African Elephant EEHV Workshop

February 24-26, 2020

INDIANAPOLIS ZOO
INDIANAPOLIS, IN

WORKSHOP PROCEEDINGS

INDIANAPOLIS ZOO

EEHV Advisory Group

African Elephant EEHV Workshop & EEHV Advisory Group Meeting
February 24-26, 2020

Monday, February 24, 2020		
1:00-5:00pm	EEVH Advisory Group Meeting	***Advisory Group Members Only***
6:00-8:00pm	Icebreaker @ Indianapolis Zoo Hulman Riverhouse	Shuttle from Fairfield Inn and Staybridge Suites to the zoo will run from 5:00-6:00pm and from 7:30-8:30pm 6:00-6:10: Welcome and opening remarks (Bill Street) *Heavy hors d'oeuvres will be served*

Tuesday, February 25, 2020		
7:00-8:00	Breakfast (provided at the hotel)	Shuttle from Fairfield Inn and Staybridge Suites to the zoo will begin at 7:00 am and will make several trips
8:00-10:15	Morning session	8:00-8:30: EEHV- The Basics (Lauren Howard, San Diego Zoo Global)
		8:30-9:00: EEHV treatment recommendations (Christine Molter, Houston Zoo)
		9:00-9:45: Training tips for young elephants (Daryl Hoffman, Houston Zoo & Steve Taylor, Louisville Zoo)
		9:45-10:15: Recent EEHV Cases at Indianapolis Zoo (Melissa Fayette and Jill Sampson)
10:15-10:30	Coffee Break	*Light refreshments will be served*
10:30-12:00	Mid-morning session	10:30-11:00: Recent EEHV Cases at Fresno Zoo (Shannon Nodolf and Vernon Presley)
		11:00-11:30: EEHV Pathology in African elephants (Jaime Landolfi, University of Illinois)
		11:30-12:00: Cross-matching in African elephants (Jennifer Kishbaugh, BodeVet)
12:00-1:00	Lunch	
1:00-4:30	Afternoon session	1:00-1:30: PR experience: How and when to share information (Judy Palermo, Indianapolis Zoo)
		1:30-3:00: Elephant training & Plasma collection demonstration
		3:00-4:30: Veterinary hospital tour & Coffee Break
4:30-5:30	Question & Answer session	Participants may submit questions using Slido. Visit **https://www.sli.do/** or download the app and enter the event code

Wednesday, February 26, 2020		
7:00-8:00	Breakfast (provided at the hotel)	Shuttle from Fairfield Inn and Staybridge Suites to the zoo will begin at 7:00 am and will make several trips
8:00-9:45	Morning session	8:00-8:30: EEHV serology (Paul Ling, Baylor College of Medicine)
		8:30-9:00: EEHV in African vs. Asian elephants (Erin Latimer, Smithsonian's National Zoo)
		9:00-9:30: Update on African EEHV Working Group (Lauren Howard, San Diego Zoo Safari Park)

		9:30-9:45: Pharmacokinetics of famciclovir in African elephants (John Griffioen, Indianapolis Zoo)
9:45-10:15	Coffee Break	*Light refreshments will be served*
10:15-11:45	Question & Answer session	Participants may submit questions using Slido. Visit **https://www.sli.do/** or download the app and enter the event code
11:45-12:00	Closing remarks and summary	
12:00	Lunch	

Transportation to/from the zoo:

For those that are driving their own vehicle to the zoo, please park on the East side of the lot (there is no parking fee) and proceed to the White River Gardens entrance. There will be a check-in table just inside the building to pick up your name tag. The meeting will take place in the Hulman Riverhouse.

We will have two 12 passenger shuttle vans that will pick-up at the Fairfield Inn & Suites and Staybridge Suites downtown. The hotels are approximately a 5-10 min drive from the zoo. Shuttles will run from 5:00-6:00pm and 7:30-8:30pm on Monday, Feb. 24th, from 7:00-8:00am and 5:00-6pm on Tuesday, Feb. 25th, and from 7:00-8:00am on Wednesday, Feb. 26th.

Transportation to/from airport:

We recommend using Uber, Lyft, or the Go Green Airport Shuttle which offers express service to/from downtown every 30 minutes from 5am to 11pm. One way ticket is $13. For advanced reservations: **https://www.goexpresstravel.com/indy_express**.

Workshop Delegates

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SAN DIEGO ZOO GLOBAL

Elephant Endotheliotrophic Herpesvirus

Lauren L. Howard, DVM, Dipl. ACZM
Director, Veterinary Services
San Diego Zoo Safari Park

Elephant Endotheliotropic Herpesvirus (EEHV)

Not *if,* but *when*

All Asian elephants shed EEHV. It is already in your herd.

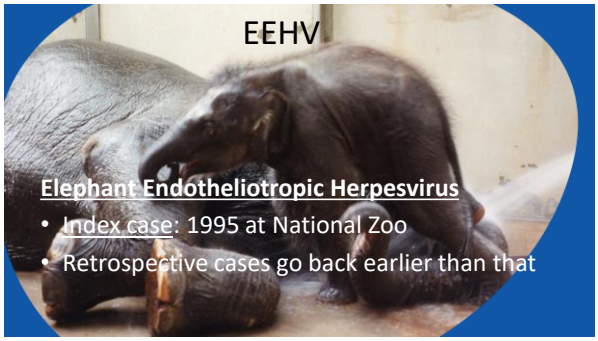
EEHV Advisory Group

Your best resource on EEHV

- Website updated regularly
- Background information on EEHV
- Professional content includes
  - sample protocols
  - recommendations on
    - anesthesia/sedation
    - diagnostics and testing labs
    - treatment for EEHV
  - *Must sign up for password to access it*

EEHV Advisory Group

eehvinfo.org



EEHV

Elephant Endotheliotropic Herpesvirus

- Index case: 1995 at National Zoo
- Retrospective cases go back earlier than that

EEHV

Elephant Endotheliotropic Herpesvirus

- Classified as a Proboscivirus
  - Placed in Betaherpesvirus family
  - should have their own virus family (Deltaherpesvirus)
- Host specific: ELEPHANTS ONLY

EEHV Epidemiology

- Most herpesviruses do not kill their host
- they create a primary infection
- Then the host becomes latently infected
- can re-activate later on.

What is EEHV HD?

EEHV

- Ubiquitous virus
- Virus is found incidentally in trunk secretions, saliva, likely other places
- May be associated with low-level viremia
- Clinically insignificant

EEHV HD

- Hemorrhagic disease
- Associated with high viremia
- Associated with abnormal CBC
- Associated with clinical signs of illness
- Life-threatening

EEHV: The Basics

Asian Elephants

- EEHV 1a and 1b
- EEHV 4
- EEHV 5

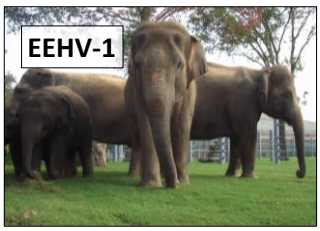
Most EEHV fatalities are in Asian elephants

African Elephants

- EEHV 2
- EEHV 3
- EEHV 6
- EEHV 7

Much to be learned about EEHV in African elephants

EEHV: The Basics



EEHV: The Basics

- Shed by asymptomatic elephants
  - PCR of trunk wash
  - Epidemiology, no clinical diagnosis
- Natural infection in elephants

EEHV: The Basics

- Hemorrhagic Disease (EEHV HD) in Asian calves
  - 1-8 years of age
  - qPCR of whole blood to detect viral DNA

EEHV: The Basics

- Hemorrhagic Disease (EEHV HD) in Asian calves
  - 1-8 years of age

Viremia ⇨ Systemic disease ⇨ Endothelial Damage ⇨ Internal Hemorrhage

EEHV: The Basics

- Hemorrhagic Disease (EEHV HD) in Asian calves
  - 1-8 years of age


Viremia ⇨ Systemic disease ⇨ Endothelial Damage ⇨ Internal Hemorrhage, Shock, DIC, Death

Why do some elephants die from EEHV HD, and some elephants survive?

EEHV Epidemiology

We believe hemorrhagic disease in calves is due to uncontrolled primary infections that lead to fulminant systemic infection in calves lacking immune protection.

EEHV in African Elephants: Prior to 2019

- Free ranging and captive
- Multiple EEHVs from lung and skin nodules
- EEHV2: 2 fatalities
  - 11 months, 13 years old
- EEHV6: 1 fatality (10 yo, in Thai Zoo)
- EEHV6, EEHV3
  - 2 cases of clinical disease, survived

North American Elephants and EEHV (including 2019)

	Asian Elephants	African Elephants
Elephants born or imported since 1980, with known follow-up	129	258
Elephants still alive	87	158
Elephants that have died	42	100
EEHV HD deaths	27	5
% of elephant deaths that are from EEHV	27/42 = 64%	5/100 = 5%

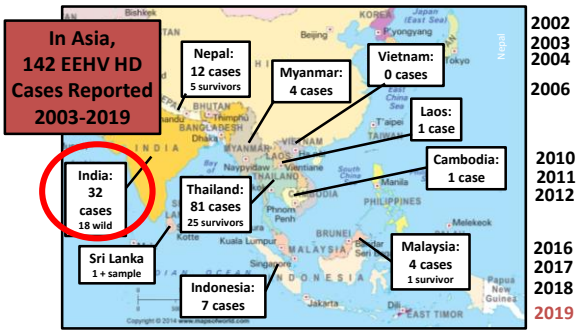
North American Elephants and EEHV (including 2019)

	Asian Elephants	African Elephants
Elephants born or imported since 1980, with known follow-up	129	258
EEHV HD Survivors	15	5
EEHV HD Deaths	27	5
Fatality Rate of EEHV HD Cases	64%	50%
% of all elephants in population that have been impacted by EEHV	42/129 = 32%	10/258 = 4%

ZOO COPENHAGEN

Asian Elephant EEHV HD in Europe

- Born between 1985-2017
- 27 cases of EEHV HD (15.12)
- Clinical illness: 0 – 6 days
- Treatments administered
  - None (3)
  - Non-specific (7)
  - Antivirals only (6)
  - EEHV HD Protocol (6)
  - Unknown (5)



What does EEHV do?

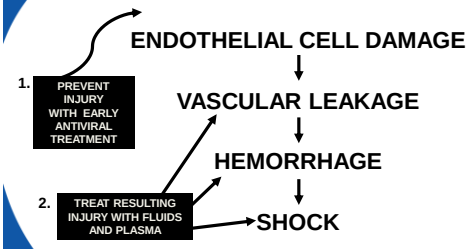
ENDOTHELIAL CELL DAMAGE
↓
VASCULAR LEAKAGE
↓
HEMORRHAGE
↓
SHOCK

What does EEHV do?

1. PREVENT INJURY WITH EARLY ANTIVIRAL TREATMENT

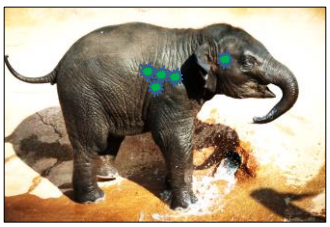
ENDOTHELIAL CELL DAMAGE
↓
VASCULAR LEAKAGE
↓
HEMORRHAGE
↓
SHOCK

What does EEHV do?



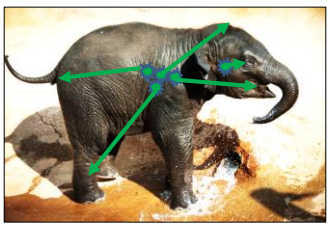
What do WE see with EEHV?

What do WE see with EEHV?



Virus is in blood up to **10 days <u>BEFORE</u>** any clinical signs of illness are observed.

What do WE see with EEHV?



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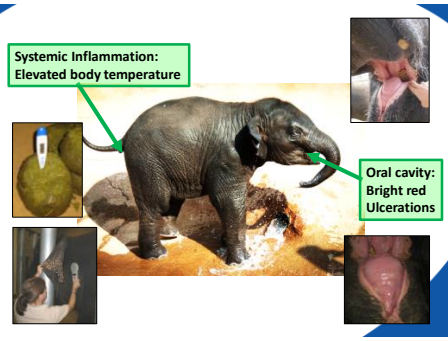
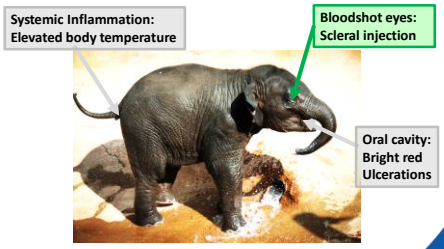
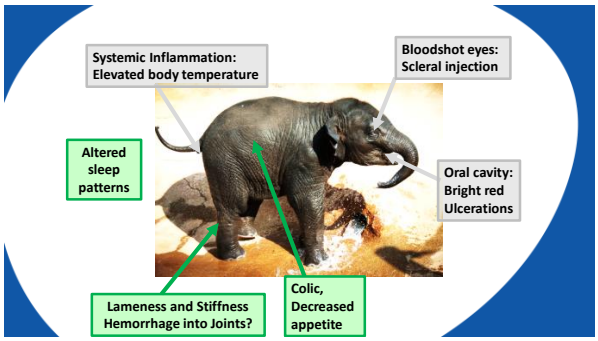
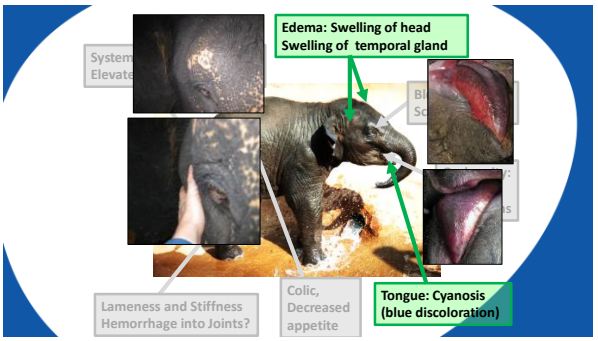
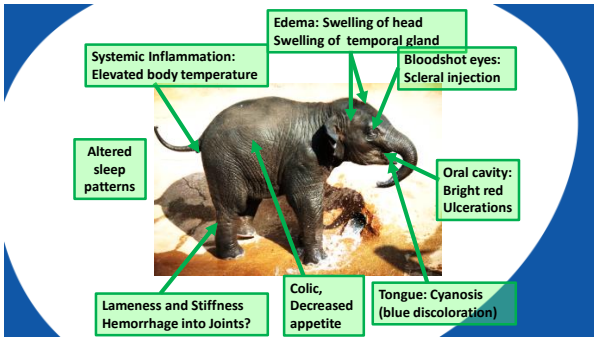


What do WE see with EEHV?







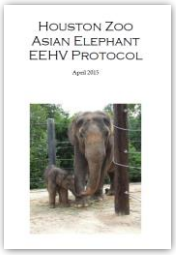


Diagnosing EEHV-HD

Clinically Ill Animals or Dead Animals

- Live animals:
  - Identify virus in blood
  - Submit whole blood (EDTA) for PCR testing
- Post Mortem:
  - Gross lesions (take photos)
  - Formalin fixed tissues: histopathology
  - PCR of tissues : preserve frozen or in DNA preservative
  - PCR of post mortem blood sample: preserve frozen or in DNA preservative

EEHV Preparedness

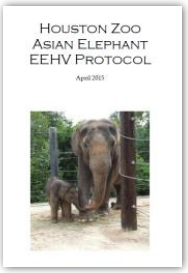


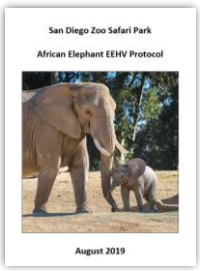
EEHV Protocol is Essential

- Monitoring
- Treatment
- Drug procurement
- Communication
- Decision tree
- Necropsy information

EEHV Preparedness

- Train your elephants.
- Train your people.
- Get the stuff.





Vigilance

- EEHV in the blood may lead to EEHV HD illness
- EEHV can be detected in blood **up to 2 weeks before illness.**
- Early changes in the CBC can indicate impending illness.

Vigilance

- EEHV i
  lead to
- EEHV c
  blood **u**
  before
- Early changes in the CBC can indicate impending illness.

Weekly blood testing in at-risk elephants saves lives.

Vigilance

- EEHV i
  lead to
- EEHV c
  blood
  before
- Early c
  can inc
  illness.

On-command blood collection in any at-risk elephant that is even slightly off.

EEHV Vigilance

CBC results

EEHV qPCR results

Clinical appearance of elephant

EEHV in the Media


Zoo should rethink elephants

Historically, EEHV was a four letter word


Zoo a hot spot for fatal elephant virus

2-year-old calf Mac the latest of 6 victims of disease


Virus top killer of young U.S. elephan

EEHV in the Media

- Shift in understanding of EEHV
- No longer a dirty word
- WE NEED TO SHARE OUR POSITIVE STORIES
- We need to be honest in our losses
- We need to support each other


HOUSTON CHRONICLE

Quests for oil coming up short

Roads shape race for mayor

A mammoth effort at the Houston Zoo

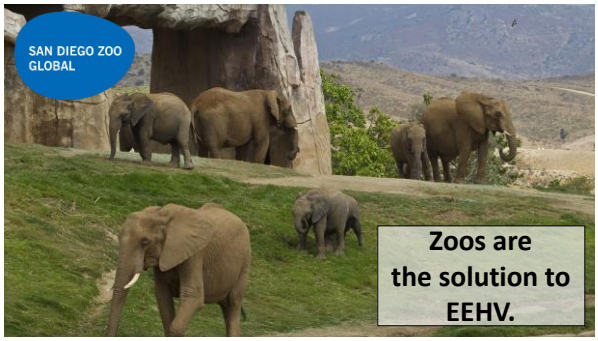
What isn't EEHV?

EEHV is NOT…….

- **A** result of mixing Asian and African elephants together.
- **A** result of being under human care.
- **A** reason to stop breeding elephants.

It starts at home

- EEHV awareness and understanding begins at home
- Resources needed to be prepared
- Time for vets and elephant staff to meet
- $$ and Time for vets and elephant staff to attend workshops and stay current
- $$ for drug procurement
- Time for elephant calf training



TREATMENT FOR ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) CASES

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ABSTRACT

Rapid and aggressive treatment is critical for any suspected or confirmed EEHV case in both Asian and African elephants. A written comprehensive plan that includes an outline for EEHV treatment, that is agreed upon by both the veterinary and husbandry teams prior to an EEHV case, is essential to facilitate immediate therapy. The following information is taken from the Houston Zoo's Asian Elephant EEHV Protocol and all drug dosages provided here are based on Asian elephants. Care should be taken if extrapolating dosages for African elephants. This document is intended to be a guideline for clinical decision making during an EEHV case. Further consultation with elephant colleagues on both the veterinary and husbandry side is encouraged.

