVA Vaccine in the United Kingdom
                J. Richard Newton

1:30 – 1:40 pm  Discussion
1:40 – 1:50 pm  Adoption of a Centralised Information System for the Equine Viral Arteritis Control Programme in Italy: Results and Evaluation Relative to the 2004-2007 Breeding Seasons  

   Marcello Sala

1:50 – 2:00 pm  Discussion

2:00 – 2:10 pm  Critical Analysis of the Current National Control Programme for Equine Viral Arteritis in Italy  

   Gian Luca Autorino

2:10 – 2:30 pm  General Discussion

2:30 – 2:45 pm  Break

2:45 – 3:30 pm  Summing Up and Recommendations  

   N. Jim MacLachlan

3:30 – 3:55 pm  Closing Remarks  

   Beverly Schmitt  
   Trevor Drew  
   Takashi Kondo  
   Peter Timoney  

   Gene Pranzo  
   (The Dorothy Russell Havemeyer Foundation, Inc.)

3:55 pm  Workshop Adjourned

4:00 – 5:00 pm  Tour of the Gluck Center [optional]

5:00 pm  Return to the Hyatt Regency Hotel
The Role of the OIE in Assay Standardization

Beverly Schmitt
Vice-President, Biological Standards Commission, OIE
National Veterinary Services Laboratories (NVSL), Ames, Iowa

The OIE, also known as the World Organization for Animal Health, is an intergovernmental organization responsible for improving animal health worldwide. It is recognized as a reference organization by the World Trade Organization (WTO) and, as of May 2008, had a total of 175 Member Countries and Territories. The main objectives of the OIE are to promote transparency, scientific exchange, international solidarity, sanitary safety, promotion of veterinary services and food safety and animal welfare.

OIE standards are recognized by the World Trade Organization as reference international sanitary rules. The OIE standard for laboratories performing testing for animals and bees is the Terrestrial Manual of Standards which is the responsibility of the Biological Standards Commission (BSC). The Manual sets laboratory standards for all OIE listed diseases as well as several other diseases of global importance. Manuscripts are requested from specialists in each of the diseases or the other topics covered. Chapters are sent to scientific reviewers and to experts at OIE Reference Laboratories. They are also circulated to all OIE Member Countries for review and comment.

The Manual specifies those “prescribed tests” that are recommended for use in health screening for international trade or movement of animals. Alternative and prescribed assays in the Manual chapters are chosen by the BSC based on review of validation data and in discussion with experts in that particular disease field. Current prescribed tests for equine viral arteritis (EVA) include virus isolation from semen and virus neutralization (VN).

OIE reference laboratories for equine viral arteritis (EVA) include the Maxwell H. Gluck Equine Research Center, Dept of Veterinary Science, Lexington, Kentucky, the Epizootic Research Center, Equine Research Institute, Tochigi, Japan and the Veterinary Laboratories Agency, Weybridge, UK. As part of their reference laboratory mandate these laboratories are asked to provide reference assistance to countries worldwide and to be involved test standardization as needed or requested. The University of Kentucky reference laboratory is the source of the international reference sera for the EVA virus neutralization assay. The availability of this serum enables laboratories worldwide to standardize their EVA VN assays and to assure reliable results to trading partners.
Session 1

Detection and Identification of Equine Arteritis Virus
A one-step fluorogenic RT-PCR (5'-nuclease probe-based) assay using a primer/Taqman probe set designed against a highly conserved region of open reading frame 7 (ORF7) of the genome of equine arteritis virus (EAV) was developed for specific detection of the virus. The assay had an analytical sensitivity of detection of EAV in clinical samples or tissue culture fluid (TCF) of about 5 PFU per 100 μl. Following intramuscular injection of two ponies with the vaccine virus (modified Bucyrus strain), shedding of virus in the respiratory tract can be detected with greater sensitivity by the fluorogenic RT-PCR than by virus isolation. A total of 456 samples were detected by the fluorogenic RT-PCR of which 6 were positive; only 2 of these were positive by virus isolation. The one-step RT-PCR method specifically detected EAV in samples from Customs and quarantine departments more rapidly, more accurately, more conveniently based on the high sample throughout using our routine diagnostic procedures of ELISA and virus isolation in cell culture. Therefore the fluorogenic RT-PCR is seen as a valuable tool to complement the routine diagnostic procedures for the diagnosis of EAV infection.

Following antigenic analysis of the EAV GL protein, one pair of primers was designed, with which the gene fragment coding the highly antigenic domain of the EAV GL protein was amplified from the EAV genome. The cloned gene was digested with Bam HI and Xho I and then inserted into pET-32a, resulting in pET-GL1. The pET-GL1 was transformed into the host cell BL21(DE3) and conditions for expression were optimized including cultivation temperature and concentration of IPTG. The cloned GL1 protein was highly expressed and was confirmed antigenic by western-blot. The recombinant GL1 protein was purified by means of the His-Bind resin protein purification procedure. An indirect ELISA was developed to detect antibody against EAV with the purified GL1 protein as the coating antigen. Results from using this assay showed that the optimal concentration of coated antigen was 9.65 μg/mL and the optimal dilution of serum was 1:80. The positive cut-off of this ELISA assay is OD of the tested serum>0.4 and OD of the tested serum / OD of the negative serum>2.0. The iGL-ELISA was evaluated against the micro-virus neutralisation (VN) test. A total of 900 sera which were stored by our lab and which had been collected during horse entry/exit inspection were tested. There was 94.1% agreement between this ELISA and the VN test and based on this result, the iGL-ELISA was considered suitable for serological screening. In a further study, 180 sera were tested by iGL-ELISA and the commercial INGEZIM ELISA kit respectively. The agreement ratio between the two methods was 95.6%.
First Detection and Characterization of Equine Viral Arteritis in France during the 2007 Outbreak Illustrating the Importance of Molecular Tools for the Diagnosis of this Infection

Stéphane Pronost1, Fabien Miszczak1, Loïc Legrand1, Udéni Balasurya2, Christel Marcillaud-Pitel3, Aymeric Hans4, Stéphan Zientara5, Guillaume Fortier1

1Frank Duncombe Laboratory, 14053 Caen, France; 2Maxwell H Gluck Equine Research Center, University of Kentucky, Lexington, USA; 3RESPE (French Network of Equine Disease Epidemiometry) 14120 Mondeville, France; 4French Food Safety Agency (AFSSA) - Laboratory for Studies and Research on Equine Diseases (LERPE)-14430 Goustranville, France; 5French Food Safety Agency (AFSSA) - Laboratory for Studies and Research on Animal Diseases and Zoonoses (LERPAZ) - 94706 Maisons-Alfort, France

Since the beginning of the 1990’s, the RT-PCR has been proposed as an alternative to virus isolation for detection of equine arteritis virus (EAV), especially for screening of semen. A recent study clearly demonstrated that differences in sensitivity exist between the two methods, emphasizing the necessity for an evaluation of these methods and harmonization between different laboratories. Educating technicians and controlling materials and processes under a quality assurance programme will also help to obtain a reliable result. Different cases of EAV detection by qRT-PCR, which have been encountered in France, will be discussed 1) before the outbreak in summer 2007, 2) during the first two days of the outbreak (critical point) and 3) during the remainder of the outbreak period. Amplification steps adapted from Balasuriya et al. (2002) were performed using a SmartCycler (Cepheid) and the different Ct values observed are discussed in relation to the epidemiological context and the type of samples analysed.

1) Before the 2007 outbreak, analyses were performed on respiratory secretions, fetal tissues and semen. Only some semen samples gave positive results and the Ct value range was mainly between 21 and 32. These seldom presented any difficulty in interpretation.

2) The first three positive samples detected during the 2007 outbreak originated from two nasal swabs from two mares as well as from the organs of a stallion, all coming from two different premises. At this stage, no suspicion of EAV infection was present. The Ct values observed were 40, 41 and 42, respectively. However subtle characteristic PCR amplification curves were observed using an amplification program of 45 cycles by the technicians running the tests. Most of the diagnostic PCR kits distributed by different companies state that Ct values over 40 should be considered negative. The conclusion of these observations is that the results in this case could have been declared negative by another laboratory performing only 40 amplification cycles which is often the “historical reference value".
3) During the outbreak, 2 foals died and were confirmed positive for EAV infection; they had significantly high Ct values (21.9 and 24.6). On another premises, semen samples from 6 stallions were confirmed positive with Ct values between 19.8 and 33.3.

Concerning the characterization of the different strains of EAV isolated, there was often consensus in the results obtained by different laboratories. Nevertheless, we observed differences between certain labs. For example we used the forward primer described by Sekiguchi et al. (1995) and the reverse primer GTGGCTATAGTTATGTTCTTTACG designed in our laboratory for sequencing ORF5. Compared to the findings of a previous French study (Zhang et al., 2007), all the strains isolated during the 2007 outbreak formed a new cluster in the European subgroup 2. (For all complementary information concerning the French outbreak, see Hans et al. 2008)
Rapid Detection of Equine Arteritis Virus by a Reverse Transcription Loop-Mediated Isothermal Amplification Assay

Takashi Kondo, Manabu Nemoto, Koji Tsujimura, Takashi Yamanaka, Tomio Matsumura

Epizootic Research Center, Equine Research Institute, JRA, 1400-4 Shiba, Shimotsuke-shi, Tochigi, 329-0412 Japan

Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid detection technique that amplifies DNA with high specificity, efficiency and speed under isothermal conditions (60-65°C). The LAMP method requires a set of four primers that recognize six distinct regions within the target gene. The final products are stem-loop DNAs with several inverted repeats of the target DNA and cauliflower-like structures with multiple loops. The LAMP method requires only simple and cost-effective reaction equipment.

