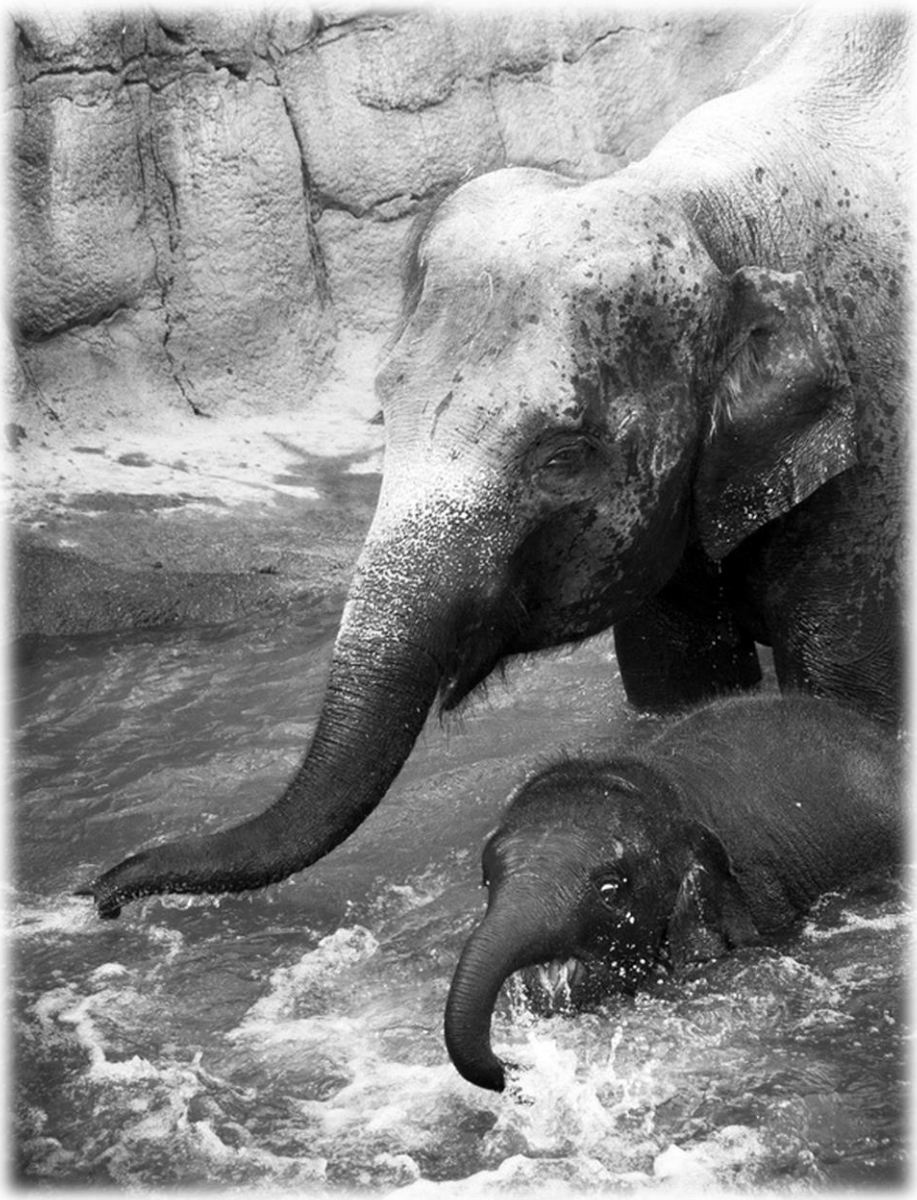
10th International Elephant Endotheliotropic Herpesvirus (EEHV) Workshop

February 17th-18th 2015

Crowne Plaza River Oaks, Houston, Texas, USA



Hosted by the Houston Zoo, Inc.

And the International Elephant Foundation

INTERNATIONAL ELEPHANT FOUNDATION.ORG

HOUSTON ZOO

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Houston Zoo, Inc.
1513 N. MacGregor
Houston, TX 77030
P) 713.533.6500
F) 713.533.6755
www.houstonzoo.org

February 17, 2015

Dear Colleagues,

It is my pleasure to welcome you to the 10th International Elephant Endotheliotropic Herpesvirus Workshop (EEHV), hosted by the Houston Zoo. This virus is the single greatest cause of death of Asian elephants born in North America and has caused significant mortality across Europe as well. Continued reports of EEHV cases in the Asian range countries indicate that this disease is a significant issue for wild and camp elephants as well.

Over the past decade, this workshop has evolved from a small, somewhat informal gathering around one table to the well-established event you see before you today. The changes in our workshop reflect the growth in our understanding of this devastating virus and the collective commitment to the fight against EEHV. Through bench-top laboratory research and "bench to barn" diagnostics, we have been successful in using science to make recommendations that are resulting in the survival of baby elephants ill from EEHV-associated disease. Each healthy elephant that reaches adulthood is a hard fought victory that we can all be proud of.

We at the Houston Zoo have continued our commitment to EEHV research and to our six year partnership with the Baylor College of Medicine and Dr. Paul Ling's virology laboratory. During the workshop this week, we look forward to sharing the details of our recent EEHV success stories and to learning about your challenges, successes, and research discoveries across North America, Europe, and Asia. With over 70 registered attendees from nine countries and 38 institutions attending our workshop, we can expect a very productive couple of days filled with information, discussion, and collaboration.

Special thanks to the International Elephant Foundation for financial support and to the Houston Zoo's Veterinary and Elephant teams for organizing and hosting this workshop.

Sincerely,

Deborah Cannon
Chief Executive Officer

AZA
American Zoo and Aquarium Association
Accredited Member

Presentation Schedule

Monday 2/16:

4:00-6:00 pm: Registration table near hotel check in

6:00-8:00 pm: Ice Breaker at Hotel, registration at Ice Breaker

Tuesday 2/17:

7:15-8 am: Breakfast

8:00-8:20: Introductions, opening remarks (D. Cannon or S. Joseph)

8:20-8:40: EEHV Advisory Group Update (L Howard)

8:40-10:00 Session I: RESEARCH UPDATES

8:40-9:00: Distribution and load of elephant endotheliotropic herpesvirus DNA in tissues from associated fatalities in Asian elephants (*Elephas maximus*)

Katharina Seilern-Moy, Dept. of Virology, Animal & Plant Health Agency, UK

9:00-9:20: Seroprevalence of IgG antibodies to elephant endotheliotropic herpesvirus (EEHV) genotype 1 in captive Asian elephants (*Elephas maximus*): Test results of 1254 samples

Byron Martina, Erasmus Medical Center & Willem Shaftenaar, Rotterdam Zoo, Netherlands

9:20-9:40: Detecting EEHV-specific T cell responses in Asian elephants

Angela Fuery, Baylor College of Medicine, Department of Virology

9:40-10:00: Question and Answer

10:00-10:30: Morning Break

10:30-12:00: Session II: EEHV AROUND THE WORLD

10:30-10:50: Update on EEHV research at the University of Nottingham, United Kingdom

Laura Bennett, Nottingham University, UK

10:50-:11:10: The occurrence of EEHV infection in Thailand: a retrospective study from 2006-2014

Supahen Sripiboon, Murdoch University

11:10-11:30: EEHV Protocol and Experiences at Twycross Zoo, United Kingdom

Sarah Chapman, Twycross Zoo, UK

11:30-11:50 Summary of EEHV-related events in Europe between February 2013 and February 2015.

Willem Schaftenaar, Rotterdam Zoo, Netherlands

11:50-12:00: Question and Answer

12:00-1:00: Lunch

Presentation Schedule

1:00-3:00: Session III: EEHV MONITORING AND SAMPLE COLLECTION

1:00-1:15: An alternative blood sampling technique in elephants for EEHV PCR testing
Javier Lopez, Chester Zoo

1:15-1:35: ABQ Biopark collaboration with the University of New Mexico to set up local EEHV diagnostic lab
Carol Bradford, Albuquerque Biopark

1:35-1:55: Refining a multiplex qPCR assay to simultaneously detect elephant endotheliotropic herpesviruses infections in Asian elephants (*Elephas maximus*)
Jonathan Haycock, Animal and Plant Health Agency, Webridge, UK

1:55-2:20: Clinical and subclinical infection of captive Asian elephants (*Elephas maximus*) with elephant endotheliotropic herpesvirus (EEHV)4 and EEHV1B
Paul Ling, Baylor College of Medicine, Department of Virology

2:20-2:40: Recommendations for Monitoring and Testing for elephant endotheliotropic herpesvirus (EEHV)
Paul Ling, Baylor College of Medicine, Department of Virology & Gary Hayward, Johns Hopkins University, School of Medicine

2:40-3:00: Question and Answer

3:00-3:30: Afternoon break

3:30-5:15: Session IV: EEHV in our Community

3:30-3:40: www.eehvinfo.org : New ! and Improved!
Deborah Olson, International Elephant Foundation

3:40-4:00: Owning the EEHV story: framing the fight against the virus.
Jill Alread, CEO/President Public Communications, Inc., Chicago

4:00-4:25: What is the evidence that EEHV causes hemorrhagic disease?
Gary Hayward, Johns Hopkins University, School of Medicine

4:25-4:45: EEHV Case Definition and what it means for us
Lauren Howard, Houston Zoo

4:45-5:15: Question and Answer, Wrap up for the day

Tuesday night: Dinner on Own

Presentation Schedule

Wednesday 2/18:

7:15-8 am: Breakfast

8:00-8:20: Wrap up from yesterday, Goals for today

8:20-10:00: Session V: EEHV Therapy

8:20-8:40: Stem cell technology and its application to EEHV
Michael Coleman, Ingeneron, Houston, TX

8:40-9:00: In vitro investigation into antiviral efficacy and EEHV
Mathias Ackermann, Institute of Virology, University of Zurich, Switzerland

9:00-9:20: Use of ganciclovir in Asian elephants for treatment of EEHV infection.
Luis Padilla, St. Louis Zoo

9:20-9:35: Penciclovir levels in Asian elephants undergoing famciclovir treatment for clinical EEHV viremia at the Houston zoo
Lauren Howard, Houston Zoo

9:35-9:45: Introduction to First EEHV Workshop in Asia (hosted by Singapore Zoo)

9:45-10:00 Question and Answer

10:00-10:30: Morning Break

10:30-11:50: Session VI: Successful Management of Clinical EEHV

10:30-10:50: Endotheliotropic herpesvirus treatments in a wild orphan baby Asian elephant (*Elephas maximus*)
Khajohnpat Boonprasert, Elephant Hospital, The Elephant Conservation Center, Thailand

10:50-11:10: Clinical EEHV1 in a juvenile Asian elephant: qPCR guided therapy
Fieke Molenaar, Whipsnade Zoo

11:10-11:30: Medical monitoring and treatment of clinical EEHV viremia in juvenile Asian elephants (*Elephas maximus*) at the Houston Zoo
Maryanne Tocidlowski, Houston Zoo

11:30-:11:45: A pharmacist's perspective on the challenges of EEHV antiviral preparedness.
Dan Loper, Pharmaceutical Specialities, Inc.

