EEHV Workshop January 2013 Houston, Texas
Summary and Report

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EEHV Workshop 2013 hosted by:
The Houston Zoo
and
The International Elephant Foundation

LARGER DISCUSSION AREAS FROM DAY 1 PRESENTATIONS

Epidemiology
Diagnostics and Immunity
Clinical management and communication

Bullet Points from the Epidemiology Lectures: (more specific details listed below)

1. 50% of all deaths in Asian elephants born in US since 1980 are due to EEHV.
2. Case definitions for EEHV death and EEHV survivor need to be developed by EEHV research community (IN PROGRESS).
3. Information presented at this workshop firmly establishes that EEHV did not cross from African elephants into Asian elephants, as was originally hypothesized in the 1990's.
4. There is no apparent association between exposure to African elephants and illness or death from EEHV in captive Asian elephants housed in North America.
5. There are 11 types of EEHVs identified to date: 1A, 1B, 2, 3A, 3B, 4, 5A, 5B, 6, 7A and 7B, proposed distribution is below:
    a. Endogenous to Asian elephants: EEHV 1, EEHV 4, EEHV 5
    b. Endogenous to African elephants: EEHV 2, EEHV 3, EEHV 6, EEHV 7
6. EEHV 1, 3 or 4, and 5 were identified in trunk washes of healthy Indian camp elephants.
7. EEHV-1 subtypes in deaths from Indian elephants have the same diversity as is found in Asian elephants in North America.
8. Elephants in Kenya have had EEHV2, EEHV3A&B, EEHV6, and EEHV 7A&B identified in lung and trunk nodules.
9. Healthy African elephants in North America have been shown to shed EEHV 2, EEHV 3A&B, and EEHV6 in saliva and EEHV 3 or 4 and 6 from trunk washes.
10. The genome of EEHV-1 has been sequenced, it is highly diverged from both gamma- and beta- herpesviruses, and should be placed in its own subfamily of mammalian Deltaherpesvirus (rather than as a Betaherpesvirus, where it is currently listed).
11. Multiple gammaherpesviruses (EGHV's) have also been identified in African and Asian elephants, details below.

Bullet points for Diagnostics and Immunity: (details below)

1. Serum amyloid (a major acute phase protein) levels were 10 fold higher in elephants with EEHV viremia, compared to those who were not EEHV viremic.
2. Based on a study of 3 newborn Asian elephants, at least some of the maternal-fetal antibody transfer occurs through the placenta, rather than through the colostrum.
3. Erasmus in Rotterdam has taken steps to develop a glycoprotein B based ELISA for EEHV antibody measurement.
4. Erasmus in Rotterdam has taken steps to develop an MVA-based gB vaccine, further evaluation of the vaccine is needed.

Bullet Points for Clinical Management and Communication: (details below):

1. EEHV-5 can cause fatal disease in Asian elephants.
2. Pericardiocentesis under sedation should be considered in calves with pericardial fluid and decreased cardiac function.
3. Methadone was effective in pain management and improved appetite in a sick calf.
4. www.eehvinfo.com website has low membership and needs more regular updates.
5. 95% of the public feels that seeing live animals gives them better appreciation for the animal and encourages them to learn more about conservation.
6. The National Elephant Herpesvirus Laboratory tests an average of 17 sick elephants each year for EEHV, free of charge. The laboratory is an important resource and is in severe need of more stable funding.

DISCUSSION TOPICS (FROM DAY 2 DISCUSSION GROUPS)

Clinical and Management Oriented Discussion
Research Oriented Discussion
Revisited Goals from 2011 EEHV Workshop
Wrap Up / Final Afternoon Collaborative Session

CLINICAL AND MANAGEMENT ORIENTED DISCUSSION

Veterinarians and Managers
Clinical Management of EEHV and EEHV-preparedness
(Facilitated by J. Flanagan and E. Wiedner)

Three good resources for more detailed information on EEHV Preparedness and Treatment:

1. Website: www.eehvinfo.com
2. Houston Zoo EEHV Protocol, updated yearly, provided to all workshop participants as a PDF on a thumb drive. This protocol is also available to anyone who needs it, contact lhoward@houstonzoo.org or call (713) 533 6632 for a copy.
3. Book Chapter (most zoo veterinarians have this book): Wiedner E, L Howard, R Isaza. 2012. Treatment of elephant endotheliotrophic herpesvirus (EEHV). In: Fowler, ME and RE Miller (eds) Zoo and Wild Animal Medicine, 7th ed: 537-543.