We have applied this method to detect equine arteritis virus (EAV) RNA. The primers were designed to amplify the regions within ORF6 and ORF7. Six EAV strains (Bibuna, Bucyrus, Kentucky84A-1, Red Mile, Vienna and Wroclaw) were used to develop and evaluate the specificity and sensitivity of LAMP method. Viral RNA was extracted from culture supernatant and was amplified with the Loopamp RNA Amplification Kit (RT-LAMP). The products were visually observed by agarose gel electrophoresis or addition of a fluorescence dye to the reaction mixture. A turbidimeter was also used for the real-time detection of the LAMP reaction.

Although the detection limit of viral nucleic acids differed among six EAV strains tested and depended on the primers tested, RNAs of all six strains tested were efficiently amplified. No amplification was observed when nucleic acids of viruses causing equine respiratory diseases other than EAV were amplified using EAV specific primers. Comparison of RT-LAMP and conventional RT-PCR assays will be presented. The RT-LAMP assay is useful not only for the rapid real-time detection of EAV in well-equipped diagnostic laboratories but also for naked-eye detection in the field.
Comparison of Two Real-Time Reverse Transcription Polymerase Chain Reaction Assays for the Detection of Equine Arteritis Virus Nucleic Acid in Equine Semen and Tissue Culture Fluid

Zhengchun Lu¹, Adam J. Branscum², Kathleen M. Shuck¹, Jianqiang Zhang¹, Edward J. Dubovi³, Peter J. Timoney¹, Udeni B. R. Balasuriya¹

¹Maxwell H. Gluck Equine Research Center, Department of Veterinary Science; ²College of Public Health, University of Kentucky, Lexington, Kentucky; ³Animal Health Diagnostic Center, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York.

Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA) in horses and other equids. Up to 10-70% of stallions infected with EAV may become carriers, and such persistently infected stallions transmit EAV to susceptible mares solely by the venereal route. Carrier stallions can be responsible for new outbreaks of EVA. The presence of EAV in the semen of carrier stallions is frequently detected by virus isolation (VI) in cell culture or by test breeding of stallions to two susceptible mares, both of which can be time consuming, expensive and cumbersome. In this study, we compared and validated two previously developed TaqMan® probe-based one-tube real-time RT-PCR assays (T1 and T2) for the detection of EAV nucleic acid in equine semen and tissue culture fluid (TCF). The specificity and sensitivity of these two molecular-based assays were compared to traditional VI in cell culture. The T1 real-time RT-PCR had a higher sensitivity (93.4%) than the T2 real-time RT-PCR (42.6%) for detection of EAV RNA in semen. However, the T1 real-time RT-PCR was less sensitive (93.4%) than the World Organization for Animal Health (OIE)-prescribed VI test (gold standard). Respective sensitivity of both PCR assays was high (100.0% [T1] and 95.2% [T2]) for detecting EAV RNA in TCF. In light of the discrepancy in sensitivity between either real-time RT-PCR assay and VI, semen that is negative for EAV nucleic acid by real-time RT-PCR should be confirmed free of virus by VI. Similarly, the presence of EAV in TCF samples that are VI-positive but real-time RT-PCR–negative should be confirmed in a one-way neutralization test using anti-EAV equine serum or by immunofluorescence assay using monoclonal antibodies to EAV. If the viral isolate is not identified as EAV, such samples should be tested for other equine viral pathogens. The results of this study underscore the importance of comparative evaluation and validation of real-time RT-PCR assays prior to their recommended use in a diagnostic setting for the detection and identification of specific infectious agents.
Detection of a Novel European Genotype of Equine Arteritis Virus in Slovenian Lipizzan Horses by One-Step MGB Real-Time RT-PCR Assay

Sara Mankoč¹, Peter Hostnik¹, Marjan Kosec¹, Darja Barlič-Maganja¹,²

¹University of Ljubljana, Veterinary Faculty, Gerbičeva 60, SI-1115 Ljubljana, Slovenia; ²University of Primorska, College of Health Care Izola, Polje 42, SI-6310 Izola, Slovenia

Recent developments in real-time PCR technology offer the potential to overcome many of the limitations of conventional PCR including improved sensitivity of equine arteritis virus (EAV) diagnostics. We have developed a one-step real-time RT-PCR with a MGB probe for the detection of different genotypes of EAV. A novel MGB probe and a reverse primer based on the multiple sequence alignment of 49 different EAV strain sequences of highly conserved ORF7 were designed. Although the ORF7 nucleotide sequences of Slovenian strains showed a high degree of nucleotide identity (97.1-98.3 %) with the most closely related Vienna strain, one mismatch was identified in seven Slovenian sequences obtained from semen samples from two persistently infected Lipizzan stallions in the region of the fluorescent TaqMan® probe used in the real-time RT-PCR developed by Balasuriya et al. (2002). For that reason, the length of our TaqMan® MGB probe was optimized to fit to a shorter more conserved region of different EAV strains that were chosen when setting up the real-time RT-PCR assay.

In this study, a total of 40 semen samples from Slovenian carrier stallions were tested by real-time RT-PCR assay for viral RNA. All 40 semen samples including two samples which were negative by classical RT-PCR were positive by real-time RT-PCR. The assay may also detect other strains of EAV as the sequence of the MGB probe was designed in silico to match the target sequences of different EAV strains.

In order to get an insight into the phylogenetic relationship of EAV strains circulating in Slovenia, a part of ORF 5 of Slovenian field strains was sequenced and compared with American and European strains selected from the GenBank database. All Slovenian isolates do not belong to any of the reported European subgroups; they form a new cluster, which has not been previously recorded in other phylogenetic studies based on the hypervariable regions of the GP5. All Slovenian sequences grouped together in an individual branch, supported by a bootstrap value of 100.

The deduced amino acid sequences of the Slovenian EAV strains were also compared with the Bucyrus reference strain. The alignment identified more than two unique amino acid changes at 11 positions in the neutralization sites C and D of some Slovenian EAV strains. When the
antigenic structure of the Slovenian strains was compared to the antigenic structure of members of the three main groups (EAV-1, EAV-2 and EAV-3), the Slovenian strains exhibited different antigenic features.

Based on analysis of sequence data coding a part of the ORF 1b and a part of the ORF 7, respectively, it was not clear whether Slovenian strains belong to any of the three large genetic groups or whether they have a unique phylogenetic position. However, the ORF 5 sequences of the virus strains present in the semen of Slovenian carrier stallions are more closely related to each other than to any other European or American EAV strain. Moreover, the phylogenetic analysis of ORF 5 showed that Slovenian EAV strains formed a distinct gene cluster.

__________________________

Equine Arteritis Virus – Virus Isolation (VI) and PCRs

David Westcott

Department of Virology Veterinary Laboratories Agency (Weybridge) New Haw, Addlestone, Surrey KT 15 3NB, UK

Abstract unavailable at time of compilation of program.
Session 2

Assays for Antibody Determination to Equine Arteritis Virus
Evidence that Use of an Inactivated EHV Vaccine Induces Serum Cytotoxicity Affecting the Equine Arteritis Virus Neutralisation Test

J. Richard Newton

Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, UK, CB8 7UU

Virus neutralisation (VN) is the current OIE prescribed test for the serological diagnosis of equine arteritis virus (EAV) infection. The Animal Health Trust (AHT) and other laboratories worldwide have increasingly experienced problems with this test when using the OIE Reference Laboratory prescribed rabbit kidney (RK-13) indicator cells, related to serum cytotoxicity. This cytotoxicity can be mistaken for viral cytopathic effect and has led to increasing difficulties in test interpretation, consequently causing disruption to both equine breeding and disease surveillance.

The hypothesis that a vaccine-induced anticellular antibody response against RK-13 cells causes this cytotoxicity was investigated by analysing vaccine histories from 93 randomly selected horses from which blood samples had been submitted for the EAV VN test. Of these sera 35 (38%) were ‘toxic’ and 58 (62%) were ‘non-toxic’.

Data analyses showed that statistically significantly greater proportions of horses with ‘toxic’ sera than with ‘non-toxic’ sera had been vaccinated with an inactivated equine influenza vaccine (PrevacPro™; 83% vs 50%; Fisher’s exact P=0.002) and/or an inactivated equine herpesvirus (EHV) vaccine (DuvaxynEHV1,4™; 100% vs 21%; P<0.001). When stratified by previous PrevacPro™ use, there remained a significant proportion of ‘toxic’ sera among horses administered DuvaxynEHV1,4™, but not vice versa. Further analyses showed

a) highly significant trend (P<0.00001) for increasing risk of toxicity with increasing numbers of doses of DuvaxynEHV1,4™ (0/1 dose, odds ratio (OR) = 1.0; 2-3 doses, OR = 42; 4-7 doses, OR = 52; ≥8 doses, OR = 96)

b) significant (P<0.001) positive linear relationship between toxicity titre and number of previous doses (X₁) of DuvaxynEHV1,4™ (Log₁₀ titre = 0.07 + 0.19X₁; R² = 55.4%)

c) significant (P<0.001) non-linear decay in toxicity titre with number of days since the last dose (X₂) of DuvaxynEHV1,4™ (Log₁₀ titre = 2.16 – 3.85[X₂/1000]⁰·⁵ + 1.71[X₂/1000]; R² = 37.5%)

The results from these field-derived data strongly suggest that EAV VN serum cytotoxicity is associated with use of a tissue culture-derived EHV vaccine and as such are consistent with demonstrable development of serum
toxicity with second and third doses of vaccine in the 9 experimental ponies used in the original evaluation of DuvaxynEHV1,4™ for protection against viral abortion. It is important to note, however, that there are no implications to date from these findings relating to any adverse effects in horses arising from use of DuvaxynEHV1,4™. The AHT continues to strongly recommend, in accordance with the HBLB Codes of Practice, use of both EHV vaccination to aid in prevention of viral abortion and pre-breeding and post-import serological testing of horses for EAV. The AHT now uses an in-house ELISA based serological assay for its first line screening test, which has the advantage of more rapid turnaround as well as fewer problems with serum cytotoxicity.