11:45-12:00: Question and Answer, Afternoon instructions

12:00-1:00: Lunch

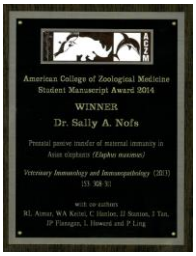
1:00-2:00: Transport to Houston Zoo

2:00-3:00: EEHV Drill Exercise at Houston Zoo's McNair Elephant Habitat Barn
Joseph Flanagan, Maryanne Tocidlowski, Maud Marin, Daryl Hoffman, Amanda Rinker

3:15-4:00: Follow up Discussion, closing Remarks at Houston Zoo Brown Education Center Auditorium

5:30-7:00: Buffet Dinner provided at Twigga Terrace in Houston Zoo's African Forest Village

7:00 pm: transportation back to conference hotel



Congratulations to Dr. Sally Nofs on being awarded the 2014 American College of Zoological Medicine Student Manuscript Award

For her paper on
Prenatal passive transfer of maternal immunity in Asian elephants (Elephas maximus)

This research was performed during her Fellowship at the Baylor College of Medicine

Congratulations to the Baylor College of Medicine team for their very recent publication in PLOS-one:

Generation and characterization of antibodies against Asian elephant (Elephas maximus) IgG, IgM, and IgA

Alan F. Humphreys, Jie Tan, RongSheng Peng, Susan M. Benton, Xiang Qin, Kim C.Worley, Rose L. Mikulski, Dar-Chone Chow, Timothy G. Palzkill, and Paul D. Ling

The article can be found online at this address:

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0116318

EEHV ADVISORY GROUP UPDATE

Lauren L. Howard, DVM, Dipl. ACZM

Denton A. Cooley Animal Hospital, Houston Zoo, Inc., Houston, Texas

Contact: lhoward@houstonzoo.org

Update

The EEHV Advisory Group was formed in the spring of 2014. An initial steering committee of seven individuals selected and recruited the remaining 21 subject matter experts.

The **Mission Statement** of the EEHV Advisory Group:

To decrease elephant deaths due to EEHV while supporting elephant holding institution programming by:

*disseminating knowledge of current best practices for prevention, diagnosis, and treatment of EEHV

*providing private and public elephant-holding facilities with technical assistance

*facilitating research by building international collaborations

The EEHV Advisory Group Goals are:

1. Recommend husbandry practices, treatments, testing protocols
2. Advise diagnostic and research goals between the NEHL Consortium and other research labs
3. Coordinate research sample requests
4. Provide media assistance
5. Assist in the identification of necropsy teams as needed
6. Coordinate EEHV fundraising
7. Assist with proposal submissions for EEHV research projects
8. Proactively provide elephant holding institutions and the general public with current EEHV information
    - Continuously update information on eehvinfo.org website (or a new website)
    - Manage EEHV listserves

The EEHV Advisory Group met for the first time in August 2014 in Fort Worth, Texas. This meeting coincided with the Elephant Tuberculosis Stakeholders Meeting and allowed many attendees to participate in both meetings without extra travel expense. Nineteen people attended the day long EEHV Advisorty group meeting and contributed to the discussions, prioritizations and decisions that were made. The meeting was financially supported by the Oregon Zoo, the International Elephant Foundation, Have Trunk Will Travel, and the Fort Worth Zoo. A few highlights of the meeting are listed below. A complete report of the meeting is available on www.eehvinfo.org or by emailing the author.

Inagural EEHV Advisory Group Meeting 8/20/14 Highlights:

1. The group selected to call clinical illness associated with EEHV "**EEHV Hemorrhagic Disease**", to distinguish it from elephants that have EEHV viremia but are not clinically ill.
2. Assignments for updating material for www.eehvinfo.org were made and all authors have finalized and contributed their sections as of February 2015. Each section was also reviewed by additional EEHV Advisory Group members. Be sure to visit the website to see all the new content!!
3. EEHV-related Research Topics were prioritized by vote:
   i. Surveillance of at-risk calves (10 votes)
   ii. Determine efficacy of anti-viral medications vs. EEHV (10 votes)
   iii. Better understanding of immunology/immune assays related to EEHV (10 votes)
   iv. Continued attempts to culture EEHV (6 votes)
   v. Investigate pathogenesis of virus, through pathology/histopath methods (5 votes)
   vi. Better understand genetics of elephants and the virus (3 votes)
   vii. Investigate novel treatments for EEHV (2 votes)
   viii. Vaccine development (0 votes)
4. Document **"Minimum Standards of Care for Elephant Calves as Related to EEHV-Preparedness"** was developed and approved via email after the meeting. A PDF of this document has been distributed throughout the zoo community and is available on www.eehvinfo.org
5. Our budget and three year plan was discussed. It was decided the EEHV Advisory Group should meet every other years, on off years when there is not an EEHV Workshop.

EEHV Advisory Group Members:

Steering Committee	Research	Pathology
Erin Latimer	Noha Abou-Madi	Michael Garner
Lauren Howard	Arun Zachariah	Tim Walsh
Ellen Wiedner	Imke Lueders	Jaime Landolfi
Debbie Olson	Akbar Dastjerdi	Daniela Denk
Harry Peachey		
Paul Ling		
Gary Hayward		

Veterinary	Elephant Management	Public Relations/Education
Dennis Schmitt	Martha Fischer	Gigi Allianic
Michele Miller	Daryl Hoffman	Brian Hill
Ramiro Isaza	Charlie Gray	Jill Allread
Willem Schaftenaar	Kari Johnson	
Jon Cracknell	Mike McClure	

DISTRIBUTION AND LOAD OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS DNA IN TISSUES FROM ASSOCIATED FATALITIES IN ASIAN ELEPHANTS (ELEPHAS MAXIMUS)

Katharina Seilern-Moy, MedVet,[1,2] Karin Darpel MedVet, MRCVS, PhD,[2] Akbar Dastjerdi, DVM, MSc, PhD[1]

[1]Animal & Plant Health Agency, KT15 3NB, UK
[2]University of Surrey, Guildford, GU2 7XH, UK

Contact: akbar.dastjerdi@apha.gsi.gov.uk

Abstract

Insight into the pathogenesis of EEHV infections can be carried out primarily by examining EEHV-infected tissues due to lack of an animal or *in vitro* cell model. Little is known about distribution and burden of the virus within the organs of fatally affected elephants, which is crucial in understanding the virus pathogenesis.

In this study, the extent of organ tropism of EEHV in fatal cases of EEHV1A, 1B, and 5 was assessed using several quantitative real-time PCRs. Viral DNA for EEHV1 and EEHV5 were detectable in all the tested tissues (aorta, blood, heart, kidney, liver, lung, lymph nodes, spleen, thymus, and tongue) although with significant differences in viral DNA load. The highest EEHV1A DNA load was observed in the liver, followed by heart, thymus, and tongue. EEHV1B and EEHV5 showed the highest DNA load in the heart, followed by tongue and liver. However, whether higher viral load in certain tissues originates from the presence of a more extensive vascular system or from the virus targeting other cells in these tissues is yet to be determined. Also, genetic variation between EEHV1A, EEHV1B, and EEHV5 may also account for the differences in tissue tropism of the viruses.

This study provides new insights into EEHV pathogenicity and has implications in choice of sample type for virus isolation and disease investigation.

Acknowledgments

This work was supported by the ZSL Whipsnade Zoo, Chester Zoo and Animal & Plant Health Agency (APHA). Special thanks to Fieke Molenaar, Nic Masters, Javier Lopez, Daniela Denk, Sharon Redrobe for provision of the samples.

SEROPREVALENCE OF IgG ANTIBODIES TO ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS GENOTYPE 1 IN CAPTIVE ASIAN ELEPHANTS (ELEPHAS MAXIMUS): TEST RESULTS OF 1254 SAMPLES

Willem Schaftenaar DVM[1], Byron Martina PhD[2], and Petra van den Doel BSc[2]

[1] Rotterdam (Blijdorp) Zoo, P.O. Box 532, 3000 AM Rotterdam, The Netherlands; [2]Erasmus Medical Center Rotterdam, Department of Viroscience, s'Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands

Contact: w.schaftenaar@rotterdamzoo.nl

Abstract

Development of the test.
The glycoprotein B of EEHV without its putative transmembrane domain and with a C-terminal histag was synthesized and cloned in the pTrcHis bacterial expression plasmid. Full length as well as truncated parts of the protein were expressed in the soluble fraction of the bacteria and was purified using the Histag. The purified proteins were then used to develop an ELISA. To this end, Costar High binding plates were coated with anti-His monoclonal antibodies. Purified gBhis was added and incubated to allow binding to the antibody. Elephant sera were diluted and added to the plates. Elephant antibodies were detected by using rabbit-anti elephant polyclonal serum and subsequently detected by a swine-anti-rabbit HRPO labelled antibody.