If you have elephants, you have EEHV.
The question is, will your animals get acute hemorrhagic disease associated EEHV?

Bullet Points for Clinical/Preparedness Discussions (see above references for more details):

1. It's not if but when
2. Check drug stocks routinely to make sure they are in date and there is enough

3. Establish communication plans and phone tree ahead of time
4. Elephants should be cross-matched prior to a blood transfusion, this can be done any time
5. Treat symptomatically if you don't have a diagnosis
6. With calves, time is of the essence, you need to treat before you know what you're treating
7. Very sick elephants don't always looks sick
8. Early signs of hemorrhagic disease are not very impressive
9. While waiting for tests to come back, start fluids and monitoring
    a. Rectal fluids are easy to give
    b. Encourage oral fluids/drinking
    c. IV fluids require catheter, difficult to maintain, sedation required to place
        i. Mila over the wire catheters recommended
10. Close monitoring of a clinical EEHV case is very important.
11. To monitor hematocrit closely, consider having a microhematocrit centrifuge stall side
12. Three treatment goals for EEHV:
    a. Treat shock (fluids)
    b. Decrease viral load (famciclovir)
    c. Provide supporting and symptomatic care.
13. Famciclovir is still the oral antiviral drug of choice for treatment of EEHV

EEHV RESEARCH ORIENTED DISCUSSION

Researchers
Prioritization of Research Needs and Goals
(Facilitated by J. Alread and L. Howard)

Identification of Research Priorities: (details below)

1. FUNDING (details below)
2. Culturing virus (details listed later in summary)
3. Immunology/ serology (details listed later in summary)
4. Vaccine development (not discussed in detail today)
5. Antiviral drug efficacy (not discussed in detail today)
6. Epidemiologic Analysis (details listed later in summary)
7. Unifying collaboration between researchers (details below)

Funding

Highest Priorities for Funding right now:

1. NEHL needs a Real time PCR machine (cost: $25,000)
    i. Ability to quantify both whole blood and trunk washes
2. ***Need full time, dedicated employees working on EEHV research****
    a. Post doc: salary $60,000/year for 2-3 year positions

b. There is space and mentoring available for these positions, just need funding
c. Can we secure donors for post doc positions?

Possible Funding Sources:

1. Have all EEHV research participants contribute information on what grants are available.
2. National Science Foundation (NSF) grant: https://www.nsf.gov/funding/
   a. evaluate genetics, population modeling, or ecology of EEHV
   b. Emerging Infectious Disease grant by NSF: due date by Dec 5ish, 2013
   c. http://www.fic.nih.gov/Programs/Pages/ecology-infectious-diseases.aspx
3. Look for Mega Grants (multi-national grants)
4. Look into funding opportunities need to focus on 100,000 to 1 million dollar grants
5. IUCN SOS grants http://sospecies.org/sos_projects/apply_for_a_grant/
6. Sime Darby grants? http://www.yayasansimedarby.com/1002101050%c2%bbConservation_of_The_Environment_and_Protection_of_Ecosystems_.aspx

Unifying Collaboration Between Researchers

1. We need ONE of the following two suggestions:
   a. A paid program manager (see Great Ape Heart Project for model: http://greatapeheartproject.org/ (hosted by Zoo Atlanta)
      i. Would require funding for salary, computer access, etc.
   b. To establish an EEHV working group (see AZA Wildlife Contraception Center for model: http://www.stlzoo.org/animals/scienceresearch/contraceptioncenter/ (hosted by St. Louis Zoo)
      i. need a zoo director to champion this and push it in AZA
2. Need a forum for researchers to communicate real time to avoid duplication, etc.
   a. Develop a specific email list serve for EEHV discussions?
   b. Or use a password protected area of www.eehvinfo.com for this?
3. Need place for centralized information, use www.eehvinfo.com for this?
   a. Open this information to everyone
      i. Clinical vets, basic researchers, human virologists, epidemiology
   b. to include bios on researchers and what they have done
      i. also contact information (this should be password protected?)
   c. All researchers at the meeting were asked to provide bio and research information to Lauren Howard to be complied (email to: Lhoward@houstonzoo.org)
4. Consider Q 3 to 6 month video conference or Skype between research institutions
5. Need a mechanism for summarizing literature, and identifying new findings and publications
6. One suggestion: Take portion of pot of money open to competitive research projects
   a. open up to other people, not just people in the room, spread the wealth
7. Should we hire a scientific journalist to spread the word on EEHV?

