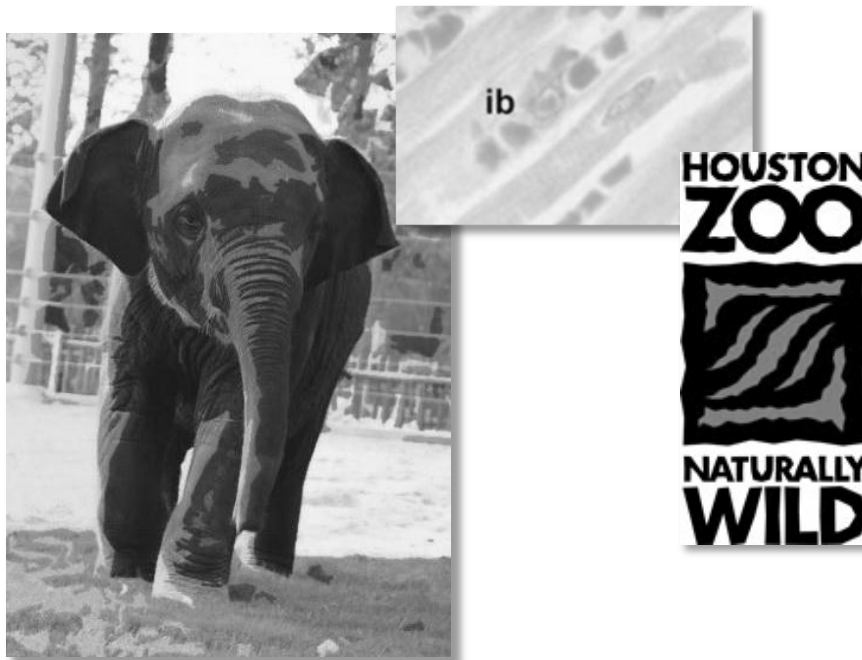


**9<sup>TH</sup> Annual International Workshop  
for  
Elephant Endotheliotropic Herpesvirus**

**Houston, Texas**

**January 27 to 29, 2013**



**Hosted by**

**The Houston Zoo Inc. and**

**International Elephant Foundation**

**INTERNATIONAL  
ELEPHANT   
FOUNDATION.ORG**

## EEHV Workshop 2013 Proceedings

### Table of Contents

Welcome Letter from Rick Barongi, Houston Zoo Director (Page 2)

Workshop Schedule (Page 3)

Abstracts from Scientific Sessions (Page 7)

Supplemental Materials: (Page 29)

Excerpts from Houston Zoo EEHV Protocol:  
EEHV Fast Plan for Emergency Treatment  
Drug dosages for Sedation of Calves  
EEHV Equipment List

EEHV Reference List (compiled Dec 2012) (Page 34)

Additional Materials on Workshop Thumb-drives:

Demographic Population Viability Investigation of the effects of EEHV 2012

EEHV Flow Chart for Treatment Options

Houston Zoo EEHV Protocol (updated June 2012)

Selected EEHV Journal Articles



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January 28, 2013

Dear Workshop Participants,

On behalf of all of us at the Houston Zoo I want to welcome you to the 9<sup>th</sup> International EEHV Workshop and the 4<sup>th</sup> such workshop hosted by the Houston Zoo. We have over 65 participants from around the world representing 21 zoos and 10 universities or medical labs.

Elephant endotheliotropic herpes virus is the most serious threat to the survival of young Asian elephants in zoos and possibly in the wild as well. As a result of all your collaborative research, we are closer to treating and eliminating this disease, but we still have a long way to go.

If you are one of the institutions that has lost an elephant to EEHV you never want it to happen again. There are no words to describe the sense of loss that affects both your staff and community. The only positive aspect of such a devastating loss is that it strengthens your resolve to eliminate this disease, no matter what it takes.

In our case we developed a unique and incredible partnership with Baylor College of Medicine and Dr. Paul Ling's team. This partnership is funded with a long-term grant from a special benefactor of the Houston Zoo who just approved a second grant allowing us to continue to grow this program for another three years.

No one can solve this problem alone but together we will defeat this deadly disease. To paraphrase a famous phrase from another Houston institution, NASA, "what appeared to be our darkest hours will one day, in the near future, be one of our finest moments."

Thank you for coming to Houston and continuing the fight.

Sincerely,

Rick Barongi  
Director

**Ninth Annual EEHV International Workshop 2013**

Houston, Texas

*Hosted by Houston Zoo, Inc., and International Elephant Foundation*

**AGENDA**

**Schedule Overview (more detailed schedule is next pages):**

**Sunday 1/27/13**

Register and Pick-Up Your Name Tag, Hotel Lobby, 5:00 to 6:30 PM

Icebreaker, Hotel Hospitality Suite,\* 6:30 to 8:30 PM

\*Name tags available in Hospitality Suite after 6:30 PM

**Monday 1/28/13**

Breakfast 7:00 to 8:00 AM

Scientific Sessions 8:30 to 12:00 PM

Lunch Break 12:00 to 1:00 PM

Scientific Sessions 1:00 to 4:40 PM

Closing Remarks 4:40 to 5:00 PM

**Tuesday 1/29/13**

Breakfast 7:00 to 8:00 AM

DIVIDED GROUP DISCUSSIONS 8:00 to 12:00 PM

Veterinarians and Managers: Clinical EEHV Management and EEHV Preparedness

Researchers: Focused Research Discussions

Lunch Break 12:00 to 1:00 PM

Final Discussions as Indicated (both groups back together)

Closing Remarks 3:30 to 4:00 PM

Adjourn 4:00 PM

**Monday 1/28/13: General Updates**

**Breakfast: 7:00 to 8:15**

**Opening Remarks: 8:15 to 8:30 Rick Barongi (Director, Houston Zoo)**

**SESSION 1: Clinical Updates on EEHV and Epidemiology (8:30 to 10:00 AM)**

Ramiro Isaza, DVM, Dipl. ACZM, University of Florida College of Veterinary medicine  
*Preliminary Results of Multiyear EEHV Epidemiology Study in Asian elephants (20 min)*

Lauren Howard, DVM, Dipl. ACZM, Associate Veterinarian, Houston Zoo  
*EEHV By the Numbers: EEHV Case Definitions and the Impact of EEHV on the Captive Asian Elephant Population in North America (20 min)*

Sharon Redrobe, BVetMed, CertLAS DZooMed, MRCVS, Zoological Director, Twycross Zoo  
*EEHV-5 Associated Calf Fatality in a Herd of Captive Asian elephants (20 min)*

Jo Mejia-Fava, Founder, Animal Necessity, Resident, University of Georgia College of Veterinary Medicine  
*Possible Anti-herpetic and Immune Supplementation using Shana Vet and Imuno-2865 in Captive Asian elephants (15 min)*

QUESTIONS AND ANSWERS (15 MINUTES)

**Mid-Morning Break (10:00 to 10:30 am)**

**SESSION 2: Updates on EEHV Sub-Types and Prevalence in the US & Range Countries (10:30 AM to 12:00 PM)**

Paul Ling, PhD, Department of Molecular Virology & Microbiology, Baylor College of Medicine  
*Detection of EEHV Infection Among Healthy Asian Elephants in South India (20 min)*

Simon Y. Long, MLAS, VMD, Pathology Post-Doctoral Fellow, Johns Hopkins University  
*High Level Genetic Variability Amongst Nine Cases of Fatal EEHV Hemorrhagic Disease in Wild and Orphan Asian Elephants in Southern India (20 min)*

Virginia Pearson, Guest Researcher, Princeton University  
*Elephant Herpesviruses EEHV2, EEHV3A, EEHV3B (a new subspecies), EEHV6, EEHV7A, EEHV7B (a new subspecies) and EGHV1A, EGHV1B (a new species), EGHV2, EGHV4 Found in Tissue Biopsies and Saliva from African Elephants In Kenya and America (20 min)*

Joseph Hicks, BS, DVM Candidate 2014, Texas A&M University (summer student at BCM)  
*Detection of EEHV in Trunk Wash Secretions from Captive African Elephants (20 min)*

QUESTIONS AND ANSWERS (10 MIN)