First test results.
In a cooperative project with European and North-American zoos, more than 1,200 blood samples were tested for the presence of detectable antibodies against EEHV1-gB.
Serum samples of North American cohorts were provided by the National EEHV serum bank of the Smithsonian Institute (Washington DC). A total of 17 zoos provided 475 serum samples, representing 63 Asian elephants (*Elephas maximus*). Five zoos had a history of one or more fatal EEHV-HD cases. Remarkably, in herds with one or more animals secreting virus did not result in 100% seropositivity. Differences were found in seropositive status of captive and wild-caught animals. More details of the results will be presented at the workshop
The samples of the European cohorts were submitted by 13 zoos. Two of these zoos had a history of fatal EEHV1-HD cases and participated in a 2-years frequent-sampling study, including 18 elephants and providing 608 samples. Another 187 samples were submitted by 11 zoos, including 49 Asian elephants. A total number of 795 serum samples from Asian elephants were tested: 206 samples had clearly positive test results in the gB-EEHV1 ELISA, while antibody titers of 101 samples were at borderline level and 488 samples tested negative.

Conclusions and trends based on the test results will be discussed at the workshop.

DETECTING EEHV-SPECIFIC T CELL RESPONSES IN ASIAN ELEPHANTS

Angela Fuery, PhD[1], Cliona M Rooney, PhD[2], George Makedonas, PhD[3, 4], Lauren L Howard, DVM, Dipl ACZM[5] and Paul D Ling, PhD[1]

[1]Baylor College of Medicine, Department of Molecular Virology and Microbiology, Houston, TX 77030 USA; [2]Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children's Hospital and Houston Methodist Hospital, Houston, TX 77030 USA; [3]Baylor College of Medicine, Department of Pathology and Immunology, Houston, TX 77030 USA; [4]Texas Children's Hospital, Department of Pediatrics, Houston TX 77030 USA; [5]Denton A. Cooley Animal Hospital,Houston Zoo Inc., Houston TX 77030 USA

Contact: fuery@bcm.edu

Abstract

Elephant Endotheliotropic Herpesvirus (EEHV) can cause lethal hemorrhagic disease in Asian elephants (*Elephas maximus*). Most EEHV-associated deaths have been in elephants aged 1-8, suggesting that an immunological component protects older elephants against the devastating consequences of the virus. Thus far, elephant immune responses towards EEHV are relatively uncharacterized. Studies of herpesviruses in other species have shown that T cells play a major role in mediating protection. In an effort to both prevent mortality associated with EEHV and understand what makes some elephants more susceptible, we are developing assays to detect antigen-specific T cell responses. Using a flow cytometry based assay, we have successfully detected IFN-γ and TNF-α secreting elephant CD3+ T cells following stimulation with the super-antigen Staphylococcal Enterotoxin B (SEB). To further optimize this assay, we are currently assessing CD3 T cell responses after vaccination with Tetanus toxoid and Rabies vaccines. The optimized assay will then be used to measure responses to peptide libraries for the EEHV Major Capsid Protein (MCP) and Major Immediate Early Protein (MIEP). By stimulating with combinations of these peptides, we hope to identify the epitopes that elicit significant functional T cell responses. Identification of these epitopes will allow us to both assess susceptibility to EEHV in some elephants and potentially design a vaccine capable of inducing a protective level of EEHV-specific T cells in susceptible elephants.

UPDATE ON EEHV RESEARCH AT THE UNIVERSITY OF NOTTINGHAM, UNITED KIGNDOM

Laura Bennett, BSc[1], Stephen Dunham BVSc, PhD[1], Lisa Yon BSc, DVM, PhD[1], Sarah Chapman, BVMedSci, MSc, CertZooMed[2], Robert Robinson, BSc, PhD[1] Megan Kenaghan, BVMedSci, Laura Purdie MSc, DPhil[1], Rachael Tarlinton BVSc, PhD[1]

[1] School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD
[2] Twycross Zoo, Burton Road, Atherstone, Warwickshire, CV9 3PX

Contact: stxlmbe@nottingham.ac.uk or Rachael.Tarlinton@nottingham.ac.uk

Abstract

Research at the University of Nottingham on whether pregnancy affected the frequency or magnitude of EEHV1 shedding in trunk washes suggests that there is no clear relationship between shedding and pregnancy. We have also demonstrated the successful culture of Asian elephant endothelial cells from elephant umbilical cord. Several further attempts were unsuccessful; it is likely that contamination and time between parturition and processing were significant factors in these failures. However, we are going to attempt to revive the successfully cultured endothelial cells from storage and attempt an EEHV infection using infected post mortem tissue.

One main focus of our research is on the relationship of EEHV and genetics, as preliminary data has shown the majority of EEHV deaths are clustered in groups of related animals. Further work will include looking at microsatellites to determine diversity in European populations compared to those in Thailand. Looking at potential co-infection pathogens, the first part of this study is to determine if elephant transmissible pathogens are present in rodent populations that are in close proximity to elephants in a UK collection.

THE OCCURRENCE OF EEHV INFECTION IN THAILAND: A RETROSPECTIVE STUDY FROM 2006 - 2014

Supaphen Sripiboon, DVM, MSc[1,3], William Ditcham, PhD[1], Carly Holyoake, BVMS, PhD[1], Lian Yeap, BVMS[1], Ian Robertson, BVMS, PhD[1], Pallop Tonkeaw, Bsc[2], Preeda Lertwatcharasarakul, BSc, PhD[3], Kristin Warren, BVMS, PhD[1]

[1] Conservation Medicine Program, College of Veterinary Medicine, School of Veterinary and Life Science, Murdoch University, Perth 6150 WA; [2] Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100 Thailand; [3] Faculty of Veterinary Medicine, Kasetsart University, NakornPrathum 73140 Thailand

Contact: S.Sripiboon@murdoch.edu.au

Abstract

Elephant endotheliotropic herpesvirus (EEHV) was first recorded in 1999[1] and has since been widely reported in North America and Europe. To date however, little research has been undertaken in Asian elephants (*Elephas maximus*) within their natural home-range countries. To obtain this information, archived tissues samples in Thailand, collected from 21 young elephants that died between 2006 and 2014, were examined. Three blood samples from suspected clinical cases were also included. Conventional PCR was used to detect the presence of EEHV[2] and 14 cases were positive for EEHV1 and two for EEHV4. Further subtyping[3] of all EEHV1 positive cases revealed 12 aligned with EEHV1A, with five different distinct strains, and two cases aligned with EEHV1B, with only one distinct strain. Positive cases ranged in age (1 to 9 years), sex, and geographical location, with cases distributed all over the country and none of them from the same facility. Negative results were obtained from a captive calf that died after an attack by its mother, and a wild elephant that was found dead in the forest. The results concur with the hypothesis that EEHV1A and EEHV1B are ancient endogenous pathogens in Asian elephants[2, 3] given that none of the cases had contact with African elephants. It is recommended that active surveillance for EEHV in captive elephants in Thailand should be undertaken in order to better understand the epidemiology and limit disease transmission. Furthermore, systematic data collection and regional collaboration/data sharing is urgently needed and should be established in the near future.

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2. Latimer, E., Zong, J.C., Heaggans, S.Y., Richman, L.K., and Hayward, G.S. 2010. Detection and evaluation of novel herpesviruses in routine and pathological samples from Asian and African elephants: Identification of two new probosciviruses (EEHV5 and EEHV6) and two new gammaherpesviruses (EGHV3B and EGHV5). *Vet Microbiol*. 147: 28-41.
3. Zachariah, A., Zong, J.C., Long, S.Y., Latimer E.M., Heaggans, S.Y., Richman, L.K., and Hayward, G.S. 2013. Fatal herpesvirus hemorrhagic disease in wild and orphan Asian elephants in Southern India. *J Wildl Dis.* 49: 381-393.

EEHV PROTOCOL AND EXPERIENCES AT TWYCROSS ZOO, UNITED KINGDOM

Sarah L. Chapman, BVM&S, MSc, DZooMed, MRCVS[1], Phillipa Dobbs, BSc (Hons), BVetMed (Hons), MRCVS[1], Sharon Redrobe, BSc (Hons), BVetMed, CertLAS, DZooMed, MRCVS[1]

[1]Twycross Zoo, Atherstone, Warwickshire, CV9 3PX, United Kingdom

Contact: sarah.chapman@twycrosszoo.org

Abstract

Twycross Zoo has a herd of four adult female Asian elephants (*Elephas maximus*) and one female calf born in March 2014 through artificial insemination. Previous monitoring has shown that all adults, within our herd, have shed EEHV1 within trunk washes at varying levels over the last three years. Lesions in the mucosa of the buccal cavity, vulva and rectum of the adults have also been seen and were found on biopsy/PCR to show the presence of EEHV1. The effect of hormone cycle, behaviour and other environmental factors, on the level of trunk wash shedding of EEHV1, has been investigated alongside the level of virus shed by using quantitative PCR techniques. Different sampling methods for testing have also been assessed e.g. trunk wash vs. conjunctival swab vs. buccal swab vs. trunk swab. The mother of the current calf gave birth in 2009 to a calf that subsequently died at 18 months of age with signs of EEHV Hemorrhagic Disease. This calf has been described as the first reported EEHV5-associated fatality[1, 2]. One other adult female gave birth in 2013 to a stillborn calf. This calf was found to have very low levels of EEHV in various tissues. Another adult female had a trunk growth which was attributed to Gamma herpes virus[3] which resolved. As EEHV can lie dormant, with clinical disease possibly triggered by stressful events or other diseases, the main stay of managing the risk in our herd is not only to monitor viral shedding and viraemias, to look at the epidemiology of the disease, but to keep the herd as healthy as possible and be vigilant to detect clinical signs early and initiate treatment in the most stress-free way possible. Positive re-enforcement training of the calf is ongoing to allow examination, regular screening for EEHV and treatment of the calf, if needed, and our facilities allow the calf to be in close contact with her mother throughout this process.