_______________

Serological Testing for Equine Arteritis Virus in Ireland 2003 to 2008

Ann Cullinane, Maura Nelly, Hannah Patterson, Rachel Kenna, Sarah Gildea
Irish Equine Centre, Johnstown, Naas, Co. Kildare

In 2003 there was serological evidence that Ireland had experienced its first incursion of equine arteritis virus (EAV) into the Thoroughbred population. The virus was not isolated and no semen shedders (carrier stallions) were identified. Since 2003, in excess of 80,000 serological tests have been performed for EAV. No serological evidence of the virus circulating in the Irish horse population has been found since 2003. The prevalence of seropositive horses in Ireland, the measures taken to counteract cytotoxicity in the virus neutralization test and evaluation of the sensitivity and specificity of a peptide-based ELISA will be discussed.
Evaluation of a Method to Reduce the Problem of Cytotoxicity in the Virus Neutralization Test during the 2008 Breeding Season and an Update on Use of an ELISA in France

L. Legrand¹, P. H. Pitel¹, G. Fortier¹, A. Hans², S. Pronost¹

¹Frank Duncombe Laboratory, 14053 Caen, France; ²French Food Safety Agency (AFSSA) - Laboratory for Studies and Research on Equine Diseases (LERPE)-14430 Goustranville, France

For a number of years, several laboratories in the world have been confronted with the problem of serum cytotoxicity in the equine arteritis virus neutralization test (EAV VNT). This phenomenon was also observed in our own laboratory and, from January to June 2007, serum cytotoxicity was present in approximately 7.4% of 15,000 samples received for examination. We previously described a method that resulted in a reduction in this problem. This study was carried out on 200 cytotoxic sera analyzed according to the VNT protocol described by the OIE and stored at -20°C. Only 11% of these sera were found to be cytotoxic using our method. To test these samples, a 24h-preformed monolayer of RK13 cells was used. The serum/virus mixture was removed after four hours and replaced by growth medium. Thus, for the 2007 breeding season, the modification of our testing technique would have enabled us to reduce considerably the frequency of cytotoxicity, from 7.4% to 0.9%. Comparative testing was performed between our laboratory and the AFSSA-LERPE to evaluate our method. The results revealed some lack of sensitivity. The protocol was further adjusted by working only with confluent monolayer cells and with the results being read at 72h. This resulted in improvement in test sensitivity. This method continued to be evaluated during the 2008 breeding season. From February to June 2008, 15,513 sera were tested by the VNT and only 21 (0.13%) were cytotoxic. This method allowed us to obtain a VNT result, which is the only method both recommended by the World Organization Animal Health (OIE) and recognized by every country and organization.

In France, an ELISA test is used when the result of the VNT can not be interpreted. During the 2008 breeding season, the warmblood studbook allowed us to check the antibody status of horses by ELISA even though a VNT had already been performed at the beginning of the season. This method has the advantage of being faster and unaffected by cytotoxicity. However, it appears to be less sensitive than the VNT as well as giving some false positive results. Nevertheless, the ELISA has been used to test sera previously analyzed by the VNT during the “French outbreak” in 2007 and all of the horses were found to be positive. This test seems to be suitable when EAV has recently circulated in an equine population but is less efficient in cases of more long-standing infection. We are trying to improve the ELISA in
order to detect low positive sera and to reduce the number of false negatives.

While these two methods allowed us to obtain serological results, only the VNT is fully reliable in evaluating the serological status of horses.

Observations on Equine Sera Giving Borderline Positive Results in the Virus Neutralisation Test for Equine Arteritis Virus (EAV)

Reinhard Böse, Julia Ehinger, Catherine Thorud-Engelke

Labor Dr. Böse GmbH, Carl-Zeiss-Str. 6, 31177 Harsum, Germany

The complement-enhanced virus neutralisation test (VN test) is still the only "prescribed test for international trade" for antibody detection as laid down in the "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2008" issued by the World Organisation for Animal Health (OIE). Therefore, standardisation of the VN test between laboratories involved in international horse trade is of great importance. In the past, particularly, borderline positive results have led to discrepancies between export and import testing.

During 2007, a total of 7346 sera from horses were submitted to our laboratory for determination of antibodies to EAV by VN test for the purpose of breeding and international trade. The VN test was performed in accordance with the OIE protocol; titers were expressed referring to the initial dilution, i.e. after addition of virus, before addition of cell suspension.

From the sera tested, 6340 (~86.3%) had no detectable neutralising antibodies (titers of <1:2), 193 sera (~2.6%) were tested 1:2, 72 sera (~1.0%) were tested 1:4 and 741 sera (~10.1%) showed titers of 1:8 or higher.

The 72 sera with titers of 1:4 were reported as borderline positive and repeat samples requested; these samples were received for 38 cases. Of these, sera from 11 horses showed an increase in titer to 1:8 or higher and are therefore likely to represent true positive results; 2 sera showed a decrease in titer to 1:2 or less and likely represent false positive results upon initial testing. Repeat samples from 25 horses showed no change, i.e. initial results of 1:4 were confirmed upon testing of repeat samples. Thus, these cases could not be resolved: based upon initial and repeat results these sera might be truly positive, possibly due to old infection or represent false positive results caused by factor(s) unknown.
Comparative Evaluation of the EVA VN-Test with a Commercial ELISA Test to Detect Antibodies Against the Major Envelope Protein (G_L) of Equine Arteritis Virus

David Westcott, Mark Horigan, Falko Steinbach, Trevor Drew

Department of Virology Veterinary Laboratories Agency (Weybridge) New Haw, Addlestone, Surrey KT 15 3NB, UK

At present the standard OIE equine arteritis virus (EAV) serological test for the detection of antibody to EAV is the VN-test. However, this test can be expensive to screen large numbers of sera, requires RK13 cell cultures, can be difficult to read end points with toxic sera, and takes three days before a result is obtained. Therefore, to eliminate these problems, a commercial enzyme-linked immunosorbent assay (ELISA) was evaluated for the detection of antibodies against the major envelope glycoprotein (G_L) of EAV. The ELISA was evaluated and compared against 500 VN-positive and 500 VN-negative EAV sera. This ELISA test has been used in several laboratories in the UK and France, and good comparative ELISA results with VN-titres have been reported.
Development of a Fluorescent Microsphere Immunoassay for Detection of Antibodies Specific to Equine Arteritis Virus and Comparison with the Virus Neutralization Test

Yun Young Go1, Susan J. Wong2, Adam Branscum3, Valerie L. Demarest2, Kathleen M. Shuck1, Mary L. Vickers4, Jianqiang Zhang1, Peter J. Timoney1, Udeni B.R. Balasuriya1

1Maxwell H. Gluck Equine Research Center, Department of Veterinary Science, 3College of Public Health, and 4Livestock Disease Diagnostic Center, University of Kentucky, Lexington, Kentucky 40546-0099, USA;2Wadsworth Center, New York State Department of Health, Albany, New York 12201, USA

Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA), a respiratory and reproductive disease of horses. Currently, diagnosis of EAV infection is based on detection of the virus either in cell culture or by real-time reverse transcription polymerase chain reaction assay, or by serologic testing of paired (acute and convalescent) sera. The virus neutralization test (VNT) is the OIE prescribed test for international trade for detection of antibodies to EAV. The assay is used for diagnosis, surveillance, trade and pre-vaccination monitoring purposes. Although highly sensitive and specific, the assay is expensive, labor intensive and time consuming. To overcome the problems associated with the VNT, a microsphere immunoassay (MIA) was developed to detect equine antibodies to the major structural proteins of EAV. This was based on cloning and expression of full-length individual major structural proteins (GP51-255; M1-162; and N1-110) as well as the partial sequence of each structural protein (GP51-116; GP575-112; GP555-98; M88-162; and N1-69) that comprise putative antigenic regions of these proteins. Purified recombinant viral proteins expressed in E. coli were covalently bound to fluorescent polystyrene microspheres and analyzed with the Luminex xMap 100 instrument. Of the eight recombinant proteins, the highest concordance with the VNT results was obtained with the partial GP555-98 protein. The latter assay was validated by testing a total of 2,500 equine serum samples previously characterized in the VNT. With use of an optimal cutoff value of 992 mean fluorescence intensity (MFI), the sensitivity and specificity of the assay were 92.6% and 92.9%, respectively. The GP555-98 MIA and VNT test outcomes correlated significantly (r = 0.84; p-value<0.0001). Although the GP555-98 MIA is less sensitive than the standard VNT, it has the potential to provide a rapid, convenient, and more economical test for screening equine sera for the presence of antibodies to EAV, with the VNT being used as a confirmatory assay.
Session 3

Epidemiology of Equine Arteritis Virus Infection
Before 1999, we did not carry out any laboratory investigations into the presence of antibodies to equine arteritis virus (EAV) nor for the presence of viral antigen. Since 2002, we have performed regular serological investigations on stud farms and riding clubs in Bulgaria. In May 2002, we estimated the seropositive rate on each of four stud farms at 40%; this percentage increased up to 82% in 2007. During the period from 2002 to 2007, we carried out detailed screening of all stallions and mares to estimate the prevalence of antibodies to EAV.

The aim of these studies was to determine the extent of EAV infection in the equine population in Bulgaria.