8. Collaborate with experts in other fields that may have resources/information
9. Consider a centralized diagnostic lab sample center to control samples and information?
10. Consider outsourcing testing to a more standard diagnostic laboratory?
    a. Then other labs (NEHL, BCM) could focus more on research projects

REVISITING OF GOALS FROM 2011 WORKSHOP

Disease and Herd Management Goals from 2011, Revisited

Completed:

- Development of online resource (www.eehvinfo.com).
- Develop list highest priority animals for potential screening.
- Review/revise TAG/SSP research documents and update.
- Distribute EEHV information to elephant facilities via workshop, website, etc.

High Priority:

- Develop case definitions for EEHV
- Form subcommittee to determine what physiologic parameters should be monitored in elephants
- Standardize recording of data on clinical cases.

Low Priority

- Design/create equipment to facilitate treatment of elephants (fluid delivery, etc.).
- Increase access to antiviral drugs to allow for treatment quickly in acute cases.
- Create a risk assessment to guide elephant transfers.
- Establish standardize testing recommendations for suspect clinical cases.

Research Goals from 2011, Revisited

Completed:

- Collect data from range country populations
- Develop real time PCR for EEHV strains other than EEHV-1

High Priority:

- Culture EEHV virus
- Develop Vaccine
- Develop ELISA
- Evaluate efficacy of antivirals
- Enhance/maintain international collaboration so resources and information can be shared

Low Priority:

- Collect more data from range country populations
- Integrate greater, more affordable access to drug therapy for EEHV

Public Messaging and Fundraising Goals from 2011, Revisited

Completed:

- Internal messaging: develop overview of EEHV (two book chapters published in 2012.

High Priority:

- Develop business model to ensure adequate funding for EEHV laboratories
- Identify new revenue streams to support research

Low Priority:

- Ensure collaboration of elephant holding institutions in the pursuit of grants
- External messaging: develop EEHV messaging for distribution outside our professional community

WRAP UP / FINAL AFTERNOON SESSION

Big Ticket Items That Need To Be Addressed:

1. **EEHV Website: how to keep it updated, whether to open up more of it to public access, make password restricted areas just for research discussions/communications**
2. **Whether to post a public list of EEHV cases**
3. **How to make NEHL sustainable**
4. **Steps needed to develop an EEHV advisory group or working group**

Some additional discussion that did not fit elsewhere:

Question arose about **mixing of Asian and African elephants**. Recent findings have established that EEHV did not cross from African to Asian elephants, so early recommendations to keep the two species separate due to EEHV risk are no longer founded. When possible it is good practice to keep the species separate, however co-mingling of elephants due to facility or social limitations does not constitute negligence.

Question arose regarding **elephant translocation relative to EEHV risk**. For AZA facilities, the AZA TAG/SSP makes breeding and transfer recommendations based upon population analysis, logistics, and dialogue with the affected institutions. With respect to the consideration of EEHV risk in translocations, elephant managers are advised to consult with their veterinarians and EEHV subject matter experts for additional information prior to the decision to relocate elephants.

Suggestion arose to develop a **consortium to secure funding for the NEHL** and establish a sound business model, committee members were suggested: Rick Barongi, Harry Peachy, Erin Latimer, Tim Walsh, Paul Ling.

Achievements Since We Wrapped up on Jan 29, 2013:

…..so, what have you done for me lately? Here is a list of some of the ongoing EEHV-related activities that have occurred since the workshop:

Post Conference Press Release was distributed by Houston Zoo to conference participants, who were encouraged to add their own details and share with their local media.

AAZV Member Spotlight and Highlight of EEHV Workshop:
http://www.aazv.org/displaycommon.cfm?an=1&subarticlenbr=904

EEHV Case Definition Committee formed:

1. Committee Members: Sharon Deem, Lauren Howard, Ramiro Isaza, Ellen Wiedner
2. *At risk population* has been defined as **Asian elephants born in North America since 1978**.
3. Abstract accepted to AAZV 2013 Conference in Salt Lake City Utah: EPIDEMIOLOGY OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS 1 (EEHV-1) IN ASIAN ELEPHANTS (*Elephas maximus*) IN NORTH AMERICA.
4. Definition of an EEHV case is underway, contact Lauren Howard if you have input lhoward@houstonzoo.org
5. Next step after case definition determined is to establish a curated master list of cases

Dr. Byron Martina and research staff at Erasmus MC in Rotterdam have developed a fully functional running EEHV gB ELISA, further testing on elephant sera is being performed.