Adaptations to the existing elephant house, over the last two years, have included a protected contact wall (with trunk ports), an elephant restraint device and a calf training area. Our protected contact programme is working towards routine trunk wash sampling of all animals, regular blood sampling of the calf and adults for EEHV qPCR and preparation for treatment of the calf in an emergency situation. Over the last five years, Twycross Zoo has contributed to EEHV research through provision of trunk washes, tissue and blood from umbilical cords, archived serum samples and tissue samples from post mortem cases to a number of European research groups including the University of Nottingham and The Animal and Plant Health Agency, United Kingdom and Erasmus University, Rotterdam, Netherlands.

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SUMMARY OF EEHV-RELATED EVENTS IN EUROPE BETWEEN FEBRUARY 2013 AND FEBRUARY 2015

Willem Schaftenaar, DVM

Veterinary Advisor European elephant TAG

Contact: W.Schaftenaar@Rotterdamzoo.nl

This presentation summarizes the clinical cases in which EEHV has played a role in Europe between 2/2013 to 2/2015. The research activities and publications during the same period will be discussed briefly.

Fatal cases of EEHV hemorrhagic disease (EEHV HD) and EEHV associated disease cases.
Since the February 2013 EEHV Workshop (Houston, TX, USA), 4 fatal cases of EEHV-HD have been diagnosed in Asian elephant (*Elephas maximus*) calves in Europe, all caused by EEHV1. These calves were treated with famciclovir but died due to the devastating hemorrhagic lesions in all organs.

Fatal EEHV-HD cases between 2/2013 and 2/2015:

Date	**Zoo**	**Name**	**Age**
24-6-2013	Pont Scorff (FR)	Arwen	1 yr 1 mo
3-6-2013	Chester (UK)	Jamilah	2 yr 5 mo
29-7-2013	Chester (UK)	Nayan	3 yr
24-11-2014	Kopenhagen (DK)	Khao Sok	2 yr

A few sick elephant calves were suspected of EEHV and treated with famciclovir: a sick, 2-yrs-old calf (Prague, CZ) died despite the treatment; hemorrhagic lesions were present in most organs and EEHV1 was confirmed at necropsy, though the viral load was considered too low to be the cause of death. A sick 2-yrs-old calf (Amersfoort, NL) had low levels of circulating EEHV1; it was treated with famciclovir, it never showed hemorrhagic symptoms and survived.

Research activities in Europe
Following the outcome of the 2013 EEHV workshop (Houston), the research activities in Europe were largely concentrated at the Veterinary Laboratory Agency (VLA) in Weybridge (UK) and the Erasmus University (EU) in Rotterdam (NL).
VLA Weybridge: TaqMan PCR's were made available for 6 serotypes. The entire genome of EEHV1A and EEHV1B was determined. An ongoing study on the viral load in healthy and sick elephants was continued.
EU Rotterdam: gB-antibody ELISA and gB-based recombinant MVA-vaccine. The gB-ELISA was developed and finalized. The presence of antibodies against EEHV-gB was determined in a total number of >1,200 serum samples from captive Asian elephants (Europe and US). A scientific paper has been submitted. Results confirm that EEHV prevalence among Asian elephants (whether captive-born or wild-caught) is much higher than initially presumed.
More research has been initiated or was continued in other institutes: *Zürich University* (CH) studied the sensitivity for famciclovir and ganciclovir in relation to the Tk gene (results to be presented separately at this meeting), *University of Glasgow et al*. (UK) determination of whole genome of EEHV5, and the *Leibnitz Institute for Zoo and Wildlife Research Berlin* (DE) recently initiated an epidemiology study.

Scientific publications from European institutes:

1. *Mathias Ackermann.* Analyses of the endotheliotropic herpesvirus of elephants and establishment of a method for surveillance. http://www.research-projects.uzh.ch/p6784.htm
2. *Gavin S. Wilkie, Andrew J. Davison, Mick Watson, Karen Kerr, Stephanie Sanderson, Tim Bouts, Falko Steinbach and Akbar Dastjerdi*. Complete genome sequences of elephant endotheliotropic herpesviruses 1A and 1B determined directly from fatal cases. Journal of Virology 3-4-2013. http://jvi.asm.org/content/early/2013/03/28/JVI.00655-13.abstract
3. *Gavin S. Wilkie, Andrew J. Davison, Karen Kerr, Mark F. Stidworthy, Sharon Redrobe,Falko Steinbach, Akbar Dastjerdi and Daniela Denk.* First Fatality Associated with Elephant Endotheliotropic Herpesvirus 5 in an Asian Elephant: Pathological Findings and Complete Viral Genome Sequence. Nature scientific reports 2014-4: http://www.nature.com/srep/2014/140909/srep06299/full/srep06299.html
4. *Yuki Furuse, Akbar Dastjerdi, Katharina Seilern-Moy, Falko Steinbach, Bryan R Cullen.* Analysis of viral microRNA expression by elephant endotheliotropic herpesvirus 1. Virology 2014. http://www.pubfacts.com/detail/24725936/Analysis-of-viral-microRNA-expression-by-elephant-endotheliotropic-herpesvirus-1.

AN ALTERNATIVE BLOOD SAMPLING TECHNIQUE IN ELEPHANTS FOR EEHV PCR TESTING

Javier López[1], LicVet MSc, Andrew McKenzie[1] Akbar Dastjerdi[2]DVM, MSc, PhD

[1] Chester Zoo,Upton-by-Chester, Chester, CH2 1LH, UK
[2] Animal & Plant Health Agency-Weybridge, New Haw, Addlestone, Surrey, KT15, 3NB, UK

Contact: j.lopez@chesterzoo.org

Abstract

Monitoring viremia is a key element in the management of EEHV infection, both in affected elephants for assessment of the clinical course and treatment response and in healthy animals for early detection of viremia. However, individuals' behaviour such as reduced compliance when ill, and management circumstances, can impose challenges for standard blood sampling.

Here we present an alternative to venepuncture, using a modified, disposable, capillary blood sampling lancet (Unistik 3 Extra, Owen Mumford Inc). Applying the Unistik 3 over an ear blood vessel allows outflow of sufficient amount of blood for a diagnostic PCR that can be collected with a sterile swab.

Two calves over 4 months of age were sampled weekly for one year using this method without adverse or pain reactions observed. The procedure is short and training was perceived to be easier and quicker than that for standard venepuncture. Similarly, a blood swab obtained from an EEHV affected animal has indicated high levels of EEHV load, verifying its use for diagnostic purposes. The EEHV DNA load can also be normalised to quantity of total nucleic acid to give a relative measure of the level of viremia and thus allow assessment of the progression of the infection over time.

Acknowledgements

We thank Katharina Seilern-Moy, Siva Karuna and Meenakshi Khatri (Animal & Plant Health Agency) for their help with the EEHV PCR and Chester Zoo's elephant team for the development of the procedure and training of the calves.

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ABQ BIOPARK COLLABORATION WITH THE UNIVERSITY OF NEW MEXICO TO SET UP LOCAL EEHV DIAGNOSTIC LAB

Carol M. Bradford M.S., DVM, Dipl. ACZM

ABQ BioPark, 903 Tenth SW, Albuquerque, New Mexico, 87102, USA

Contact: cbradford@cabq.gov

Abstract

In 2009, the ABQ BioPark had the joy of the birth of a female Asian elephant (*Elephas maximus*) calf but was then faced with the worry and challenges of EEHV diagnostic testing; the need for local testing became clear. The reality of a 24 hour turn-around time regarding the shipment of samples to either Houston, TX or Washington, D.C. was not ideal, considering the distinct possibility of acute or peracute onset of illness and even mortality reported in other elephant calves. Through a unique collaboration with the University of New Mexico (UNM), round the clock, immediate access to PCR testing for EEHV became a reality. Since its development in 2010, the ABQ BioPark has utilized the EEHV diagnostic lab at UNM for routine screening as well as at times of concern for potential EEHV symptoms.

REFINING A MULTIPLEX QPCR ASSAY TO SIMULTANEOUSLY DETECT ELEPHANT ENDOTHELIOTROPIC HERPESVIRUSES INFECTIONS IN ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)

Jonathan Haycock, BSc(Hons), BVetMed, MSc,[1] Katharina Seilern-Moy, DVM,[1,2] Fieke Molenaar, DVM, MSc, GPCert(ExAP),[3] Akbar Dastjerdi, DVM, MSc, PhD[1]

[1]Animal and Plant Health Agency, Weybridge, KT15 3NB, UK; [2]University of Surrey, Guildford, GU2 7XH, UK; [3]Whipsnade Zoo, Dunstable, LU6 2LF, UK

Contact: Akbar.Dastjerdi@apha.gsi.gov.uk

Abstract

Recent documented cases of elephant endotheliotropic herpesvirus (EEHV) infections indicate a detectable viraemia several days prior to the clinical signs. A more favourable disease prognosis is also suggested if veterinary intervention is carried out immediately following detection of this early viraemia[1]. Therefore, there are considerable interests in developing rapid diagnostic tests to identify viraemic animals as early as possible, alongside the application of such diagnostic tools to investigate fatal cases and for epidemiological studies.

This study aimed to refine a multiplex TaqMan qPCR assay for simultaneous detection of EEHV genotypes most commonly associated with fatalities in Asian elephants (*Elephas maximus*) i.e. EEHV1, 3, 4 and 5. DNA polymerase (U38) and Terminase (U60) genes of published sequences of EEHV1, 3, 4 and 5 were targeted for consensus primer and probe design. Performance of each primer and probe set was initially verified in simplex assays before being further optimised in the multiplex format. The triplex qPCR assay delivered comparable sensitivity to the simplex qPCR assays and was applied successfully to test archived clinical specimens.