The first case of reproductive failure and a significant respiratory syndrome in equines was observed in February 2002 on a horse-riding club having 30 animals. The same picture was observed in the spring and summer of 2002 on several other premises of the same type. Epizootiological investigation confirmed that horses on these premises had been vaccinated against equine herpesvirus and equine influenza. The results from the serological investigation of possible herpesviral infections and influenza were negative. The stallions and mares involved had not taken part in any competitions in the previous year. In 2000 and 2001, it was established that some semen had been imported from stallions and stallions had bred with mares from different part of neighboring regions. The outcome of infection with EAV is determined by the strain and infectious dose of the virus, the age and physiological condition of the infected horse, the environmental conditions and the route of infection. Clinical signs of disease included: fever, anorexia, depression, oedema of the limbs, conjunctivitis with lacrimation, rhinitis and nasal discharge, urticaria of the head, neck and trunk, and leucopenia. Occasionally, infection of pregnant mares may result in abortion during the first four months of gestation. This can occur 10 - 30 days after virus infection even if no clinical signs are noted in the mare. The first clinical signs observed were depression and anorexia and next, conjunctivitis with tearing from the medial canthus. The body temperature increased to approximately 40-40.2°C. Problems with estrus have been observed. The percentage of cases of abortion increased to 18%. Abortions were mostly in mares during the first part of gestation and then recovered without treatment. In some animals dermatitis was noted. Sick animals were retarded in growth and frequently experienced secondary bacterial infections, such as colienteritis, salmonellosis, and pneumonia. Pathological
changes included: cyanosis of skin; typical lesions of interstitial pneumonia with multiple nodular tumor masses in the lungs which were pink to bloody-red in color. The trachea and bronchi contain frothy fluid. On occasion, changes were found in the lungs that were caused by other infectious processes.

The diagnosis of EAV infection was confirmed by ELISA, virus neutralization test and based on negative results for other viral and bacterial equine infections.

EVA has to be considered as a major problem of veterinary and economic importance in Bulgaria.

Based on the study of the disease in horses in Bulgaria, our results would indicate that EAV infection occurs and is probably the result of using virus-infective semen imported into the country.
Epizootiology of Equine Arteritis Virus (EAV) Infection in the Horse Population in Poland

Magdalena Larska, Jerzy Rola
National Veterinary Research Institute, Virology Department,
Al. Partyzantow 57, 24-100 Pulawy, Poland

The epizootiology of equine viral arteritis (EVA) in horses in Poland was analysed based upon the results of serological surveillance carried out on both national and private horse studs and a phylogenetical study of EAV strains isolated from persistently infected stallions from 19 different locations. A total of 7903 equine sera were tested between 1998 and 2005 and EAV antibodies were found in 18.2% of the animals tested. In 1998-2000, the percentage of seropositive horses was 22.1%, over the next three years it decreased to 12.2 - 15% and then it started to increase dramatically, reaching over 30% in 2004-2005. The possible cause of the increase in percentage of EAV infections was the amending of the regulations concerning the health control of stallions at the reproduction centers that repealed the requirement to test stallions for EAV. The size and type of the stable, age, gender and manner of horse use were found to be the most significant factors in EAV distribution among horses. The highest percentage of seropositive animals was found in large national studs (38.2%) and in national stallion studs (31.5%), whereas only 10.6% of horses originating from small studs had antibodies to EAV. The percentage of seropositive animals among tested stallions was 12.1%, whereas it was considerably higher in mares reaching 39.0%. The percentage of seropositive horses increased proportionally to the age of the animals tested. A high percentage of positive animals, over 30%, was found among Hucul, Wielkopolski, Pure Arabian and Angloarabian breeds.

Phylogenetic analysis was performed on 44 Polish isolates of EAV that were recovered from the semen of stallions from 19 different studs and stallion depots. These isolates were compared to 41 reference EAV strains commonly used in phylogenetic studies of this virus. On the basis of the nucleotide sequence analysis of the ORF5 gene encoding GP5 glycoprotein, it was found that the Polish EAV isolates belonged to two subgroups, showing closest relationship to other European strains. Similar results to those were obtained based upon the nucleotide sequences of the ORF7 gene. The homology of ORF5 nucleotide sequences of all Polish isolates ranged from 80.1 – 99.0%, whereas the homology of the ORF7 sequences fell within the range of 93.6 – 100%. Additionally, ORF5 sequence analysis proved to be helpful in a retrospective epizootiological investigation. Moreover, computer comparison of the hydropathy and antigenicity profiles of GP5 protein showed significant differences at the sites of the neutralization ectodomain among the EAV isolates tested.
Prevalence of Equine Arteritis Virus and Other Infectious Microorganisms Causing Abortion among Mares in Hungary

Ákos Hornyák¹, Gyula Balka², Tamás Bakonyi¹, Levente Szeredi³, Miklós Rusvai³

¹Department of Microbiology and Infectious Diseases, and ²Department of Pathology and Forensic Veterinary Medicine, Faculty of Veterinary Science, Szent István University, Budapest, Hungary; ³Central Veterinary Institute, Budapest, Hungary

Over the past year, the health control for 2000 mares was managed by our team at the Faculty of Veterinary Science, Budapest. These animals were kept on different studs or farms, and their gynecological and pregnancy care was organized and managed on a common basis. Out of 49 recorded abortions only 1 (2.0%) proved to be caused by equine herpesvirus 1 (EHV-1), bacterial abortion was detected in 8 (16.3%) cases; in 4 (8.1%) cases pathogenic Leptospira spp. played a role in causing abortion, in the other 4 cases Mycoplasma spp. were implicated. Equine arteritis virus (EAV) was not detected in any of the specimens. Although the overwhelming majority of the abortions were non-infectious, sporadic cases, the low rate of viral abortion can be explained by the effectiveness of control and preventive strategies like serological surveillance, quarantine regulations, vaccination programs and rapid diagnosis of the abortion cases. The very low rate of EHV-1 abortion may be attributed to the regular vaccination programs applied on all farms managed by our group. Comparative analysis of the aforementioned period with an earlier six year-period revealed that both of the two major viral diseases that can cause abortion and perinatal foal losses, EAV and EHV-1, are manageable. Between 1998 and 2003 according to our earlier publication, out of 248 aborted foals EAV was detected on 4 (1.6%) occasions. This compared with 26 (10.5%) cases proven to be caused by EHV-1. In each case, the results were confirmed by 3 different laboratory methods; virus isolation, PCR and immunohistochemistry (IHC). The striking difference between the incidence of the two abortigenic viral agents confirmed that EAV was of marginal importance in Hungary compared to EHV-1. Additionally, both viral diseases can be managed by appropriate rules and regulations, together with implementation of a vaccination program. In the case of EAV, compulsory vaccination was prescribed by the Hungarian veterinary authority with respect to all breeding stallions in 2003. The consequence was a marked decrease in EVA positive laboratory results even from semen samples. Since 2005, only three specimens were diagnosed EAV positive: two semen samples, one of them originating from a newly imported stallion, and one from an abortion case. Regular vaccination can also significantly reduce EHV-1 abortion storms on the managed farms. In the last breeding season, only one case of EHV-1 abortion occurred on a poorly managed farm. In contrast to these farms, the average rate of EHV-1 abortion in Hungary over the past 30 years has fluctuated between 10 and 17%.
French 2007 Equine Viral Arteritis Outbreak: Epidemiological and Pathological Features

A. Hans¹, D. Gaudaire¹, J. Tapprest¹, E. Guix¹,², C. Marcillaud-Pitel², L. Legrand³, S. Pronost³, G. Fortier³, C. Laugier¹, S. Zientara⁴

¹French Food Safety Agency (AFSSA) - Laboratory for Studies and Research on Equine Diseases (LERPE) - 14430 Goustranville, France; ²RESPE – Institut de Pathologie du Cheval – 14430 Dozulé, France; ³Animal Health Department Frank Duncombe Laboratory, 1 route du rosel – 14053 Caen Cedex 4, France; ⁴French Food Safety Agency (AFSSA) - Laboratory for Studies and Research on Animal Diseases and Zoonoses (LERPAZ) - 94706 Maisons-Alfort, France

During the spring and summer of 2007, an equine viral arteritis (EVA) outbreak occurred in the Normandy region of France. This outbreak was the most important since the one of 1994, and was characterized by rapid viral spread in the equine population and by the severity of clinical signs.

The first cases of EVA were diagnosed in a stud of draught horses in June 2007 and the last case was reported in August 2007. During the summer, important measures were taken to prevent further viral spread in the horse population. Indeed, several horse breeding meetings were cancelled over the summer and a veterinary certificate was required of each participant before participating in any horse show. Those measures helped to restrict further spread of the disease.

During this outbreak, 30 premises were reported affected. Exposure to the virus occurred through artificial insemination, by direct contact between sick horses or by indirect contact (human). In total around 200 horses were infected with the virus and clinical signs associated with infection ranged from oedema, rashes, nasal discharge to death of foals and abortion. About 80 horses exhibited clinical signs during this outbreak, of which 8 died. At necropsy, it has been found that the death of 5 out of 8 horses, was due to equine arteritis virus infection (4 foals and 1 abortion). It is the first time that foal deaths were reported during an EVA outbreak in France.

A large number of stallions have been tested; more than 30 virus isolates coming from semen, which were identified by PCR (see Pronost et al. abstract for more detail). The chronology of the French 2007 outbreak, associated pathology as well as its epidemiological features will be covered in this presentation.
Attitudes to control of EVA within Europe could simplistically be thought of as being polarised between those that effectively have ‘zero tolerance’ of it and therefore all reasonable measures are taken to maintain infection/disease freedom and those that do not recognise a clinical problem and are satisfied to ‘live and let live’. It is important to note, however, that such polarisation of views is not necessarily along national or regional lines but more likely represents different strata of the European equine industry. This complexity is probably best evidenced by the differences in level of EVA control adopted for example between the UK, with its statutory EVA Order and Thoroughbred predominance and elsewhere in Europe where other breeds predominate that do not employ such powers. Closer examination shows that the Thoroughbred breeding industry across Europe vigorously adopts a Common Code of Practice for the disease, whereas other breeds do not. It may be argued that this difference in attitude is evidenced through the breed specific seroprevalence data from a cross sectional surveillance study conducted in the UK in the mid 1990’s (Figure 1).