Baltimore Zoo successfully diagnosed and treated a 5 year old African elephant bull calf ill from EEHV-3, laboratory testing by both NEHL/Johns Hopkins and BCM was critical to medical management and decisions on treatment (to date calf is no longer viremic and is off treatment). Baltimore Zoo officials publicly announced the situation when the calf was still on treatment and weathered the good and bad publicity that followed, kudos to the whole team for a job well done. Dr. Ellen Bronson will be presenting the clinical case at the IEF/IRF meeting in Aug 2013.

Lauren Howard and Paul Ling were interviewed by Thayne Maynard (Field Notes with Thayne Maynard http://wvxu.org/programs/field-notes) on 4/23 to promote EEHV awareness on his Sunday radio show. Transcript of interview will be available via a link through Houston Zoo website once it has aired (air-time TBA).

Antiviral Drug Procurement:

Pharmaceutical Specialties, Inc.(PSI) has committed to keeping 1 kilogram of ganciclovir powder* and 1 kilogram of famciclovir powder on hand at their pharmacy in Georgia for any institution that may need to treat an elephant ill from EEHV-associated disease. PSI will provide ganciclovir and famciclovir at a fair market price, emergency delivery can be provided at an additional cost. Contact must be made prior to any drug requests, and institution representatives **MUST** contact Dan Loper, DVM, RPh, at the following number **BEFORE** an EEHV emergency occurs: dan@psipharmacy.com, (800) 818 6486, no commitment required.

*Note: While ganciclovir sodium powder is the salt form used to manufacture the FDA approved injection, this active pharmaceutical ingredient is not sterile (powder only) and is NOT for IV-injection and is only intended for use in an oral ganciclovir syrup recipe. This oral form has not been confirmed to have oral bioavailability in elephants.

EEHV-Related Publications Since January 2013:

- Stanton, J et al. 2013. Kinetics of viral loads and genotypic analysis of elephant endotheliotropic herpesvirus-1 infection in captive Asian elephants. J Zoo Wildl Med 44(1): 42-54.
- Atkins, L et al. 2013. Elephant endotheliotropic herpesvirus 5, a newly recognized elephant herpesvirus associated with clinical and subclinical infections in captive Asian elephants. J Zoo Wildl Med 44(1): 136-134.
- Sripiboon, S et al. 2013. The occurrence of elephant endotheliotropic herpesvirus in captive Asian elephants: First case of EEHV-4 in Asia. J Zoo Wildl Med 44(1): 100-104.
- Zachariah A, et al. 2013. Fatal herpesvirus (EEHV) hemorrhagic disease in wild and orphan Asian elephants in Southern India. J Wildl Dis 49(2): 381-393.
- Ling, PD, et al. 2013. The complete genome sequence of elephant endotheiotropic herpesvirus 1A. Genome Announcements March/April 1(2): 1-2.
- Nofs S, et al. 2013. Prenatal passive transfer of maternal immunity in Asian elephants. Vet Immunology and Immunopathology. *In press*.
- Stanton, JJ et al. 2013. Detection of elephant endotheliotropic herpesvirus infection amongst healthy Asian elephants in South India. J Wildl. Dis. *In press*.

DETAILS ON PRESENTATIONS FROM DAY 1

Epidemiology
Diagnostics and Immunity
Clinical Management and Communication

EEHV EPIDEMIOLOGY

North American Asian Elephant Epidemiology

1. *Preliminary results of multiyear EEHV epidemiology study in Asian Elephants.* (R. Isaza) Study from 2007 to 2012 to determine if EEHV in Asian elephants is associated with exposure to African elephants, and to identify any risk factors associated with EEHV.

In captive born Asian elephants born between 1978 and 2012 in the US, there were 21 deaths from EEHV, and 9 EEHV survivors. Of these 30 total EEHV cases, 15 calves were not treated with antiviral therapy and all died. Fifteen calves were treated with antiviral therapy and 9 survived. The median age was 2.5 years, and 75% of cases occurred between 1.5 to 6 years of age. Twenty institutions that bred Asian elephants were evaluated for risk factors associated with EEHV (11 had a history of EEHV, 9 did not.). **There was no apparent association between exposure to African elephants and illness or death from EEHV in captive Asian elephants in North America**. Of 76 husbandry risk factors evaluated, none were associated with illness or death from EEHV in Asian elephants.