Initiation of treatment is triggered by one or more of the following:

- Clinical signs
- WBC and/or monocyte and/or platelet count has dropped significantly below elephant's normal range
- 5,000 to 10,000 VGE/ml or greater on qPCR
- Rapidly increasing VGE/ml on qPCR
- Animal is viremic for EEHV strain and is known to be seronegative for it and/or is known to not have experienced a primary infection yet

TREATMENT FOR SUSPECTED OR CONFIRMED EEHV CASES

The two most important treatments for any EEHV case are rectal fluids and antiviral medications.

For a suspected EEHV case, rectal fluids and/or antiviral medications may be started immediately. There is no need to wait for qPCR laboratory results if the clinical suspicion is high and/or if there are abnormalities in the CBC.

Rectal Fluid Therapy

Rectal fluids can be administered to any EEHV-suspect elephant in an abundance of caution immediately, while awaiting results. Rectal fluids may also be given to any elephant that is dehydrated, but ambulatory, or to partially rehydrate an elephant prior to anesthesia and placement of an intravenous (IV) catheter. Moreover, rectal fluids *must* be given after the administration of IV fluids, which are hypertonic for elephants,

to aid in the redistribution of fluid in the elephants intracellular and extracellular spaces.

Rectal fluids should be administered a minimum of 3-4 times per day, up to every 2 hours. A bolus treatment of 10 to 20 ml/kg dose is often used. If an animal expels the rectal fluids when a large volume is given, give smaller volumes more frequently. If the animal is absorbing the rectal fluids given, larger or more frequent boluses may be administered.

Rectal fluids given may be from the barn hose and should be warm, not hot. It is strongly encouraged to determine the temperature and flow rate of the hose in an elephant barn prior to an EEHV illness to help best determine appropriate administration volume.

Antiviral Therapy

Antiviral medications are recommended in most suspect or and all confirmed EEHV cases to, in theory, decrease or eliminate viral replication and thus reduce the viral load in the patient. However, the antiviral medications do not reverse the damage the virus has already done to internal organs.

Famciclovir

In the United States, famciclovir is the most common antiviral drug used to treat EEHV. The typical Famciclovir dosage for Asian elephants is 15 mg/kg three times daily (TID) orally (PO) or per rectum. This dosage may decrease to 15 mg/kg two times daily (BID) after improvement in clinical signs or decline in viral load has been observed. The decision to of when to decrease the frequency of antiviral treatment should be made based upon dialogue between veterinary and husbandry staff.

It is known that famciclovir given at 15 mg/kg PO or per rectum at least every 8 hours results in penciclovir (active form of famciclovir) concentrations that are considered therapeutic in humans (*Brock et al. 2012*). The maximum plasma concentration following PO or per rectum administration of famciclovir is about 1 hour (*Brock et al. 2012*).

Famciclovir is available in a powder or tablet form. Famciclovir powder (1 g/g) is available through compounding pharmacies and can be ordered in large quantities with advanced notice. Famciclovir tablets (500 mg tablets) may be ordered from compounding or commercial pharmacies as well. In general, compounding and commercial pharmacies do not stock large quantities of famciclovir, so having an in-date supply that can cover vulnerable animals for several days, is important to prevent treatment delay. Budgeting to maintain this adequate supply of in-date famciclovir in-

house is necessary for any institution with at risk calves, as a treatment delay could be fatal. Expired famciclovir stock should be retained for emergencies. If famciclovir is needed emergently, contacting other elephant holding facilities to obtain famciclovir is an option.

When dosing famciclovir, the powder form should be weighed out into individual doses and dispensed in individual containers or plastic bags.

It is believed that the bitter taste of famciclovir is unpalatable for some elephants. To achieve oral compliance the following has been anecdotally suggested: training to accept oral medications prior to illness, mixing famciclovir in a flavored beverage and administering it to a young calf conditioned to take a bottle, or putting famciclovir into gel caps coated in coconut oil or peanut butter or other palatable coating. To achieve rectal administration the following has been successful at the Houston Zoo: clean out the rectum, mix famciclovir powder with saline or water to create paste (or grind tablets with a mortar and pestle to make a powder to then create a paste), use 60 ml syringe attached to long lubricated stomach tube to instill medication as deeply into the rectum as possible, chase medication in tube with saline or water to flush the tube, kink the tube, remove tube, and hold tail down to discourage expulsion. This should be done at least 1 hour before (ideal) or 1 hour after rectal fluid administration. When administering during a sedation, famciclovir should be administered rectally as soon as possible at the start of the procedure.

Ganciclovir

Ganciclovir (Cytovene IF) may be elected to treat an EEHV case; however, this medication may only be given IV and does not offer the flexibility for other routes of administration. Moreover, this medication has the potential to cause hematologic toxicity and must be used with care. This medication may cause phlebitis and should not be given intra-arterially. Ideally, it would be given in a large gauge IV catheter in a large peripheral vessel to reduce risk of phlebitis.

If it is determined that the elephant will be treated with Ganciclovir, it should be delivered according to the following specifications: give 5 mg/kg IV BID to be given slowly over an hour in 1-2 liters of sodium chloride (NaCl) fluids. Once dose is started, should be administered BID for minimum three days.

Ganciclovir is stored in sterile vials containing 500 mg of powder and is available from commercial and compounding pharmacies.

Acyclovir

Acyclovir is not commonly used in the United States to treat EEHV, but is more widely used in other parts of the world where it is more readily available (*Sripiboon et al. 2017*). This medication may be given IV, PO or per rectum BID.

Reported doses range from 12-15 mg/kg IV, PO, or per rectum BID. Like famciclovir, this medication may be tapered with clinical and bloodwork improvement.

TREATMENT FOR A CONFIRMED EEHV CASE

Once an EEHV case is confirmed, further intensive management is required.

INTENSIVE CARE OF THE EEHV PATIENT

Aggressive and immediate rectal fluids, antiviral medications, supportive therapies, and close monitoring of the patient is necessary in any confirmed EEHV case and especially in animals that develop EEHV-hemorrhagic disease (EEHV-HD).

Calves that develop EEHV-HD may die from the virus within several hours of the onset of visible clinical signs. A delay in treatment may result in a fatal outcome, as clinical signs are the *last* indicator of EEHV to appear. Do not wait to start treatments until clinical signs appear.

To facilitate treatments, standing sedations may and, in most cases, will be necessary. Sedations may be needed more than once daily and may be needed during off-hours. Do not hesitate to sedate a calf to deliver potentially life-saving treatments.

During an EEHV-HD case, staff from either the elephant husbandry and/or veterinary team should monitor the animal constantly for changes in clinical condition. Careful, frequent observations should be shared between the husbandry and veterinary teams to adjust treatments as needed based on the animal's overall condition. Overnight staff may be necessary and video equipment is very helpful for monitoring.

It is important to note that while observing a sick calf, human discretion is advised to allow the animal to rest and recover. Allow the calf to rest and spend as much time as possible with the mother or favored companion between treatments for comfort, nursing, transfaunation opportunity, and to reduce stress. Keep the barn calm and as normal as possible.

VITAL PARAMETER AND BEHAVIOR MONITORING

Vital parameters and behaviors should be monitored by the elephant husbandry team multiple (minimally 2-4) times a day and in severe cases, overnight either with direct observation or live video surveillance.

Photos of any abnormalities should be taken for documentation purposes.

Vital parameter and behavior monitoring should include, but not be limited, to the following:

- Body weight (should be obtained by keepers daily, if possible)
- Temperature (fecal bolus), pulse, respiratory rate, and blood pressure
- Evaluation of mucous membranes (tongue, oral mucosa, palpebrae, sclera, vulva)
- Evaluation of head, limbs, ventrum for edema
- Evaluation of any prior injection or venipunctures sites for swelling or phlebitis
- Evaluation of fecal quality and quantity
- Evaluation of food consumption and nursing
- Evaluation of sleep and rest patterns
- Evaluation of overall mentation

STANDING SEDATION

To facilitate treatments, standing sedations may and, in most cases, will be necessary. General anesthesia is not necessary to accomplish EEHV treatments and is not commonly practiced.

Standing Sedation of Asian Elephant Calf

Calves may be sedated in the same stall as the mother and then separated once drugs have been administered prior to effects or sedated separately. Drugs may be hand injected, pole syringed, or darted into the animal with ideal placement being in a large muscle belly, such as just above the stifle in the caudal aspect of the rear limb.

Prior to or once effects are seen by about 15-20 minutes post-drug administration, keepers should work to place ropes on the limbs and secure the animal near bollards or secure the animal in an elephant restraint device. Keepers should also place a blind fold to decrease visual stimulation. A blindfold is easily made from a large bath towel on ropes to be secured around the head.

Ideally, a sawhorse stance (stable, widely placed legs supporting a relaxed body) should be achieved during the sedation and this level of sedation should last 60-90 minutes. Supplements may be given as needed to increase depth and duration of the sedation.

In some cases, at the beginning and end of sedation procedures, the calf may be a little light and have "munchie" behavior where the animal goes through the motions of eating and will eat if food is available. Houston Zoo has taken advantage of this to provide both oral medications and moisture rich foods (in small quantities) to sedated anorexic calves. This can be a very helpful aspect of the sedation for the animal, but giving anything orally to a sedate calf should be done with a high level of caution.

Drug dosages for Asian elephant calf:

- Induction
    - Butorphanol 0.045–0.075 mg/kg IM
        i. Average dosage is 0.06 mg/kg IM
    - Detomidine 0.011–0.022 mg/kg IM
        i. Average dosage is 0.015 mg/kg IM
        ii. Higher doses of Detomidine may result in lateral recumbency
    - Initial doses of Butorphanol and Detomidine lasts about 60-90 minutes, then supplemented as needed
- Supplements
    - Supplements of Butorphanol and/or Detomidine may be given IV or IM
    - Start low and titrate up to effect
        i. Satisfactory supplementations with 2 mg Detomidine IV (0.002 mg/kg) in a 1000 kg calf sedated with Butorphanol and Detomidine has been noted at Houston Zoo.
- Reversal
    - Naltrexone reverses Butorphanol at 2.5–5 times the Butorphanol dose
    - Atipamezole reverses Detomidine at 5 times the Detomidine dose
    - Reversal with Naltrexone and Atipamezole may be performed at the end of the procedure, but will affect subsequent sedations performed same day
        i. Plan to reverse if there are no additional sedations planned for the same day
        ii. Consider not reversing if there are additional sedations planned for the same day
            1. Residual effects of the reversal agents will impact subsequent sedations performed during the same day, resulting in higher doses and/or additional supplements being required to achieve a working plane of sedation

Light Sedation in Adult Asian Elephant

It may be necessary to sedate the dam or other adult herd mates, so they are not stressed during calf treatments. Drugs may be hand injected, pole syringed, or darted into the animal with ideal placement being in a large muscle belly, such as just above the stifle in the caudal aspect of the rear limb.

Effects are seen by about 15-20 minutes post-drug administration and should achieve a light, relaxed plane of sedation for approximately 60-90 minutes. The adult should be calm and may want to eat during this period. A keeper should stay with this adult to monitor the animal to provide food items and reassurance. Ropes may be placed on the limbs to secure animal to bollards.

The Houston Zoo has performed multiple calf treatments both with and without lightly sedating the dam or other herd mates. The decision to sedate an adult should be made between the husbandry and veterinary staff prior to sedating the calf.

Standing sedation protocols have been published for African elephants in the literature and may be a useful reference (*Neiffer et al. 2005*).

Drug dosages for Asian elephant adult cow:

- Induction
    - Butorphanol 20-50 mg/cow IM
        i. Average starting dose is 20 mg/cow IM
        ii. Higher dose of 50 mg/cow IM has been used without adverse effects
    - Detomidine 10-50 mg/kg IM
        i. Average starting dosage is 10 mg/cow IM
        ii. Higher dose of 15 mg/cow IM has been used without adverse effects
    - Initial doses of Butorphanol and Detomidine lasts about 60-90 minutes, then supplemented as needed
- Supplements
    - Supplements of Butorphanol and/or Detomidine may be given IV or IM
    - Start low and titrate up to effect
- Reversal
    - Naltrexone reverses Butorphanol at 2.5–5 times the Butorphanol dose
    - Atipamezole reverses Detomidine at 5 times the Detomidine dose
    - Reversal with Naltrexone and Atipamezole may or may not be performed at the end of the procedure

i. May elect to not reverse adults, to allow for prolonged calming effects

<u>Anesthesia Support and Monitoring for Standing Sedation</u>

During a standing sedation, there should be a dedicated veterinarian or veterinary technician designated to monitor and record vital parameters, anesthetic effects, and treatments. Likewise, there should be at least one dedicated elephant keeper monitoring the animal's behavior and depth, as well as for human safety.

- Support
  - Nasal oxygen should be provided via nasal cannula if possible, at 2-4 L/m, as tolerated
    - Supplemental oxygen may not be tolerated based on depth of animal
- Monitoring
  - Manual temperature, pulse, respirations (TPR), and anesthetic depth should be monitored routinely throughout the procedure
    - Temperature may be challenging to monitor as fecal boluses are not usually available due to rectal treatments and fluids
    - Pulse can usually be palpated over an aural artery
    - Respiratory rate may be obtained visually
    - Anesthetic depth may be assessed by the following
      - Body position (sawhorse stance or movement)
      - Trunk relaxation
      - Relaxation or dropping of the vulva or penis
      - Eyelid drooping (palpebral reflex will usually be retained)
      - Relaxation of the lower lip
  - Multiparameter monitors and/or portable monitors should be used to facilitate monitoring when possible and practical
    - Blood pressure
      - Cuff on tail
    - Oxygen Saturation (SPO2)
      - Probe may be used on any mucosal surface including on nasal frenulum, rectum, vulva etc.
    - EKG
      - Clips or sticky pads may be used, references on placement and interpretation are available in the literature (*Bartlett et al. 2009, Chai et al. 2016*)
  - Documentation

- Vital parameters, exam events, drug administration, and other notes should be recorded on an anesthesia sheet by the dedicated recorder

PHYSICAL EXAM

A physical exam should be performed by a veterinarian at least once daily and during every sedation during an active EEHV case. During sedations, the exam may be performed at the same time as IV catheter placement and other initial treatments.

Photos of any abnormalities should be taken for documentation purposes.

The physical exam should include, but not be limited, to the following:

- Body weight (should be obtained by keeper prior to sedation, if possible)
- TPR and blood pressure
- Evaluation of mucous membranes (tongue, oral mucosa, palpebrae, sclera, vulva)
- Evaluation of head, limbs, ventrum for edema
- Evaluation of any prior injection or venipunctures sites for swelling or phlebitis
- Evaluation of fecal quality
- Ultrasound of heart to look for pericardial effusion
- Ultrasound of abdomen (transabdominal) to look for free abdominal fluid
    - Transrectal ultrasound may be performed, but should not interfere with or significantly delay rectal famciclovir or fluid therapy

INTRAVENOUS (IV) CATHETER PLACEMENT AND MAINTENANCE

Placement of a temporary IV catheter is one of the first tasks to be performed after a calf is sedated. An ear vein is generally selected and attempts to use a peripheral vessel (vs. a central vessel) is advised to preserve the health and integrity of the aural vasculature should a hematoma form or an ischemic event occur. Recording which vessels have been used and having a plan to rotate which are to be used next, if available, is also advised. Furthermore, blood for diagnostic tests may be obtained from a secure IV catheter prior to treatments to reduce punctures to other ear veins that may be needed later. This is particularly important as a case progresses and as vascular options become more limited. In certain cases, IV catheters may be placed in both ears. Rear leg veins may also be utilized.

Venous access is normally achieved using the vasculature on the caudal aspect of the ear or rear limb. If using an ear vein, a dedicated keeper should hold the ear perpendicular to the body during IV catheter placement and throughout treatment to provide as much stability as possible. The skin should be prepped with chlorhexidine

scrub and alcohol using standard aseptic procedures. Although there will be variations in gauge and length, in general, a 10-20 ga, 3" intravenous catheter should be placed and stabilized with tape and skin staples. The largest gauge IV catheter possible is preferred and catheter type is as veterinary discretion.

Milacath (Mila International) catheters have been suggested when longer term placement is needed, when blood pressure is low, or when vasculature has been damaged due to prior treatment.

Once placed and secure, the IVC may have a t-set placed or be attached directly to fluid lines. Heparin-locking (hep-locking) the catheter may be useful while getting set up.

Elephants in an intensive care environment can be subject to secondary infections, such as MRSA (*Janssen et al. 2009*). Attention to hygiene and biosecurity is very important in elephants being treated for EEHV, particularly due to their immuno-compromised status. Frequent hand washing, prompt removal of waste products, and regular sanitizing of equipment are recommended. Any handling of the intravenous catheter or associated fluid lines should be done with clean hands, gloves, and aseptic technique.

When a catheter is ready to be removed, it may be done following routine procedures with care to hold off the vessel completely to prevent hematoma formation.

INTRAVENOUS FLUID THERAPY

Elephant plasma is hypo-osmotic relative to standard crystalloid intravenous fluids, hence, standard intravenous fluids given to elephants work like hyperosmotic or hypertonic saline most frequently used in large animal medicine.

Fluids can be given through a small or large animal IV line, using a fluid pump or fluid bag under pressure to speed delivery. It is helpful to use at least one extension set between the fluid's administration line and the catheter to facilitate changing fluids and administration of medications along with the IV fluids.

Crystalloid Fluids

Intravenous fluids are recommended to support circulation and hydration. An initial bolus of crystalloid IV fluids (0.3 to 4 ml/kg in a calf) can be given to a dehydrated or shocky elephant as a resuscitative measure; this bolus could be repeated up to three times with re-evaluation of the patient and vital signs after each bolus. However, as most IV fluid therapy is performed under a single sedation event, repeated IV fluid boluses may not be possible, so a total volume to be delivered should be determined at the onset of the procedure.

Asian elephants have very low serum osmolarity and are hyponatremic and hypochloremic compared to other species. African elephants have similarly low serum osmolarity. The normal osmolality of Asian elephants ranges from 252-270 mOsm/L. Therefore, even a relatively small volume of fluids can make a difference to the elephant's response. Minimally one liter/450 kg seems to give visible results.

Commercial fluids such as Plasmalyte or Norm-R are actually hypertonic for elephants. It is recommended to use crystalloid fluids in elephants as actual hypertonic solutions would be used in other species; in other words, very small amounts (1 L fluids/450 kg) are given through an IV catheter and followed afterwards with large volumes of rectal fluids.

Rectal fluids *must* always be given in conjunction with IV crystalloid fluids.

Crystalloid fluid osmolality:
5% Dextrose: 252 mOsm/L
Lactated Ringers Solution: 273 mOsm/L
Normosol-R, Plasma-Lyte: 294 mOsm/L
0.9% Sterile Saline: 308 mOsm/L

Crystalloid dosage:

- Crystalloid fluid bolus 0.3-4 ml/kg IV

Synthetic Colloid Fluids: Hetastarch
Synthetic colloids, such as Hetastarch (6% Hetastarch in 0.9% Sodium Chloride), when used at low dosages (0.25 – 0.5 ml/kg IV), may be more effective for volume expansion in viremic or seriously ill animals compared to plain crystalloids. The larger molecules in these fluids do not leak out of capillaries as easily, increase plasma volume rapidly, and may help to reduce edema. It is possible in other species, that Hetastarch may cause coagulation abnormalities or renal injury, but usually only when recommended dosages are exceeded. These effects have not been recognized in elephants, but should be monitored for.