**Lunch Break (12:00 to 1:00 PM)**

**SESSION 2: Updates on EEHV Sub-Types and Prevalence in the US & Range Countries, continued**

**(1:00 to 1:50 PM)**

Gary S. Hayward, PhD, Johns Hopkins University  
*Multiple New Variants and Sub-types of Elephant Gammaherpesviruses (EGHVs) and Deltaherpesviruses (EEHVs) (20 min)*

Gary S. Hayward, PhD, Johns Hopkins University  
*Both Benign Lung and Skin Nodules from African Elephants Contain Multiple Strains and Sub-types of EEHV2, EEHV3, EEHV6, and EEHV7 that are Genetically Very Distinct from EEHVs Found in Asian Elephants (20 min)*

QUESTION AND ANSWERS (10 min)

**SESSION 3: EEHV Advances in the Laboratory (1:50 to 3:00 PM)**

Carolyn Cray, PhD, Professor of Clinical Pathology and Microbiology & Immunology, University of Miami Miller School of Medicine  
*Acute Phase Protein Expression During EEHV-1 Viremia in Asian Elephants (20 min)*

Sally Nofs, DVM, Department of Molecular Virology & Microbiology, Baylor College of Medicine  
*Prenatal Passive Transfer of Maternal Immunity in Asian Elephants (20 min)*

Dr. Byron E.E Martina, Erasmus MC Rotterdam, Netherlands  
*Development of an ELISA for Detection of Antibodies Against EEHV (20 min)*

QUESTIONS AND ANSWERS (10 min)

**Mid-Afternoon Break (3:00 to 3:30 PM)**

**SESSION 4: Public Messaging on EEHV and Funding Needs (3:30 to 4:40 PM)**

Jon Cracknell, BVMS CertVA CertZooMed MRCVS, Director of Animal Operations, Longleat Safari and Adventure Park  
*[www.eehvinfo.com](http://www.eehvinfo.com): Update on the Successes and Failings of the EEHV Website (20 min)*

Jill Alread, APR, President, Public Communications, Inc.  
*The EEHV Story..... That You Should Help Tell (20 min)*

Paul Ling, PhD, Department of Molecular Virology & Microbiology, Baylor College of Medicine  
*Financial Needs for EEHV Research (15 min)*

QUESTIONS AND ANSWERS (15 min)

**Closing Remarks 4:40 to 5:00 PM**

**Dinner on Own**

**Tuesday 1/29/13: Focused Discussions/Activities**

**Breakfast 7:00 to 8:00 AM**

Veterinarians and Managers

**Clinical and Management Oriented Discussion**

**Mid-morning Break 10:00 to 10:30 AM**

**EEHV-preparedness discussion and EEHV-drill**

Researchers: Focused Discussions (in smaller group, invitation only):

**Discussion Goal:** To have focused, facilitated discussion about the state of these areas, the current findings, why things aren't happening or working, and what specifically can be done to get answers (with to-do lists and follow-up).

**8:00 to 10:30 AM : Four topics to discuss:**

Antiviral efficacy

Vaccine development

Serologic Evaluation and Immune Response

Virus cultivation

**Mid-morning Break 10:00 to 10:30 AM**

**Discussion Continued.**

**Lunch at Hotel (12:00 to 1:00 PM)**

**Afternoon (1:00 to 3:30 PM) Both Groups Back Together at Hotel**

Summary of EEHV Drill Experience and Discussion

Summary of Research Discussion

Revisit Goals from Last Workshop

Further Discussions as Indicated

**Closing Remarks (3:30 to 4:00 PM)**

**Adjourn (4:00 PM)**

## PRELIMINARY RESULTS OF MULTIYEAR EEHV EPIDEMIOLOGY STUDY IN ASIAN ELEPHANTS (*Elephas maximus*)

Ramiro Isaza, DVM, Dipl ACZM, MPH<sup>1</sup> and Carla Bernal, BS<sup>1</sup>

<sup>1</sup> Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, FL 32610, USA

### Abstract

The primary objective of this case-controlled epidemiological study was to determine if elephant endotheliotropic herpesviruses (EEHV) in Asian elephants (*Elephas maximus*) are associated with exposure to African elephants. The secondary objective was to identify additional husbandry and individual risk factors associated with EEHV cases in captive Asian elephants. Site visits were conducted at 20 collections within the United States that had bred at least one calf and consented to the terms of the study. At each collection, a complete historical census of all the elephants in the collection was conducted, all available Asian elephant medical records were abstracted, and a survey of husbandry practices was administered. The data analysis was split into three phases. First, a descriptive analysis was used to identify the EEHV cases and assess the impact of EEHV in the North American SSP population. This initial assessment was used to categorize the collections and identify all the EEHV cases in the American SSP population. Next, a case-controlled analysis of the 11 sampled collections with at least one case of EEHV was compared to 9 control collections to evaluate husbandry 76 risk factors. Finally, the medical records from 17 sampled calves with EEHV were compared with 25 controls. In both the collections and the individual analysis, we found no significant association between EEHV occurrence and exposure to African elephants. We also failed to identify any secondary risk factors associated with EEHV. Our findings suggest that the husbandry and individual risk factors we studied, including exposure to African elephants, are not different enough to merit further investigation as individual causal factors. This finding is in agreement with other studies suggesting that the epidemiology of EEHV is more complicated than originally thought.



# **EEHV BY THE NUMBERS: EEHV CASE DEFINITIONS AND THE IMPACT OF EEHV ON THE CAPTIVE ASIAN ELEPHANT (*Elephas maximus*) POPULATION IN NORTH AMERICA.**

*Lauren L. Howard, DVM, Dipl. ACZM*

*Denton A. Cooley Animal Hospital, Houston Zoo, Inc., 1513 Cambridge, Houston, TX 77030 USA*

## **Abstract**

Elephant endotheliotropic herpesvirus (EEHV) causes acute and often fatal hemorrhagic disease in young Asian elephants (*Elephas maximus*), with the majority of deaths occurring in elephants between 2 and 9 years of age. Intuitively, we know this condition has a major negative impact on our captive North American Asian elephant collection, however little research has been done to confirm or document this. Standardized classifications of EEHV-associated illness and fatalities are needed to facilitate the development of a universally accepted roster of EEHV cases. This roster can be used in future epidemiologic and also can be referred to in grant applications and research publications. The goal of this lecture will be to outline some decisions that need to be made as far as classification of cases, to present a starting list of EEHV-associated cases, and to set the group up for a more in-depth discussion on the second day of the workshop.

Based on the North American Asian elephant studbook, there are currently 143 Asian elephants housed in North America. A 2011 ZooRisk population analysis (Lisa Faust, 2011), identified a target population size of 220 individuals for the population to be self-sustaining. With only 3.2 births/year, average inter-birth intervals of 5 to 7 years, and many older females soon aging past their reproductive years, the current population is experiencing an annual decline of 1.6%. Thus, it will not reach the projected target population size without significant management changes and/or an importation of new animals. A population modeling report performed in 2012 (see addendum) evaluated the impact of EEHV on the captive North American Asian elephant population. This model found that eliminating EEHV-related mortality, along with increasing breeding rates, would improve the population growth rate from declining annually to becoming stable, ultimately doubling the total population size in 100 years. By increasing the probability of breeding and eliminating EEHV-related mortality, the population has a 97% chance of reaching its target population size, a much higher percentage than under any other scenario evaluated.

Baseline numbers have been established for this abstract but further discussion will determine if these are the final numbers or if revisions need to be made. Three groups of EEHV-related mortalities and illnesses in Asian elephants will be considered, **HISTORIC DEATHS**, **CURRENT EEHV DEATHS**, and **EEHV SURVIVORS**.

The first population of EEHV-related mortalities is from elephants that were born before 1980 and are considered **HISTORIC DEATHS**. Because histopathology and cause of death were not recorded as frequently in the past, these cases add valuable information historically but should not be used to in mortality percentage calculations since the denominator (elephant population as a whole, including causes of death) was less documented.

**HISTORIC DEATHS** include 4 elephants born before 1980. In these cases, EEHV was confirmed as the cause of death through histopathology lesions and/or PCR of banked tissues.

Not all elephants were necropsied in this era, therefore there are potentially other EEHV cases that have not been identified. There have been no identified or confirmed EEHV survivors in this group, though in 2011 a 42 year old wild caught female at the Houston Zoo recovered from moderately severe clinical signs associated with EEHV-viremia, and may be considered a survivor. These 4 known deaths and the potential EEHV survivor are listed in Table 1.