2. *EEHV by the numbers: EEHV case definitions and the impact of EEHV on the captive Asian elephant population in North America.* (L. Howard)

A 2011 Zoo Risk Analysis for Asian elephants identified the North American Asian elephant population as declining 1.6%/year, and approaching a significant bottleneck within 15 to 30 years. An informal population analysis in December 2012 found that the combination of eliminating EEHV and improving breeding is the best way to reach a target population size. Of 130 total Asian elephants in North America that were born on/after 1980 (both captive and wild born), 42 elephants have died and 22 of these were due to EEHV (**50% of all deaths in Asian elephants born in North America since 1980 are due to EEHV**). An additional 8 elephants were ill from EEHV-associated disease but survived infection. Survivors may be defined by the amount of virus present in their blood at the height of their illness.

ADDENDUM: The differences in numbers listed by these two presentations are due to the fact that one EEHV case was in a wild born elephant, thus not included in Isaza's list. One EEHV survivor counted by Isaza was not significantly ill and was not included in Howard's list. **These differences highlight the need to come up with a universally accepted case definition for EEHV-associated death and EEHV survivor.** This was addressed by a smaller discussion group on the second day of the workshop and it was decided that a case definition needed to come *after* a closer evaluation of all the cases. Howard, Isaza, and Sharon Deem will be working

on this through an abstract to be submitted for the AAZV conference and a subsequent peer-reviewed publication.

Indian Asian Elephant Epidemiology

1. *Detection of EEV infection among healthy Asian elephants in South India* (P. Ling)
Study to evaluate whether in situ Asian elephants shed EEHVs in trunk secretions, similar to captive elephants, and what types they shed.

Evaluated 46 healthy camp elephants in 3 regions of South India using real time PCR of trunk washes. **EEHV 1 was seen in 6.5% of trunk washes, EEHV 3 or 4 (BCM test is unable to distinguish between these two) in 8.7%, and EEHV 5 in 19.6%.** EEHV 2 and 6 were not identified in any trunk washes, consistent with the observation that these are associated primarily with African elephants.

2. *High level genetic variability amongst nine cases of fatal EEHV hemorrhagic disease in wild and orphan Asian elephants in Southern India*. (S. Long)
Genetic subtyping of 9 different EEHV 1 viruses identified from Asian elephant calves (5 orphans, 4 wild) that died due to EEHV-associated illness.

Two orphans had the same EEHV 1 subtype, the other 7 samples included 6 distinct strains of EEHV 1A and one of EEHV 1B. **The major EEHV 1 gene subtype distribution patterns from India show the same diversity as is found in Asian elephants in North America and Europe, suggesting EEHV 1 is an endogenous virus in the Indian population**.

Additional Report: EEHV 1 was identified in a female calf under 2 years old at the Chitwan Elephant Breeding Center in Nepal (consistent gross/histopathologic lesions and PCR positive.) Previous screening at this center through blood, trunk secretions and oral swabs had failed to identify EEVH-1.

African Elephant Epidemiology

1. *Elephant herpesviruses EEHV2, EEHV3A, EEHV3B (a new subspecies), EEHV6, EEHV7A (a new subspecies), and EGHV1A, EGHV1B (a new subspecies), EGHV2, EGHV4 found in tissue biopsies and saliva from African elephants in Kenya and America.* (V. Pearson)
Study to determine which elephant herpesviruses are endogenous in African elephants, by evaluating skin nodules from wild African elephants and saliva samples from captive African elephants for the presence of EEHV.

Five Kenyan juvenile elephants with trunk nodules, and one killed elephant with lung nodules, had between 3 and 6 of the following herpesviruses detected via PCR/gel electrophoresis/DNA sequencing: EEHV2, EEHV3A, EEHV3B, EEHV6, EEHV7A, EEHV7B, and EGHV1B.

One year of weekly saliva samples from 2 captive African elephants in New Jersey, shed more than one herpesvirus at a time and have shed EEHV2, EEHV3A, EEHV3B, EEHV6, EGHV1A, EGHV1B, EGHV2 and EGHV4.No EEHV1A, EEHV1B, EEHV4 or EEHV5 have

been found in these samples. Next phase of study is to determine if hemorrhagic deaths occur in wild African elephants, and if co-infection with elephant gammaherpesviruses affects pathogenesis of EEHV.

2. *Detection of EEHV in trunk wash secretions from captive African elephants*. (S. Nofs)
Used quantitative real time PCR to assess type and prevalence of EEHV in trunk washes from 2 healthy captive African elephant herds.