Hetastarch is not a replacement for crystalloid fluid, plasma or blood therapy.

Rectal fluids *must* always be given in conjunction with IV synthetic colloid fluids.

Hetastarch osmolarity:
Hetastarch: 308 mOsmol/L

Hetastarch dosage:

- Hetastarch fluid bolus 0.25-0.5 ml/kg IV

Natural Colloid Fluids: Whole Blood and Plasma

Natural colloids, such as whole blood and fresh or frozen plasma, are often more effective than crystalloid fluids for volume expansion in viremic or seriously ill animals. Like synthetic colloids, the larger molecules in these fluids do not leak out of capillaries as easily, increase plasma volume, and help to reduce edema.

Additionally, natural colloids have several advantages beyond providing oncotic support making them a critical component of EEHV-HD treatment.

First, fresh whole blood and fresh plasma have essential clotting factors that are important for calves bleeding from EEHV-HD. Plasma and whole blood are considered "fresh" if administered or processed within 8 hours of collection. After 8 hours, labile clotting factors deteriorate. Plasma that was processed and frozen within 8 hours of collection is considered "fresh frozen" plasma. Plasma that was processed and frozen after 8 hours of collection is just considered "frozen" plasma.

Second, fresh whole blood contains red blood cells that are important for increasing the oxygen carrying capacity in anemic animals bleeding from EEHV-HD. This is essential to preserve oxygenation to all parts of the body.

Third, animals with active infection are not expected to have antibody to the virus making them sick. If it is available, a whole blood or plasma transfusion from a donor with high antibody titers to EEHV may be of benefit.

At the Houston Zoo, whole blood, fresh plasma, fresh frozen, and frozen plasma have been administered alone and in combination without adverse effect. Moreover, famciclovir fortified whole blood and fresh plasma have been administered (see more information below).

Additionally, there is on-going research and development in alternative forms of blood products, including freeze dried platelets and packed red blood cells. These may be highly useful therapies in the future. For further information on this, please contact Dr. Jennifer Kishbaugh (JKishbaugh@bodevet.com).

Prior to Whole Blood or Plasma Transfusion

Blood products should only be administered intravenously after crossmatching donor and recipient blood samples to assure compatibility. A minor crossmatch is needed for a plasma donation and a major crossmatch is needed for a whole blood donation. Ideally, cross matching should be performed well in advance of a clinical illness. Animals that received repeated transfusions should be re-crossmatched to ensure continued compatibility.

If an animal is seriously ill, is a first-time recipient, and time cannot be spared to crossmatch the animal emergently, administer diphenhydramine 0.5 mg/kg IM prior to transfusion and monitor for adverse effects.

If an animal has received multiple transfusions, diphenhydramine 0.5 mg/kg IM may be elected in an abundance of caution prior to additional transfusions, even with acceptable crossmatching results.

Any donor animal should meet the following requirements for donation:

1. Be clinically healthy and in good condition
2. Have a normal CBC and Chemistry
3. Be free of EEHV viremia on whole blood qPCR at the time of donation
4. Be an acceptable donor on minor and/or major crossmatch for the recipient

<u>Whole Blood or Plasma Administration and Adverse Reactions</u>

Whole blood and plasma should be administered through an appropriately sized blood filter to remove fibrin clots. Terfusion Blood Administration Sets, 20 drops/ml, B type (manufactured by Terumo Medical Corporation, Somerset NJ) have been used successfully as filters in the past.

The first 100 ml should be given slowly, and heart rate, respiratory rate, and temperature should be monitored. In domestic animals, parameters are generally measured prior to the transfusion and then every 15 – 30 minutes throughout the process. However, because elephants are often sedated for treatment, the rate of transfusion is more rapid and monitoring frequency should be adjusted to the projected duration of time to complete the transfusion.

Possible transfusion reactions include fever, rash, or anaphylaxis. Mild signs can be treated with antipyretics or antihistamines and by decreasing the rate of transfusion. More severe reactions should be addressed by stopping the transfusion. If no reaction is seen, the transfusion rate can be increased.

In most cases, fresh plasma will be the preferred natural colloid; however, whole blood transfusions may also be needed. The amount of blood needed to transfuse in elephants is unknown. Overall small volumes of blood products are very low compared to transfusion recommendations for other species, but seems to yield positive clinical effects. Clinical benefits have been seen even with administration of less than 1 L whole blood for clinically ill calves.

In the literature, it is recommended that if hematocrit (HCT) falls below 14%, blood transfusion should be considered (*Fowler and Mikota eds. 2006*). However, consideration for a blood transfusion should be given well before this extremely low HCT. In EEHV-HD, indications for a whole blood transfusion include, but are not limited to, anemia, hypoproteinemia, and hypoalbuminemia or downward trending HCT, total proteins, and albumin.

Blood Product Administration Examples

In a case at the Houston Zoo, a 1000 kg calf with clinical EEHV-HD demonstrated a daily precipitously decreasing packed cell volume (PCV), from 31% to 27%, which was mirrored by low and decreasing total protein and albumin, indicating active bleeding. Whole blood transfusions (1-1.5 units; total 450-675 ml/1000 kg calf) from 2 different donors were given daily for 3 days without adverse effects. This was in addition to 17 units of fresh and frozen plasma from 2 different donors over 5 days. Diphenhydramine was not given prior to the first plasma transfusion, but diphenhydramine 0.5 mg/kg IM was given prior to every subsequent transfusion event, even though donors had acceptable major and minor crossmatches to the recipient and to each other. Several days into treatment, the calf began to feel warm to the touch, which was the only possibly associated adverse effect noted and strengthened the justification to give diphenhydramine. The diphenhydramine was given approximately 30 minutes prior to blood product administration at the onset of the sedation prior to IV catheter placement.

In a different 1,500 kg calf at the Houston Zoo, two units of plasma (approximately 650 ml total) were administered IV over the course of 30 to 45 minutes, on multiple occasions with no ill effects. In elephants, very small amounts of plasma (2-4 liters/elephant) have been quite beneficial for sick elephants. At the Houston Zoo, clinical improvement was seen at a plasma dose of 0.5 ml/kg in this 1,500 kg elephant.

Blood Product dosage:

- Whole blood 0.5-2 ml/kg IV
- Plasma 0.5-2 ml/kg IV

Famciclovir Fortified Natural Colloid Fluids: Whole Blood and Plasma

It has been anecdotally reported in several EEHV-HD cases to administer famciclovir fortified blood products to an ill calf. The theory is that a dose of famciclovir is administered PO or per rectum to a healthy donor and blood is collected about 1 hour later when the famciclovir is converted to the active form of penciclovir at peak plasma concentrations. Then, the blood product containing penciclovir is administered IV to the recipient. No adverse effects to the donor or recipient have been reported.

At the Houston Zoo, multiple units of both famciclovir fortified whole blood and famciclovir fortified fresh plasma from 2 different donors have been administered to a 1000 kg calf without adverse effect noted in either the donors or recipient. One donor animal received three famciclovir doses at 30 mg/kg over the course of 5 days without adverse effect.

It is also anecdotally reported that famciclovir on the cusp of expiration has been administered to donors to in order to collect for famciclovir fortified frozen plasma.

Famciclovir dosage for donor:

- Famciclovir 30 mg/kg PO or per rectum, then collect blood ~ 1 hour later

Famciclovir Fortified Blood Product dosage for recipient:

- Whole blood 0.5-2 ml/kg IV
- Plasma 0.5-2 ml/kg IV

SUPPORTIVE THERAPIES

Oxygen Therapy

Supplemental oxygen therapy should be administered, when possible, to all patients undergoing treatment for EEHV. Oxygen can be administered at 2-4 L/m via a flexible plastic tube passed into one side of the trunk as tolerated.

Oxygen dosage:

- Oxygen 2-4 L/m nasally

Antibiotics

Antibiotics have no effect in treating EEHV directly. However, the animal's immune system will be severely compromised, and the clinical situation could be complicated by secondary opportunistic infections. Therefore, antibiotic therapy should be considered, and dosages of potential antibiotic treatments are listed below. Initial doses should be administered IV for immediate effect, if possible, and should be given

through an IV catheter to spare the vasculature. When appropriate, route of administration may be changed to PO, IM, or SQ as indicated by the antibiotic itself and animal's clinical condition. Gastrointestinal dysbiosis resulting in diarrhea is a potential sequela to antibiotic therapy in an elephant, therefore antibiotics should be used after considering risks and benefits.

For EEHV-HD cases without overt diarrhea or specifically known secondary infection, Houston Zoo treats for secondary infections prophylactically with ceftiofur sodium (Naxcel) IV and ceftiofur crystalline free acid (Excede) IM both administered on the first day of treatment. Excede does not have instantaneous action in the body but lasts for approximately 7 days (*Adkesson et al. 2012*), which is why Naxcel is given IV, in order to provide immediate antibiotic coverage.

Clostridial Co-infections

Specific co-infections with EEHV-HD and *Clostridium spp* have been reported in the literature (*Boonsri et al 2018*) and may become a secondary infection in any sick elephant. A *Clostridium spp* infection may manifest as diarrhea, hematochezia, colic, dehydration, etc. Fecal cytology, fecal culture, fecal occult blood, and fecal molecular testing may be useful in diagnosis. Treatments used at the Houston Zoo for possible *Clostridial spp* infection include injectable penicillin for a week over the course of EEHV-HD treatment, in conjunction with other supportive therapies. Diagnostics did not confirm a *Clostridial spp* co-infection in this case, but penicillin was elected in an abundance of caution when the animal presented with scant feces to diarrhea while combating EEHV-HD.

It is advised to be highly vigilant for any signs of a Clostridial co-infection during EEHV cases.

Moreover, if a calf does develop diarrhea, allowing opportunities for the calf to transfaunate or consume feces from a healthy adult with normal stool is important. It is also thought that calves will eat feces when experiencing any sort of gastrointestinal distress. EEHV replicates well in the gastrointestinal (GI) tract and bleeding throughout the GI tract should be presumed in EEHV-HD cases.

Antibiotic dosages:

- Amikacin 3-5 mg/kg IV, IM SID
- Ampicillin 8 mg/kg IV, PO BID - TID
- Ceftiofur sodium (Naxcel) 1.1-2.2 mg/kg IV SID or give single dose concurrently with Excede on the first day of treatment

- Ceftiofur crystalline free acid (CCFA, Excede) 6.6 mg/kg IM q 7 days
- Enrofloxacin 2.5 mg/kg PO SID
- Penicillin (Dual-Pen) 2275-4545 IU/kg IM SID

Analgesia and Anti-inflammatory Treatments

Non-steroidal Anti-inflammatories

Although EEHV is thought to be a vasculopathy as opposed to a vasculitis, anti-inflammatories are indicated as part of the analgesic regime as well as reducing secondary inflammation resulting from peripheral edema and hemorrhage. Non-steroidal anti-inflammatories (nSAIDs) may play a useful role in early management of the disease. However, it should be noted that in human medicine nSAIDs are contraindicated in cases where peripheral edema or hemorrhagic diathesis is present due to the decreased glomerular filtration rate and the effects on coagulation seen when using nSAIDs. The analgesic and anti-inflammatory effects of these drugs should be weighed against these side effects. Flunixin meglumine, meloxicam, or other nSAIDS should be administered only to patients that appear hydrated or are receiving rectal and/or IV fluids.

At the Houston Zoo, low (anti-endotoxemic) doses of flunixin meglumine (0.15-0.5 mg/kg) have been used both IM and IV in multiple EEHV-HD cases. In an EEHV-HD case with persistent azotemia, nSAIDs were stopped completely after initial treatments with low dose flunixin meglumine. After a 1 day wash out, meloxicam at a low dose 0.03 mg/kg IV was given to help decrease inflammation. Meloxicam is a more selective nSAID than flunixin meglumine and is a safer choice in azotemic or significantly compromised EEHV-HD cases.

Non-steroidal anti-inflammatory dosages:

- Flunixin meglumine (Banamine) 0.15-0.5 mg/kg IM SID-BID, use lower dosage for endotoxemia
- Meloxicam (Metacam) 0.0.3-0.06 mg/kg IV, IM, PO

Opioids

Opioids are also a useful adjunct to provide pain relief and, in some cases, mild sedation to assist in the management of animals being treated. Butorphanol has been the main opioid used for analgesia in elephants. There is the possibility of behavioral changes in the elephant when using opioids and trained behaviors may well be lost or less responsive. Opioids may be a preferred to non-steroidal anti-inflammatory analgesics in azotemic animals in order to avoid adverse effects on the urinary system.

Moreover, high dosages of opioids may result in ileus manifested by colic, decreased fecal production, and anorexia.

If an animal is being sedated with butorphanol, an additional dose of this drug will cause deeper or longer duration of sedation. Moreover, if an animal has received naltrexone butorphanol following a sedation, any butorphanol dosed within a few hours for analgesia will be less effective.

In a 1,400 kg Asian elephant treated at the Houston Zoo (for signs of colic during EEHV viremia), 9 mg (0.006 mg/kg) seemed to cause more sedation than desired. Based on that experience, it was recommended to start with 7 mg (0.005 mg/kg) IM for future cases, up to BID.

Opioid analgesia dosages:

- Butorphanol 0.005-0.14 mg/kg IV, IM SID-BID

Steroids

The anti-inflammatory properties of steroids have been used in multiple cases reported in the literature; however, use remains controversial due to immunosuppressive potential. Dexamethasone 0.05 mg/kg IM daily during treatment has been used previously (*Wissink Argilaga et al. 2019*). Steroids and non-steroidal anti-inflammatory medications should not be given simultaneously due to potentially harmful effects.

Gatroprotectants

During EEHV-HD, it is common for the animal to experience bleeding within the gastrointestinal tract, even in the absence of frank blood or melena in the stool. Fecal occult blood tests may be used diagnostically, but results may be false negative due to the elephant's very absorptive colon. Therefore, gastroprotectants should be considered in EEHV-HD cases. Omeprazole and famotidine are medication options, but encouraging the calf to eat and nurse, as well as providing opportunities for transfaunation are also important to maintain GI health.

Omeprazole

Administration of omeprazole (Gastrogard) for gastrointestinal protection during any clinical EEHV case, but especially during EEHV-HD and during nSAID treatments should be considered. The equine dose for omeprazole is 4 mg/kg PO SID, but the dosage used in Asian elephants is much lower at 0.7 - 1.4 mg/kg PO once daily. If the animal is anorexic, the paste formulation may be placed within the oral cavity during the end of sedation events. Omeprazole is also commercially available in pill form and

is an alternative if compliance with paste is poor. Omeprazole should also be continued as clinical signs resolve for on-going protection.

Gastroprotectant dosages:

- Omeprazole 0.7-1.4 mg/kg PO SID

Famotidine

Famotidine (Pepcid) and omeprazole may be given simultaneously during EEHV-HD treatment. Famotidine has the advantage of being readily available in an injectable form that may be given by multiple parenteral routes including IV, which is preferred to avoid IM or subcutaneous injections. Oral famotidine is available in a pill form that may be an alternative gastroprotectant to be used as clinical signs resolve for on-going protection if the animal is not compliant with oral omeprazole. The dose for famotidine used at the Houston Zoo is 0.5 mg/kg IV once daily during sedations for treatment.

Gastroprotectant dosages:

- Famotidine 0.5 mg/kg IV, slow, or PO

Vitamin Supplements

Vitamin B Complex

Vitamin B Complex contains multiple water-soluble B vitamins that are important for metabolic function. B vitamins may be especially beneficial to anorexic or anemic calves. Doses are highly variable, but the Houston Zoo has elected a 5 ml/elephant dose of Vitamin B Complex IM, given every other day during EEHV-HD treatment.

Vitamin B complex dose:

- Vitamin B complex 5 ml/elephant calf IM q 48 hrs

Vitamin C

Vitamin C is a water-soluble vitamin that has anti-oxidant and immune system boosting properties. It is also becoming more widely used in human medicine to treat sepsis and may also be beneficial for microvasculature health. Houston Zoo uses the dose advocated for by Thailand's EEHV Task Force of Vitamin C 6 mg/kg IV or PO daily during treatment (*Sripiboon et al. 2017*).

Vitamin C dosages:

- Vitamin C 6 mg/kg IV, PO SID

Vitamin E

Vitamin E is a fat-soluble vitamin that has anti-oxidant and cell membrane protective properties. The dosage range is variable. The Houston Zoo uses a vitamin E dose 10 ml/elephant IM every other day during EEHV treatment.

Vitamin E dosages:

- Vitamin E 10 ml/elephant IM q 48 hrs

Clotting Aids

Aminocaproic Acid

Aminocaproic Acid (EACA) is an anti-fibrinolytic drug that is most often used in hemorrhagic diseases of humans and domestic animals, but may be of some benefit in severe EEHV-HD cases. It has been studied *in vitro* in elephant models (*Kaye et al. 2016*). This drug is a protein that helps the body make blood clots properly, but should be used with caution in patients with renal insufficiency. Ideally, this drug would be given when thromboelastography (TEG) profiles can be monitored and compared to normal parameters to best direct treatment (*Perrin et al. 2018*). If TEGs are not available, microscopic evaluation of blood cells should be done to roughly evaluate agglutination or clumpiness. There is much to be learned about using this drug in vivo in elephants. Its use should be limited to severe clinical cases of EEHV only and then be used with caution.

The Houston Zoo has used this drug at 15 mg/kg, diluted into 1 L LRS IV once daily for 4 days without ill effect in a calf with severe clinical disease. The drug was stopped when the calf started to show signs of hypercoagulability on TEG and increased blood cell clumpiness on major and minor crossmatching.

Aminocaproic Acid dosages:

- Aminocaproic Acid 15 mg/kg IV diluted in 1 L crystalloid fluid, given slowly and monitor for hypercoagulability

Stem Cells

Stem cells are an emerging therapy for EEHV due to their antimicrobial potential and ability to decrease cytokine storms and inflammation, which may help to improve clinical outcomes. Stem cells have been safely administered to both African and Asian elephants, including calves with EEHV with no apparent ill-effect. Adverse effects in other species include a transient tachycardia, tachypnea, fever, lethargy, and anorexia. Treatment for adverse effects include fluids, antibiotics (in case of stem cell bacterial contamination), and supportive care.

Although stem cells do not have an immediate effect, they likely support the body during the recovery phase. Stem cells do not need to be autogenous and can be from any same-species donor. Crossmatching does not need to be done prior to a stem cell treatment. Developing antibodies to allogenic stem cells may occur over time, but in general anaphylaxis has not been an issue.

In general, the younger the donor, the better the stem cells. Stem cells may be obtained from the umbilical cord at the time of a calf birth or may be derived from whole blood.