The second group of EEHV-related deaths are the **CURRENT EEHV DEATHS** and includes elephants in North America, born on or after 1980, that were ill or died from EEHV-associated disease. EEHV infection was confirmed via histopathology +/- PCR on post-mortem tissues and/or pre- or post-mortem whole blood samples. This list includes wild caught elephants as well as captive bred elephants.

According to the current North American Asian elephant studbook, there are 130 elephants in North America that were born on/after 1980, and 88 of these are still alive in 2013. *(There have also been 35 stillbirths or immediate neonatal deaths (died on first day of life) since 1980, which, while important to document in general, will not be considered in this discussion since EEHV has not been associated with abortions, stillbirths, or immediate neonatal deaths in North America).* In deaths of North American Asian elephants born since 1980, 15 have died for reasons other than EEHV, and 5 have died for undocumented reasons. The remaining 22 elephants have died from illness related to EEHV, rendering 22 of the 42 deaths of Asian elephants since 1980 due to EEHV (52%). This makes EEHV the single greatest cause of death in this cohort of elephants. The 22 **CURRENT EEHV DEATHS** are listed in Table 2.

The final group to discuss is **EEHV SURVIVORS**. Elephants in this group, born on or before 1980, were diagnosed with illness associated with documented EEHV viremia and overcame illness to become clinically normal. The degree of illness, level of viremia, and confirmation of recovery from infection are all parameters that need to be discussed to develop a firm definition of EEHV SURVIVOR. To date, there are 8 identified potential EEHV SURVIVORS, and several others that could be considered as survivors. These are listed in Table 3. By the current numbers, there have been 30 total EEHV cases in elephants born on/after 1980, with 8 survivors and 22 deaths, leading to a fatality rate of 22/30 or 73%.

The tables below are left with blank spaces to allow for workshop participants to supply information as a group to complete the information known on each EEHV case. The final results will be reported in an addendum made available on the [www.eehvinfo.com](http://www.eehvinfo.com) website.

**Proposed Definitions for EEHV Roster:**

EEHV Case: illness or death in elephant confirmed by DNA/PCR or histopathology.

EEHV Death: EEHV is confirmed cause of death of elephant by histopathologic lesions +/- PCR/DNA.

EEHV Survivor: elephant has clinical illness consistent with EEHV and has EEHV viremia confirmed by PCR/DNA of whole blood.

Non EEHV-associated Death: confirmed cause of death is something other than EEHV, confirmed by histopathology. (NOTE: may still be EEHV PCR positive)

Unknown Death: cause of death is unknown or not confirmed

<b>Table 1. HISTORIC DEATHS of EEHV in Elephants Born Before 1980.</b>						
<b>Studbook</b>	<b>D.O.B.</b>	<b>M/F</b>	<b>Age @ death</b>	<b>Clinical illness?</b>	<b>Histopathology?</b>	<b>PCR?</b>
170	1960	F	42 years			
431	1958	F	8 years			
434	1928	F	52 years			
436	1971	F	6 years			
Possible EEHV Survivor Listed Below:						
127	1969	F	42 when ill	Yes	no (still alive)	yes

<b>Table 2. CURRENT EEHV DEATHS in Elephants Born On/After 1980.</b>						
<b>Studbook</b>	<b>D.O.B.</b>	<b>M/F</b>	<b>Age @ death</b>	<b>Clinical illness?</b>	<b>Histopathology?</b>	<b>PCR?</b>
128	1984	M	4 years			
207	1981	M	2 years			
262	1986	M	7 years			
277	1987	M	11 years			
301	1988	F	3 years			
311	1991	F	13 years	Yes	Yes	yes
312	1991	F	2 years			
326	1991	F	8 years			
350	1993	F	2 years			
352	1993	F	7 years			
508	1998	M	7 years			
509	1998	M	2 years			
520	1999	M	3 years			
521	2000	F	3 years			
528	2000	F	7 years			
599	1982	F	5 years			
616	1998	F	5 years			
638	2006	M	2 years			
641	2006	F	1 year			
645	2006	M	2 years	Yes	Yes	yes
647	2006	M	4 years			
648	2007	F	1 year			

<b>Table 3. EEHV SURVIVORS in Elephants Born on/after 1980.</b>						
<b>Studbook</b>	<b>D.O.B.</b>	<b>M/F</b>	<b>Age @ illness</b>	<b>Clinical illness?</b>	<b>Viremia?</b>	<b>Treatment?</b>
385	1996	F				
420	1997	M				
424	1997	F				
615	2003	M				
646	2007	F				
653	2008	M				
656	2009	M				
687	2009	M				
Possible Additional EEHV Survivors Listed Below:						
642	2006	F				
308	1990	F				
632	2005	M				

Questions to Consider in Defining EEHV Survivors:

1. Are there specific clinical signs that should be observed to qualify as a survivor?
2. Is treatment with anti-viral medications required to qualify as a survivor?
3. Is there a minimum level of EEHV viremia that should be documented to qualify as a survivor? (similarly, are low levels of viremia NOT considered a clinical concern)
4. At what point is EEHV-associated illness considered resolved? (resolution of clinical signs, resolution of viremia, or both?).
5. Should strain of EEHV (EEHV1A or 1B, EEHV5, etc.) be considered when documenting survivors?

Addendum: A Demographic Population Viability Investigation of the Effects of EEHV (12/2012) by Talia Gazlay and Lisa Faust, Lincoln Park Zoo, Chicago, IL, is included on the proceedings thumb drive. Or contact [lhoward@houstonzoo.org](mailto:lhoward@houstonzoo.org) for a copy.

Acknowledgements: Asian elephant studbook information and updates provided by Mike Keele of Oregon Zoo. Population modeling study on EEHV impact provided by Lisa Faust and Talia Gazlay of Lincoln Park Zoo. Initial EEHV cases list compiled by Debbie Olson of IEF and Martha Fischer of St. Louis Zoo.

## **EEHV-5 ASSOCIATED CALF FATALITY IN A HERD OF CAPTIVE ASIAN ELEPHANTS (*Elephas maximus*)**

**Sharon Redrobe,<sup>1</sup> Sarah Chapman,<sup>1</sup> Jonathon Cracknell,<sup>2</sup> Stephen Dunham,<sup>3</sup> and Rachael Tarlinton<sup>3</sup>**

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<sup>3</sup>*School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK*

### **Abstract**

Elephant endotheliotropic herpesviruses (EEHVs) cause acute hemorrhagic disease with high mortality rates in Asian elephants (*Elephas maximus*). A captive UK elephant herd of four adult female Asian elephants had been repeatedly tested negative for EEHV-1 using standard trunk wash techniques at a UK laboratory. A 20-month old captive-bred Asian elephant developed a rapidly progressive disease characterized by severe edema, hemorrhage of mucosal membranes and cyanosis. This herd had been closed for 10 years and had been regularly tested negative for EEHV via trunk washes. However, a provisional diagnosis of EEHV infection was made based upon clinical signs and treatment followed protocols for EEHV infection, including extensive antiviral therapy and drainage of the pericardial effusion under ultrasonographic guidance. Despite initial improvement the health of the animal deteriorated necessitating euthanasia within 10 days of the onset of clinical signs.

Postmortem findings were consistent with previously reported fatal cases of EEHV, revealing marked edema and multiorgan haemorrhages. Given the clinical and post-mortem findings samples were submitted to several teams to further investigate the possibility of EEHV infection. The presence of the EEHV-5 nucleic acid was detected at AHVLA, UK using both published and unpublished PCR methods, and results were additionally confirmed by the National Elephant Herpesvirus Laboratory, USA.

Work to investigate EEHV in the surviving herd is continuing with the University of Nottingham. We have established qPCR tests for viral DNA based on a published method and used this test to determine viral excretion in trunk wash samples from the adult animals at Twycross Zoo. Low and variable levels of EEHV-1 have been detected from the adults. Cells have been grown from an elephant umbilical cord in the lab (from another calf) and we have “Deep sequenced” a trunk wash sample containing EEHV-1 DNA. Ongoing work includes a comparison of the specificity and sensitivity of the 3 published qPCR tests for EEHV-1 viral DNA and comparison of excretion and detection of virus from different sites that could be used for diagnostic testing (trunk washes, oral, nasal, conjunctival swabs).