Results show that the highest EAV seroprevalence (25%) was amongst Standardbred breeding animals, which was related to a particularly high prevalence (41%) in American-bred shedding stallions but did not apparently result in clinical signs of EVA. There was also an increased prevalence of EAV seropositivity in some non-indigenous breeds that were imported from mainland Europe. There was a very low level of seropositivity (<0.3%) among Thoroughbreds. The study demonstrated that 18 of 50 seropositive stallions that were identified in the UK in 1994 and 1995 and 5 of 9 confirmed EAV shedding stallions, were non-Thoroughbreds that originated from European Union countries. This has been supported in recent years in that several non-Thoroughbred stallions that have been transiting the UK during export from mainland Europe to destinations outside the European Union have been detected as positive for EAV during quarantine in England and this has invoked statutory powers of control through the EVA Order 2005.

See Figure 1 next page.
Figure 1: EAV seroprevalence (%) for different breeds and types of Standardbred horses in the UK (data from 1995 seroprevalence survey)
Historically, there has been no clinical and serological evidence of equine viral arteritis (EVA) in Japan. The infection free status of Japan has been maintained and confirmed by a strict quarantine system and seroepidemiological surveillance. Serological diagnosis of EVA is carried out using the virus neutralization (VN) test and the ELISA. The ELISA, in which 6xHis-N-G\textsubscript{L} fusion protein expressed by a recombinant baculovirus system is the antigen, is used for the initial screening of horse sera. The VN test is used as the definitive test for the diagnosis of EVA. The VN test procedure is almost the same as that described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE 2004). In recent years, several thousands of horses have been imported from foreign countries every year. All imported horses are required to be tested for EVA during the quarantine period by the Animal Quarantine Services (AQS) of the Ministry of Agriculture, Forestry and Fisheries (MAFF). From 1991 to 2007, evidence of equine arteritis virus infection was serologically confirmed in 52 horses and these horses were denied entry.

We have performed serological surveillance in horses including stallions, breeding mares bred to newly imported stallions with a history of EVA vaccination and pregnant mares that aborted on Hokkaido island, the major breeding area for Thoroughbred horses in Japan every year. During the last ten years, a total of 1627 horse sera were tested and no EVA antibodies were detected among horses without an EVA vaccination history. The majority of horses which were vaccinated before importation into Japan have antibodies to equine arteritis virus.

In Japan, four diagnostic laboratories (one in AQS, two in the National Institute of Animal Health, and our institute) have the ability to diagnose EVA. Comparative studies have been carried out to standardize the VN test procedure and to evaluate the quality of testing undertaken by each laboratory. The results of these studies indicated that the quality of testing by all four laboratories appeared to be satisfactory.
The infrequent occurrence of recorded outbreaks of equine viral arteritis (EVA) in the US over the years has been responsible for much of the complacency with which this disease has been viewed by many horse owners, breeders, and even veterinarians. Prior to 2006, EVA had never been reported in the American Quarter horse breed, the single most numerous equid population in the country. That changed dramatically in 2006, however, with widespread occurrence of the disease primarily in American Quarter horses, but also in horses of several other breeds. There was confirmed evidence that equine arteritis virus (EAV) spread to a total of 10 states, with New Mexico and Utah having the greatest number of affected premises. Secondary and tertiary transmission of infection from primary affected premises occurred in Utah and also possibly in New Mexico. A total of 69 direct exposures were identified of which 48 (69.5%) were mares inseminated with shipped fresh-cooled semen from a single infected stallion on the index premises in New Mexico, with the remaining 21 (30%) involving mares and foals that visited the same index premises. With the exception of AL, all of the affected states (CA, CO, ID, KS, MT, NM, OK, TX and UT) were in the Western Region. The overall clinical attack rate was variable with no fatalities reported in foals or older horses. Abortion was a notable feature on certain farms, with abortion rates as high as 55% being recorded. The gestational age of aborted fetuses ranged between 90 and 214 days. The carrier state was established in a variable percentage of infected stallions. Over the course of the outbreak, a total of 23 premises in New Mexico involving an estimated 1,081 horses were placed under official or voluntary quarantine. Some 21 premises were considered affected in Utah with 591 horses placed under official quarantine. There was a very high seroprevalence (≥90%) of antibodies to EAV on many affected farms, confirming efficiency of virus spread by the respiratory route, under the conditions of close physical contact between horses that existed on these premises. Equine arteritis virus was readily detected in aborted fetuses, blood leukocyte cultures, serum and semen from infected stallions. Inadequate supplies of the modified live viral vaccine against EVA (ARVAC®) was considered a significant impediment to curtailing spread of the disease in 2006/07. Also, the absence 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 infection in certain states during this occurrence.
Molecular Characterization of Equine Arteritis Virus Isolates Associated with the 2006/2007 Multi-State Disease Occurrence in the USA

Jianqiang Zhang, Kathleen M. Shuck, Gong Seoul, Yun Young Go, Zhengchun Lu, Barry J. Meade, David G. Powell, Peter J. Timoney, Udeni B.R. Balasuriya

M.H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546-0099

In 2006/2007, a multi-state occurrence of equine viral arteritis (EVA) was confirmed for the first time in Quarter horses in the State of New Mexico (NM) and 5 other states (UT, AL, OK, KS, and MT). Furthermore, circumstantial evidence of equine arteritis virus (EAV) infection was found in horses in 4 additional states (CA, CO, ID, TX). Over the duration of the 2006/2007 occurrence of EVA, every effort was made to collect tissues from aborted fetuses, blood from acutely infected horses and semen samples from stallions that became virus shedders during the outbreak. A total of 108 isolates of EAV were obtained from NM, UT, KS, OK, ID, CA and TX, including 71 sequential isolates from the semen of 15 carrier stallions, 13 isolates from aborted fetuses, 18 isolates from peripheral blood mononuclear cells and 6 isolates from serum. This collection of strains constitutes a unique resource with which to investigate the origin and spread of EAV during the 2006/2007 EVA occurrence and to study the evolution of EAV during separate outbreaks and during carriage of the virus in stallions.

Viral RNAs were extracted from 52 EAV isolates (including 15 isolates from the semen of 15 carrier stallions, 13 isolates from aborted fetuses, 18 isolates from peripheral blood mononuclear cells and 6 isolates from serum). RT-PCR amplification and nucleotide sequencing of the structural protein genes (ORFs 2-7) and the 3’ untranslated region (UTR) were performed. All 50 isolates obtained from NM, UT, KS, OK and ID had 99-100% nucleotide identity to each other (based on ORFs 2-7 and 3’UTR) and all of them contained an identical 12 in-frame nucleotide insertion in ORF3, encoding the structural protein GP3. Such an insertion was absent from all previously sequenced EAV strains either reported in the scientific literature or deposited in GenBank. These findings are highly indicative that EVA occurrence in these states was initiated by the same virus strain. In contrast, two EAV isolates, obtained from TX and CA, had only 89-90% nucleotide identity to the outbreak isolates and did not contain the characteristic 12 nucleotide insertion in ORF3, confirming that the EAV infections in TX and CA were unrelated to the major occurrence involving the other states.

In late 2005, an outbreak of EVA was diagnosed retrospectively on another large Quarter Horse breeding farm in NM. The nucleotide sequences of ORFs 2-7 and 3’UTR of four EAV isolates obtained in connection with the
2005 outbreak (one isolate from the placenta of an aborted fetus on December 11, 2005 and 3 sequential isolates collected from one carrier stallion on November 8, 2005, January 12, 2007, and January 29, 2008, respectively) were determined and compared to those of the 2006/2007 isolates. Interestingly, these four isolates had 99-100% nucleotide identity to the isolates made in 2006/2007 and all four contained the unique 12 nucleotide insertion in ORF3. These findings provide strong evidence that the 2005 outbreak may well have been the source of virus for the 2006/2007 multi-state occurrence of EVA. For this to have been the case, EAV would most likely have had to have been spread through the movement of an infected mare or the use of infective semen.

Phylogenetic analysis based on the nucleotide sequences of ORF5 encoding the structural protein GP5 clearly demonstrated that EAV isolates from both the 2005 outbreak and the 2006/2007 multi-state occurrence of EVA are phylogenetically very closely related to each other. The isolates belong to the European subgroup-1. However, they form a separate cluster and are distinctly different from any previously sequenced strains of EAV either reported in the scientific literature or deposited in GenBank.
Isolation of Equine Arteritis Virus (EAV) From Stallions Whose Semen was Imported into the United States, 1996-2007

Barry Meade1, Peter Timoney2, Udeni Balasuryia2, Kathy Shuck2

1United States Department of Agriculture, Animal Plant Health Inspection Service (USDA-APHIS), Frankfort, Kentucky; 2Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546-0099, USA

Import permit data obtained from the United States Department of Agriculture (USDA), Animal Plant Health Inspection Services (APHIS), Veterinary Services (VS) Import/Export database for the years 1996 through 2007 were compared to information abstracted from sample submissions recorded by the Office International des Epizooties (OIE) Equine Viral Arteritis (EVA) reference laboratory at the University of Kentucky, Gluck Equine Research Center.

From 1996 through 2007, a total of 3,779 permits were issued for the purpose of importation of processed, frozen or fresh cooled, equine semen into the United States (US). Of these, 483 were issued for stallions which resided in the European Union (EU). During this time frame, a total of 246 imported semen samples from 151 individual stallions were submitted to the OIE laboratory at the University of Kentucky for EAV examination by attempted viral isolation in cell culture. Seventy-three percent (180/246) of the samples were submitted from February through May and all originated from stallions residing in seven countries within the EU. Ninety-three percent (230/246) of samples were submitted from stallions residing in Germany and the Netherlands; these two countries also had the largest number of EAV positive stallions. Specimens were obtained from eleven broadly categorized breeds, of which 51.2% (126/246) were designated as European warmblood stallions. Of the 78 import permits from which samples were evaluated at the Gluck Center, 26 (33.3%) had at least one EAV positive stallion within the batch of samples submitted.