Stem cells from the umbilical cord have been processed by Ingeneron, Inc. (8205 El Rio, Houston, TX USA 77054, (713) 440-9900, www.ingeneron.com) for the Houston Zoo herd. There are very specific steps to take for umbilical cord sampling and the sampling must be expedited after the placenta is passed. Stem cells collected by this method take several weeks to grow and may be frozen once grown for future use. There is a 24-hour long process to prepare frozen stem cells prior to administration. Administration also has specific administration techniques that must be followed as well. It is strongly recommended, that Ingeneron, Inc. be contacted well in advance of an elephant birth to plan for umbilical stem cell collection.

Stem cells from whole blood have been processed by Dr. Valerie Johnson (Colorado State University, Valerie.Johnson@colostate.edu). Stem cells may be grown from a minimum of 40 ml whole blood in EDTA (purple top) tubes, which are then shipped overnight to Dr. Johnson. Stem cells collected by this method take several weeks to grow. There are specific processing and administration techniques to follow as well. It is strongly recommended, that Dr. Johnson be contacted by any institution with at risk calves well in advance of a clinical illness.

There may be other companies or institutions, such as a university, that may be able to process and provide stem cells. It is strongly recommended that any institution with at risk calves seek a source of stem cells prior to clinical illness. Having locally available stem cells is advantageous.

At the Houston Zoo, a single severe case of EEHV-HD was treated with umbilical cord derived stem cells at a dose of 21.28 million cells IV from a half-sibling donor cord without ill effect. Ideally, a dosage of 1 million cells/kg would be given, but it is rarely possible to grow that volume of cells, so a dose of 100 – 500 million cells (or the more the better) would be ideal according to current research (Dr. Johnson, personal communication).

EEHV Advisory Group Website

Up to date information on EEHV background, history, diagnostics, monitoring, and treatment can be found at www.eehvinfo.org. Each individual person who wishes to have full access to the website needs to sign up for an individual password. If there are questions regarding this, contact Erin Latimer (LatimerE@si.edu).

The EEHV Advisory Group has a Veterinary and Management Subcommittee that maintains current EEHV Advisory Group recommendations in a downloadable document. This committee may be contacted should any clinical needs arise. Subcommittee chairperson: Dr. Noha Abou-Madi (na24@cornell.edu).

References

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Elephant Calf Training, Foundation is Everything

Daryl Hoffman
Houston Zoo

Abstract

A strong training foundation could be the difference between life and death of a young elephant, especially when EEHV is involved. A lot goes into treating and saving an elephant infected with EEHVHD and a solid training foundation is vital to maximizing preparedness and minimizing stress during these life threatening events. A trained elephant is more cooperative for routine blood collection, more participatory in treatments, and more quickly to recover behavioral compliance after a lengthy treatment.

Solid training foundations are based on an expanded version of the AZA behavioral components that all elephants in AZA facilities are required to know. The foundation is constructed and strengthened through daily practice and repetition.

This presentation will briefly outline these behaviors which the Houston Zoo has made a priority to train all baby elephants by the time they reach one year of age. These behaviors have played an important role in the Houston Zoo success with early detection and treatment of EEHV HD.

Acute Hemorrhagic Disease due to EEHV-3 Infection in Five Captive African elephants (*Loxodonta africana*)

Melissa A. Fayette, DVM & Jill Sampson

1

Case #1: Day 1

- **Presenting clinical signs**
  - Acute lethargy
  - Anorexia
  - Abdominal discomfort
  - Loose stool
- **Initial blood work**
  - Elevated Hematocrit (50%)
- **Treatment**
  - Flunixin meglumine (1.1 mg/kg IM)
  - Butorphanol (0.02 mg/kg IM)

NYAH
6.5 year old female

2

Case #1: Day 2

- **Clinical signs**
  - Continued lethargy and anorexia
  - Hematuria
  - Diarrhea
  - Vomiting
  - Tremors
  - Scleral hyperemia
  - Skin ulcerations
- **Blood work**
  - Elevated Hematocrit (63%)
  - Band neutrophilia (4%)
  - Thrombocytopenia
  - Azotemia
  - ↑AST and ↑GGT
  - Hyperbilirubinemia
  - Hyperkalemia
- **Treatment**
  - IV and rectal fluids
  - Excede (6.6 mg/kg SQ)
  - Banamine (1.1 mg/kg IM)

3

Case #1: Day 3

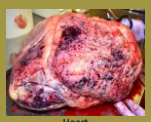
- **Clinical signs**
  - Severe depression
  - Dyspnea
  - Marked abdominal distension
  - Subcutaneous edema in the head and forelimbs
- **Blood work**
  - Elevated hematocrit (58%)
  - Band neutrophilia (2%)
  - Monocytosis
  - Thrombocytopenia
  - Worsening Azotemia
  - ↑AST, ↑GGT, ↑CK
  - Hyperbilirubinemia
  - Hypoproteinemia
- **Treatment**
  - Furosemide (1 mg/kg IV)
  - Ceftiofur sodium (2.2 mg/kg IV)
- **Outcome**
  - Death in <72 hrs. from the onset of clinical signs

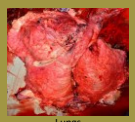
4

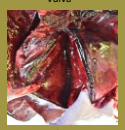
Case #1: Necropsy Findings

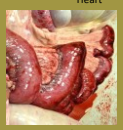

Skin ulcerations


Vulva


Heart


Lungs


Liver

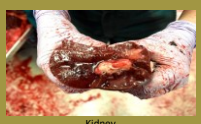

Intestine

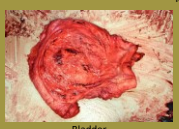

Stomach

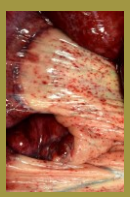
5

Case #1: Necropsy Findings


Spleen


Kidney


Bladder


Messentery

6

Histopathology

- Disseminated vascular necrosis with edema, hemorrhage, and endothelial cell intranuclear inclusions within the liver, kidney, spleen, and ovary
- Severe renal tubular necrosis
- Mild rhabdomyolysis

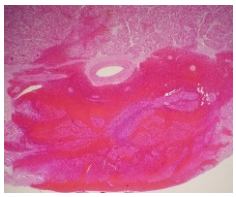


Photo Courtesy of Michael Garner, DVM, Dipl ACZM

7

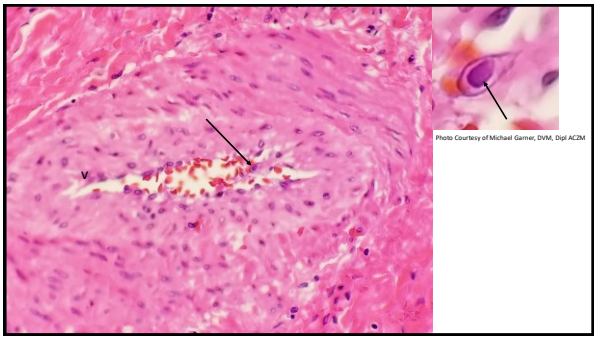
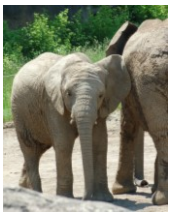


Photo Courtesy of Michael Garner, DVM, Dipl ACZM

8

EEHV Testing

- **Quantitative PCR** (performed postmortem on banked whole blood samples)
  - Day 1: 2,000,000 vge/ml (EEHV-3/4)
  - Day 2: 3,000,000 vge/ml (EEHV-3/4)
- **Whole genome sequencing**
  - Performed on frozen lung tissue
  - Strain identified as EEHV3A



9

Case #2: Day 1

- **Presenting clinical signs**
  - Loose stool
  - Hematuria
- **Initial blood work**
  - Band neutrophilia (1%)
  - Monocytopenia
  - Thrombocytopenia
- **Treatment**
  - Famciclovir (12 mg/kg PO q 8 hr)
  - Butorphanol (0.02 mg/kg IM q 8 hr)
  - Ceftiofur sodium (2.2 mg/kg IM q 24 hr)
  - Rectal fluids (q 6 hr)

Kalina
7.5 year old female

10

Case #2: Day 2

- **Clinical signs**
  - Lethargy
  - Anorexia
  - Stiff gait
  - Abdominal pain
  - Frank hemorrhage in urine
- **Blood work**
  - Band neutrophilia (2%)
  - Monocytosis (69%)
  - Thrombocytopenia
  - Hypoalbuminemia
  - ↑AST and ↑GGT
  - Hyponatremia
- **Treatment**
  - Famciclovir (12 mg/kg PO q 8 hr)
  - Butorphanol (0.02 mg/kg IM q 8 hr)
  - Ceftiofur sodium (2.2 mg/kg IM)
  - Rectal fluids (q 6 hr)

11

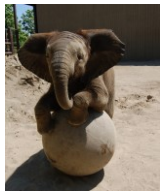
Case #2: Day 3

- **Clinical signs**
  - Depression
  - Edema along ventrum and in hind limbs
  - Tremoring of the trunk
  - Diarrhea
- **Blood work**
  - Band neutrophilia (1%)
  - Thrombocytopenia (18,496/μL)
  - ↑AST and CK
  - Azotemia
  - Hyponatremia
  - Hypoproteinemia
  - Hyperglycemia
- **Treatment**
  - Dexamethasone SP (0.25 mg/kg IV)
  - IV fluids (crystalloids + Hetastarch)
  - 2L of plasma collected from dam
  - Famciclovir (12 mg/kg PO q 8 hr)
  - Butorphanol (0.02 mg/kg IM q 8 hr)
  - Ceftiofur sodium (2.2 mg/kg IV)
  - Rectal fluids (q 6 hr)

12

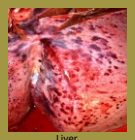
Case #2: Day 4

- **Clinical signs**
  - Rectal hemorrhage
  - Tachypnea
  - Tachycardia
- **Blood work**
  - Band neutrophilia (4%)
  - Thrombocytopenia
  - ↑AST and CK
  - Worsening Azotemia
  - Hyponatremia
  - Hypoproteinemia
  - Hyperglycemia
- **Outcome**
  - Death in <72 hrs. from the onset of clinical signs



13

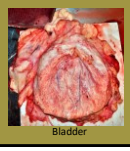
Case #2: Necropsy Findings


Liver


Stomach


Intestine


Lymph node


Bladder

14

EEHV Testing

- **Quantitative PCR** performed on whole blood
  - Day 1: 1,000,000 vge/ml (EEHV-3/4)
  - Day 2: 3,700,000 vge/ml (EEHV-3/4)
  - Day 3: 1,600,000 vge/ml (EEHV-3/4)
  - Day 4 (antemortem): 920,000 vge/ml (EEHV-3/4)
  - Day 4 (postmortem): 2,300,000 vge/ml (EEHV-3/4)

15

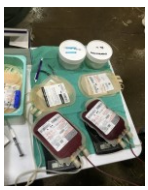
Indianapolis Zoo EEHV Surveillance Program

	ROUTINE MONITORING	CLINICAL SUSPECT	CONFIRMED CASE	MANAGEMENT OTHER CALVES
Purple top (whole blood)	Calves: Biweekly: qPCR (NEHL) Adults: Weekly: qPCR (NEHL)	ASAP: qPCR (NEHL)	Daily: EEHV qPCR (NEHL)	Daily: EEHV qPCR (NEHL)
Purple top (whole blood)	Calves: Weekly: CBC (In-house + Idexx) Adults: Monthly: CBC (In-house and Idexx)	ASAP: Daily In-House CBC	Twice daily: In-house CBC	Daily: In-house CBC
Red top / tiger-top (serum)	Calves: Weekly: In-house Chemistry panel, SAA/haptoglobin (UM) and serum banking Quarterly: CP (Idexx) Adults: Quarterly: CP (Idexx), SAA (UM) Serum banking	ASAP: In-house CP, SAA/haptoglobin (UM) Pre-meds once: 50- 100mls for serum banking	Twice daily: In-house CP (priority. BUN/Creatinine) Weekly: SAA/haptoglobin -(UM) Save rest for serum banking	Daily: In-house CP
Green top (plasma)	N/A	ASAP: Blood gases /electrolytes	Twice daily: Blood gases / electrolytes	N/A
Urine	Calves: Monthly: In-house UA Adults: Quarterly	ASAP: In-house UA Save rest for research*	Daily: In-house UA Once: Research	Daily: In-house UA
Trunk Wash	Weekly (banked)			
Royal blue top	Quarterly sample for NS	N/A	Weekly: Mineral panel (MSU)	N/A

16

Preparation For Future Cases

- Creation of a plasma bank
- Blood cross-matching for all elephants in the collection to identify donor compatibility
- Acquisition of anti-viral medication to keep in stock (3 day supply of oral famciclovir for each of our younger elephants and 1 dose of intravenous ganciclovir per elephant)
- Establishment of normal reference ranges for blood parameters for each individual elephant



17

Equipment Purchases

- IDEXX ProCyte DX for automated CBCs: $22,500
- J-15R Centrifuge, Tube sealer, and Separator stand for processing plasma: $14,900
- Ultra-low freezer for sample storage: $9520
- Blood refrigerator and Plasma freezer: $21,750

18

Case #3

- **Presenting clinical signs**
  - Loose stool
  - Lethargy
- **Initial blood work**
  - Mild anemia
  - Monocytopenia
  - Thrombocytopenia
  - ↑ Acute phase proteins (SAA, haptoglobin)
- **EEHV qPCR**
  - Day 1: 5000 vge/ml (EEHV-3/4)
  - Day 2: 17,250 vge/ml (EEHV-3/4)
  - Day 3: 115,000 vge/ml (EEHV-3/4)
  - Day 4: 265,000 vge/ml (EEHV-3/4)

Kedar
13 year old male

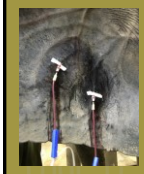
19

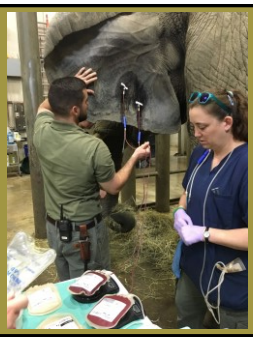
Case #3: Treatment Summary

- **Transfusions**
  - Plasma (~2 L) on Days 2 and 3
  - Whole blood (2-3.5 L) one or two times/day on Days 4-7 (Total of 6 transfusions)
- **Antibiotics**
  - Ceftiofur sodium (2.2 mg/kg IM q 24 hr) Days 2-9
- **Anti-inflammatory**
  - Flunixin meglumine (0.3 mg/kg IM q 24 hr) Days 2-7
  - Meloxicam (0.03 mg/kg PO q 24 hr) Days 8-10
- **Gastroprotectant**
  - Omeprazole (1 mg/kg PO q 24 hr) Days 4-11
- **Anti-viral**
  - Famciclovir (15 mg/kg PO or rectally q 8 hr) Days 2-25
- **Fluids**
  - Rectally q 6 hrs. Days 2-10
  - IV crystalloids on Day 3 with plasma transfusion
  - Hetastarch (2L with each whole blood transfusion)
- **Other**
  - Vitamin C (6 mg/kg IV) on Day 3
  - Stem cells (100 x 10^6 in 10 ml) IV on Day 7

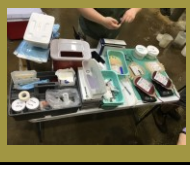
20

Blood Collection From Donor Elephant



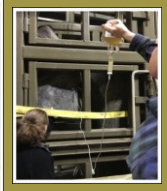


- Standing position on tethers
- Place multiple catheters if possible
- May take 2-3 hrs. to collect a large volume!



21

Plasma & Whole Blood Transfusions



Plasma rate: ~2L/hr.
Whole blood rate: First unit slowly over ~30 mins (while monitoring TPR), then 1.5-2L/hr.
Total time for transfusion (including prep): 2-4 hours

22

Preparing Kedar for Transfusion

- Standing sedation with butorphanol (0.025 mg/kg IM) in ERD
- Placed 14-gauge catheter in auricular vein

23

Treatment

24

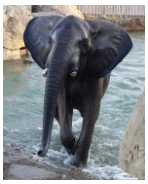
Treatment (continued)

Lite Raspberry Vinaigrette Chaser! ☺

25

Case #3: Outcome

- **Day 4**: Viral load peaked at 265,000 vge/ml (EEHV-3/4)
- **Day 6**: Most severe changes in blood work seen
- **Day 7**: Clinical improvement and platelet number starting to increase
- **Day 10**: Behaviorally normal
- **Day 12**: All blood parameters within the normal range
- **Day 12**: Viral load decreased to <1000 vge/ml (EEHV-3/4)
- **Day 16**: EEHV-3/4 qPCR was negative
- **Day 17 to Present**: Intermittent low level viremia (<1000 vge/ml) of EEHV-2, 3/4, 6
- **Day 25**: Treatment discontinued, but monitoring blood work daily
- **Day 36**: Resumed routine blood work monitoring (2 times per week)



26

Case #4

- **Presenting Clinical Signs**
  - None
  - Lethargy and loose stool on Day 5
- **Initial blood work**
  - All normal on Days 1 and 2
  - Day 3:
    - Severe thrombocytopenia
    - Band neutrophilia (7%)
    - Mild anemia
    - ↑ SAA
- **EEHV qPCR**
  - Day 1: 21,000 vge/ml (EEHV-3/4)
  - Day 2: 115,000 vge/ml (EEHV-3/4)
  - Day 3: 115,000 vge/ml (EEHV-3/4)

Zahara
12 year old female

27

Case #4: Treatment Summary

- **Transfusions**
  - Plasma (1.5-2.6L) on Days 3, 6, and 7
  - Whole blood (1.8L) twice daily on Days 4-6
- **Antibiotics**
  - Ceftiofur sodium (2.2 mg/kg IM q 24 hr) Days 3-10
  - Marbofloxacin (2 mg/kg PO q 24 hr) Days 6-17
- **Anti-inflammatory**
  - Flunixin meglumine (0.3 mg/kg IM q 24 hr) Days 3-6
- **Gastroprotectant**
  - Omeprazole (1 mg/kg q 24 hr) Days 3-10
- **Anti-viral**
  - Famciclovir (15 mg/kg PO q 8 hr) Days 3-25
- **Fluids**
  - Rectally (q 6 hr) Days 3-10
  - Hetastarch (2L with whole blood)

28

Treatment

Zahara prefers Gatorade!