Through reducing time and costs associated with detecting multiple EEHV genotypes in a sample, this single-tube triplex qPCR assay could improve monitoring of at-risk juvenile elephants for early EEHVs viraemia to reduce their significant impacts on Asian elephant conservation.

Acknowledgments

This work was supported by the ZSL Whipsnade zoo, Chester zoo, Animal and Plant Health Agency (APHA) and the Royal Veterinary College, University of London. We also thank Paul Ling and Twycross zoo for provision of clinical materials.

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CLINICAL AND SUBCLINICAL INFECTION OF CAPTIVE ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*) WITH ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) 4 AND EEHV1B

Angela Fuery, PhD[1], Geoffrey Browning, BS[2], Jie Tan, BS[1], RongSheng Peng, BS[1], Lauren Howard DVM, Dipl ACZM[3], Jian-Chao Zong, PhD[4], Gary Hayward, PhD[4], and Paul D. Ling, PhD[1]

[1]Department of Molecular Virology and Microbiology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas, 77030, USA

[2]Virginia-Maryland College of Veterinary Medicine, 205 Duck Pond Drive, Blacksburg, Virginia USA

[3]Houston Zoo Inc., 6200 Hermann Park Drive, Houston, Texas, 77030, USA;

[4]Viral Oncology Program, Johns Hopkins School of Medicine, Baltimore, Maryland, 21231, USA

Contact: pling@bcm.edu

Abstract

Elephant endotheliotropic herpesvirus (EEHV) is the single largest cause of death among North American juvenile Asian elephants (*Elephas maximus*) born after 1980. The vast majority of cases have been associated with two EEHV subtypes known as EEHVs 1A and 1B. Only two cases of EEHV4-associated death have been documented, one in the United States and the other in Thailand. There is also evidence that EEHV4 is shed in trunk secretions from some normal healthy elephants in Southern India. Here we report the serendipitous detection of EEHV4 shedding from a 9-year old male elephant at the Houston zoo, which temporally matches the subsequent infection of two of his juvenile 4-year old herdmates. One of the juveniles developed a short lived EEHV4 viremia, which was followed by high levels of shedding in his trunk secretions. His 4-year-old herdmate then developed an extended viremia peaking at more than 300,000VGE/ml with associated clinical signs. Significant blood viremia with EEHV4 was associated with a transient drop in white blood cell counts (WBCs) and mild thrombocytopenia. Limited sequence analysis of the EEHV4 strain circulating amongst these elephants indicates that it is different from the original North American index case. Following clearance of EEHV4 from the blood, both elephants then developed subsequent infections with EEHV1B with a similar profile of short lived viremia in the 4-year-old male followed by significant shedding in trunk secretions that led to an extensive viremia with associated clinical signs in the 4-year-old female. These results add to a small but growing body of knowledge for infections caused by EEHV4 and also indicate that prior infection with this virus was not sufficient to protect the elephants from significant infection with EEHV1.

RECOMMENDATIONS FOR MONITORING AND TESTING FOR ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV)

Erin Latimer[1], Paul Ling[2], PhD, and Gary Hayward[3], PhD

[1]National Elephant Herpesvirus Laboratory, Smithsonian Conservation Biology Institute, Washington, DC 20008; [2]Baylor College of Medicine, Houston, TX, 77030; [3]Viral Oncology Johns Hopkins University, Baltimore, MD 21287

Contact: LatimerE@si.edu

Abstract

Routine monitoring of elephant calves for EEHV is very useful and can detect low levels of EEHV in the blood before clinical signs occur, allowing increased monitoring and early treatment if viral levels increase (Stanton, 2013). The increased sensitivity of qPCR and multiple rounds of cPCR as well as the ability to quantify whole blood viral levels with qPCR allows for better management of calves with regard to possible EEHV Hemorrhagic Disease (EEHV HD). It is now possible to pick up low levels of EEHV in the blood and monitor closely for rapid increases in viral levels to distinguish between a calf's "normal" primary herpes infection and the much more serious EEHV HD. Elephants can have low levels of EEHV in the blood and show no or minimal clinical signs (Stanton, 2013) for up to two months, but possibly for as long as one year (Latimer, pers comm).

Trunk wash or saliva screening can also detect shedding of virus (as DNA by PCR) for several months during convalescence after primary viremic infection or occasionally from reactivation from latency. While there may be some overlap between high levels of viremia and shedding, viremia is the only parameter that correlates most consistently with disease. Only high levels of EEHV in blood are associated with disease.

Standard monitoring and testing protocols for elephant owning institutions should be developed, to maximize the knowledge gained by appropriate testing while optimizing the use of resources (time and test reagents).

www.eehvinfo.org: New! And Improved!

Deborah Olson

International Elephant Foundation

Introduction to our newly updated and formatted website. Contact Erin Latimer at LatimerE@si.edu with comments, input, suggestions or questions. Thanks for visiting our website!

OWNING THE EEHV STORY: FRAMING THE FIGHT AGAINST THE VIRUS

Jill Alread, APR

Public Communications, Inc. Chicago, IL

Contact: jallread@pcipr.com

WHAT IS THE EVIDENCE THAT EEHV CAUSES HEMORRHAGIC DISEASE?

Jian-Chao Zong[1], Sarah Y.Heaggans[1], Simon Y. Long[1], Erin M. Latimer[2], Virginia R. Pearson[3], and Gary S. Hayward[1]*

[1]Johns Hopkins University, School of Medicine, Baltimore, Maryland

[2]National Elephant Herpesvirus Laboratory, Smithsonian National Zoo, Washington, D.C.

[3]Fox Chase Cancer Research Institute, Philadelphia.

Contact: gary.s.hayward@gmail.com

Abstract

Now that we know that the EEHVs (including EEHV1) are essentially ubiquitous and associated largely with inapparent infections in all elephants, do we have to revisit this question? A skeptical herpesvirologist reviewer of our paper about multiple EEHVs in African elephant (*Loxodonta africana*) lung nodules recently challenged us by saying "How do you know that these little bits of viral DNA really represent intact viral genomes, that they ever actually exist as infectious viruses, and indeed if they are so ubiquitous do they really even cause the hemorrhagic disease you anecdotally attribute to them?" Of course, this reviewer was not familiar with all of the old unpublished (i.e. anecdotal) data that has been accumulated over the years about EEHV cases. Also, he was coming from an historical position that bedeviled human herpesvirus research for decades about how can we prove that ubiquitous viruses such as Epstein-Barr virus (EBV) in particular cause all the different diseases that it does rather than just being a uninvolved passenger. Obviously we can't fulfill Koch's postulates here and the recognition that many elephants also routinely shed one or more EGHVs as well as EEHVs in saliva and trunk washes, including sometimes being positive in the blood, also greatly complicates the situation for herpesvirologist experts.

The traditional view of herpesviruses was and still is that they are largely well adapted to and harmless in their natural hosts. Back in 1999, when we were only aware of two types of EEHV and had only detected them in the blood and tissues of lethal or surviving cases of disease, and also thought that EEHV1 was jumping species from natural host African elephants to unnatural Asian elephant (*Elephas maximus*) hosts, these questions didn't come up. This is especially so with the precedent of the closely related rhesus B-virus and human HSV both causing severe disease in rare cases of cross-species infection in the opposite hosts, but being largely innocuous in their own natural hosts. The apparent effectiveness of famciclovir at least (and maybe

ganciclovir) in saving ten or so young Asian calves with the disease also seemingly unambiguously boosted the case for a role of EEHV. But now that we clearly know that EEHV1 is a natural infection of Asian elephants (not of African elephants), that it and the many other types of EEHVs are likely all highly prevalent even in the majority of asymptomatic elephants, and that the supporting evidence that the drugs are actually effective substrates for the EEHV TK or CPK enzymes is quite weak, it seems a worthwhile exercise to reassess what it is about EEHV hemorrhagic disease that makes us so sure that EEHV is indeed the cause. We will try to make the case that the best arguments come from: (a) the huge viral DNA loads found in all tissues, blood and even serum of numerous evaluated cases; (b) the fact that viral inclusion bodies (i.e. lytic infection) are observed in vascular endothelial cells in association with massive extravasive RBCs (i.e. hemorrhaging); (c) the lower viral load in blood seen in survivors compared to lethal cases, together with the observed decline in blood virus levels during convalescence; and (d) the half dozen cases of near simultaneous infection in pairs of calves with identical viruses. However, with the exception of just a few original signal examples, most of this data (whilst known to many of us) is not in fact published – which was part of the problem for our reviewer – we could not convince him because it was just anecdotal. Here we shall review and try to put in context much of that critical relevant unpublished data from our laboratories, as well as assess potential mechanisms in comparison with other herpesvirus diseases.

EEHV CASE DEFINITION AND WHAT IT MEANS FOR US

Lauren L. Howard, DVM, Dipl. ACZM

Denton A. Cooley Animal Hospital, Houston Zoo, Inc., Houston, TX

Contact: lhoward@houstonzoo.org

Abstract

Research has demonstrated that elephant endotheliotropic herpesvirus (EEHV) viremia in Asian elephants (*Elephas maximus*) can be detected up to two weeks before elephants show clinical signs associated with EEHV hemorrhagic disease (EEHV HD)[1]. This ground-breaking discovery has led to the recommended practice of screening at-risk elephants (Asian elephants from 1 to 8 years of age) routinely, often weekly, via whole blood EEHV PCR testing, with the hope that early detection of low level viremia will allow early intervention and successful treatment. Simultaneously, since the development of a quantitative PCR test that can detect low levels of virus in elephant blood, subclinical EEHV viremia has been detected in young Asian elephants that appear, and remain, otherwise healthy. While this information adds to our overall knowledge base of this virus, it also muddies the waters when it comes to understanding and interpreting clinical signs and predicting the disease process.