# POSSIBLE ANTIHERPETIC AND IMMUNE SUPPLEMENTATION USING SHANA VET™ AND IMUNO-2865™ IN CAPTIVE ASIAN ELEPHANTS (*Elephas maximus*)

Johanna Mejia-Fava, D.V.M.<sup>1\*</sup>

<sup>1</sup>Zoological Medicine Resident, Department of Small Animal Medicine and Surgery, University of Georgia, College of Veterinary Medicine, Athens, Georgia 30606, USA

## Abstract

Elephant endotheliotropic herpesviruses is an acute hemorrhagic disease in endangered Asian (*Elephas maximus*) elephants that is fatal. The disease is difficult to manage as variables unrelated to treatment have an effect on clinical outcome such as viral load, strain virulence, and immune status.<sup>1</sup> The most common antiviral drugs used are famciclovir, ganciclovir, and acyclovir. Phosphorylation by viral thymidine kinase is required by all drugs into their active metabolites.<sup>1</sup> Currently, it is unknown whether all strains of EEHV have a functioning thymidine kinase gene.<sup>1</sup> In humans, prophylactic antiherpetic and immune supplementation has been used for herpes virus.<sup>2,3,4,5,6</sup> Shana Vet™ (Animal Necessity LLC, New York, NY, 10018) contains docosanol which is a saturated 22-carbon aliphatic alcohol which inhibits enveloped viral replication by interfering with the early intracellular events surrounding viral entry into target cells.<sup>2</sup> Another main ingredient includes triacontanol which exhibits anti-inflammatory properties mediated through inhibition of lipid peroxidation.<sup>6</sup> Imuno-2865™ (Animal Necessity, LLC) is a natural hemicellulose made of arabinoxylan, arabinogalactan, and fatty acid mixture extracted by a proprietary process from a natural blend of gramineae, poaceae, and dioscoreaceae. Pilot studies have shown encouraging findings in improving lymphocyte activation and interleukin activity.<sup>3,4,5,7,8</sup> A PCR assay using trunk wash samples can detect the presence of pathogenic EEHVs in healthy Asian elephants.<sup>1,2</sup> There is probably value in studying the effects of these supplements on viral shedding and immune function in elephants.

## LITERATURE CITED

1. Wiedner E, Howard L, Isaza R. Treatment of Endotheliotropic Herpesvirus. In: Miller RE, Fowler ME. Fowler's Zoo and Wild Animal Medicine Current Therapy. Volume 7. St. Louis: Elsevier Saunders, 2012; 537-543.
2. Katz DH, Marcelletti JF, Khalil MH, et al. Antiviral activity of 1-docosanol, an inhibitor of lipid-enveloped viruses including herpes simplex. Proc Natl Acad Sci 1991; 88(23):10825-9.
3. Chavoustie S, Perez P, Fletcher M, et al. Pilot study: Effect of PDS-2865® on natural killer cell cytotoxicity. Journal on Nutraceuticals and Nutrition 2003; 6(2):39-42.
4. Weeks BS, Perez. The hemicellulose preparation, Natramune™ (PDS-2865®), increases macrophage phagocytosis and nitric oxide production and increases circulating human lymphocytes levels. Med Sci Monit, 2009; 15(2): 43-46.

5. Weeks BS, Woo Lee S, Perez P, et al. Natramune and PureWay-C reduce xenobiotic-induced human T-cell  $\alpha 5\beta 1$  integrin-mediated adhesion to fibronectin. *Med Sci Monit* 2008; 14(12): 279-285.
6. Ramanarayan K, Bhat A, Shripathi V, et.al. Triacontanol inhibits both enzymatic and nonenzymatic lipid peroxidation. *Phytochemistry* 2000; 55(1): 59-66.
7. Rodriguez MM, Mejia JC, Blanchard MT, Stott J, Pestano N, Perez PP. 2007. Pilot Study: The Affects of Natramune™ (PDS-2865®), a New immunostimulator supplement on different cetacean species; *Tursiops truncatus*, *Lagenorhynchus obliquidens*, and *Orcinus orca*. IAAAM 38th Annual Conference Proceedings, Orlando, FL; Pp. 44-45.
8. Rodriguez MM, Mejia JC, Blanchard MT, Stott J, Pestano N, Perez PP. 2007. Natramune™(PDS-2865®), an immuno-stimulator supplement in cetaceans. IAAAM 39th Annual Conference Proceedings, Pomezia, Italy; Pp. 26.
9. Stanton JJ, Zong J, Latimer E, et al. Detection of pathogenic elephant endotheliotropic herpesvirus in routine trunk washes from healthy adult Asian elephants (*Elephas maximus*) by use of a real-time quantitative polymerase chain reaction assay. *Am J Vet Res* 2010; 71(8): 925-933.

**DETECTION OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS INFECTION AMONG HEALTHY ASIAN ELEPHANTS (*Elephas Maximus*) IN SOUTH INDIA**

**Jeffrey J. Stanton,<sup>1,2</sup> Sally Nofs,<sup>2</sup> Arun Zachariah,<sup>3</sup> N. Kalaivannan,<sup>4,5</sup> and Paul D. Ling<sup>2\*</sup>**

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<sup>3</sup> *Wildlife Disease Research Laboratory, Department of Forest and Wildlife, Kerala, India*

<sup>4</sup> *Department of Forests and Wildlife, Tamil Nadu, India*

Elephant endotheliotropic herpesviruses (EEHVs) can cause fatal hemorrhagic disease in Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. While cases of fatal EEHV1-associated hemorrhagic disease have been reported in range countries, the prevalence of subclinical EEHV infections in these countries is relatively unknown. To address this question, we used EEHV-specific quantitative real-time polymerase chain reaction (qPCR) assays to detect subclinical EEHV infection in three regionally distinct Asian elephant cohorts in South India during October and November 2011. Using DNA prepared from trunk washes, we found evidence for shedding of EEHV1, EEHV3/4, and EEHV5. None of the trunk washes were positive for EEHV 2 or 6. At least one EEHV species was detectable in about one third of the samples that were screened. These data suggest that subclinical EEHV infection amongst *in situ* Asian elephants occurs and that Asian elephants may be natural hosts for EEHV1, EEHV3 or 4, and EEHV5, but not EEHV2 and EEHV6.



## HIGH LEVEL GENETIC VARIABILITY AMONGST NINE CASES OF FATAL EEHV HEMORRHAGIC DISEASE IN WILD AND ORPHAN ASIAN ELEPHANTS (*Elephas maximus*) IN SOUTHERN INDIA.

\*Simon Y. Long,<sup>2</sup> Arun Zachariah,<sup>1</sup> Jian-Chao Zong,<sup>2</sup> Erin M. Latimer,<sup>3</sup> Sarah Y. Heaggans,<sup>2</sup> Laura K. Richman,<sup>3</sup> and Gary S. Hayward<sup>2</sup>

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### Abstract

Up to 65% of the deaths of young Asian elephants between 3 months and 15 years of age in Europe and North America over the past twenty years have been attributed to hemorrhagic disease associated with a novel DNA virus called Elephant Endotheliotropic Herpesvirus (EEHV). To evaluate the potential role of EEHV in suspected cases of lethal acute hemorrhagic disease with similar pathology occurring in Southern India, we studied tissue DNA samples collected from field necropsies. Nine convincingly positive cases amongst both orphaned camp and wild Asian elephants were identified by conventional diagnostic PCR for PAN-EEHV POL and EEHV1-specific POL. These were then subjected to detailed gene subtype DNA sequencing at six key PCR loci, U38/POL, U48/gH, U51/vGPCR, U60/TER, U71-72/gM and U76-77/HEL. The results revealed seven distinct strains of EEHV1A and one of EEHV1B. Two orphan calves that died within three days of one another at the same training camp proved to have identical EEHV1A DNA sequences to one another indicating a common epidemiological source. However, the high level of EEHV1 gene subtype diversity found amongst the other Indian strains matches that observed amongst over 30 EEHV1 strains that have been evaluated from Europe and North America.