Comparisons of import permits with at least one EAV positive semen sample to the total number of permits evaluated by the Gluck Center suggest that there is a decreasing trend that is statistically significant (p < 0.007) in the risk of isolating EAV from foreign origin semen. As an example, for the most recent five-year period 2003 through 2007, the risk for a single import permit having at least one EAV positive sample is 2.3 times less than for the period 1996 through 2000. The basis for this trend is undetermined. It could be due to an actual decrease in the number of EAV positive stallions whose semen is imported to the US; or perhaps the stallions are being evaluated at other laboratories, including those in the EU.
A comparison of the number of import permits issued to the historical rate of exchange of the British Pound to the US dollar suggests that economic factors may also influence the volume of foreign origin equine semen imported into the US.
Serosurveillance of the US Camelid Population for Evidence of Equine Arteritis Virus Infection

J. Hennig\textsuperscript{1}, P. Timoney\textsuperscript{1}, K. Shuck\textsuperscript{1}, U. Balasuriya\textsuperscript{1}, R. Baker\textsuperscript{2}, L. Williamson\textsuperscript{3}, A. Niehaus\textsuperscript{4}

\textsuperscript{1}M. H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546-0099; \textsuperscript{2}Veterinary Diagnostic Laboratory, Oregon State University, Corvallis, OR 97339-0429; \textsuperscript{3}The University of Georgia, College of Veterinary Medicine, Athens, GA 30602-7385; and \textsuperscript{4}College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210

Equine viral arteritis (EVA) is a contagious viral disease of equids. Evidence of infection with the causal agent equine arteritis virus (EAV) has been found not only in horses but also in other members of the family \textit{Equidae} such as ponies, donkeys, mules and zebras. Viral spread is through direct or indirect contact with infective respiratory secretions and through the semen of carrier stallions. Serological evidence of EAV infection has been found in many countries throughout the world. It is an important factor when considering importing and/or exporting stallions or semen as it can have significant economic repercussions for an industry if certain testing and management requirements are not adhered to.

The objective of this study was to explore the possible exposure of members of the family \textit{Camelidae} specifically, Alpacas and Llamas, within the United States to determine if there was evidence of EAV infection and if so, what its clinical significance might be.

The serological assay used was the virus neutralization test (VNT). A total of 650 camelid serum samples (alpacas and llamas) have so far been screened for evidence of EAV infection. Firstly, sera to be screened were heat inactivated and tested in 96-well plates at 1:4 and 1:8 dilutions, with known positive and negative equine sera serving as controls. Any positive camelid samples were then retested by titrating them out to determine their respective end-points. Out of 650 camelid serum samples tested to this point, 6 tested positive and all had low antibody titers; two were positive at 1:8 (female alpaca from Oregon with no history of abortion and one camelid with no known demographics) and four were positive at 1:4 (a 3 year old female alpaca from Washington state with no history of abortion, a 7 year old male alpaca, and 2 camelids with no known demographics).

Less than 1\% of camelids tested were positive for antibodies against EAV. Although the prevalence of infection is very low, veterinarians, alpaca and llama owners and breeders should be aware that EAV can infect both species. Notwithstanding the absence of any information on the possible clinical significance of this finding, it would seem prudent to recommend avoiding direct contact between alpacas and llamas and horses or other equid species on farms on which both are kept, until more is known about the outcome of EAV infection especially in pregnant alpacas and llamas.
Session 4

National/International Control of Equine Viral Arteritis
Equine Viral Arteritis: Current Status in Argentina

A. Vissani1, M. Iglesias2, M.G. Echeverría3, S. Tordoya1, J. La Torre2, G. Metz3, L. Becerra1, S. Serena3, C. Olguin Perglione1, M. Barrandeguy1

1Instituto de Virología, CICV, INTA-Castelar, CC 25, Castelar 1712, Buenos Aires, Argentina; 2CEVAN CONICET; 3Cátedra de Virología, Facultad de Ciencias Veterinarias UNLP

Equine viral arteritis (EVA) is considered a notifiable disease by OIE. In Argentina the virus neutralization test (VNT) is the official test used as part of an EVA surveillance program (all registered stallions of all breeds must be EVA-tested once a year-Resolution N° 434/01), as a requirement for horses to enter the country and also for the export of Argentinean horses (depending on the import requirements of the destination country). In addition, virus isolation/test mating must be carried out on all vaccinated seropositive stallions and imported semen before use.

Stallions testing positive by VNT without an official certificate of EVA vaccination must be withdrawn from commercial breeding. This presentation summarizes the findings of the stallions' surveillance program, obtained from the three officially approved laboratories that carry out the EVA test, over the last three years. We also present the results obtained from all horses EVA-tested at the INTA laboratory during 2007. The following number of stallions were tested: 1208 in 2005, 1362 in 2006 and 1283 in 2007. No EVA-seropositive stallions were found among local breeding stallions. However, 7, 2 and 10 thoroughbred stallion, all of which had been imported several years before and had been EVA-vaccinated in the country of origin (The United States and France), were found EVA-seropositive in 2005, 2006 and 2007 respectively.

We tested 4133 horses (either import/export animals) at the INTA laboratory during 2007, 49 of which were found positive. These included 25 thoroughbred shuttle stallions that had entered the country for the breeding season, 1 Arabian stallion acquired in Europe for breeding, 7 jumping mares imported from Europe, and 16 locally bred jumping horses belonging to breeding farms which had experienced equine arteritis virus infection. Even though there is still a small number of EVA-seropositive horses among native Argentinean horses, all of them are on jumping horse breeding farms where active infection was confirmed (by serology and virus isolation) and controlled several years ago.

From these data, it is possible to conclude that the EVA situation on Argentinean breeding farms, equine sport clubs, stables and hippodromes is not a cause of current concern. These results also reinforce the need to continue with the control of EVA in all horses and semen entering the country as well as with the national surveillance program in order to maintain the current EVA "free status" in Argentina.
Equine Viral Arteritis Screening Methods to Ensure Disease-Free Status of the Horse Population in Israel

Zvia Mildenberg

Section of Equine Veterinary Medicine, Division of Virology, Kimron Veterinary Institute, 52050 Bet Dagan, Israel

Equine viral arteritis (EVA) is a contagious viral disease of horses that causes a variety of clinical signs, most significantly abortion. The disease can be transmitted through both the respiratory and reproductive systems. Horses may be asymptomatically infected or exhibit flu-like clinical signs for a short period of time. Abortion in pregnant mares is often the first, and in some cases, the only sign of infection. **EVA has not been diagnosed in Israel.**

In cases of equine abortion or cases of flu-like manifestations, samples are obtained from affected horses and tested at the equine viral disease laboratory of the Kimron Veterinary Institute.

During the last 4 years, samples from horses presenting with clinical signs suggestive of EVA were tested by virus isolation and/or serological methods. In the case of aborting mares, the aborted fetus and placenta are used for virus isolation. Nasal swabs are taken from horses that show flu-like signs and semen is collected from suspect carrier stallions. Serum antibody detection is performed using the gold standard test, the virus neutralization test (VNT) and the modified live vaccine strain of EAV (ARVAC®) as reference virus. Virus is grown and titrated in monolayers of rabbit kidney cell cultures (RK-13, ATCC-CCL37). Positive controls samples provided by the Gluck Equine Research Center in Kentucky are included in every test.

Over the past 4 years, no positive virus isolations were obtained from the equine population in Israel and no horses were found sero-positive for antibodies to EAV. Samples that had a low titer were retested and in case of a repeated low titer (1:8), the samples were sent to VLA laboratory (Weybridge, UK) for testing. Retesting performed in the UK confirmed that all 3 sera were negative.

Stallions imported to Israel have to test negative for EAV prior to importation. Stallions are tested either by serological methods (VNT) or by virus isolation from semen. Semen imported into Israel is also required to be tested by virus isolation and confirmed negative for EAV.
Equine arteritis virus (EAV) entered Australia following the international movement of horses from the USA to New Zealand. The health certification and testing of these horses was compromised and infected horses entered New Zealand. As Australia and New Zealand were both free of EAV at that time, there were reciprocal agreements for the movement of horses that did not require testing for EAV. As a consequence, infected horses entered Australia. There is a moderate prevalence of infection in warmblood horses but the disease is rare. The prevalence of infection in the thoroughbred population is extremely low.

Laboratory testing for EAV is almost exclusively related to the international movement of horses. Most frequently there is a need to conduct serology using the virus neutralisation test (VNT), although from time to time there is a requirement to perform virus isolation on semen. The majority of samples are tested without problem but an annoying proportion of sera give toxic reactions in the VNT. At times this can occur to quite high dilutions, rarely, to a dilution of 1/32 – [starting dilution], often at dilutions of 1/8-1/16 and commonly at a dilution of 1/4. Depending on the country to which the horses are being shipped, this can cause considerable problems if a negative result is required at a low dilution of serum. Shipping agents have attempted to minimise such problems by insisting on avoidance of the use of specific equine herpesvirus vaccines close to the time of sampling where this is possible.

In order to minimise disruption to movements and at the same time provide appropriate safeguards to importing countries there is a need for modification of international health protocols to allow acceptance of a negative result at a higher serum dilution in the VNT, and to support the use of rapid diagnostic procedures including ELISA for the detection of antibodies and real time PCR for detection of virus. These assays require urgent validation in a range of environments and circumstances so their use is quickly accepted by authorities globally.
The annually updated Horserace Betting Levy Board (HBLB) Code of Practice (available online at http://www.hblb.org.uk/sndFile.php?fileID=%203) continues to be the practical means by which prevention of EVA is implemented in the UK and some other parts of Europe, which is done particularly but not exclusively by the Thoroughbred breeding industry. This is based on annual pre-breeding serological screening of both stallions and mares and use of a killed vaccine (Artervac®; Fort Dodge Animal Health) in stallions only. This approach has shown its worth on at least two occasions when previously infected mares have been detected following subclinical outbreaks among Thoroughbreds in France in 2000 and Ireland in 2003 and highlights the importance of sero-surveillance to detect infected animals in the absence of clinical signs. This has subsequently led to heightened control measures among imported animals on some UK Thoroughbred stud farms.