Since the Houston Zoo has implemented weekly whole blood PCR testing on our herd of 3.4 Asian elephants, we have detected subclinical, pre-clinical, and clinical EEHV1, 4, and 5 viremia in elephants ranging from 4 to 42 years of age (See Table 1). As more zoological institutions implement the same recommended screening practice, the elephant community will be detecting more cases of EEHV viremia at more zoos. It is likely some of these viremias will be associated with clinical signs and EEHV HD, and it is possible some of these viremias may never become clinically significant.

This mantra has not changed: **An Asian elephant between 1 and 8 years of age that demonstrates any degree of lethargy or illness, and does not have recent EEHV PCR testing available, should be considered ill with EEHV HD until proven otherwise, and treatment with antiviral therapy and supportive care started immediately and continued until EEHV PCR results are available.**

The conundrum lies with the elephants that appear and remain clinically normal, or only very transiently, mildly ill, in spite of having a measurable EEHV viremia. Should each of these elephants be considered an EEHV survivor? Should they be considered a confirmed case of EEHV? Was the viremia detected in these elephants part of the normal cycle of exposure and survival that happens in almost every elephant?

It is up to us in the EEHV community to develop tools to tease apart the clinically affected survivors of EEHV HD from the elephants that were incidentally detected as mildly viremic through routine screening. With the development of a case definition, we can begin a valid epidemiologic investigation of our elephant populations, to evaluater impact and assess risk.

A working group of veterinarians and virologists was developed after the 9th Annual International EEHV Workshop in 2013 to develop an EEHV case definition. Several times we have come close to finalizing a case definition for EEHV, only to be stymied by confounding factors and fear that our definition might be interpreted as a recommendation not to treat clinically ill elephants. The highs and lows of the case definition conundrum will be addressed in this talk, with the Houston Zoo elephants in the table below as examples of subclinical, preclinical, and clinical viremia. The reader is encouraged to visit www.eehvinfo.org in the future for a final version of the EEHV HD case definition.

Table 1. Summary of EEHV viremic episodes in Asian elephants (*Elephas maximus*) at the Houston Zoo, from 2010 to 2014.

Elephant Information	Year	EEHV, peak vge/ml	Clinical findings, WBC changes	Treatment
Shanti ♀ 19 y.o. pregnant	2010	EEHV1B, 2,432 vge/ml	No clinical signs observed Monocytosis	Famciclovir TID X 10 days
Tucker ♂ 5 y.o.	2010	EEHV1B, 407 vge/ml	No clinical signs observed WBC normal throughout	No treatment
Methai ♀ 42 y.o.	2011	EEHV5A, 18,561 vge/ml	Swollen temporal glands, hyperemic oral mucosa Leukopenia, then monocytosis	Famciclovir TID X 12 days rectal fluids Flunixin, ceftiofur
Baylor ♂ 9 months old	2011	EEHV5A, 18,518 vge/ml	One day of swollen temporal glands WBC normal throughout	No treatment
Tucker ♂ 6 y.o.	2011	EEHV5B, 35,538 vge/ml	No clinical signs observed WBC normal throughout	No treatment
Baylor ♂ 4.5 y.o.	2014	EEHV4, 98,000 vge/ml	Subdued behavior, hyperemic oral mucosa, Monocytopenia, leukopenia	Famciclovir X 1 day, rectal fluids
Tupelo ♀ 4 y.o.	2014	EEHV4, 501,271 vge/ml	Colicy, scleral injection, dry feces, poor sleeping, Leukopenia/monocytopenia	Famciclovir X 12 days, rectal fluids, IV plasma/fluids
Baylor ♂ 4.5 y.o.	2014	EEHV1B, 16,504 vge/ml	Hyperemic oral mucosa one day, otherwise no signs Leukocytosis, monocytosis	Famciclovir X 2 days, rectal fluids
Tupelo ♀ 4 y.o.	2014	EEHV1B, 453,784 vge/ml	Scleral injection, subdued behavior, hyperemic oral mucosa, Leukopenia, monocytopenia	Famciclovir X 21 days, rectal fluids, IV plasma/fluids

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STEM CELL TECHNOLOGY AND ITS APPLICATION TO EEHV

Michael E. Coleman, PhD[1], Rudy Martinez, MS[1], Raul Trujillo, MD[1], Timo Nazari-Shafti, MD[2], Ivone Bruno, PhD[2], Ana Elena Kadala, JD[1], Eckhard Alt, MD, PhD[1], Lauren L. Howard, DVM, Dipl ACZM[3]

[1]InGeneron, Inc., Houston, TX, USA; [2]Current affiliation Methodist Hospital Research Institute, Houston, TX, USA; [3]Denton A. Cooley Animal Hospital, Houston Zoo, Inc., Houston, TX 77030 USA

Contact email: mcoleman@ingeneron.com

Abstract

In veterinary medicine, use of autologous stem and regenerative cells (SRCs) is an emerging treatment for modulating inflammation and autoimmunity and promoting regeneration in diseased and damaged tissue[1]. Stem cells, just one type of regenerative cell found in tissue, can differentiate into multiple cell types to form new functional tissue. These stem cells can be found in a perivascular niche in all organs of the adult[2] as well as in perinatal tissues such as placenta and umbilical cord[3]. Adipose tissue in the adult and the umbilical cord matrix (UCM) have particularly high concentrations of these stem cells, and they can be isolated at point-of-care with high yield and viability in approximately 90 minutes using the InGeneron ARC™ system. UCM stem cell isolated with the ARC™ system can be efficiently differentiated to cells of all three germ layers[4].

Elephant endotheliotropic herpesvirus (EEHV) is found in Asian (*Elephas maximus*) and African elephants (*Loxodonta africana*) and can be shed from asymptomatic, healthy elephants as well as lead to severe hemorrhagic disease (EEHV HD). In animals ill from EEHV HD, the virus damages capillary endothelial cells and leads to widespread internal hemorrhage, vascular collapse and shock. Recent research has suggested that early detection of EEHV viremia through routine screening of at-risk calves (primarily Asian elephants, 1 to 8 years of age) can facilitate early, aggressive treatment prior to the onset of clinical signs, which will reduce mortality.

InGeneron received and processed umbilical tissue from two Asian elephant calves born in 2014. Autologous SRCs from the calves are now stored in a cryobank. These SRCs will be available as potential research tool for EEHV and/or adjunct therapy for these elephants in the future, if either develops significant EEHV viremia or evidence of EEHV HD.

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IN VITRO INVESTIGATION INTO ANTIVIRAL EFFICACY AND EEHV

Mathias Ackermann[1], Nelli Schetle[1]

[1]Institute of Virology, University of Zurich, Switzerland

Contact: mathias.ackermann@vetvir.uzh.ch

Abstract

In recent years, the Zoo in Zurich, Switzerland, has lost two male baby elephants due to confirmed elephant endotheliotropic herpesvirus (EEHV1) infection. Worldwide, however, at least 10 survivors of symptomatic EEHV-associated disease are known, which had been treated with anti-herpesvirus nucleoside analogues. Yet, the effectiveness of these drugs remains uncertain, since both survivors and non-survivors have been treated and direct action against EEHV has not yet been proven, due to the fact that these viruses cannot be propagated in cell culture.

We have begun establishing a strategy to address these issues by using recombinant Herpes Simplex Viruses (HSV-1) technology. For this purpose, we knock out the original HSV-1 genes that confer susceptibility against the above-mentioned drugs. Then, we transfer selected EEHV genes into the disabled viruses in order to test for gain of susceptibility against individual drugs. Presently, three EEHV genes are in our focus, namely the EEHV thymidine kinase (ETk, U48.5), the conserved herpesvirus protein kinase (ECPK, U69), and the EEHV DNA polymerase (Epol, U38). As of today, we can report that ETk does not phosphorylate the prodrug Penciclovir (PCV), thus, not contributing to its antiviral activity. In contrast, ECPK seems to confer a mild susceptibility against PCV.

At the meeting, I will provide an update on these ongoing studies, addressing the problems associated with the drugs that are presently being in use to treat EEHV-diseased elephants. Moreover, I will discuss the difficulties that have to be overcome in order to address these issues more profoundly.

USE OF GANCICLOVIR IN ASIAN ELEPHANTS FOR TREATMENT OF EEHV INFECTION

Luis R. Padilla, DVM, Dipl ACZM

Saint Louis Zoo, Saint Louis, MO 63110, USA

Contact: padilla@stlzoo.org

Abstract

In 2009, two approximately 2 yr old Asian elephant calves were treated with ganciclovir following diagnosis with EEHV infections. One individual underwent two courses of therapy, the other one was treated once. In all cases, antiviral administration was one of many supportive care measures and treatments administered in response to diagnosis. Both calves recovered without apparent permanent effects. In each instance, ganciclovir was initially administered at 5mg/kg intravenously twice daily for at least three days, but was subsequently given orally in a prepared formulation. Both individuals have been monitored with weekly bloodwork over the last 6 years. Recently, three individuals, including one of the previously treated animals, were treated with a single intravenous injection of ganciclovir at 1 mg/kg in an attempt to elucidate the pharmacokinetics of this compound in this species. Preliminary results suggest significant individual variation in levels achieved and relatively rapid clearance.

PENCICLOVIR LEVELS IN ASIAN ELEPHANTS UNDERGOING FAMCICLOVIR TREATMENT FOR CLINICAL EEHV VIREMIA AT THE HOUSTON ZOO

Lauren L. Howard, DVM, Dipl. ACZM[1], Sherry Cox, MS, PhD[2]

[1]Denton A. Cooley Animal Hospital, Houston Zoo, Inc., Houston, TX

[2]University of Tennessee, College of Veterinary Medicine, Knoxvillle, TN

Contact: lhoward@houstonzoo.org

Abstract

From September to November 2014, two young Asian elephants (*Elephas maximus*) at the Houston Zoo underwent antiviral treatment for clinical and subclinical EEHV1 and EEHV4 viremia. In addition to other supportive treatments, famciclovir (FCV) powder was administered rectally (15 mg/kg two to three times daily) to Tupelo (female, 4 years old, 1400 kg) and Baylor (male, 4.5 years old, 1600 kg) for a total of 38 days of treatment between them.