Ten adult elephants total, collection schedules ranged from 3 consecutive days (n=4) to twice weekly for 8 weeks (n=6), samples screened for EEHV 1, 2, 3/4, 5 and 6 (BCM test does not differentiate between 3 and 4). **Four elephants tested were positive for EEHV 3/4, four elephants were positive for EEHV 6, and one elephant was positive for EEHV 1 (had been previously housed with Asian elephants).** Future research to include sequencing and characterization of the detected EEHV's, and test development to differentiate between EEHV 3 and 4.

Summary of Strains and African and Asian EEHVs:

1. *Multiple new variants and sub-types of elephant gammaherpesviruses (EGHVs) and deltaherpesviruses.* (G. Hayward)
Discussion of 19 existing elephant herpesviruses and their taxonomic distribution.

The EEHV-1A genome (source elephant: Kimba, Houston Zoo) has been sequenced by a consortium at the Houston Genome Center (J. Petrosino group), Baylor College of Medicine (P. Ling Group), and Johns Hopkins (G. Hayward group); publication is pending. **EEHV is highly diverged from both mammalian gammaherpesviruses and betaherpesviruses (**by greater than 50% at the protein level**) with more than half the genes being novel and not present in other herpesviruses, and should be placed in its own subfamily of mammalian Deltaherpesvirus,** rather than as a Betaherpesvirus, where it is currently listed.

There are 19 types of elephant herpesviruses, 11 EEHVs (Proboscivirus: 1A, 1B, 2, 3A, 3B, 4, 5A, 5B, 6, 7A and 7B) and 8 EGHVs (Gammavirus: 1A, 1B, 2, 3A, 3B, 4, 5A, 5B). Of the 8 types of elephant gammaherpesviruses, evidence suggests so far that Asians carry one subtype (EGHV1A, 3A, and 5A), while Africans carry another (EGHV1B, 3B, and 5B).

There have been 35 distinct strains of EEHV1A and 6 strains of EEHV 1B identified in Asian elephants. No two facilities have shared the same strain. There are 6 facilities that have had disease cases involving both 1A and 1B strains, with 2 separate primary infection events. This suggests infection with one subtype does not provide immunologic protection against *infection* by the other subtype, but may protect against catastrophic disease.

This is a very old virus family unlike any other known herpesvirus with a complex recombinational history.

2. *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.* (G. Hayward)
This talk firmly establishes that EEHV did not cross from African elephants into Asian elephants.

Numerous cases (40-60) of hemorrhagic death associated with EEHV infection have been found in both orphan and wild calves in Asian range countries, many of these confirmed via PCR. To date EEHV1A and EEHV1B have been identified in India, plus EEHV1A in Thailand, Nepal, Indonesia, and one case of EEHV4 in Thailand. The pathology of these cases is identical to North American and European pathologic findings.

It appears that EEHV1A &1B, EEHV4 & EEHV5 are native to Asian elephants.

To date, EEHV2, EEHV3, EEHV6 and EEHV7 have been found in both lung and skin nodules in wild Kenyan and South African elephants and in one euthanized USA African adult.

The old idea of EEHV1 crossing species from Africans to Asians in zoos is disproved by these findings. However, there was one cross species event of an Asian elephant becoming ill from EEHV3, which has been more closely associated with African elephants.

DIAGNOSTICS AND IMMUNITY

1. *Acute phase protein expression during EEHV-1 viremia in Asian elephants*. (C. Cray)
Study to evaluate the use of inflammatory markers in elephants with EEHV infection.

Major acute phase proteins (serum amyloid and haptoglobin) promote healing and homeostasis and are produced by the liver within the first 24 hours of tissue damage or infection. These proteins are measured in the serum, increase rapidly compared to traditional markers such as fibrinogen or white blood cell count, and have short half-lives. **Of 59 samples from 8 Asian elephants with known EEHV status, serum amyloid levels were 10 fold higher in elephants with EEHV viremia, compared to those who were EEHV whole blood PCR negative.** Measurement of serum amyloid may have elephant wellness/surveillance implications, may assist with prognostic evaluation of EEHV cases, and will contribute to further understanding of the elephant immune system and vaccine development.

2. *Prenatal passive transfer of maternal immunity in Asian elephants*. (S. Nofs)
Study to evaluate how elephant calves receive immunity from their dams.

Asian and African elephants have endotheliochorial placentation, which is similar to carnivore placentation. Measurement of rabies and tetanus antibody levels in 3 newborn Asian calves right after birth showed antibody levels equivalent to or higher than their dams, **indicating the majority of maternal-fetal antibody transfer occurs through the placenta, rather than through the colostrum.** This is different from domestic horses, which is a commonly used domestic model for veterinary work on elephants.