In the event that EVA is confirmed, the Code of Practice recommends that the local Divisional Veterinary Manager of the Department for the Environment, Food and Rural Affairs (DEFRA) be immediately notified in accordance with The EVA Order 1995 (available online at http://www.opsi.gov.uk/si/si1995/Uksi_19951755_en_1.htm). In addition, all movements and breeding is stopped, all cases and contacts are traced, sampled and isolated and all other horses on the affected premises are screened and grouped according to infectious status. It is also important that good communication exists between interested parties including premises that have received animals (and semen if relevant) from the infected stud, those that are due to send animals and the breeder’s association. Testing and screening should continue on all possible affected premises until the end of the outbreak, seropositive animals and pregnant mares should be isolated for four weeks after first sampling and stallions must have their shedding status investigated.

The most important aspect of control programs after an outbreak of EVA has been diagnosed on a stud is to stop covering immediately. If a stallion is not infected, then attempts must be made to prevent infection of all stallions. If a stallion has already been infected, then it will be the most efficient means of maintaining the spread of the infection around the stud. The rates of infection on the index stud in the 1993 outbreak in the UK closely followed the rate of covering in the preceding week.
All horse and semen movement should be stopped on and off the stud and recently departed animals should be traced, placed in isolation and tested. The control of outbreaks of EVA can be based on the fact that virus is most unlikely to be present in the horses (other than stallions) for more than three weeks after exposure. It is sometimes impossible to determine the precise day of infection during an outbreak. Thus, horses should be kept in isolation for at least three weeks after their first positive blood test. They then are likely to be free from infection. As EVA usually spreads poorly between animals via the respiratory route, it should be possible to stop the spread of infection around a stud once covering has ceased.
Vaccination Against Equine Viral Arteritis in Ireland

P. Lenihan

Department of Agriculture, Fisheries and Food, Central Veterinary Research Laboratory, Backweston Campus, Stacumny Lane, Celbridge, Co. Kildare, Ireland

Since 1994, the Department of Agriculture, Fisheries and Food has licensed the inactivated equine viral arteritis (EVA) vaccine “Artervac®” annually for restricted use in stallions. Vaccination of all thoroughbred and non-thoroughbred stallions is recommended. However, vaccination has largely been confined to thoroughbreds. To date, 1,191 horses have been vaccinated.

Primary vaccination consists of two doses of vaccine, with a 3-6 week interval between the inoculations, followed by a single dose every 6 months. To comply with legislation, a serum sample must be collected for antibody surveillance before vaccination or revaccination, and again 14-21 days thereafter. All vaccinations, and evidence of a negative serum-neutralization (VN) test before primary vaccination, must be recorded on official documentation.

These requirements allow:
- The serological status of stallions seeking approval to be vaccinated in Ireland to be investigated. Since 2004, 51 imported seropositive stallions that did not have a history of vaccination, or were vaccinated without evidence of being sero-negative before primary vaccination, have been detected. These were subjected to test mating and/or examination of semen for equine arteritis virus (EAV), when necessary.
- The monitoring of post-vaccination serological responses to detect horses that do not seroconvert and to advise the administration of an additional dose of vaccine.
- The maintenance of a database to facilitate epidemiological studies.
- The facilitation of equine imports and exports when the EVA status of animals may be in doubt.

There is an average seroconversion rate of 80% after primary vaccination with 50% of horses having neutralization titres in the range 1/16 - 1/1024. One year later, the majority of these are still seropositive, many with high titres. Seropositivity increases to 88% and 93% in horses that have been revaccinated annually once and twice respectively after primary vaccination. More than 60% of these animals have titres between 1/16 and 1/1024.
Challenge studies have not been performed by this laboratory to ascertain the protection afforded by Artervac®. However, extensive surveillance after an asymptomatic episode of EAV infection in 2003 found no evidence that vaccinated stallions potentially exposed to the virus had become persistently infected.

A study that compared a commercial ELISA with the VN test, using sera from vaccinated and non-vaccinated horses, will be discussed in the context of using this ELISA for diagnostic and surveillance purposes.
Use of an Inactivated EVA Vaccine in the United Kingdom

J. Richard Newton

Animal Health Trust, Lanwades Park,Kentford, Newmarket, Suffolk, UK, CB8 7UU

Following the EVA outbreak in the UK in 1993, a formalin inactivated vaccine (Artervac®; Fort Dodge Animal Health) has been used. In order to provide protection from the commercially devastating effects that long term EAV shedding would incur, vaccination has almost exclusively been restricted to breeding stallions, with the majority of Thoroughbred stallions receiving vaccine. However, although an archive of data exists to demonstrate that the vaccine is capable of inducing long lasting neutralising antibody levels in animals receiving repeated booster vaccinations, no definitive direct evidence from its use in the field is available on which to assess its efficacy in preventing the establishment of the carrier state in infected stallions. As EAV infection has not been widely associated with abortion in Europe, no data are available as to the safety of this inactivated vaccine during pregnancy or its effectiveness in preventing EVA-related abortion. The decision not to adopt EAV vaccination among breeding mares, in which the carrier state does not occur, in combination with requirements for routine pre-breeding serological screening has effectively provided a sentinel population in which on-going surveillance for EVA can be conducted. As described previously this has proved valuable on several occasions for alerting to new subclinical infections.

Sero-surveillance of stallions vaccinated using Artervac® conducted at the AHT demonstrated that to achieve and maintain levels of immunity required to protect against developing semen shedding, stallions required several boosters in addition to the two or three dose primary course (Figure 1). This indicated that many first season sires are probably inadequately protected against EVA infection by use of killed vaccine. However, practically this could be overcome by vaccination and subsequent boosting of potential stallions whilst they are still racing. This was particularly highlighted in the 2003 breeding season when there were problems with availability of Artervac® in Europe. The consequence of this was that first season sires were left completely susceptible to EAV infection, whereas previously well vaccinated stallions had good levels of residual immunity as evidenced by high VN antibody levels. The outbreak in Ireland resulted in infection of a first season sire, which did not subsequently shed virus in its semen.

See Figure 1 next page.
Figure 1: EAV VN serological status of 108 stallions measured between 320 and 400 days after last vaccination using a killed virus vaccine: a) log_{10} titre vs number of previous boosters and b) proportion with protective titres (>1.9 log_{10}) vs number of previous boosters
Adoption of a Centralised Information System for the Equine Viral Arteritis Control Programme in Italy: Results and Evaluation Relative to the 2004-2007 Breeding Seasons

Marcello Sala, Maria Teresa Scicluna, Gian Luca Autorino

A National Reference Centre for Equine Diseases (CeRME)– Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Via Appia Nuova 1411 – 00178 Roma - Italy

A National Control Programme for equine viral arteritis (EVA) has been implemented in Italy since 1994, which requires annual verification of the infectious status of stallions before they are officially approved for commercial breeding purposes.

The control programme consists of serological screening, followed up by a series of virological tests on semen in the case of seropositive stallions.

Due to the difficulties encountered during the first 10 years of the programme in the collection of consistent disease related information and in the monitoring of the effectiveness of the control programme, a central information system was introduced in 2004 that was based on the transfer of all data obtained by the network of ten regional institutes to CeRME.

The primary aim was to obtain consistent validated data for evaluation of the control programme as well as for decision making, allowing corrective interventions and future sanitary planning.

The system relies on a standardized information flow, fed by data, recorded on a pro forma questionnaire completed by veterinary officers on sample collection (serum and/or semen). Each questionnaire specifically requires information on horse identification, ownership and location, reason for sampling and previous EVA status.

The questionnaires, along with the laboratory sample results, are recorded on a local database supported by a software programme specially created and distributed by the CeRME. On receiving the quarterly updates from the network of institutes, the National Centre integrates the information into the Central Database, for data clean-up, data mining and data analysis at a national level.

The results of the serological screening, relative to the 2004-2007 breeding seasons, reveal an increase in the prevalence of infection (8.2% to 10% of tested stallions) while the number of cases of EVA is stable throughout the years (4.5%). On the other hand, the results of virological examination performed on the semen of the seropositive stallions highlight a
clear decrease in the prevalence of virus shedders during the period under evaluation and indicate that occurrence of cases of the disease has become sporadic. In conclusion, a summary of the main critical points encountered in the data management (missing data and unavailable information) and the resulting limitations on analysis will be discussed.
Critical Analysis of the Current National Control Programme for Equine Viral Arteritis in Italy

Maria Teresa Scicluna, Marcello Sala, Gian Luca Autorino

National Reference Centre for Equine Diseases (CeRME) – Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Via Appia Nuova 1411 – 00178 Roma - Italy

A comprehensive evaluation of the National Control Programme (NCP) for equine viral arteritis (EVA) was possible following the adoption in 2004 of a centralised information system (CIS).

Several critical points relative to the sequence of activities and procedures, encompassed by the NCP are presented.

The primary focus of the NCP is to control only stallions to be approved for breeding (around 2,700 stallions/year), therefore, excluding other categories of horses at risk. In addition, authorized stallions are tested only once, i.e. at the beginning of each breeding season, at the risk of missing the early diagnosis of new cases that may occur during the rest of the breeding period.

Once semen shedders (carriers) are identified, field information would indicate that there are objective difficulties in verification of the appropriate containment measures, further complicated by lack of compliance, also at this level, with the Equine National Registry.