Though a full pharmacokinetic study could not be performed, opportunistic blood samples were collected from both elephants following FCV treatment, to be submitted for penciclovir (PCV, the metabolite of famciclovir) analysis. A total of 11 serum samples were submitted to the University of Tennessee for measurement of PCV concentration. The ideal sample for PCV measurement is heparin plasma (minimum volume: 0.25 ml), however only serum samples were available for analysis in this study.

In a previous study of healthy young Asian elephants, the mean maximum plasma concentration of PCV was 3.6 ug/ml at 0.66 hours after rectal administration of 15 mg/kg FCV[1]. This PCV level is considered therapeutic in humans; there is no data on therapeutic efficacy of FCV in elephants. In 2000, a 16 month old elephant ill from EEHV HD was treated with famciclovir orally in dosages ranging from 6.4 to 12 mg/kg and PCV levels post treatment were 0.097-4.36 ug/ml[2]. In our elephants, maximum serum concentration of PCV (6.1 ug/ml) was recorded at one sample taken 8 hrs post treatment in the female elephant. Four samples yielded results between 2.0 and 3.0 ug/ml (0.3 hrs, 1.3 hrs, 1.3 hrs, and 3.3 hrs post treatment). The remaining samples yielded results between 0.3 and 1.95 ug/ml (1.2 hrs, 7.2 hrs, 14 hrs, 14.3 hrs, and 15 hrs post treatment) and one sample whose results were not reliable due to analysis problems.

To our knowledge, this is the first instance where PCV levels were measured in juvenile Asian elephants with active EEHV viremia, given rectal FCV in a realistic treatment setting. The PCV levels measured were highly variable, and the sample size was too small for significant conclusion. The variability of results could be due to possible expulsion of rectal medication after administration, differences in individual elephant drug metabolism, the impact of EEHV viral insult to the gastrointestinal tract and its ability to absorb, or other unknown factors. Veterinarians treating elephants with FCV are encouraged to collect blood samples to submit for PCV measurement, to provide further insight into future treatment recommendations.

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ENDOTHELIOTOPIC HERPESVIRUS TREATMENTS IN A WILD ORPHAN BABY ASIAN ELEPHANT (*ELEPHAS MAXIMUS*)

Khajohnpat Boonprasert*[1], *Sittidet Mahasawangku1*[1], *Taweepoke Angkawanish*[1], *Saran Jansittiwej*[1], *Warangkhana Langkaphin*[1], *Petthisak Sombutputorn*[1], *Pitikarn Bampenpol*[1] *Waraporn Mahanil,

[1]Elephant hospital, Thai Elephant Conservation Center, Hangchart, Lampang, Thailand 52190

Contact: khajohnpat@gmail.com

Abstract

A wild, orphaned Asian elephant (*Elephas maximus*) was successfully treated for Endotheliotropic Herpesvirus (EEHV) infection with acyclovir. The elephant was first rescued from the forest at the age of 4 months. He was then translocated to hospital and has been taken care at Thai Elephant Conservation Center where EEHV has never been reported. Two years later, the elephant showed EEHV-like clinical signs, including lethargy, anorexia, high fever, facial edema and tongue cyanosis. EEHV infection was highly suspected and treatment was immediately conducted. Acyclovir (Vilerm®, Siam Bheach CO.,Ltd, Thailand, 12mg/kg, BID, IV) and Penicillin G (M&H Manufacturing Co., Ltd, Thailand, 50,000 IU/kg, SID, IV) were given for 15 and 7 days respectively. Supportive treatment including Vitamin C and fluids such as Normal saline, Dextrose 5% in saline were administrated intravenously. Clinical signs improved after treatment and resolved after 10 days. A blood sample was collected during the clinical period and tested for EEHV using conventional PCR, which revealed EEHV1A infection. The elephant was monitored for three months, and no other abnormal signs were observed. This is the first time, we used injectable acyclovir (IV) and result was very satisfying. Giving an antiviral drug via an oral or rectal route could be difficult if the animal is not cooperative, and the absorption rates of those two routes are lower when comparing to intravenous route. Therefore, this drug could be another good option to choose for EEHV treatment.

CLINICAL EEHV1 IN A JUVENILE ASIAN ELEPHANT: qPCR GUIDED THERAPY

***Fieke Molenaar** DVM MSc GPCert(ExAP) MRCVS[1] & Katharina Seilern-Moy DVM[2]*

[1]Zoological Society of London Veterinary Department, ZSL Whipsnade Zoo, Whipsnade, Dunstable, Bedfordshire, LU6 2LF, UK; [2]Animal and Plant Health Agency Weybridge, Virology Department, Woodham Lane, New Haw, Addlestone Surrey KT15 3NB, UK

Contact: fieke.molenaar@zsl.org

Abstract

A 1.5-year-old male Asian elephant (*Elephas maximus*) at ZSL Whipsnade Zoo presented with sudden onset lethargy and an abnormal gait. Considering history of EEHV1 fatality in this herd and non-pathognomonic signs of early EEHV1 infection, famcyclovir treatment was initiated on the first day of clinical signs (prior to confirmation of viraemia) and consisted of 15 mg/kg per rectum three times daily after a thorough rectal enema using warm water from a hose. This was followed by laboratory confirmation of the infection. As a preventative measure, the calf‘s four-year old female and three-year old male siblings were treated simultaneously with an initial dosing regime of famcyclovir 15 mg/kg TID per rectum. Different methods of famciclovir delivery were trialled, and suspension of ground famciclovir tablets in handwarm water proved the most efficient and practical. No other fluid therapy was given other than thorough rectal enemas prior to each famciclovir treatment.

Q-PCR monitoring of EDTA blood samples, collected at regular intervals under trained behaviour, indicated a correlation between EEHV genome quantity and disease development. The highest viral load in blood, approximately 10K vgc per millilitre of blood, coincided with clinical signs. Monitoring of EEHV infection using a qPCR assay proved valuable by early disease detection and in guiding therapy in an attempt to improve survival rate and reduce the overall anti-viral therapy cost.

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We thank Nic Masters (ZSL Head of Veterinary Services), Akbar Dastjerdi (Animal & Plant Health Agency) and Karin Darpel (University of Surrey) for reviewing the abstract . The assistance of Jo Dodds and Karla Berry of the Whipsnade Zoo Veterinary Department and the training and handling of the elephants by Lee Sambrook and his team of elephant keepers at Whipsnade are highly appreciated.

FURTHER READING

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MEDICAL MONITORING AND TREATMENT OF CLINICAL EEHV VIREMIA IN JUVENILE ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*) AT THE HOUSTON ZOO

Maryanne E. Tocidlowski, DVM, Dipl. ACZM[1], Maud Marin, DMV, MSc, Dipl. ACZM[1], Joe P. Flanagan, DVM[1], and Lauren L. Howard, DVM, Dipl. ACZM[1]

[1]Denton A. Cooley Animal Hospital, Houston Zoo, Inc., Houston, TX 77030 USA

Contact: mtocidlowski@houstonzoo.org

Abstract

The Houston Zoo analyzes the blood of our three young at-risk Asian elephants (*Elephas maximus*) weekly and our adults monthly, for changes in their complete blood cell count (CBC) and for qPCR detection of EEHV1 virus. Whole blood and trunk washes are analyzed via PCR by Dr. Paul Ling and the virology department at the Baylor College of Medicine. Between September 2 and November 17, 2014, two young Asian elephants (Tupelo, female, 4 years old) and Baylor (male, 4.5 years old) experienced clinically significant viremia with EEHV4 and with EEHV1, resulting in husbandry-based supportive therapy (close observation and nursing care, rectal fluids, increased physiological parameter monitoring), rectally administered antiviral therapy, and veterinary treatment. Veterinary treatment required administration of butorphanol (0.045-0.075 mg/kg IM) and detomidine (0.011-0.022 mg/kg IM) for standing sedation to allow intravenous therapy. Calves were treated with intravenous crystalloids (Normosol, 1.5 to 3 ml/kg IV bolus) and with plasma collected from herdmates (0.5 ml/kg administered IV over 30 to 45 minutes). Water (given via hose) and famciclovir (15 mg/kg, TID) were administered rectally by elephant staff throughout the duration of clinical viremia. This presentation will give you an idea of the veterinary thought process and procedures that were followed to treat our young elephants during their illnesses.

A PHARMACIST'S PERSPECTIVE ON THE CHALLENGES OF EEHV PREPAREDNESS

Dan Loper, DVM, RPh

PSI Pharmacy, Atlanta, GA

Contact: dan@psipharmacy.com

EEHV DRILL EXERCISE AT HOUSTON ZOO'S ELEPHANT BARN

NOTES:

POSTER

NEW EVIDENCE FOR ANATOMIC TESTING SITES TO DETECT SHEDDING OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUSES EEHV1 AND EEHV4 IN ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*) AT THE HOUSTON ZOO

Geoffrey R. Browning, MS[1], Lauren L. Howard, DVM, Dipl. ACZM[2], and Paul D. Ling, PhD[3]

[1]Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, 24060 USA; [2]Denton A. Cooley Animal Hospital, Houston Zoo, Inc., Houston, TX 77030 USA; [3]Baylor College of Medicine, Department of Molecular Virology and Microbiology, Houston, TX 77030 USA

Contact: gbrowni@vt.edu

Abstract

Elephant endotheliotropic herpesviruses (EEHVs) can cause fatal hemorrhagic disease in elephants. Some evidence for multiple shedding sites has been elucidated, so this study explored their use in EEHV screening. Swab samples of various anatomic sites were compared with trunk washes in order to determine optimal sampling methods. This would ideally help to improve epidemiologic studies globally. Concurrent trunk wash and swab samples were collected from seven Asian elephants for six weeks between May and August 2014. Swabs were taken of the distal trunk, hard palate, saliva, and conjunctiva. DNA was screened for EEHV1, 4, and 5 using qPCR. EEHV1 was detected most frequently in trunk wash samples. This may be the optimal method for EEHV1 screening; however, conjunctival and distal trunk swabs produced a few positive samples. EEHV4 was detected most reliably in hard palate samples. This may be a novel sampling method for EEHV4 screening. EEHV4 was also found in saliva, conjunctival, and trunk wash samples. This study also demonstrated the first non-fatal clinical cases of EEHV4 in North America. An epidemiologic link between shedding and clinical infection is suspected in these cases. This illustrates the potential of swabs in both epidemiology and clinical utility.