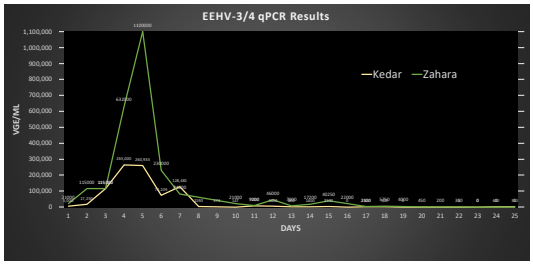
29

Outcome

Day 5: Viral load peaked at 1,100,000 vge/ml (EEHV-3/4)
Day 6: Most severe changes in blood work seen
Day 8: Clinically normal and blood work starting to improve
Day 14: All blood parameters returned to normal
Day 20: Viral load <1,000 vge/ml
Day 23: EEHV-3/4 q PCR negative
Day 25: Treatment was discontinued
Day 37: Resumed routine monitoring (2X per week)

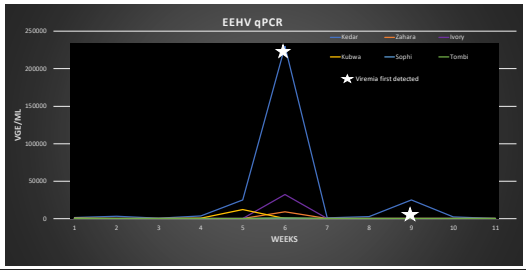
30

Viral Testing (Whole Blood)



31

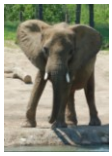
Viral Testing (Trunk Wash)



32

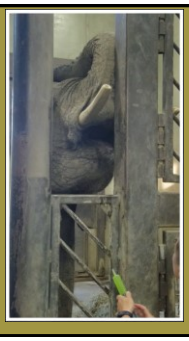
Case #5: "Ivory"

- **37 yr old female**
- **Presenting clinical signs**
  - Moving slower than normal
- **Blood work**
  - 40% reduction in platelets initially
  - Marked elevation in SAA
  - EEHV-3/4 q PCR: 3,200 vge/ml
- **Treatment**
  - Famciclovir (15 mg/kg PO q 8 hr) x 11 days
  - Naxcel (2.2 mg/kg IM q 24 hr) x 5 days
  - Omeprazole (1 mg/kg PO q 24 hr) x 4 days
  - Flunixin meglumine (1.1 mg/kg IM) x 4 days
  - Rectal fluids (q 6 hr)
- **Outcome**
  - Viral load peaked at 12,600 vge/ml on Day 3
  - Platelets decreased by 63% on Day 3, returned to normal by Day 5
  - Viral load undetectable by Day 10
  - SAA gradually returned to baseline by Day 13



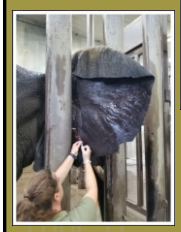
33

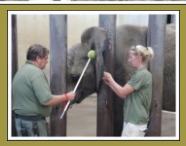
Ivory in the ERD for administration of injectable medications and rectal fluids

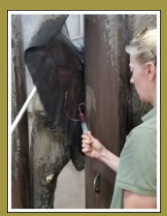


34

Training: Venipuncture







35

Training: Accepting Injections

36

Training:
Oral Medications &
Tether Restraint

37

Take Home Points

- **Surveillance is the key!**
- Clinical signs may be absent or subtle
- Establish normal reference ranges for blood parameters for each individual elephant
- **Be prepared** to provide **early and aggressive** treatment
- Training of staff and elephants is essential

Kedar & Zahara
EEHV Survivors 2019

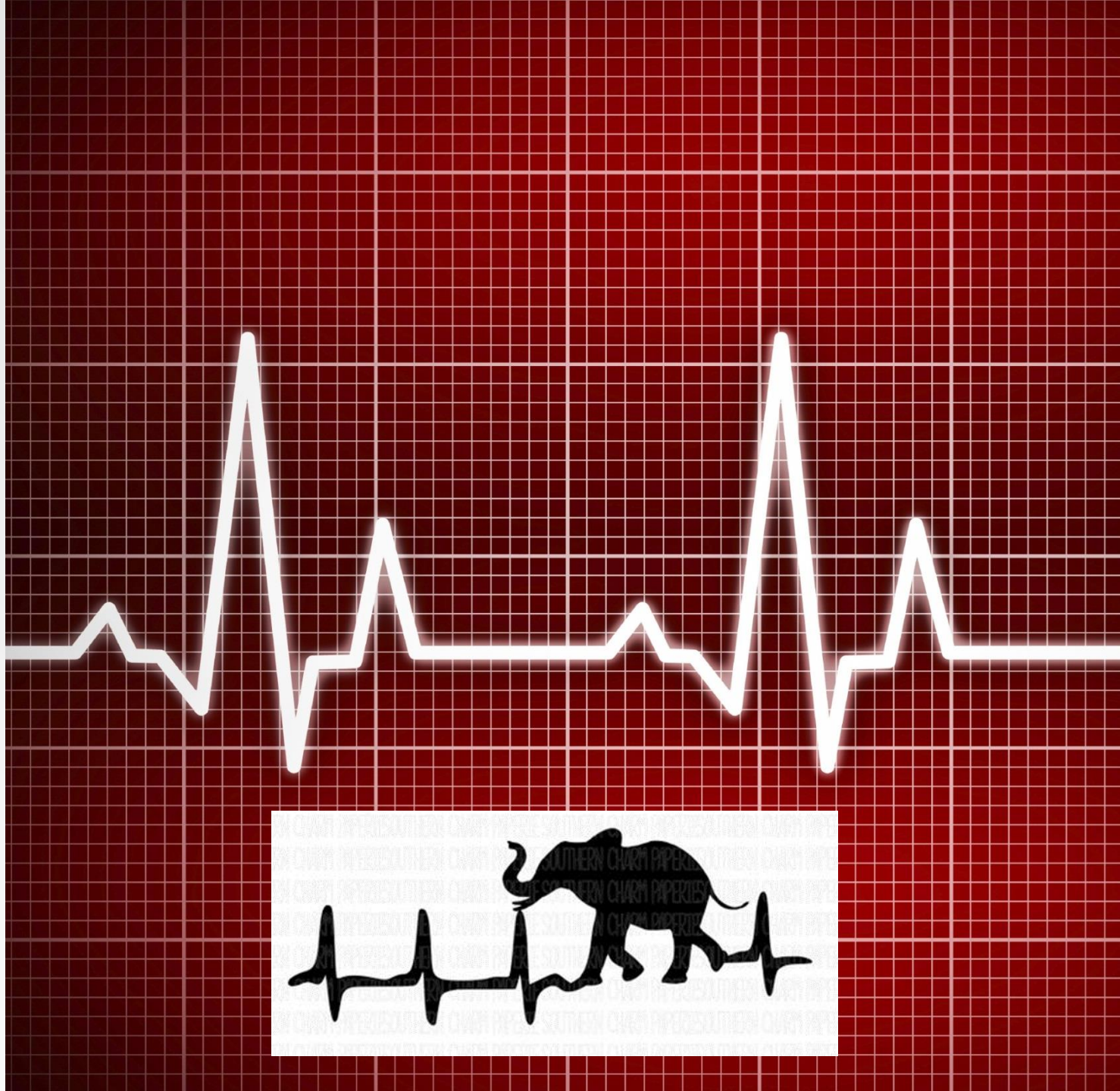
38

39

Elephant Endothelial Herpes Virus at Fresno Chaffee Zoo 2019

SHANNON NODOLF, DVM

VERNON PRESLEY



Prior to 2019…

- Herd of 1.3 **African** elephants all over the age of **10 years**
- Guess how much we though about EEHV???

Miss Bets

- 11 year old female African Elephant
- Born at Riddle's Elephant & Wildlife Sanctuary
- Dam 'Amy' was wild caught as juvenile in Zimbabwe
- Miss Bets was index case for EEHV6 when at Riddle's
- Arrived with Amy to Fresno in May 2015

February 8, 2019

- Miss Bets appeared uncomfortable with hunched abdomen and slow choppy gait
- Eating produce during training but refusing hay
- Subtle shaking present intermittently
- Treated for suspected colic/gi discomfort
  - Omeprazole
  - Ranitidine
  - Banamine
- Seemed improved by afternoon
- Limited historical blood work for normal values but initial bloodwork considered unremarkable at the time

February 9, 2019

- AM still off but willing to train for blood collection which showed static chemistry values
- Clinical picture began to rapidly decline throughout the morning
  - Developed shaking/tremors
  - Facial grimacing
  - Erratic trunk swinging
  - Disoriented mentation
  - Intermittent aggression
  - Progressive ataxia

Miss Bets

- Animal stopped participating in training but did willingly go into ERD
  - Administered diazepam due to suspected focal seizure activity
    - Continued agitation, muscle fasiculations, and she removed all attempts at catheterization
  - Elected to sedate with butorphanol 35mg, detomidine 35 mg and midazolam 26 mg IM
  - Bets calmed down significantly and allowed for rectal fluid enema
  - Placed catheter in coccygeal vein and started IV fluids (even with sedation she removed all other catheters)
  - Diagnostics collected for: Encephalitidies, Sarcocystis, Neospora, Toxoplasma, Coccidiodes, EEHV, EMC, Lepto, Heavy Metals
  - Bets remained calm in ERD and started playing with food and eating some hay
  - ~2 hours later she went down in the ERD, arrested and died.
  - Remains packed on ice (~2 tons) while awaiting coordination with pathologist for necropsy

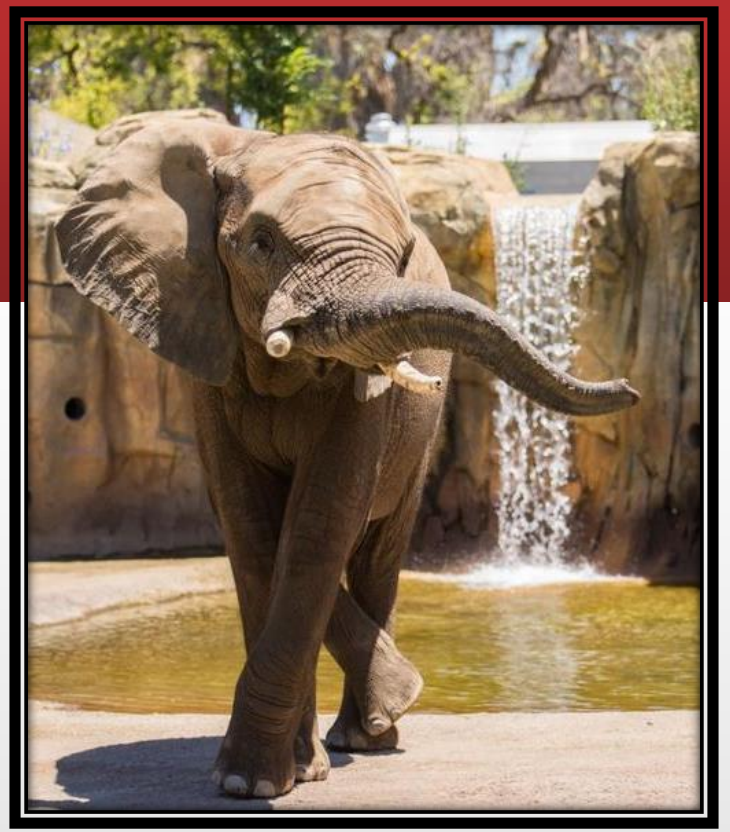
Bloodwork Changes

	Bets Normal	2/9/19	2/9 compared to a healthy Bets
RBC x10^3/uL	3.0	3.5	
HCT	34.5%	43%	
WBC x10^3/uL	9.4	7.4	21% ↓
Neutrophil x10^3/uL	3.1; 32.5%	5.0; 68%	35% ↑
Monocyte x10^3/uL	2.8; 32%	1.4; 19%	13% ↓
Lymphocyte x10^3/uL	3.5; 35%	0.89; 12%	23% ↓
Eosinophil	0	0.074; 1%	
Platelets x10^3	**1300**	**173**	**86%** ↓
Monocyte:neutrophil	32:32.5	19:68	80% ↓

Miss Bets

- Necropsy performed at UC Davis extension: California Animal Health & Food Safety Laboratory
- Antemortem diagnostic results
  - 0 gross hemorrhage or petechiation
  - Marked inflammation of colon and distal GI tract: Cultures came back growing bacteria Clostridium sordelli and C. perfringes
  - Toxin and Infectious disease testing all initially negative
  - No EEHV inclusions identified histologically

Miss Bets confounders



- Samples submitted to the National Elephant Herpesvirus Laboratory for EEHV testing prior to death initially tested 0 vge/ml
- Tissues submitted at necropsy for additional EEHV testing came back markedly positive:
  - Liver:
    - **EEHV 3-4: 100,000 vge/rxn**
  - Lung:
    - **EEHV 3-4: 300,000 vge/rxn**
- Retested initial serum and WB that was pulled prior to death
  - **EEHV 3-4 EDTA WB: 14,000,000 vge/ml**
  - Serum: 200,000 vge/ml
  - Sent additional whole blood to Baylor College of Medicine which confirmed EEHV 3-4 positive

Miss Bets confounders

- Second cutting histology slides sent to Dr. Jaime Landolfi with University of Illinois:
- “widespread endothelial cell hypertrophy, rare endothelial cell necrosis and several **intranuclear inclusions**”
- “appreciated the acute inflammation and erosion in the sections of rectum, however, I did not think these lesions were of sufficient severity to have accounted for clinical course and death. Mucosal inflammation and damage were limited to the rectum in sections I examined. The large intestine and, to a lesser extent, small intestine did have mural edema and some hemorrhage, but I believe these lesions could be attributed to EEHV3-mediated endothelial damage. I think the Gram positive bacilli noted in the large intestinal and rectal glands represented postmortem colonization/overgrowth, or at most, opportunistic infection. C. sordelli, like most Clostridium sp., is a common postmortem invader. I think **EEHV was the primary contributor to clinical course and death in this case**.”

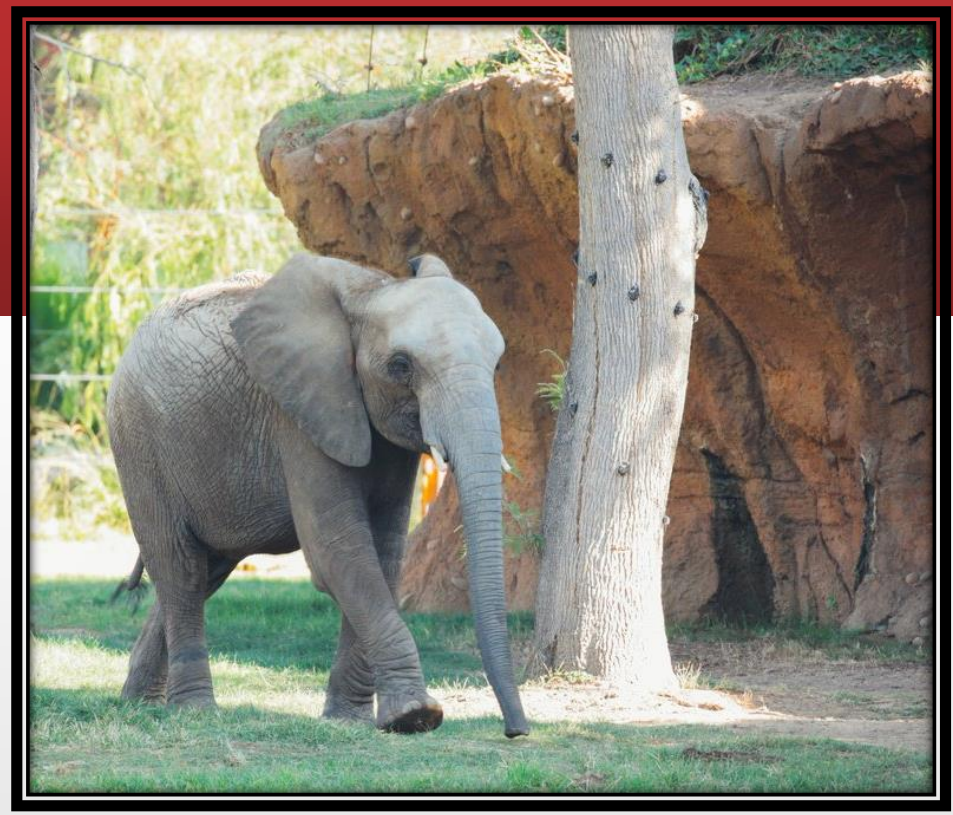
Amahle

- Part of a Swaziland cull rescue in 2016
- Arrived with her mother Nolwazi to Fresno from Dallas in Oct 2018
- Estimated to be 11 years old

April 29, 2019

- During afternoon training session noted to be slower in her movements compared to her normal
- When asked for front foot present she had difficulty reaching the same heights she normally does for her stretches
- Heightened sensitivity due to similar clinical signs mentioned by other facilities with EEHV calves.
- Infectious and toxin screening tests sent out
- In-house bloodwork fairly unremarkable compared to limited historical normal
- Started contacting folks for advice and asking all of you experts to weigh in as we anxiously waited for EEHV results to return
- Elected to start her on antiviral therapy famciclovir 30,000mg PO TID, meloxicam 120mg PO SID

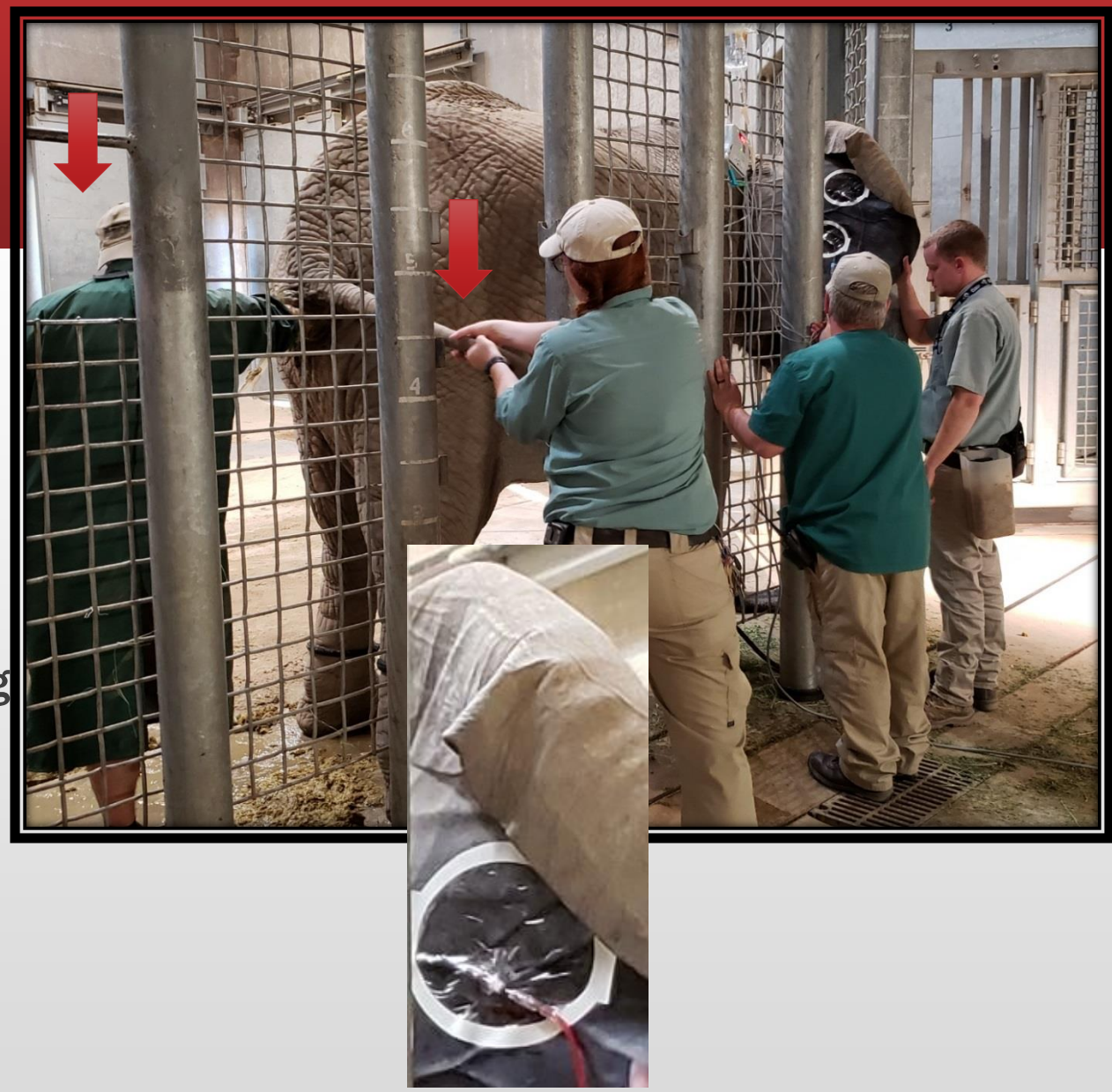
April 30, 2019



- ~20 hours after first symptoms observed initial EEHV results showed that despite her relatively stable lab work we were unfortunately correct in our fears and Amahle was EEHV 3-4 positive with **80,000 vge/ml** of whole blood
- Amahle continues to be BAR, eating/drinking and generally behaving totally normally aside from slight stiffness/decreased leg extension.
- Despite clinical appearance elected for aggressive medical management