These results argue strongly against the previously suggested notion that this is just a disease of captive elephants and that the EEHV1 virus has crossed recently from African elephants (*Loxodonta africana*) hosts to Asian elephants (*Elephas maximus*). Instead, both the EEHV1 virus and its associated hemorrhagic disease are evidently widespread in Asian range countries and therefore, despite the unexpected disease severity, Asian elephants themselves appear to be the ancient natural endogenous hosts of both EEHV1A and EEHV1B. Based on the identification of one Asian elephant death from EEHV4 in Thailand as well as one in the USA, two other EEHV1A cases in Sumatra and the detection of several distinct strains of EEHV5 in captive elephants in both USA and Europe, we anticipate that all four of these viruses EEHV1A, EEHV1B, EEHV4 and EEHV5 may be natural endogenous viruses that evolved together with Asian elephant hosts. Nevertheless, the relative abundance and pathogenicity of these different EEHV types in both wild range elephants and in captive elephants, as well as their ability or otherwise to provide immune protection against infection or disease caused by the other types, all remain to be determined in the future.

**ELEPHANT HERPESVIRUSES EEHV2, EEHV3A, EEHV3B (A NEW SUBSPECIES), EEHV6, EEHV7A, EEHV7B (A NEW SUBSPECIES) AND EGHV1A, EGHV1B (A NEW SUBSPECIES), EGHV2, EGHV4 FOUND IN TISSUE BIOPSIES AND SALIVA FROM AFRICAN ELEPHANTS (*Loxodonta africana*) IN KENYA AND AMERICA.**

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## **Abstract**

Determining which species of elephant herpesviruses are endogenous to African elephants and to Asian elephants is critical to understanding these ancient viruses that co-evolved with the elephant lineage for one hundred million years. Herpesviruses in general are species-specific; however, if inter-species infection occurs, unusually severe disease in the non-natural host can be the outcome. To address the hypothesis that elephant herpesviruses EEHV1A and EEHV1B, the most frequent causes of hemorrhagic death in juvenile Asian elephants, could be jumping from the African genus, *Loxodonta africana*, to the Asian genus, *Elephas maximus*, we set out to answer the question, “Which elephant herpesviruses are endogenous viruses in African elephants?” In January/February 2011, in collaboration with Save The Elephants and Kenya Wildlife Service Veterinary and Capture Services Department, we immobilized twelve wild Kenyan elephants: five juveniles with visible herpes-like skin nodules, three mothers of the youngest juveniles, two adults for GPS collaring, and two adults for emergency veterinary attention. We collected skin biopsies from five juvenile elephants; saliva and other exudates from all twelve elephants; and, opportunistically, lung and heart necropsy samples from a thirteenth elephant that had recently died. The biological samples we collected and prepared in Kenya were stored in reagents that we had tested previously with EEHV positive samples for effectiveness in preserving and recovering viral and genomic DNA in field conditions at ambient temperatures. PCR and viral DNA sequencing in two independent laboratories, first at Princeton University and subsequently at Johns Hopkins School of Medicine, proved that all five juvenile wild Kenyan elephants and the dead elephant were infected with at least one type of EEHV, and five of them with between three and six different species or subtypes of elephant herpesviruses,

including EEHV2, EEHV3A, EEHV3B (*a new subspecies*), EEHV6, EEHV7A, EEHV7B (*a new subspecies*), and EGHV1B (*a new species of elephant gammaherpesvirus*).

In a corollary study, we are analyzing weekly saliva samples collected over a one year period beginning in January, 2012, from two African elephants from Zimbabwe and Uganda at Six Flags Wild Safari, Jackson, New Jersey, USA. We have tested several collection reagents for preservation of DNA in saliva at ambient temperature. For comparison, we collected saliva samples twice from each of five other elephants (all from Uganda) at the same park, and once from two African elephants from South Africa and two Asian elephants from Bhutan and India at Six Flags Discovery Kingdom, Vallejo, California, USA. PCR and viral DNA sequencing to date have revealed: 1) that one or more species of elephant herpesvirus including EEHV2, EEHV3A/3B, EEHV6, EGHV1B (*first discovered in this study*), EGHV2 and EGHV4 are shed in saliva from all nine captive African elephants; 2) that several elephants periodically shed two or more herpesviruses in the same saliva sample; and 3) that EGHV1A is shed in saliva from the two captive Asian elephants. To date, no EEHV1A, EEHV1B, EEHV4 or EEHV5, likely endogenous viruses of and the most common causes of hemorrhagic deaths in wild and captive Asian elephants, have been found in our tissue biopsies and saliva samples from either wild or captive African elephants. However, because EEHV2 and EEHV3 have caused hemorrhagic deaths in two captive African and one captive Asian elephant respectively, we need to ask the questions, “Do the elephant herpesviruses we suspected as natural endogenous viruses of African elephants (EEHV2, EEHV3A, EEHV3B, EEHV6, EEHV7A, EEHV7B) cause hemorrhagic deaths in wild African elephants and does co-infection with elephant gammaherpesviruses (EGHV1B, EGHV2, EGHV3B, EGHV4B, EGHV5B) affect pathogenesis of this disease?”

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## DETECTION OF EEHV IN TRUNK WASH SECRETIONS FROM CAPTIVE AFRICAN ELEPHANTS (*Loxodonta africana*)

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### Abstract

Elephant Endotheliotropic herpesvirus (EEHV) can cause fatal disease in endangered Asian elephants (*Elephas maximus*) though latent infection and shedding appears common in normal animals in captivity and in range countries. EEHV's have been noted in captive African elephants (*Loxodonta africana*) in necropsy samples and in wild African elephants recently surveyed for endogenous viruses via trunk nodule biopsy. The prevalence and type of latent EEHV infection in healthy captive African elephants is relatively unknown. To address this issue, trunk wash samples were collected from two African elephant herds. DNA extraction and qPCR was performed for EEHVs 1, 2, 3/4, 5, and 6. Preliminary results indicate that in one herd, 4 of 6 elephants were positive for EEHV 3/4. One elephant in this herd was positive for EEHV1 and had previously been housed with Asian elephants. Each animal in the second herd was only positive for EEHV6, although the sample timeline was very limited. Further collection for DNA sequencing will differentiate between EEHV 3 and 4, and characterize it along with the EEHV1 and EEHV6 shed by the other elephants.

## MULTIPLE NEW VARIANTS AND SUB-TYPES OF ELEPHANT GAMMAHERPESVIRUSES (EGHVS) AND DELTAHERPESVIRUSES (EEHVS)

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### Abstract

Expanding genetic analysis of Asian and African elephant herpesvirus genomes has both greatly clarified the evolutionary relationship between EEHVs and other mammalian herpesviruses and revealed even more distinct types. Previous studies of the best known Proboscivirus genome EEHV1B (Kiba) revealed only that the closest related mammalian herpesviruses were HHV6A, HHV6B, HHV7 and PigCMV in the Roseolovirus genus. However, our extensive evaluations of two strains each of EEHV1A, EEHV1B and EEHV2 (totaling 60 to 65-kb each) and two of EEHV5 and EEHV6 (totaling 22 to 25-kb each) have revealed that all five types have a large inversion of a 40-kb core segment of the genome relative to the Roseoloviruses and all Betaherpesviruses. They also encode Alphaherpesvirus-like TK, RRB, OBP genes and Ori-Lyt that are absent in Betaherpesviruses. Furthermore, in phylogenetic trees all known EEHV genes and proteins that have orthologues in other herpesviruses are highly diverged from and fall into a monophyletic clade branching intermediate between the mammalian Gammaherpesvirus and Betaherpesvirus sub-families. Therefore, we propose that the Probosciviruses/EEHVs should be considered the prototypes of a new fourth Deltaherpesvirus sub-family of mammalian herpesviruses.