3. *Development of an ELISA for detection of antibodies against EEHV*. (B. Martina)
Ongoing research to develop a serological assay and vaccine against EEHV

Glycoprotein B (gB) is a membrane glycoprotein on EEHV that is involved in binding and entry of virus into target cells, it is one of the most immunogenic proteins and is highly conserved among herpesviruses. Development of an ELISA antibody test for EEHV will help to determine immune status of elephants and to evaluate future vaccine responses. To develop an ELISA, this group has cloned gB into several expression systems, including the adenovirus , the leishmania, the baculovirus and the prokaryotic E-coli expression system. Only the E-coli system has shown promising results, although this system has some disadvantages. The preliminary results indicate that groups of animals can be identified with high, moderate, and low titers of gB-specific antibodies. In addition, animals with no antibodies were detected. Further validation of the ELISA will be conducted using sera from samples banked by NEHL, particularly the ones from the Houston zoo. Additionally, a recombinant MVA-vaccine based on gB is being developed. The preliminary results suggest that the protein is unstable in transfected cells. Studies are ongoing to further study the protein stability and expression levels Next steps will include increasing producing of antigen for the ELISA and using it to evaluate the European and North American serum banks, confirming gB expression in the MVA vaccine, and working on a vaccination plan.

4. *Possible antiherpetic and immune supplementation using Shana Vet and Imuno-2865 in captive Asian elephants*. (J. Mejia-Fava)

There may be value in studying the effects of two immune-modulating supplements on EEHV shedding and immune function in elephants. Shana Vet™ contains docosanol (which inhibits enveloped viral replication by interfering with viral entry into target cells) and triacontanol (which exhibits anti-inflammatory properties). Imuno-2865™ is a natural mixture made of arabinoxylan, arabinogalactan, and fatty acid, pilot studies in humans and cetaceans have shown encouraging findings in improving lymphocyte activation and interleukin activity.

CLINICAL MANAGEMENT AND COMMUNICATION

1. *EEHV-5 associated calf fatality in a herd of captive Asian elephants*. (S. Redrobe)

In April 2011, a 19 month old male Asian calf at Twycross Zoo had reduced appetite progressing to severe edema, hemorrhagic mucus membranes, and cyanosis. This herd had been closed for 10 years and all trunk wash screens had been negative for EEHV-1. Though blood sample on the calf was negative for EEHV-1, a provisional diagnosis of EEHV was made based on clinical findings, and famciclovir and supportive care were started. **The use of methadone 15 mg for analgesia greatly improved calf's attitude and appetite.** On day 6 a pericardiocentesis was performed under butorphanol/detomidine sedation using aseptic catheter placement. 150 ml of straw colored fluid was removed from the sac around the heart. The calf improved initially but was euthanized 1 day later due to deteriorating condition. **Further testing identified the virus**

as EEHV-5, not previously associated with fatal disease. The authors would recommend the pericardiocentesis procedure again in a similar situation.

2. www.eehvinfo.com: *Update of the successes and failings of the EEHV website* (J. Cracknell)

This website went live Sept 2011 with the aim: "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." There are currently 60 registered users (42% veterinarians, 20% managers, 17% researchers), with only 16 institutions in the US and 9 in the UK represented. **Major failings of the website thus far include lack of updates on new material since 2011 and low membership.**

Actions to be taken or discussed include: the creation of summary pages on each topic, with details available as a download, making more (or all) of the website accessible without a password, and openly listing the names/dates of all EEHV deaths and survivors on the website to provide accurate information to the public.

ADDENDUM: a link from EMA has been established, and a temporary link from AAZV has been established along with information on our workshop.

3. *The EEHV story…. That you should help tell.* (J. Alread)

A discussion of the communication and public relations side of EEHV in zoos.

Our current EEHV research and partnerships offer the best hope for managing EEHV in zoos and the wild. EEHV is a challenging but hopeful story, and it is OURS to tell. **In a public research poll, 95% of the people surveyed felt that seeing live animals gave them a better appreciation for the animal and encouraged them to learn more about conservation.** It is up to zoos to share their good news and trumpet their triumphs, to get their stories (good or bad) to the public first, and to engage stakeholders, visitors, and potential funders in our programs.