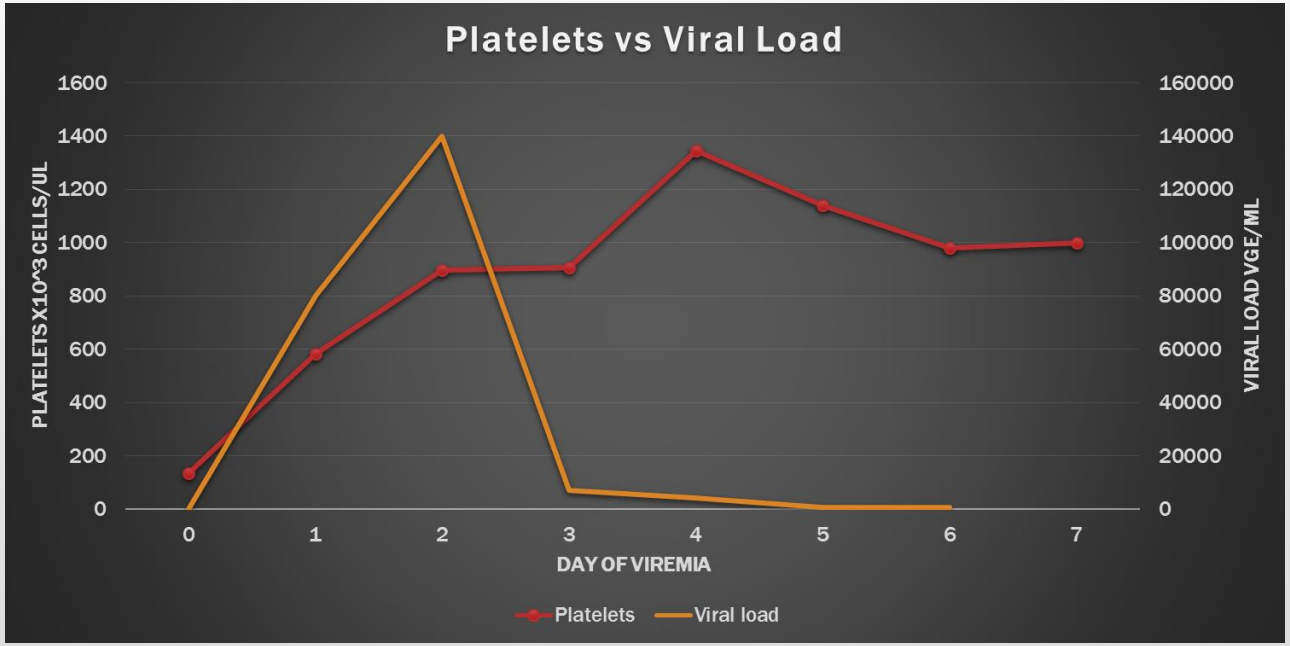
Medical Management

- 24 hour monitoring
- TID Visual exams-scouring for petechiation or evidence of hemorrhage
- TID antivirals- Famciclovir
- Daily sedation for administering IV and rectal fluids
- Cross matching Amahle to our other elephants for blood and plasma transfusions
- Famciclovir loading plasma donor prior to collecting and administering donor plasma
- Continued meloxicam for anti-inflammatory analgesia
- Twice daily assessment of blood and urine
- Vit C
- Administration of stem cells kindly donated from two different sources.
- Continued recruitment from colleagues for additional potential plasma donors, medications – ganciclovir, additional advice and support

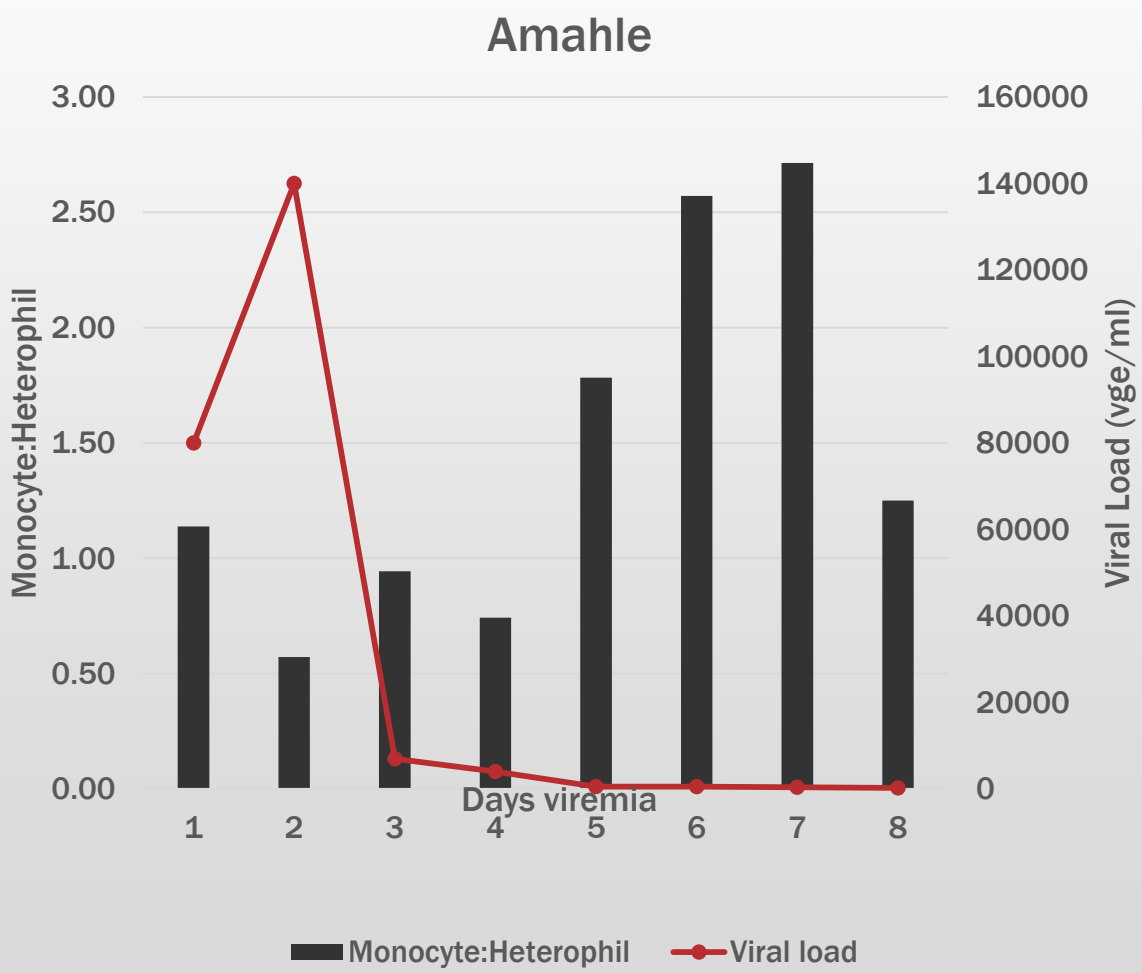


Medical Management

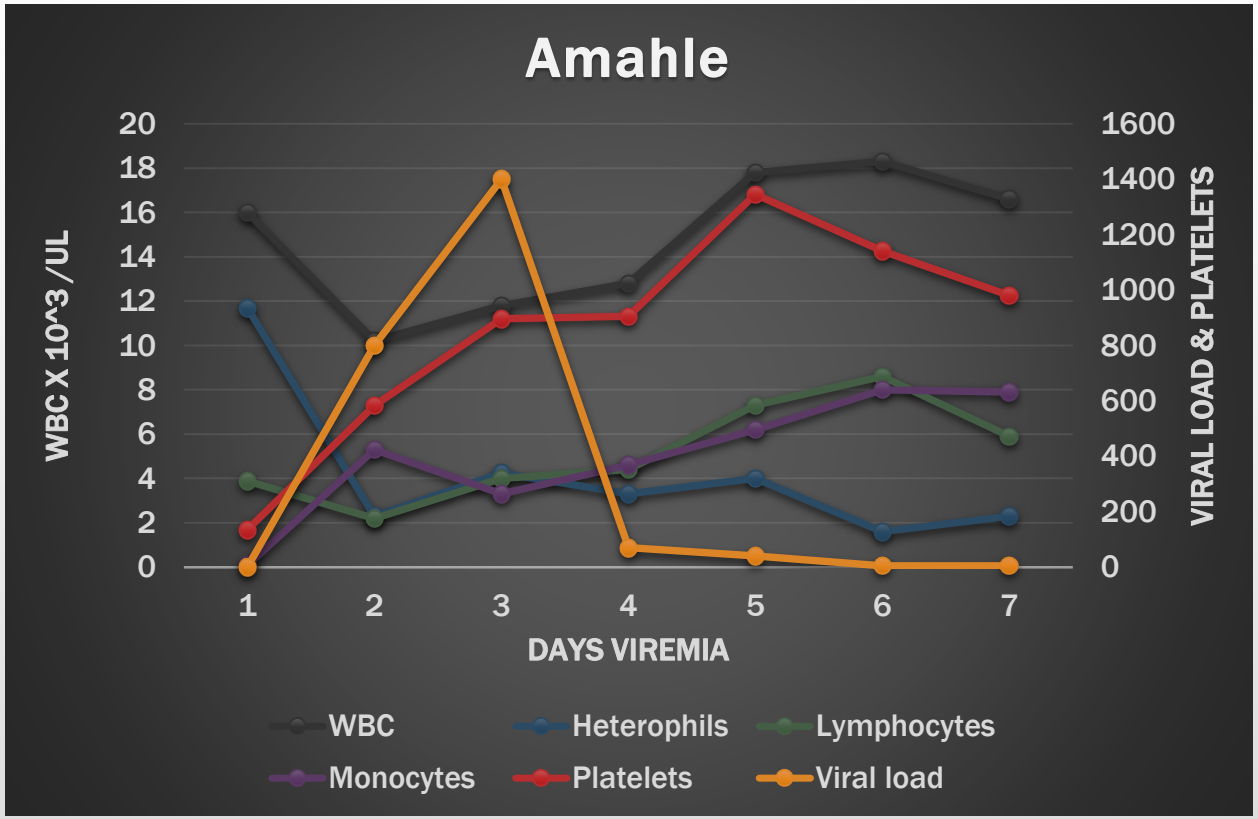
- 5 days after initiation of supportive care Amahle declined to participate in restraint behaviors
- Viral load down to 500 vge/ml
- Platelet count maintaining over 1,000,000 cells/uL
- Elected to discontinue daily sedation for enema administration
- 10 days after initial EEHV 3-4 detected, low levels of EEHV 2: 400 VGE/ml detected
- 22 Days after initial EEHV detection and 10 days of EEHV below 1000vge/ml elected to discontinue famciclovir

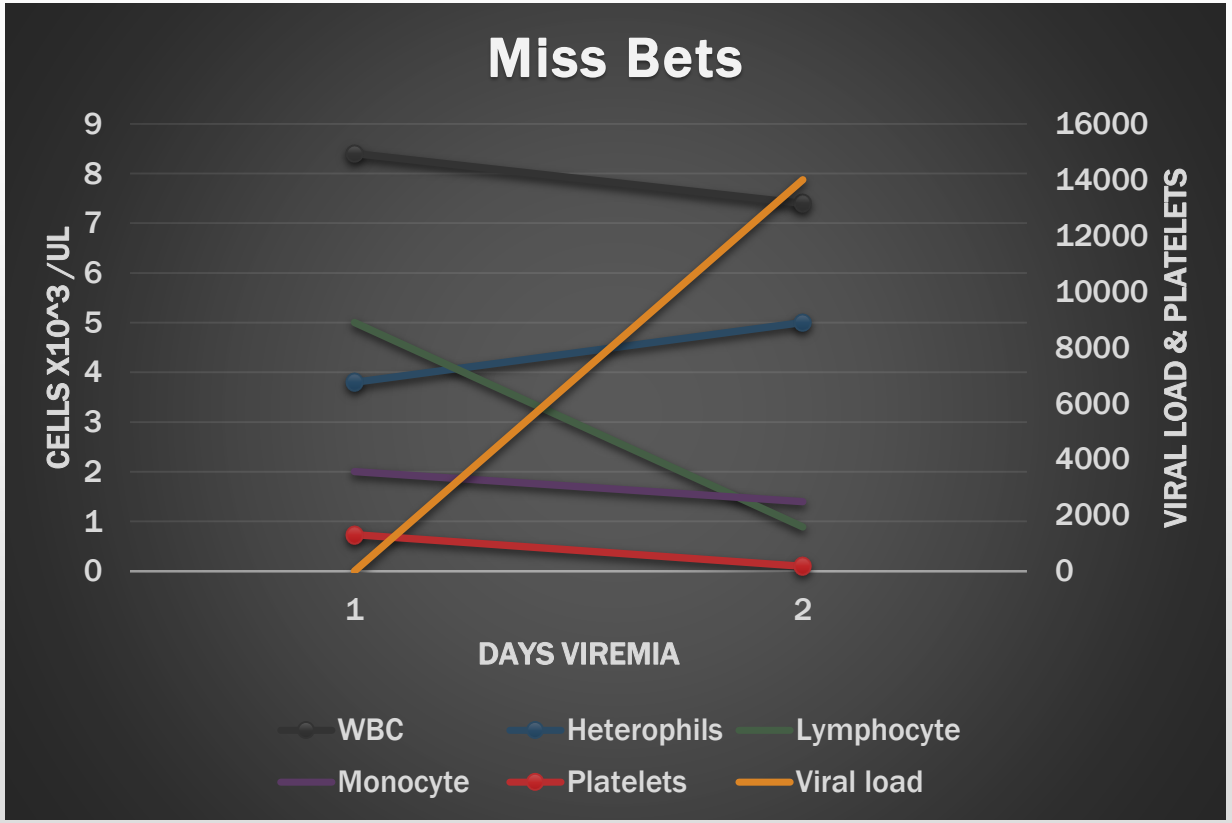


Monocyte: Heterophil ratio



- Early in viremia monocyte:heterophil ratio dropped <1, during recovery ratio increases to >2.
- Average juvenile elephant ratio: 2.37 based on Wissink-Argilaga reference to Molenaar unpublished data.
- Amhale healthy avg 1.6

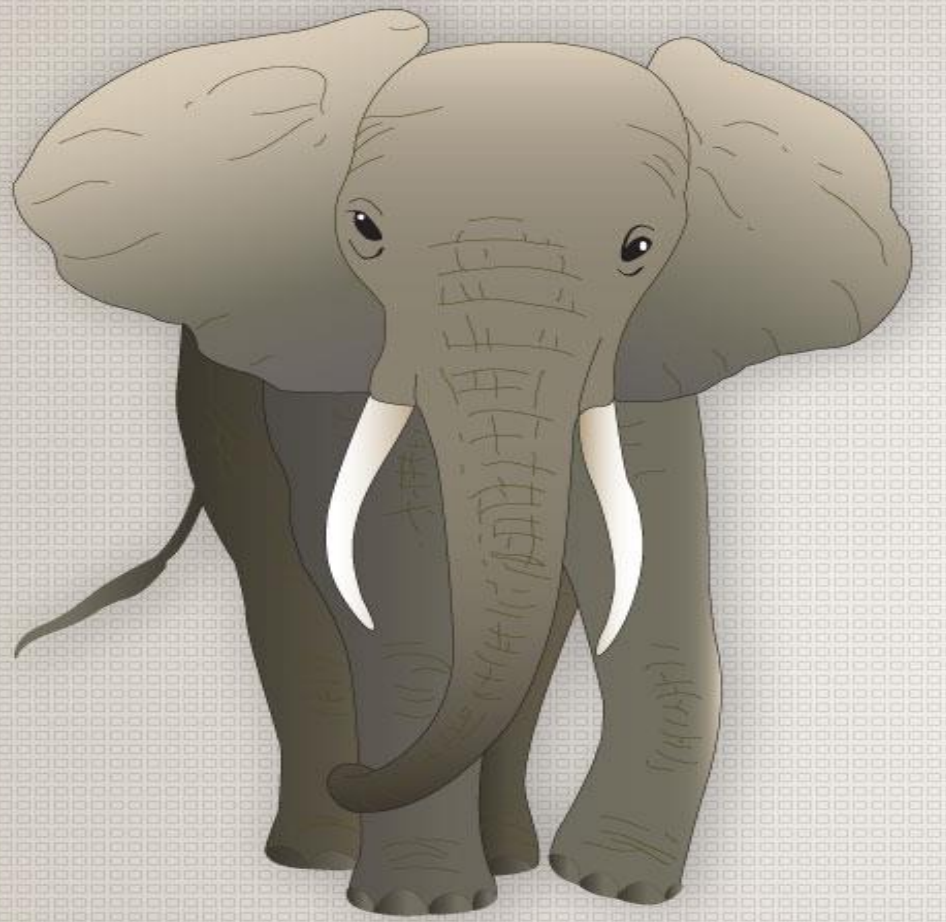

Amahle
WBC X 10^3 /UL
VIRAL LOAD & PLATELETS
DAYS VIREMIA
WBC
Heterophils
Lymphocytes
Monocytes
Platelets
Viral load


Miss Bets
CELLS X10^3 /UL
VIRAL LOAD & PLATELETS
DAYS VIREMIA
WBC
Heterophils
Lymphocyte
Monocyte
Platelets
Viral load

What does this mean for elephants at Fresno Chaffee Zoo?