We have also identified chimeric features and variants in many EEHV species. All 36 known EEHV1 strains fall into just two major clusters EEHV1A and EEHV1B that differ mostly at two loci of 3.0-kb (gB-POL) and 3.8-kb (gN-gO-gH-TK) that are diverged by 17% and 32% at the DNA level, but vGPCR1 and gH also cluster into 4-5 additional subtypes. Whilst this indicates an ancient chimeric origin for EEHV1B, at least one hybrid virus (Haji) is a simple modern recombinant between EEHV1A and EEHV1B. Similar analyses revealed two sub-types of EEHV2 (that differ only in the vGPCR), as well as two of EEHV6 (differing by 15% in gH) and similarly two of EEHV5 (differing by 10-20% in gB-POL, TK-U49 and UDG). Additional comparative data based on the collaborative project with Paul Ling (BCM) and Joe Petrosino (HGC) to identify all 117 core and novel genes in the intact 177-kb draft genome of EEHV1A(Kimba) has also revealed other hypervariable loci in EEHV1A (vGPCR4 and two types of captured cellular vOX2 genes) plus evidence of even more chimeric recombination patterns. Thus, this is a very old virus family unlike any other known herpesviruses and with a complex recombinational history.

We now also recognize 8 types of elephant gammaherpesviruses that fall into five groups. Two new EGHVs have been found in African elephants, one (EGHV5B) associated with oral mucosal “papilloma-like” lesions and the other (EGHV1B) in saliva. Data from the 480-bp POL locus suggest that Asian elephants carry one subtype (EGHV1A, EGHV3A and EGHV5A), whereas African elephants carry another (EGHV1B, EGHV1B and EGHV5B). In each case, the

pairs differ by between 3.5 to 5% at the DNA level (ie 15 to 23-bp). If this pattern holds at other genetic loci, it is likely that each member of these pairs will become recognized as a distinct virus species. The pathology of the three types of herpesvirus associated nodules carrying EEHVs (lung and skin) versus EGHVs (oral) will be compared.

**BOTH BENIGN LUNG AND SKIN NODULES FROM AFRICAN ELEPHANTS (*Loxodonta Africana*) CONTAIN MULTIPLE STRAINS AND SUBTYPES OF EEHV2, EEHV3, EEHV6 AND EEHV7 THAT ARE GENETICALLY VERY DISTINCT FROM THE EEHV'S FOUND IN ASIAN ELEPHANTS (*Elephas maximus*).**

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**Abstract**

Herpesvirus-like particles were first recognized in the 1970s by light and electron microscopy in benign lung nodules in culled adult South African elephants and again in the 1980s in skin nodules observed in orphaned calves imported from Zimbabwe to Florida. Initial PCR analysis in the late 1990s suggested that the lung nodules contained EEHV2 and the skin nodules contained EEHV1. However, the latter result could not be confirmed in subsequent re-examination of archival skin nodule specimens. Evaluation of two large frozen South African lung nodules did confirm the present of very abundant EEHV2 in one and also of EEHV3 in both. Four small lymphoid nodules were later also dissected from the lung of a euthanized USA zoo adult African elephant. Remarkably, these proved to contain multiple different combinations of EEHV6>EEHV3>EEHV2 as well as another newly identified virus EEHV7. Sequence data at five PCR loci (POL, TER, U71/gM, OBP, HEL) totaling over 3000-bp have now been acquired for the prototype EEHV7 genome. The novel EEHV7 is 14-17% different from both EEHV3 and EEHV4, but the three branch together as a separate GC-rich group of the Probosciviruses, which is 35% diverged from the AT-rich branch containing EEHV1A, EEHV1B, EEHV2, EEHV5 and EEHV6.

In recent collaborative studies, carried out jointly with Save the Elephants and The Kenya Wildlife Service, these same four species EEHV2, EEHV3, EEHV6 and EEHV7 have now also been found in multiple samples collected from healthy African elephants in Kenya. EEHV6, EEHV3 and EEHV2 were all present in random lung samples from an adult necropsy and EEHV3, EEHV7 and EEHV2 were present in a variety of combinations in biopsied skin lesions from five immobilized juveniles. Furthermore, in many of these nodules, two distinctive subtypes of either or both EEHV3 and EEHV7 were identified that we refer to as EEHV3A, EEHV3B, EEHV7A and EEHV7B. The latter two new sub-type pairs were both distinguishable at and resolved further into multiple strains at all four or five PCR loci examined so far. Although the 3A/3B and 7A/7B pairs were found to differ from each other by between 5 to 17% at the U71/gM and U73/OBP loci, the differences are no more than 1 to 3% at the POL, HEL or TER loci so they probably do not qualify as distinct species. Overall, the discovery of several independent lung and skin nodules that each contained between three and five different EEHV types simultaneously makes a powerful argument implying that most likely all six viruses (EEHV2, EEHV3A, EEHV3B, EEHV6, EEHV7A and EEHV7B) are natural endogenous infections of African elephants, and that most African elephants both ex situ and in situ likely carry multiple dormant or latent species of EEHVs. Furthermore, these are all very different

from the several other EEHV types found in both lethal hemorrhagic disease cases in Asian elephants and in many asymptomatic Asian elephants, and only a single example is known of a cross-species infection event (with EEHV3A).



**ACUTE PHASE PROTEIN EXPRESSION DURING ELEPHANT  
ENDOTHELIO TROPIC HERPESVIRUS-1 VIREMIA IN ASIAN ELEPHANTS (*Elephas  
maximus*)**

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**Abstract**

The acute phase response forms the cornerstone of the inflammatory response and is mediated by acute phase proteins (APP). Major APP are synthesized by the liver within the first 24 hours post stimuli and can increase in magnitude rapidly in comparison to traditional markers of inflammation such as fibrinogen and total white blood cell count. In addition, the half life of these markers is quite short so they are often utilized for prognostication. APP have been demonstrated to increase with a variety of infectious etiologies in domesticated mammals but have not yet been described in elephants. In this study, whole blood and trunk wash samples representing repeated measures from 10 elephants were examined for the presence of EEHV1 using a qPCR assay. Elephants were classified into three groups: 1) EEHV1 negative; 2) EEHV1 trunk wash positive, but whole blood negative; 3) EEHV1 whole blood positive (viremic). Serum amyloid A (SAA) and haptoglobin (HP) levels were compared between the three groups of elephants. A significant difference in SAA was observed with nearly 10 fold higher mean values during periods of viremia. Higher values of SAA were associated with >10,000VGC/ml in whole blood. A significant increase in HP was observed in elephants which were whole blood negative but trunk wash positive. These data indicate that an inflammatory process is stimulated during EEHV1 viremia. APP quantitation may aid in monitoring the health status of Asian elephants and provide a platform for future EEHV research.

## PRENATAL PASSIVE TRANSFER OF MATERNAL IMMUNITY IN ASIAN ELEPHANTS (*Elephas maximus*)

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### Abstract

Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants exhibit characteristics of endotheliochorial placentation, which is common in carnivore species and is associated with modest maternal to fetal transplacental antibody transfer. However, it remains unknown whether the bulk of passive immune transfer in elephants is achieved prenatally or postnatally through ingestion of colostrum, as has been documented for horses, a species whose medical knowledgebase is often extrapolated for elephants. To address this issue, we took advantage of the fact that many zoo elephants are immunized with tetanus toxoid and/or rabies vaccines as part of their routine health care, allowing a comparison of serum antibody levels against these antigens between dams and neonates. Serum samples were collected from 3 newborn Asian elephant calves at birth (before ingestion of colostrum); 2-4 days after birth; and 2-3 months of age. The findings indicate that the newborns had anti-tetanus toxoid and anti-rabies titers that were equivalent to or higher than the titers of their dams from birth to approximately 3 months of age, suggesting that the majority of maternal-to-fetal transfer is transplacental and higher than expected based on the architecture of the Asian elephant placenta.

**Keywords:** Asian elephant, endotheliochorial, passive immunity, rabies, tetanus

### Acknowledgments

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## DEVELOPMENT OF AN ELISA FOR DETECTION OF ANTIBODIES AGAINST ELEPHANT ENDOTHELIO TROPIC HERPESVIRUS (EEHV)

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### **Abstract**

Since the identification of elephant endotheliotropic herpes virus (EEHV) in 1995 as a causative agent of fatal hemorrhagic disease of Asian elephants (*Elephas maximus*), the virus has claimed more than 50 deaths of newborn and young Asian as well as African elephants (*Loxodonta africana*) both in captivity and in the wild. Despite the devastating disease that it may cause and the great risk that it poses to elephant conservation programs, little is known about the epidemiology, prevalence and occurrence of EEHV. The department of virology of the Erasmus University in Rotterdam, The Netherlands, is involved in a project to develop a serological assay for detecting EEHV specific antibodies. To this end, the glycoprotein B of EEHV was cloned into the baculovirus and the E-coli expression systems. Semi-purified proteins were used to test the antibody sero-prevalence in sera of elephants from the Blijdorp zoo in The Netherlands. Challenges to develop the gB-based ELISA system will be discussed.