Other factors contributing to maintenance of EAV infection could be the lack of a passive surveillance system, the possible co-existence of breeding centres together with reproductive farms and uncontrolled movements of animals and, finally, the omission from the NCP of an undetermined fraction of the sexually active population. These last critical points often imply a lack of awareness of the risk of virus transmission. To overcome the aforementioned issues, for the control of EVA, continuous monitoring and surveillance inclusive of all categories of the equine population is needed.

These problems compromise the completeness, appropriateness and promptness of information that is processed by the CIS, impacting on the immediate application of control measures and on the quality of data analysis and final evaluation of the NCP.

Also relevant to the critical analysis is laboratory proficiency and efficiency of the national diagnostic network in providing results predictive of
the true status of the animals. This should extend to all laboratories involved in the authorisation of animal movement.

In analysing these points, one of the main objectives to achieve, remains the harmonization of the sanitary procedures for the control of EVA, both within the EU as well as among third countries.

Should we continue to believe that EVA control is an unilateral problem and is it still hypothetically feasible to prevent new cases without resorting to vaccination?
Contact List

Dr. Gian Luca Autorino
National Reference Centre for Equine Diseases (CeRME),
Istituto Zooprofilattico Sperimentale del Lazio e della Toscana,
Via Appia Nuova 1411,
00178 Roma, Italy
Ph. +39-06-79099-449448
E-mail: gianluca.autorino@izslt.it

Dr. Udeni Balasuriya
Department of Veterinary Science,
Gluck Equine Research Center,
University of Kentucky,
Lexington, KY 40546-0099, USA
Ph. +1-859-257-4757, ext. 81124
E-mail: ubalasuriya@uky.edu

Dr. Maria Barrandeguy
Instituto de Virología
CICV-INTA, Castelar,
Buenos Aires, Argentina
Ph. +54-11-4621-1447
E-mail: mbarrandeguy@cicv.inta.gov.ar

Dr. Reinhard Böse
Labor Dr. Böse GmbH,
Carl-Zeiss-Str. 6,
31177 Harsum, Germany
Ph. +49-5127-90205-0
E-mail: boese@labor-boese.de

Dr. Ivaylo Chenchev
National Diagnostic and Research Veterinary Medical Institute (NRRVMI),
15A “Pencho Slaveikov” Blvd.
1606 Sofia, Bulgaria
Ph. +359-2-934-54-02
E-mail: romiiv@yahoo.com

Dr. Ann Cullinane
Virology Unit,
Irish Equine Centre,
Johnstown,
Naas,
Co. Kildare, Ireland
Ph. +353-45-866266
E-mail: acullinane@equine-centre.ie

Dr. Trevor Drew*
Department of Virology,
Veterinary Laboratories Agency (Weybridge),
New Haw,
Addlestone, Surrey KT15 3NB,
United Kingdom
*OIE Designated Specialist for EVA
Ph. +441-932-357637
E-mail: t.w.drew@vla.defra.gsi.gov.uk

Dr. Guillaume Fortier
Animal Health Department Manager,
Frank Duncombe Laboratory,
1, route de Rosel,
14053 Caen, France
Ph. +33-231-471-954
E-mail: g.fortier@cg14.fr
Dr. Yun Young Go  
Department of Veterinary Science,  
Glück Equine Research Center,  
University of Kentucky,  
Lexington, KY  40546-0099, USA  
Ph.  +1-859-257-4757, ext. 81110  
E-mail:  go.yun@uky.edu

Dr. Aymeric Hans  
Virology Laboratory,  
AFSSA-LERPE-Dozulé,  
Equine Pathology and Disease  
Research Laboratory,  
14430 Goustranville, France  
Phone:  +33-2-31-79-79-07  
E-mail:  a.hans@AFSSA.FR

Ms. Jessica Hennig  
10424 SMC,  
1 Otterbein College,  
Westerville, OH 43081, USA  
Ph.  +1-513-720-8384  
E-mail: Jessica.Hennig@otterbein.edu

Dr. Ákos Hornyák  
Szent István University  
2103 Gődöllő, Páter Károly utca 1,  
Budapest, Hungary  
Ph.  +36-1-251-99-00  
E-mail:  akos1526@freemail.hu

Dr. Peter Kirkland  
Elizabeth Macarthur Agricultural Institute,  
PMB 8 Camden, NSW 2570, Australia  
Ph.  +61-2-4640-6331  
E-mail:  peter.kirkland@dpi.nsw.gov.au

Dr. Takashi Kondo*  
Epizootic Research Center,  
Equine Research Institute,  
Japan Racing Association,  
1400-4, Shiba, Shimotsuke-shi,  
Tochigi 329-0412, Japan  
*OIE Designated Specialist for EVA  
Ph.  +81-285-44-0090  
E-mail:  kondo@epizoo.equinst.go.jp

Dr. Magdalena Larska  
Virology Department, National Veterinary  
Research Institute,  
Al. Partyzantow 57, Pulawy  
24-100 Poland  
Ph.  +48-818893068  
E-mail:  m.larska@piwet.pulawy.pl

Dr. Patrick Lenihan  
Virology Division  
Central Veterinary Research Laboratory,  
Department of Agriculture and Food  
Laboratories,  
Backweston Campus,  
Stacumny Lane,  
Celbridge, Co. Kildare, Ireland.  
Ph.  +353-1-615-7316  
E-mail:  pat.lenihan@agriculture.gov.ie

- 58 -
Dr. Chengzhu Liang  
Equine Disease Research  
Laboratory of STCIQ,  
No. 70, Qutangxia Road, Qingdao,  
266002 Shandong, P.R. China  
Ph. +86-532-80885601  
E-mail: liangchengzhu@yahoo.com  

Dr. Zhengchun Lu  
Department of Veterinary Science,  
Gluck Equine Research Center,  
University of Kentucky,  
Lexington, KY 40546-0099, USA  
Ph. +1-859-257-4757, ext. 81213  
E-mail: Zhengchun.lu@uky.edu  

Dr. N. Jim MacLachlan  
Equine Viral Diseases Laboratory,  
Dept. of Vet. Pathology, Microbiology & Immunology,  
School of Veterinary Medicine,  
University of California – Davis,  
One Shields Avenue,  
1112A Haring Hall,  
Davis, CA 95616-8739, USA  
Ph. +1-530-752-1385  
E-mail: njmaclachlan@ucdavis.edu  

Dr. Sara Mankoc  
Virology Unit, Institute for Microbiology and Parasitology,  
University of Ljubljana,  
Gerbiceva 60,  
SI-1115 Ljubljana, Slovenia  
Ph. +3896-1-4779-846  
E-mail: Sara.Mankoc@vf.uni-lj.si  

Dr. Barry Meade  
Department of Veterinary Science,  
Gluck Equine Research Center,  
University of Kentucky,  
Lexington, KY 40546-0099, USA  
Ph. +1-859-257-4757, ext. 81216  
E-mail: barry.meade@aphis.usda.gov  

Dr. Elizabeth Medcalf  
Centre of Preventive Medicine,  
Animal Health Trust,  
Lanwades Park,  
Kentford,  
Newmarket,  
Suffolk, UK, CB8 7UU  
Ph. +44-1638-750659 x1299  
E-mail: Elizabeth.medcalf@aht.org.uk  

Dr. Zvia Mildenberg  
Division of Virology,  
Kimron Veterinary Institute,  
52050 Bet-Dagan, Israel  
Ph. +972-3-9681698  
E-mail: zviami@moag.gov.il  

Dr. Richard Newton  
Animal Health Trust,  
Lanwades Park,  
Kentford,  
Newmarket,  
Suffolk,  
CB8 7UU, United Kingdom  
Ph. +44-1638-555395  
E-mail: Richard.newton@aht.org.uk
Mr. Gene Pranzo  
The Dorothy Havemeyer Foundation, Inc.,  
60 East 42nd Street, 40th Floor,  
New York, New York 10165-0006, USA  
Ph. +1-212-682-3700  
E-mail: genepranzo@verizon.net

Dr. Stéphane Pronost  
Frank Duncombe Laboratory,  
1, route de Rosel,  
14053 Caen, France  
Ph. +33-231-471-954  
E-mail: s.pronost@cg14.fr

Ms. Jena Richards  
Department of Veterinary Science,  
Gluck Equine Research Center,  
University of Kentucky,  
Lexington, KY 40546-0099, USA  
Ph. +1-859-257-4757  
E-mail: jena.richards@uky.edu

Dr. Marcello Sala  
National Reference Centre for Equine Diseases (CeRME),  
Istituto Zooprofilattico Sperimentale del Lazio e della Toscana,  
Via Appia Nuova 1411,  
00178 Roma, Italy  
Ph. +39-06-79099473  
E-mail: marcello.sala@izslt.it

Ms. Kathy Shuck  
Department of Veterinary Science,  
Gluck Equine Research Center,  
University of Kentucky,  
Lexington, KY 40546-0099, USA  
Ph. +1-859-257-4757, ext. 81170  
E-mail: kmshuc2@pop.uky.edu

Mr. David Schaengold  
The Dorothy Havemeyer Foundation, Inc.,  
60 East 42nd Street, 40th Floor,  
New York, New York 10165-0006, USA  
Ph. +1-212-682-3700  
E-mail: david@sgoldcpa.com

Dr. Beverly Schmitt*  
Diagnostic Virology Laboratory,  
USDA, APHIS, VS, 
NVSL,  
P.O. Box 844,  
Ames, IA 50010, USA  
*Vice President, OIE Standards Commission  
Ph. +1-515-663-7551  
E-mail: Beverly.J.Schmitt@aphis.usda.gov

Dr. Maria Teresa Scicluna  
National Reference Centre for Equine Diseases (CeRME),  
Istituto Zooprofilattico Sperimentale del Lazio e della Toscana,  
Via Appia Nuova 1411,  
00178 Roma, Italy  
Ph. +39-06-79099473  
E-mail: teresa.scicluna@izslt.it