- Officially joined the EEHV Consortium at the National Elephant Herpesvirus Laboratory- Gold Members
- Knowledge that our herd intermittently sheds EEHV2, 3-4, and 6.
- Updated our EEHV protocols (and by update I mean we pilfered most of yours to create ours thanks all!!).
  - Blood testing:
    - Calves 0-7yr & Juveniles 8-15 yrs
      - EEHV 2, 3, 4, and 6 PCR weekly
      - CBC weekly
      - Serum biochemistries monthly

      Adults 16yrs and older
      - EEHV 2, 3, 4, and 6 PCR as indicated
      - CBC monthly
      - Serum biochemistries monthly
- In the event of an active EEHV case; non clinical herd mates:
  - Calves 0-7yr & Juveniles 8-15yrs
    - EEHV 2, 3, 4, and 6 PCR twice weekly
    - CBC twice weekly
    - Serum biochemistries weekly
  - Adults screened for potential donors and active EEHV shedding
  - Behavioral training



EEHV!
I thought that was an Asian elephant thing?

Amahle

Keys to success

Early Detection

Training

Elephant Community

Training

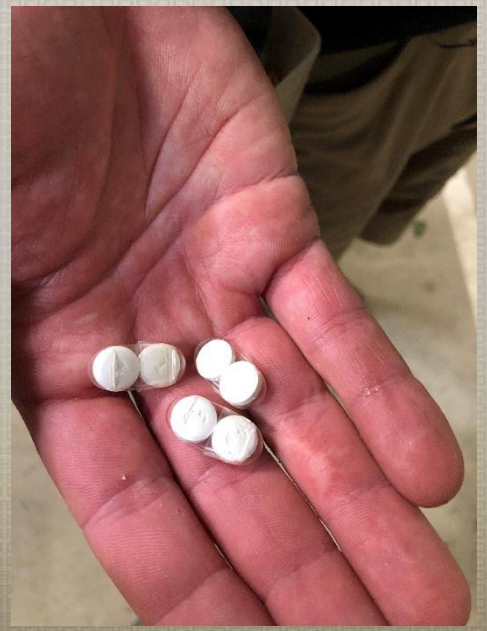
3 main behaviors that allowed us to be successful

- Oral medication
- Blood draws
- Restraints

Oral Medication

- Awful tasting stuff, could not hide it
- Trained to swallow pills

Oral Medication



Oral Medication

Oral Medication

Oral Medication

Oral Medication

Blood Draws

- Critical for the vets to gather information
  - Viral counts
  - Normal blood chemistry

Restraints

- Amahle had not completed her ERD training
- Went to “restraint school” real fast
- Used what she knew
- Practiced over and over

Restraints

Restraints

Take Home Messages

TRAIN YOUR ELEPHANTS!!!!!!

Monitor blood

Look for subtle signs

Use your elephant community

Thank You Elephant Community!!

- FCZ elephant keepers
- FCZ hospital team
- Dallas Zoo
- San Diego Zoo
- Oakland Zoo
- Houston Zoo
- Oklahoma City Zoo
- CAHFS-Tulare
- Erin Latimer
- Daryl Hoffman
- Jill Sampson
- Dr. Ellen Wiedner
- Dr. Lauren Howard
- Dr. Paul Ling
- VetStem Biopharma
- Dr. Valerie Johnson
- Dr. Jaime Landolfi

The End

- Questions?

Pathology of Elephant Endotheliotropic Herpesvirus (EEHV) infections in African elephants

Jaime Landolfi, DVM, PhD, DACVP
University of Illinois Zoological Pathology Program, c/o Chicago Zoological Society, 3300 Golf Road, Brookfield, Illinois 60513

Elephant endotheliotropic herpesviruses (EEHV) cause EEHV hemorrhagic disease (EEHV-HD), a significant cause of mortality in young elephants. Disease is reported in both wild and zoo animals, and in susceptible individuals, infection manifests as an acute, multisystemic, often fatal hemorrhagic syndrome (Long et al, 2016; Ossent et al, 1990; Richman et al, 1999, Zachariah et al, 2013). EEHVs are ubiquitous in healthy adult elephants; Asian elephants harbor EEHV1A, 1B, 4, and 5, and African elephants have EEHV2, 3, 6, and 7. EEHVs are endotheliotropic with infection resulting in endothelial cell damage, vascular compromise and resultant hemorrhage, edema, and coagulopathy. The pathogenesis of endothelial cell damage has not yet been elucidated. Direct virus-mediated injury is considered the most likely mechanism; other hypotheses include induction of apoptosis, immune-mediated insult, and secondary damage associated with coagulopathy. Death results from hypovolemic shock due to cardiac and circulatory collapse. Disease is most common in 1-8 year-old Asian elephants due to infection with EEHV1A. In cases of "classic" EEHV-HD in Asian elephants, capillary endothelium, particularly in the heart, is the target of the virus. Gross lesions are manifestations of vascular damage secondary to viral endothelial cell infection and include: lingual cyanosis; widespread edema affecting the trunk, head, neck, limbs and dependent abdomen, intestinal wall, mesentery and mesenteric root, omentum, and lungs; pericardial effusion; cardiac petechial to ecchymotic hemorrhages; and visceral petechial hemorrhage (Long et al, 2016, Richman et al, 2001). Hemorrhage with intralesional endothelial cell nuclear viral inclusions are diagnostic. In African elephants, lesions due to EEHVs are most common in clinically healthy adults and consist of incidental pulmonary and distal reproductive tract mucosal lymphoid nodules and cutaneous papillomas (Jacobsen et al, 1986; Masters et al, 2011; McCully et al 1971; Munson et al 1995; Richman et al, 1999; Richman et al, 2001; Zong et al 2016). Hemorrhagic disease has occurred less commonly, though a sharp uptick in incidence of EEHV-HD in African elephants in the past year is of grave concern. Historic cases with mortalities in African elephants have been attributed to EEHV2 and EEHV6, and lesions were similar to those in Asian elephant cases of EEHV1A. Recent African elephant cases have been due to infection with EEHV3. A few cases of EEHV-HD due to EEHV3 have previously occurred in Asian elephants, and the disease in African elephants appears comparable. Though EEHV3 still targets endothelial cells, larger caliber vessels (venules, arterioles) are involved. With EEHV3, cardiovascular manifestations are noted, however, additional hemorrhagic lesions in the urinary, gastrointestinal and lymphoid systems are more prevalent than with other EEHVs. Lesions in cases of African elephant EEHV-HD appear dependent on the EEHV type; no host species-specific variations have been noted.

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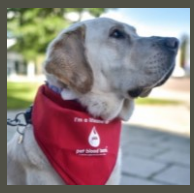
TRANSFUSION MEDICINE AND EEHV-HD: DEVELOPING SUSTAINABLE PROGRAMS

Jennifer Kishbaugh
BodeVet, Inc.
African Elephant EEHV Workshop
Indianapolis, IN

1

SMALL ANIMAL TRANSFUSION MEDICINE DEVELOPMENTS

- Component therapy administration and availability
  - Rapid growth of private blood banks
  - Lyophilized canine albumin
  - Lyophilized canine platelets and plasma
- Advances in technology
  - Therapeutic apheresis
  - CAR-T cancer research
- Influx of research and discussion extrapolating from human medicine

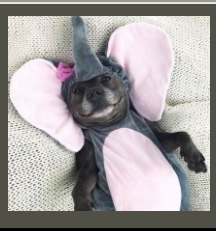


2

RESOURCES FOR THE SMALL ANIMAL PRACTITIONER

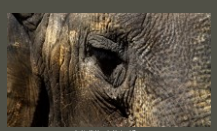
3

BUT WHAT ABOUT THE ELEPHANTS??



4

EEHV AND TRANSFUSIONS


Jackie Kishbaugh, National Zoo

- Coagulopathic, hemorrhagic, hemostatically unstable animals
  - Thrombocytopenia, edema, vasculitis
  - Internal hemorrhages with severe lesions often reported at necropsy
- Indications for fresh plasma, platelets, or whole blood
  - Only a few published reports of plasma transfusions given with a wide range of dosages

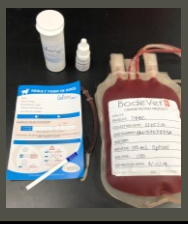
5

WHAT WE NEED TO KNOW

- OPTIMIZE COLLECTION AND STORAGE
  - Best collection methods from adult donor elephants
  - How to choose your donors
  - Accurate lifespan of stored blood products
  - Zoo preparedness for necessary equipment
- GOALS
  - Patient population
  - Component therapy?
  - Focus on banking or including emergency collections

6

DONOR SELECTION AND COLLECTION



- Regular training for animals and staff is VITAL
  - Technical comfort for staff
  - Removes stress from the animal
  - Optimizes collection method
  - Allows for banking of blood products
- Know the collection systems available
- Try various sites and methods for collecting from each animal

7

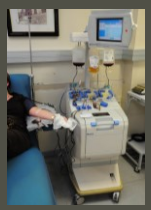
BLOOD COLLECTION SYSTEMS

- **Standard 450mL with nutrient solution**
  - PROS: pre-filled with anti-coagulant, satellite bag attachments for component processing, centrifuge to separate plasma easily
  - CONS: Limits size of volume collected with each draw
- **1 to 5 L bag without nutrient solution**
  - PROS: Able to collect larger blood volumes in a single collection event
  - CONS: Have to add nutrient solution, may be more technically demanding to utilize, can only separate via refrigerated gravity system
- **Apheresis**
  - PROS: Collect and process large volumes storage, minimal long-term animal impact
  - CONS: Require advanced training and facility willingness to explore novel collection technique

8

WHY PURSUE APHERESIS?

- Large volume collection and patient-side separation of components
- Provides a large volume of healthy platelets and plasma
- With funding available, can generate elephant lyophilized blood products for use on demand
- Requires facility interest, willingness to explore novel collection methods, animal intravenous catheterization for long-term (45 minute or longer) collection



9

BLOOD PROCESSING EQUIPMENT

- **Crossmatch** supplies
- **Blood collection bags** and well-trained donor animals
- **Refrigerated centrifuge** to provide thermal control during friction-related heat generation
- **Press** to separate plasma from red cells
- **Refrigerator/freezer** to store products

10

ONGOING RESEARCH ENDEAVORS AT BODEVET

- **Asian elephant blood typing** – completing final analysis and preparing for publication
- **African elephant blood typing** – sourcing resources and interested participants
- **Elephant lyophilized platelet product manufacture** – sourcing resources and interested donor facilities
- **Elephant lyophilized plasma** – exploring the potential based on ongoing canine research
- **Facility transfusion survey** – pending co-researcher review and distribution to interested facilities

11

BODEVET PROJECT UPDATES: ASIAN ELEPHANT CROSSMATCHING

- OVER 60 SAMPLES SUBMITTED!
- Manuscript in preparation for publication
- Interesting finds:
  - Previous plasma donations = no antibody generation
  - High degree of rouleaux in stored samples = many +1 agglutination reactions
- For future studies:
  - Interested in evaluating animals receiving other blood products
  - African elephant project in development

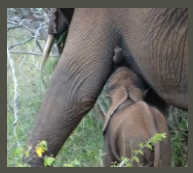
12

WE NEED YOUR INPUT!

- Zoo veterinarian participation is VITAL to helping gather information for the community
  - Suggesting additional areas that need research attention
  - Blood sample submissions
  - Whole blood donations
  - Medical record data and case reviews
  - Answering questions
  - Funding source recommendations
  - Grant collaborations

13

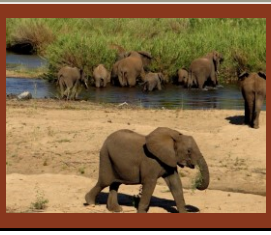
COMING SOON, TO A ZOO NEAR YOU:



- Updated transfusion guidance
  - Survey of transfusion practices in elephant care facilities
  - Cross-matching and compatibility study
- Updated EEHV treatment projects
  - Exploring objective scoring systems
  - Providing freeze-dried blood products to the elephant community
  - Evaluating antiviral activity of platelet therapy and antiviral-impregnated lyophilized blood products

14

THANK YOU!



15

FOR MORE INFORMATION:

- http://www.avhtm.org/ - Transfusion and hematology resources
  - ($35 membership! Totally worth it!)

AVHTM
ASSOCIATION OF VETERINARY HEMATOLOGY AND TRANSFUSION MEDICINE

- jkishbaugh@bodevet.com – Research projects for elephant transfusions, blood collection and banking guidance for zoological species
- ahale@bodevet.com – Facilities interested in discussing apheresis, ideas for funding support, general transfusion inquiries

BodeVet®

16

Managing a Crisis
Public Relations

1

Managing a Crisis

Today's Presentation:

- What constitutes a crisis
- PR first steps
- Your role in a crisis
- Review of PR during EEHV crisis

2

What is a Crisis

Anything that can damage a zoo's image, credibility or reputation.

- Death or injury
- Mismanagement
- Natural disaster
- Animal escape
- Protests
- Environmental
- Disgruntled employee
- Active shooter/violence
- Technology failure
- Issue at another zoo/aquarium can damage your reputation

3

What do we have the most control over?

The message!

We are:

- Assuming that all major facts will become public knowledge, but avoiding sending media in a direction they wouldn't have taken
- Understanding that people will believe what they hear
- Responding to misinformation
- Responding to media in a timely and honest manner
- Assuring public safety
- Not speculating
- Monitoring all social channels
- Owning ***our*** own ***story***

4

What are the first steps during a crisis?

Accurate information is the key

- PR is the hub for information gathering and dissemination
  - Gathering facts (who, what, when, where, why and how) – 3 main points
  - Preparing statements, releases, social media copy and responses
  - Assuring the public in a timely manner especially when safety is a concern
  - Communicating to stakeholders including board members, staff, donors, members, volunteers and public
  - Answering media questions and follow up
  - Identifying the best Zoo representative to talk (people who have been trained)
  - Deciding the approach for information release (written statement, interview, press conference)
  - Conducting follow up responses in the following hours, days and weeks
  - Being truthful and calm throughout the crisis
  - If we don't tell our story, it forces media to seek information from other sources

5

What is your role in support of PR during a crisis?

Accurate information is the key

- When you safely can, do:
  - Report what you know to your assigned leader according to your crisis plan
  - Never speculate
  - If you can, write down the times and actions that took place
  - Stay calm when talking with guests, volunteers and other staff members during a crisis
  - Inform PR if you are hearing inaccurate details either from guests, media or social media, etc

6

What is your role in support of PR after a crisis?

Consistency in messaging is key

- PR should provide talking points
- You may be asked to help with facts for those talking points
- In the days and weeks following a crisis, you are the one with direct access to the public so make sure to let PR know if you are getting unusual questions.
- Continue to not speculate
- If you can't answer a question, that's ok – refer to PR
- Don't get bored with the talking points – keep to the message
- Stay calm if the public questioning borders on humorous – this can be emotionally tough if they are joking about something happening where an animal was involved

7

What is your role in support of PR after a crisis?

- Ways to deflect/extricate yourself when you should not be commenting:
  - Watch out for an ambush – a reporter may easily come on grounds without identifying themselves and seem like a guest.
    - Watch what you say – never speculate
    - After the crisis is over, you should have approved talking points provided by PR
  - It's ok to politely excuse yourself if the conversation is becoming uncomfortable or inappropriate.
  - If you are approached by a reporter, let them know you would be happy to get them in touch with PR.
  - Be aware of off-hours questioning – In informal settings, you might be discussing a situation and you can later be quoted as an "inside source"
  - Watch what you post on social media

8

The time to plan for a crisis is before it occurs!

Your team should have a plan, have a process, and practice

- Decide on what crisis response model to use – Incident Command, IAAPA
- Phone numbers programmed ahead of time, list updated regularly, laminated card
- First responsibility is to safety – of guests, animals, volunteers and staff
- Identified all possible crisis situations
- Understand that mobile phones turn anyone into a citizen journalist – you should always assume you are being recorded or possibly live on social media
- Always be honest in your communications to the media. Sometimes there are proprietary details that we can't discuss.

9

After the Crisis

Understanding and learning from what went right – what went wrong

- Once the immediate crisis has passed, a post incident review with all involved can be helpful
- Remember, you are all part of one big team – support each other and remain as positive as possible

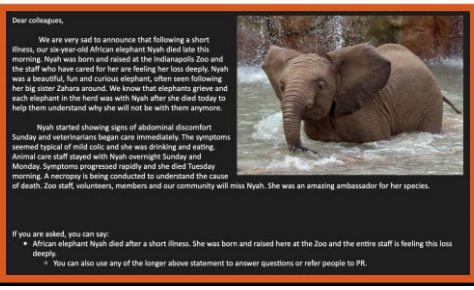
10

Elephant Nyah's Illness

- March 17 – Nyah started showing signs of discomfort and veterinarians started immediate care.
  - Symptoms seemed like mild colic, she was drinking and eating – the thought was colic or salmonella – antibiotics being used
  - Veterinarians and animal care team stayed with Nyah overnight Sunday and Monday.
    - (note our knowledge base at the time: EEHV not known to strike African Elephants)
- March 18 – PR was communicated to about the issue
  - Elephant history was gathered into one place
  - Talking points formulated in preparation of a possible death – outside PR firm & AZA notified
  - Photos were organized
- March 19 – Nyah died late morning.
  - By 2pm a note to the Board of Trustees was approved (reviewed by Collections & Vet)
  - Communication tree went into place: Staff, donors, volunteers, members, media
  - Decided to stay with same statement to put on Facebook.
  - All local media responded using the statement and photo. PR did interviews
  - National and International media and social media took continued PR time

11

Elephant Nyah's Illness


Dear colleagues,

We are very sad to announce that following a short illness, our six-year-old African elephant Nyah died late this morning. Nyah was born and raised at the Indianapolis Zoo and the staff who have cared for her are feeling her loss deeply. Nyah was a beautiful, fun and curious elephant, often seen following her big sister Zahara around. We know that elephants grieve and each elephant in the herd was with Nyah after she died today to help them understand why she will not be with them anymore.

Nyah started showing signs of abdominal discomfort Sunday and veterinarians began care immediately. The symptoms seemed typical of mild colic and she was drinking and eating. Animal care staff stayed with Nyah overnight Sunday and Monday. Symptoms progressed rapidly and she died Tuesday morning. A necropsy is being conducted to understand the cause of death. Zoo staff, volunteers, members and our community will miss Nyah. She was an amazing ambassador for her species.

If you are asked, you can say:

- African elephant Nyah died after a short illness. She was born and raised here at the Zoo and the entire staff is feeling this loss deeply.
  - You can also use any of the longer above statement to answer questions or refer people to PR.