**[WWW.EEHVINFO.COM](http://WWW.EEHVINFO.COM): UPDATE ON THE SUCCESSES AND FAILINGS OF THE EEHV WEBSITE**

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**Abstract:**

At the 7<sup>th</sup> Annual International Elephant Endotheliotropic Herpes Virus (EEHV) Workshop, 2011, a proposal was made to develop and deploy a website dedicated to EEHV. The stated aim was “To provide a peer reviewed website dedicated to communicating current information on all aspects of EEHV to both veterinarians, animal husbandry staff and any other interested parties that care for both captive and wild elephant species”. A team of veterinarians, curators, researchers, pathologists and field researchers provided input and the website went live in September 2011.

Whilst the website does meet the aims it set out to do it has failed in some areas. Following the initial interest updates have not been provided, either through lack of communication, lack of knowledge of the website or due to failure of the web provider to chase up authors. This is an area that needs to be addressed and consideration to this challenge will be reviewed during the presentation.

The website still provides accurate and current information. However membership is low, despite links through websites such as Elephant News and through publicity at national and international veterinary meetings such as AAZV in 2011. Trying to reach a wider audience, e.g. EMA would be useful.

[www.eehvinfo.com](http://www.eehvinfo.com) continues to be supported and this brief overview will consider what we want this invaluable tool to do for the elephant community.

Final two presentations on Monday 1/28/12:

**THE EEHV STORY.... THAT YOU SHOULD HELP TELL**

*Jill Alread, APR, Public Communications, Inc.*

NOTES:

**FINANCIAL NEEDS FOR EEHV RESEARCH**

*Paul D. Ling, Ph.D., Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, 77030, USA*

NOTES:

## EEHV “Fast Plan”

Initiate treatment if :

- clinical symptoms present
- 5000 VGE/ml or greater
- rapidly increasing VGE/ml

This is intended to be an instruction sheet to get therapy initiated as quickly as possible. Background information, details, and reasoning for these steps are present in the EEHV protocol.

1. Decision to treat an infected elephant (see decision tree page)
  - TPR
  - BP
  - Blood collection (12 ml in purple tops 30 ml in tiger tops)
2. Administer rectal fluids
3. Administer 15 mg/kg Famciclovir
  - Orally or
  - Rectally (grind with mortar and pestle, mix with water to make a paste)
4. Standing sedation with Butorphanol 0.06 mg/kg IM followed in 15 minutes by detomidine 0.015 mg/kg IM (can reverse with 2.5 X dose naltrexone and 5 X dose atipamezole)
  - Provide supplemental oxygen via nasal cannula when possible
5. Place 3” long (or longer) large bore catheter in saphenous vein (with injection cap) (consider multiple venous catheters if reversing sedatives immediately)
6. Administer 5 mg/kg ganciclovir mixed in 1 liter of fluids over the course of an hour
7. Administer up to 10 ml/kg plasma
8. Maintain fluids at rate of 2-4 ml/kg/hour
9. Administer Naxcel at 1.1 mg/kg IV

## EXCERPTS FROM HOUSTON ZOO EEHV PROTOCOL (updated June 2012):

### Standing Sedation of Calf/Clinical Suspect

- Butorphanol 0.045 – 0.075 mg/kg butorphanol IM – reverse with Naltrexone 2.5 – 5 X Butorphanol dose
- Followed 15 – 20 minutes later by Detomidine 0.011 – 0.022 mg/kg IM – reverse with Atipamezole 5 X Detomidine dose
- This results in a good standing sedation “sawhorse stance” which allows placement of catheters in the ear or front and rear leg.
- Initial dose lasts about 2 hrs, then supplemented as needed.
- Higher doses of Detomidine resulted in lateral recumbency.
- Reversal w/Naltrexone and Atipamezole was complete.

### Light Sedation in Adult elephant

- It may be necessary to sedate the dam or other adult herd mates so they are not stressed during manipulations of the calf
- We have given 2 adult female Asian elephants (Shanti and Methai) Butorphanol 20 mg (0.006 mg/kg) and Detomidine 10 mg (0.0026 mg/kg) intramuscularly
  - calming/light sedative effect occurred within 10 to 15 minutes and lasted 1-2 hrs
  - no adverse side effects were seen
- Sedation can be reversed as described above but is not necessary

## EQUIPMENT AND SUPPLIES

The following equipment and supplies will need to be on hand for support during therapy. One staff member will be designated to move these supplies in an organized manner into the hay room of the barn. Supplies used on a daily basis in the area will be left in their normal storage locations.

### Elephant Barn supplies:

- Assortment of ropes, slings and belly bands
- Calf harness
- Flashlights
- Mortar and pestle
- Towels
- Inner tubes (various sizes)/ gym mats —to be used for cushioning and support in the event of a full immobilization procedure

### Clinic Supplies:

#### 1) **Oral or rectal administration of Famciclovir**

- Famciclovir 500 mg tablets, 30 tablets/bottle: 15,000 mg / bottle
- Mortar and pestle (to grind in case rectal administration is necessary)
- Ultrasound gel (for mixing with famvir for rectal administration)
- OB sleeves and lube
- Duct tape

- Hydrogen peroxide (minimum 1 bottle)
- Orasweet OTC syrup (minimum on hand 4,500 ml)
- Exam gloves (all sizes)
- Towels (10-12)

## 2) Rectal Fluids

- Staff should wait a minimum of one hour after fluids to administer Famciclovir. Excess water in the rectum should be raked out before the rectal administration of meds.
- To be administered by elephant team via warm water hose
  - Adults elephants at HZI receive ~3 gallons of water per minute from the old cow barn
  - Rectal fluid treatments average 8 minutes (time is dependent on warm water avail.)
  - Methai averaged 25.2ml/kg per treatment in 2011
  - (initial bolus 20 ml/kg recommended)

## 3) Standing sedation

- Print out medication dosage chart from P drive
- Veterinarians will grab from safe:
  - Butorphanol (minimum 4 bottles, 5 ml/bottle, 30 mg/ml)
  - Detomidine (Minimum 3 bottles 5 ml/bottles, 10 mg/ml)
  - Naltrexone (minimum 4 bottles, 50 mg/ml, 30 ml/bottles)
  - Atipamezole (minimum 13 bottles, 10 ml/bottle, 5 mg/ml)
- Tech box
- Drugs: ceftiofur, amikacin, flunixin meglumine (see dosage chart for amounts)
- Emergency box (make sure there is enough of the following drugs):
  - Large animal atropine
  - epinephrine
- Anesthesia clip board
- Calculator
- Pole Syringe
- Syringes (Box each of 60, 35, 20, 12, 6, 1 ml sizes)
- Needles (14g, 16g, 18g, 20g, 22g 1.5", 23g, 25g; one box each)
- Butterfly catheters 19g, 21 ga. (1 box each)
- Supplies to make up Heparin flush
  - 250 ml bag of NaCl
  - 500 U Heparin added to 250 ml of NaCl

## 4) Oxygen-portable anesthesia machine

- Portable oxygen tanks
  - Plastic tubing for nasal oxygen administration
- ET tubes (40, 30, 24, 22, 20)
- Catheter type stylets for intubation
- Laryngoscope w/long blade
- Y piece (nasal administration)
- Ropes (open mouth)
- Blocks (open mouth)



- Pulse oximeter and capnograph
- I-stat
- Stethoscope
- Thermometer
- Head lamp
- Flash light
- Endoscope and associated equipment (intubation)

#### 5) Placement of IV Catheter

- Sonosyte Ultrasound and 5 MHz probe
- Large animal surgery pack
- Clear instrument pack
- 10-14 GA catheters
- Injection caps, T port
- Large Animal IV (bungee type) line (3 complete sets)
- Standard IV administration set (3 complete sets)
- Large Animal IV extension set (3 complete sets)
- Standard Extension set (3 complete sets)
- Scalpel blades: 22, 10, 15
- Surgical prep: chlorhexidine scrub and alcohol
- Sterile Gloves (6 1/2, 7, 8, 8 1/2)
- Lidocaine, bupivacaine
- Tourniquet (bungee cords, or Daryl suggested innertubes)
- Drapes
- Sharps container
- Suture (0, 1, 2 prolene or similar with cutting needle)
- Skin stapler
- Tissue glue
- Duct tape (3-4 rolls)
- White tape (1 inch, 2 inch, 4 inch; 5 rolls each)
- Vetwrap (2-6", multiple rolls each size)
- Elastica (2-4", multiple rolls each size)
- Rolled cotton (3 rolls)
- 4X4 gauze (6 packages)
- 5 liter fluids (all in stock)
- IV pump
- IV pole
- Flagpole holders (called parade belts, ~\$20-\$30 each)
- Ropes/wire to hang bags
- Extension cord
- Towels
- Flashlights/head lamps
- Plasma (placed in elephant office freezer, keep frozen till needed)
- Plasma administration filter
- Hetastarch (acquire later?)