POSTER

ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS TYPE 6 INFECTION IN A CAPTIVE AFRICAN ELEPHANT (*Loxodonta africana*) IN THAILAND

Piyaporn Kongmakee, DVM[1,5], Suttipong Suttiyaporn, DVM[2], Wirongrong Changpetch, DVM[2], Wichit Kongkham, DVM[2], Chalisa Mongkolphan, BSc[3], Kanittha Tonchiangsai[3], Preeda Lertwatcharasarakul, BSc, PhD[4], Supaphen Sripiboon, DVM, MSc[4], Boripat Siriaroonrat, DVM, PhD[1], Wijit Banlunara, DVM, PhD[5]

[1] Bureau of Conservation, Research and Education, Zoological Park Organization, Bangkok 10300 Thailand; [2] Nakhon Ratchasima Zoo, Nakhon Ratchasima 30000 Thailand; [3] The Monitoring and Surveillance Center for Zoonotic disease in Wildlife and Exotic animals, Mahidol University, NakonPrathom 73170 Thailand; [4] Faculty of Veterinary Medicine, Kasetsart University, NakornPrathom 73140 Thailand; [5] Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330 Thailand

Contact: piyaporn.kmk@gmail.com

Abstract

A 10-year-old male captive-born African elephant (*Loxodonta africana*) kept with his parents in zoo collection showed signs of anorexia, severe depression and weakness for a few days. No treatment was given at that point, and unfortunately the elephant died within 2 days. Necropsy and sample collection were conducted immediately. Gross pathology showed the remarkable lesions including tongue cyanosis, severe extensive patchy hemorrhages on coronary fat and endocardium of left ventricle. Mild pulmonary congestion with renal and hepatic swelling was noted. Tissue samples were collected and preserved in 10% neutral buffered formalin, processed for histological examination under microscopy. There was severe diffuse myocardial congestion with severe hemorrhagic endocarditis and basophilic intranuclear inclusion bodies in cardiomyocytes. Moreover, diffuse pulmonary, hepatic and renal congestion was noted. Frozen heart and kidney samples were preserved and submitted to diagnostic laboratories for Elephant endotheliotropic herpes virus (EEHV) nucleic acid detection using polymerase chain reaction (PCR) and sequencing. The heart sample revealed a homology DNA sequence to EEHV6.

This is a first report of fatal case of EEHV6 infection in captive African elephant. This elephant lived in his own area and did not contact with other animals except his family member. Therefore, the source of infection and factors that introduce the virus into the area are under investigation. None of the elephant in this herd had been tested for EEHV before, however, EEHV monitoring within this herd is planned to conduct in near future.

Conference Attendees

Name	Institution or Affiliation	Email
Mathias Ackermann	University of Zurich Institute of Virology	mathias.ackermann@uzh.ch
Jill Allread	Public Communications Inc	jallread@pcipr.com
Robert Atmar	Baylor College of Medicine	ratmar@bcm.edu
Lisa Marie Avendano	Houston Zoo	lavendano@houstonzoo.org
Priya Bapodra	Columbus Zoo and Aquarium	priya.bapodra@columbuszoo.org
Jesse Bautista	Houston Zoo	jbautista@houstonzoo.org
Laura Bennett	The University of Nottingham	stxlmbe@nottingham.ac.uk
Diana Boon	Denver Zoo	dboon@denverzoo.org
Khajohnpat Boonprasert	Thai Elephant Conservation Center, Thailand	Khajohnpat@gmail.com
Laura Bottaro	Oklahoma City Zoo	lbottaro@okczoo.com
Tim Bouts	Pairi Daiza SA	tim.bouts@pairidaiza.eu
Ellen Bronson	Maryland Zoo in Baltimore	ellen.bronson@marylandzoo.org
Ann Buchanan	Caldwell Zoo	veterinarian@caldwellzoo.org
Deborah Cannon	Houston Zoo	dcannon@houstonzoo.org
Shannon Cerveny	San Antonio Zoo	shannon.shaw@gmail.com
Jacob Chaffee	Houston Zoo	jchaffee@houstonzoo.org
Sarah Chapman	Twycross Zoo	sarah.chapman@twycrosszoo.org
Michael Coleman	Ingeneron	mcoleman@ingeneron.com
Maren Connolly	Dallas Zoo and Children's Aquarium at Fair Park	maren.connolly@dallaszoo.com
Joseph Cotropia, MD	BioClonetics Immunotherapeutics, Inc.	Cotropia@gmail.com
Kelsey Crandall	San Antonio zoo	kelsey.crandall605@gmail.com
Jennifer Dagostino	Oklahoma City Zoo	jdagostino@okczoo.com
Akbar Dastjerdi	Animal and Plant Health Agency	Akbar.Dastjerdi@apha.gsi.gov.uk
Daniela Denk	International Zoo Veterinary Group	d.denk@izvg.co.uk
Joseph Fava	Animal Necessity	jofava13@gmail.com
Paul Fellegy	BioClonetics Immunotherapeutics, Inc.	PDFellegy@gmail.com
Joe Flanagan	Houston Zoo	jflanagan@houstonzoo.org
Angelina Fontenot	Houston Zoo	afontenot@houstonzoo.org
Sonia Fontes	Leibniz Institute for Zoo & Wildlife Research	fontes@izw-berlin.de
Shannon French	HZI Veterinary Student	frenchs@mail.uoguelph.ca
Angela Fuery	Baylor College of Medicine	fuery@bcm.edu
Alicia Hahn	Pittsburgh Zoo & PPG Aquarium	ahahn@pittsburghzoo.org
Aaron Halling	Houston Zoo	ahalling@houstonzoo.org
Jon Haycock	Animal & Plant Health Agency	jhaycock@rvc.ac.uk
Gary Hayward	Johns Hopkins University	Gary.S.Hayward@gmail.com
Alan Herron	Baylor College of Medicine	herron@bcm.edu
Daryl Hoffman	Houston Zoo	dhoffman@houstonzoo.org
Lauren Howard	Houston Zoo	lhoward@houstonzoo.org
Sharon Joseph	Houston Zoo	sjoseph@houstonzoo.org
Wendy Keitel	Baylor College of Medicine	wkeitel@bcm.edu
Matt Kinney	Saint Louis Zoo	kinney@stlzoo.org
Wendy Kiso	Ringling Bros. Center for Elephant Conservation	Wkiso@feldinc.com
Andrea Lee	Houston Zoo	alee@houstonzoo.org
Paul Ling	Baylor College of Medicine	pling@bcm.edu

Kathryn Lippman	**Houston Zoo**	klippman@houstonzoo.org
Simon Long	**Johns Hopkins University School of Medicine**	sylong@jhmi.edu
Dan Loper	**PSI**	dan@psipharmacy.com
Javier Lopez	**Chester Zoo**	j.lopez@chesterzoo.org
Maud Marin	**Houston Zoo**	mmarin@houstonzoo.org
Byron Martina	**Erasmus University Rotterdam**	b.martina@erasmusmc.nl
Fieke Molenaar	**Zoological Society of London**	Fieke.Molenaar@zsl.org
Jeanette Nash	**Houston Zoo**	jnash@houstonzoo.org
Don Nevitt	**Fort Worth Zoo**	dnevitt@fortworthzoo.org
Jeyoung Oh	**Dallas World Aquarium**	jyogoon@gmail.com
Deborah Olson	**International Elephant Foundation**	dolson@elephantconservation.org
Jim Oosterhuis	**San Diego Zoo Safari Park**	joosterhui@sandiegozoo.org
Luis Padilla	**Saint Louis Zoo**	padilla@stlzoo.org
Kathryn Perrin	**Copenhagen Zoo**	kap@zoo.dk
Kelcie Pletch	**Houston Zoo**	kpletch@houstonzoo.org
Andrea Pohlman	**Houston Zoo**	apohlman@houstonzoo.org
Kim Putnam	**Houston Zoo**	kputnam@houstonzoo.org
Mandy Rinker	**Houston Zoo**	arinker@houstonzoo.org
Sam Rivera	**Zoo Atlanta**	srivera@zooatlanta.org
Ian Ross	**Houston Zoo**	iross@houstonzoo.org
Carlos Sanchez	**Fort Worth Zoo**	csanchez@fortworthzoo.org
Willem Schaftenaar	**Veterinary Advisor European elephant TAG**	W.Schaftenaar@rotterdamzoo.nl
Dennis Schmitt	**Ringling Bros. Center for Elephant Conservation**	dschmitt@feldinc.com
Katharina Seilern-Moy	**Animal and Plant Health Agency**	katha_seilern@hotmail.com
Eva Smoot	**Houston Zoo**	esmoot@houstonzoo.org
Supaphen Sripiboon	**Murdoch University**	s.sripiboon@murdoch.edu.au
Maryanne Tocidlowski	**Houston Zoo**	mtocidlowski@houstonzoo.org
Kurt Volle	**Buffalo Zoo**	kvolle@buffalozoo.org
Tarren Wagener	**Fort Worth Zoo**	twagener@fortworthzoo.org
Kristin Windle	**Houston Zoo**	kwindle@houstonzoo.org
Libby Woodruff	**Houston Zoo**	lwoodruff@houstonzoo.org
Jessie Ziegler	**Feld Entertainment**	jziegler@feldinc.com