4. *Financial needs for EEHV research*. (P. Ling and E. Latimer)

A discussion of the status of the National Elephant Herpesvirus Laboratory (NEHL)

The NEHL is a critical resource for EEHV research. It was funded by the Smithsonian Institution in 2004, by Feld Entertainment from 2005 to 2007, and by the International Elephant Foundation (IEF) from 2008 to 2012. For 2013, the Smithsonian Institution has pledged partial support.

The NEHL does not currently charge for EEHV testing, and is exploring ways to make the lab sustainable. The NEHL receives an average of 17 samples from sick elephant calves each year, with 2 to 3 positive results. Additionally, it receives 10-15 routine samples per year (from necropsies, biopsies, prior to moves, etc.) Additionally, the lab screens a few herds with young calves weekly or monthly for EEHV. Initial PCR on 1-3 samples from a sick elephant costs $450 (with follow up sequence to confirm diagnosis and determine subtype costing an additional $200). Follow up testing on herd-mates and clinical cases costs additional $400-$700. To date,

the NEHL does not have a real-time PCR machine, which is important to quantifying viremia in clinical cases. The NEHL has a bank of samples from previous cases, and weekly to quarterly serum and whole blood samples from most of the N American Asian elephants. These have been used in several studies by researchers outside of the Smithsonian.

DETAILS ON RESEARCH DISCUSSIONS FROM DAY 2

Virus cultivation
Serologic and Immune Response
Epidemiologic Analysis

Virus cultivation

1. Cultivation of virus would also help to determine antiviral efficacy
2. Group discussion on specific techniques that have been tried, different options for cell virus
3. Need to continue to have multiple people trying to culture virus at the same time
   a. Could we try growing it in hepatic cell lines (other human viruses are grown in these)
   b. Should we try to make three dimensional cell cultures? (Akbar has experience with this).
   c. Paul suggests: Isolate virus from trunk washes if we can figure out how to filter and optimize it.
4. Limitations to address:
   a. Need access to viable virus, how to get the samples where they need to go, keep husbandry manual and SSP protocols updated regularly
   b. Need money/time to focus on this, this was listed as our #1 priority but no one is doing this full time

Serologic Evaluation and Immune Response

1. Need to establish a base of what is known on elephant immunology, then determine what else we need to know
   a. Dennis knows a researcher at Illinois that looked at T helper cells as it relates to TB immunity in elephants, may be a good starting point.
2. Do we need to evaluate neutralization ability of elephant antibodies to EEHV?
   a. Assume based on other herpesviruses that both humoral/antibody and CMI is important
      i. ELISA is in development in Rotterdam (antibody)
      ii. Protein based chip needs funding for development by Johns Hopkins University (antibody)
      iii. CMI may be most important: can develop these assays at BCM
         1. Immunologist at BCM has resources to supervise a researcher to develop different CMI assays
         2. would need financial support for this $300,000 for 3 years
3. Influence of age on immunity?
   a. In laboratory mice, immunity is very different for different age classes
   b. Carolyn Cray is looking into different questions to be asked in this regard
      i. May help to get a neonatal immunologist involved
4. Paul suspects it is the primary infection that is the problem
   a. Hypothesis: Calves have protective immunity up to 1 year of age?

i. Exposure to virus and shedding when early/protected is a good thing?

Epidemiologic Analysis (retrospective and prospective)

1. GOAL: To move from understanding EEHV (getting there but not done!) to application of what knowledge we do have to elephants in the barn
   a. Projects should be more translational/applied: vaccines, treatments, serologic assays
2. Prospective Evaluation: use our current and future calves to learn more
   a. Follow calves from birth and collect samples to monitor exposure, shedding, etc.
   b. This would be to monitor EEHV exposure
      i. but could also include samples for immunology studies
   c. Oregon, OKC and Houston Zoo already monitoring calves
   d. Should we require/recommend monitoring of all calves and if we do, can we afford it?
3. Retrospective: Focus in on 1 to 6 year olds that are dying of this disease
   a. Refine good diagnostics for that age group, prognostic indicators
   b. What is proper form of treatment, which antiviral if any
   c. Use information we already have (medical records of survivors vs. deaths) to look for trends
4. Should we use our limited money/resources in Range country surveys?
   a. We have already established that EEHV is present in wild populations
   b. Do we need a laundry list of cases/countries at this point?
   c. This information could be used to apply to focused epidemiology so has its place, but is a lower priority than the above items.
   d. We should continue to work closely with Range country colleagues and encourage them in their own work and field studies, offer intellectual assistance whenever possible.

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