#### 6) IV Ganciclovir (Cytovene)

- Ganciclovir 500 mg vials, 25 vials / box: 12,500 mg/ box
  - Minimum 3 day supply for Tucker: 11,500 mg PO BID 2 boxes/day = 6 boxes
- 100 ml bottle of sterile water to mix with ganciclovir powder
- 2 liters NaCl to administer during ganciclovir
- Large syringes (20-60 ml) for mixing up ganciclovir

#### 6) Monitoring

- ICU flow sheet, pens, clipboard, watch
- Sonosite U/S Machine
- Doppler
- Blood pressure cuff
- ECG (cerclage wire contacts vs. sticky pads?)
- Digital camera
- Video camera
- Ophthalmoscope (1)
- Ophthalmoscope extra battery
- Culturettes

#### 7) Drugs (see chart)

#### 8) Equipment for pericardiocentesis:

- Sonosite U/S machine
- Scrub and alcohol
- 60 cc regular tipped syringes
- 3 way stop cocks (2)
- Extension sets(2)
- 5 ¼ “ IV catheter, smallest gauge available
- 100 mm dart needles (2)
- Sterile urine cup to save for culture
- 50 ml conical vials for storage of fluid

## EEHV References Updated December 2012

- Richman LK, Montali RJ, Cambre RC, Schmitt D, Hardy D, Hildbrandt T, Bengis RG, Hamzeh FM, Shahkolahi A, Hayward GS. 2000. Clinical and Pathological Findings of a Newly Recognized Disease of Elephants Caused by Endotheliotropic Herpesviruses. *J. Zoo and Wildlife Medicine* 36(1): 1-12.
- Schmitt D, Hardy D, Montali RJ, Richman LK, Lindsay WA, Isaza R, West G. 2000. Use of Famciclovir for the Treatment of Endotheliotropic Herpesvirus Infections in Asian Elephants (*Elephas maximus*). *J. Zoo and Wildlife Medicine* 31(4): 518-522.
- Hayward G, Latimer E, Richman L, Olson D. 2009. International Elephant Foundation's Progress Report on Elephant Endotheliotropic Herpesviruses. On Association of Zoos and Aquariums Elephant TAG/SSP Website: [www.elephanttag.org](http://www.elephanttag.org)
- Schaftenaar W, Reid C, Martina B, Fickel J, Osterhaus ADME. 2010. Nonfatal Clinical Presentation of Elephant Endotheliotropic Herpes Virus Discovered in a Group of Captive Asian Elephants (*Elephas maximus*). *J. Zoo and Wildlife Medicine* 41(4): 626-632.
- Richman LK. 2008. Elephant Herpesviruses. *In: Fowler ME and Miller RE (eds.). Zoo and Wild Animal Medicine, Current Therapy 6.* Saunders Elsevier, St. Louis, MO. Pp. 349-354.
- Fowler ME. 2006. Elephant Endotheliotropic Herpesvirus (EEHV) Infection. *In: Fowler ME and Mikota SK (eds.). Biology, Medicine, and Surgery of Elephants.* Blackwell Publishing, Ames, IA. Pp. 132-133.
- Reid CE, Hildebrandt TB, Marx N, Hunt M, Thy N, Reynes JM, Schaftenaar W, Fickel J. 2006. Endotheliotropic Elephant Herpes Virus (EEHV) Infection. The First PCR-confirmed Fatal Case in Asia. *Vet Q.* 2006 Jun; 28(2): 61-64.
- Isaza R, Hunter RP, Richman LK, Montali RJ, Schmitt DL, Koch DE, Lindsay WA. 2003. Famciclovir Pharmacokinetics in Young Asian Elephants (*Elephas maximus*). *Proceedings American Association of Zoo Veterinarians 2003.*
- AZA-TAG Protocols on Elephant Necropsies and Tissue/Samples for research (Oct 2010 updates).
- Stanton JJ, Zong JC, Latimer E, Tan J, Herron A, Hayward GS, Ling PD. 2010. Detection of pathogenic elephant endotheliotropic herpesvirus in routine trunk washes from healthy adult Asian elephants (*Elephas maximus*) by use of a real-time quantitative polymerase chain reaction assay. *Am J Vet Res* 71(8): 925-933
- Latimer E, Zong JC, Heaggans SY, Richman LK & Hayward GS. 2011. 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.
- Brock AP, Isaza R, Hunter RP, Richman LK, Montali RJ, Schmitt DL, Koch DE, & Lindsay WA. 2012. Estimates of the pharmacokinetics of famciclovir and its active metabolite penciclovir in young Asian elephants (*Elephas maximus*). *Am J Vet Res* 73(12): 1996-1999.

- Wiedner E, L Howard, R Isaza. 2012. Treatment of elephant endotheliotropic herpesvirus (EEHV). *In: Fowler, ME and RE Miller (eds) Zoo and Wild Animal Medicine, 7th ed: 537-543.*
- Richman LK & Hayward GS. 2012. Elephant herpesviruses. *In: Fowler, ME and RE Miller (eds) Zoo and Wild Animal Medicine, 7th ed: 496-502.*
- Stanton JJ, Zong JC, Eng C, Howard L, Flanagan J, Stevens M, Schmitt D, Wiedner E, Graham D, Junge RE, Weber MA, Fischer M, Mejia A, Tan J, Latimer E, Herron A, Hayward GS & Ling PD. 2013. Kinetics of viral loads and genotypic analysis of elephant endotheliotropic herpesvirus-1 infection in captive Asian elephants (*Elephas maximus*). *J Zoo Wildl Med* 44(1): 42-54.
- Atkins L, Zong JC, Tan J, Mejia A, Heaggans SY, Nofs SA, Stanton JJ, Flanagan JP, Howard L, Latimer E, Stevens MR, Hoffman DS, Hayward GS & Ling PD. 2013. Elephant endotheliotropic herpesvirus 5, a newly recognized elephant herpesvirus associated with clinical and subclinical infections in captive Asian elephants (*Elephas maximus*). *J Zoo Wildl Med* 44(1): 136-143.
- Zachariah A, Zong JC, Long SY, Latimer EM, Heaggans SY, Richman LK & Hayward GS. 2013. Fatal herpesvirus (EEHV) hemorrhagic disease in wild and orphan Asian elephants in Southern India. *J Wildl Dis, in press*
- Hardman K, Dastjerdi A, Gurralla R, Routh A, Banks M, Steinbach F & Bouts T. 2012. Detection of elephant endotheliotropic herpesvirus type 1 in asymptomatic elephants using TaqMan real-time PCR. *Vet Rec* doi: 10.1136/vr.100270
- Denk D, Redrobe S, Latimer E, Hayward GS, Cracknel J, Classens A, Steinbach F, McGowan S & Dastjerdi. 2012. Fatal elephant endotheliotropic herpesvirus type 5 infection in a captive Asian elephant. *Vet. Rec* doi: 10.1136/vr.e6833
- Stanton JJ, Nofs SA, Peng R & Ling PD. 2012. Development and validation of quantitative real-time polymerase chain reaction assays to detect elephant endotheliotropic herpesviruses-2, 3, 4, 5 and 6. *J Virol Meth* 186: 73-77
- Hayward GS. 2012. Conservation: clarifying the risk from herpesvirus to captive Asian elephants. *Vet Rec* 170: 202-203.