12

Elephant Kalina's Illness

- March 23 – Kalina started to show symptoms similar to Nyah's.
  - PR was notified, preparations began on talking points, photos, etc
  - EEHV suspected as Nyah tested with high levels of EEHV
  - PR researching EEHV, working with Houston Zoo, Chester and Smithsonian
  - PR had to start working on messaging for the worst-case
  - Outside PR/crisis firm notified again (and AZA) and talks began on communication plan
    - Transparency, explain EEHV, quick action by staff, African elephant lack of EEHV, public safe, devastating to staff, no vaccine, explain daily testing of other elephants and changing protocols, no understanding of why it becomes active, affects elephants in the wild and in human care
- March 26 – Kalina died
  - Communication:
    - Board, staff, donors, volunteers, members, media
    - Press Conference
      - Dr. Rob Shumaker – written statement and then took questions
      - Local, national and international media and social media coverage for days

13

Elephant Kedar tests positive for EEHV

- March 27 – EEHV Learning session in Houston
  - Zoo sent veterinarian to Houston for the EEHV session. Houston Zoo PR was prepared to help our staff if approached by media.
- May 6 – Kedar tests positive for EEHV and aggressive treatment begins
- May 9 – Communication plan set into motion – no interviews for media
  - Transparency and including the community was essential
  - Timeline:
    1:30pm Board (Dr. Rob send)
    1:35 Staff (Judy send)
    1:40 Volunteers, Donors (Kristin send)
    1:45 Media (Judy send)
    1:50 Social Media (Carla send)

14

Elephant Kedar tests positive for EEHV

Note to News Media:

We felt it was important to keep the community informed and updated on any new development with our elephant herd in connection with Elephant Endotheliotropic Herpesvirus (EEHV). Blood test results reveal a third elephant, Kedar, has EEHV. Zoo veterinarians already had antiviral medication on hand and started aggressive treatment immediately. Kedar is a 13-year old male and had until this week, tested negative for the virus. Following the EEHV deaths of elephants Nyah and Kalina in March, we started testing all of our elephants twice a week for the virus. We conducted a blood test on Kedar on Thursday, May 2, and the results came back negative on Friday, May 3. We tested Kedar again on Monday, May 6, and the results came back positive for EEHV.

Please click on this link for our free file share where you can find photos, video, press release, and that question & answer sheet. We will not be doing any media interviews at this point. I will keep you up to date if I receive any new information on Kedar's health.

15

Elephant Zahara tests positive for EEHV

The New Normal

- May 18 – Blood test taken on Zahara
- May 20 – Zahara blood platelets drop and staff starts immediate antivirals
- May 21– Zahara's blood test came back positive for EEHV. She has a quick recovery
- We never discussed her case with media as it became our new normal.

16

Questions about the PR Process

Questions?

17

DEVELOPMENT OF EEHV-SPECIFIC SEROLOGY ASSAYS IN ASIAN AND AFRICAN ELEPHANTS

PURSELL, T[1], FUERY, A[1], HAYWARD, GS[2], HEAGGANS SY[2], MENON, VK[3], QIN, X[3,4], WORLEY, KC[3,4], LING, PAUL D[1]

[1]Baylor College of Medicine, Department of Molecular Virology and Microbiology, Houston, Texas 77030; pling@bcm.edu
[2]Johns Hopkins University School of Medicine, Viral Oncology, Baltimore, Maryland, 21287
[3]Baylor College of Medicine, The Human Genome Sequencing Center, Houston, Texas 77330
[4]Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, Texas 77030

Elephant endotheliotropic herpesviruses (EEHVs) can cause hemorrhagic disease (HD), particularly in captive juvenile Asian elephants between the ages of 2-8. Recent cases of EEHV HD in African elephants have raised the concern for EEHV in this species too. Understanding antibody responses towards EEHV in elephants, especially to the different types of EEHVs, could provide information about the prior history of infections with these viruses in individual animals. Moreover, robust anti-EEHV serology assays will be needed to evaluate potential EEHV vaccines, which our laboratory is working to develop. Evidence will be presented that we have developed a first generation EEHV serology assay that can distinguish whether an elephant has already been infected with EEHV1A or 1B versus 4 and/or 5. In addition, we have recently determined the complete genomic sequence EEHV3A, which was associated with some recent African elephant EEHV HD cases. We have used this knowledge (along with the EEHV4 genome sequence) to begin generating serology assays for the GC-rich branch EEHVs; EEHV3A in African elephants and EEHV4 in Asian elephants. The implications for how the serology assay might provide useful knowledge for herd management and vaccine development and evaluation will be reviewed.

EEHV IN AFRICAN ELEPHANTS (*Loxodonta africana*)

Erin Latimer

Wildlife Health Sciences, Smithsonian's National Zoological Park, Washington DC 20008, USA

Please direct correspondence to Erin Latimer (latimere@si.edu)

Abstract

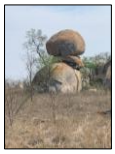
Elephant Endotheliotropic Herpesvirus (EEHV) is most known for causing an acute hemorrhagic disease (EEHV HD) in mostly young Asian elephants (*Elephas maxim*us), but it has also been implicated in morbidity and mortality in older Asian elephants and in African elephants. There has been increased interest in EEHV in the African elephant community because of recent deaths and disease due to EEHV in African elephants in the US. This report will review what is known about EEHV in African elephants, including saliva, lung, and skin nodules in otherwise healthy animals in human care and in the wild, the historical deaths and illness due to EEHV2, 3, and 6, and will briefly touch on the 2019 EEHV cases. Over the past year, EEHV shedding in trunk secretions and detection of low levels of EEHV2, 3-4, and 6 in blood and necropsy samples have been common; comparisons to EEHV in Asian elephants will be made.

Routine monitoring of young elephants, both Asian and African, is recommended by the EEHV Advisory Group. Weekly/twice weekly polymerase chain reaction (PCR) of blood can provide early detection of viremia; testing of herd trunk secretions might provide information on viral types circulating in the herd.

Recent capacity-building and opportunities for research projects in African range countries will be discussed.

Update on African EEHV Working Group and our work at San Diego Zoo Global

Lauren L. Howard, DVM, Dipl. ACZM
Director of Veterinary Services, San Diego Zoo Safari Park
San Diego Zoo Global
Steering Committee, North American EEHV Advisory Group

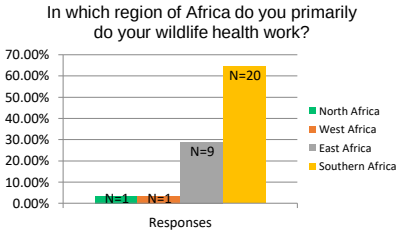


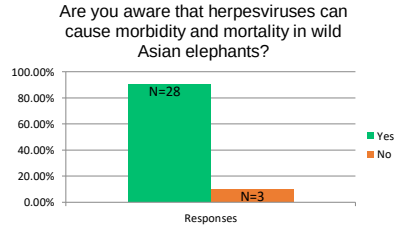
~120 Participants
Africa
Asia
US
UK
Europe

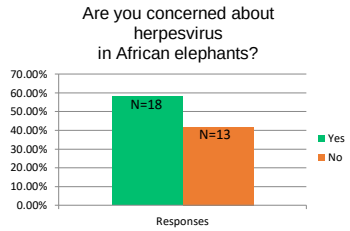
EEHV Survey Results of Wildlife Health Professionals Working in Africa

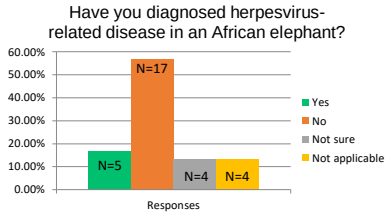
Responders:
WDA Africa and Middle East Section and Wildlife Vetnet Listservs

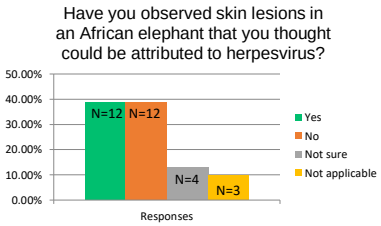
N=31 (Aug-Sep 2019)

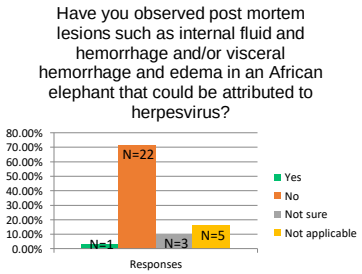


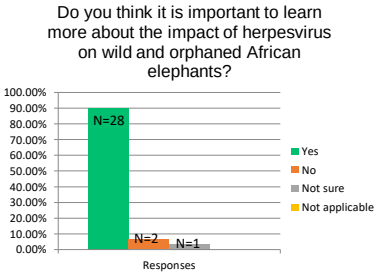



Are you concerned about herpesvirus in African elephants?
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
N=18
N=13
Yes
No
Responses


Have you diagnosed herpesvirus-related disease in an African elephant?
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
N=5
N=17
N=4
N=4
Yes
No
Not sure
Not applicable
Responses


Have you observed skin lesions in an African elephant that you thought could be attributed to herpesvirus?
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
N=12
N=12
N=4
N=3
Yes
No
Not sure
Not applicable
Responses


Have you observed post mortem lesions such as internal fluid and hemorrhage and/or visceral hemorrhage and edema in an African elephant that could be attributed to herpesvirus?
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
N=1
N=22
N=3
N=5
Yes
No
Not sure
Not applicable
Responses


Do you think it is important to learn more about the impact of herpesvirus on wild and orphaned African elephants?
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
N=28
N=2
N=1
Yes
No
Not sure
Not applicable
Responses


First EEHV in Africa Workshop
25 participants from 10 countries

Six African Countries Represented

- Kenya
- South Africa
- Zimbabwe
- Zambia
- Namibia
- Uganda
- *Botswana travel fell through at last minute*

Meeting Highlights

- Formed African EEHV Working Group
  - Communication group established on Whats App
  - Working Group led by Dr. Edgar Simulunda (Zambia)
  - Managed by Rachel Harris (Namibia)
  - African page on www.eehvinfo.org to be provided
- Biggest Need:
  - Information on what samples to collect, how to store them
- Also requested Gross Necropsy Photos of EEHV deaths
- PCR Testing Needs
  - Now: Zambia Vet School
  - Soon: Kruger, Zimbabwe
  - Needed: Kenya, Uganda, Namibia
  - Unknown: Botswana, Tanzania

Meeting Highlights

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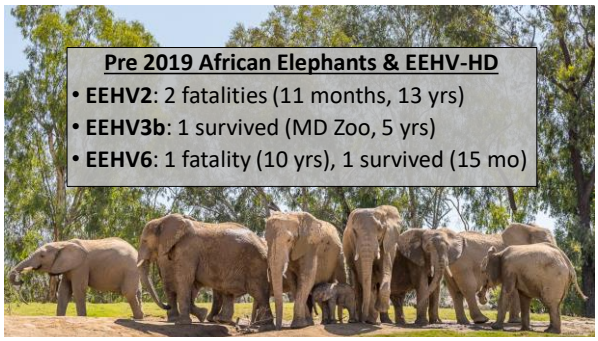
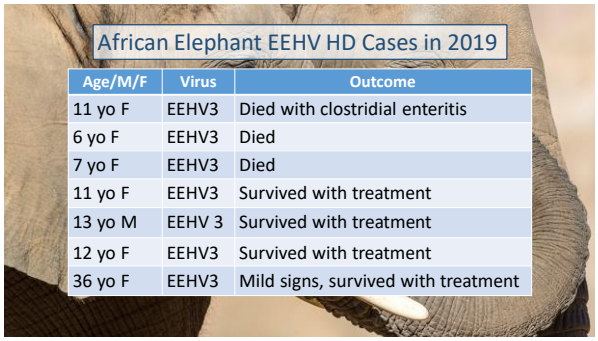
North American Elephants and EEHV 2019

	Asian Elephants	African Elephants
Elephants born or imported since 1980, with known follow-up	129	258
Elephants still alive	87	158
Elephants that have died	42	100
EEHV HD deaths	27	5
% of elephant deaths that are from EEHV	27/42 = 64%	5/100 = 5%

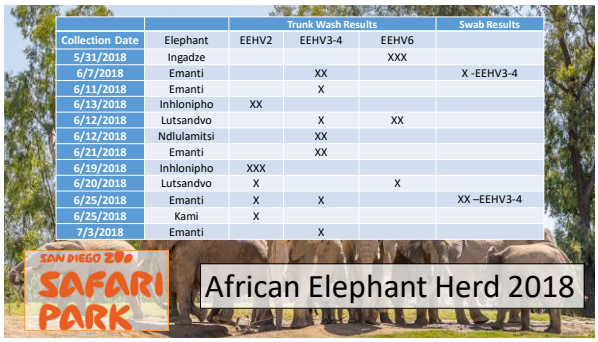
North American Elephants and EEHV 2019

	Asian Elephants	African Elephants
Elephants born or imported since 1980, with known follow-up	129	258
EEHV HD Survivors	15	5
EEHV HD Deaths	27	5
Fatality Rate of EEHV HD Cases	64%	50%
% of all elephants in population that have been impacted by EEHV	42/129 = 32%	10/258 = 4%

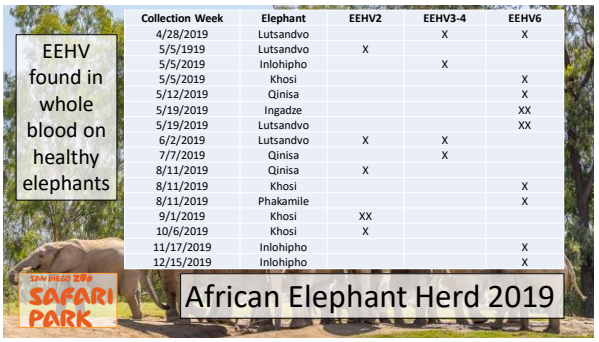




Age/M/F	Virus	Outcome
11 yo F	EEHV3	Died with clostridial enteritis
6 yo F	EEHV3	Died
7 yo F	EEHV3	Died
11 yo F	EEHV3	Survived with treatment
13 yo M	EEHV 3	Survived with treatment
12 yo F	EEHV3	Survived with treatment
36 yo F	EEHV3	Mild signs, survived with treatment



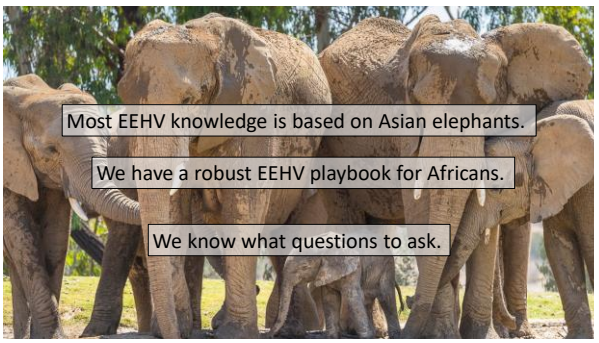
		Trunk Wash Results			Swab Results
Collection Date	Elephant	EEHV2	EEHV3-4	EEHV6	
5/31/2018	Ingadze			XXX	
6/7/2018	Emanti		XX		X -EEHV3-4
6/11/2018	Emanti		X		
6/13/2018	Inhlonipho	XX			
6/12/2018	Lutsandvo		X	XX	
6/12/2018	Ndlulamitsi		XX		
6/21/2018	Emanti		XX		
6/19/2018	Inhlonipho	XXX			
6/20/2018	Lutsandvo	X		X	
6/25/2018	Emanti	X	X		XX –EEHV3-4
6/25/2018	Kami	X			
7/3/2018	Emanti		X		

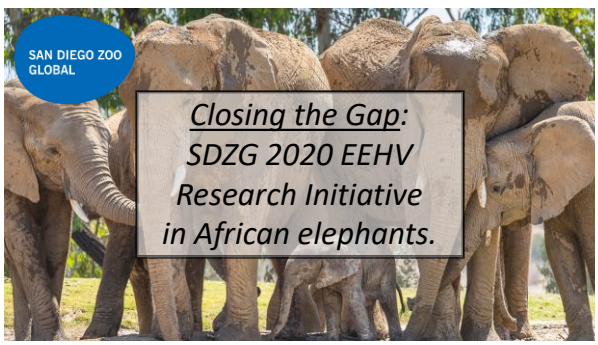


Collection Week	Elephant	EEHV2	EEHV3-4	EEHV6
4/28/2019	Lutsandvo		X	X
5/5/1919	Lutsandvo	X		
5/5/2019	Inlohipho		X	
5/5/2019	Khosi			X
5/12/2019	Qinisa			X
5/19/2019	Ingadze			XX
5/19/2019	Lutsandvo			XX
6/2/2019	Lutsandvo	X	X	
7/7/2019	Qinisa		X	
8/11/2019	Qinisa	X		
8/11/2019	Khosi			X
8/11/2019	Phakamile			X
9/1/2019	Khosi	XX		
10/6/2019	Khosi	X		
11/17/2019	Inlohipho			X
12/15/2019	Inlohipho			X

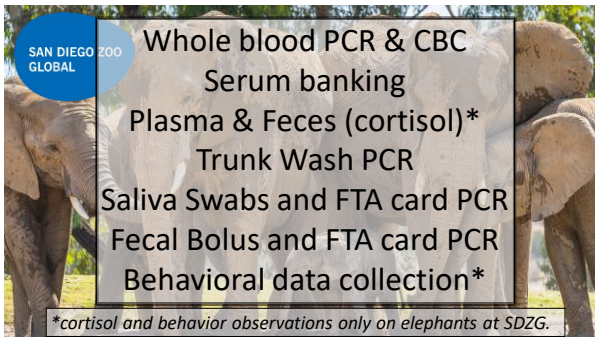

Most EEHV knowledge is based on Asian elephants.


Most EEHV knowledge is based on Asian elephants.
We have a robust EEHV playbook for Africans.


Most EEHV knowledge is based on Asian elephants.
We have a robust EEHV playbook for Africans.
We know what questions to ask.


SAN DIEGO ZOO GLOBAL
Closing the Gap:
SDZG 2020 EEHV Research Initiative in African elephants.


SAN DIEGO ZOO GLOBAL
1 year of weekly sample collection on up to 30 elephants.



When do African elephants shed EEHV?

When does EEHV get into African elephant blood (viremia)?

Is viremia (EEHV in the blood) detrimental to African elephant health?

How do African elephants shed EEHV?

EEHV: Closing the Gap

How can we predict viremia and EEHV shedding in African elephants?

How will we test elephant samples in Africa?

How does EEHV behave in a herd with both Asian and African elephants?

When do African elephants shed EEHV?
When, how frequently and how much is the virus shed?

When does EEHV get into African elephant blood (viremia)?
When, how frequently, and how much of the virus gets in the blood?

Is viremia (EEHV in the blood) detrimental to African elephant health?
What is the significance of viremia? When should we worry?

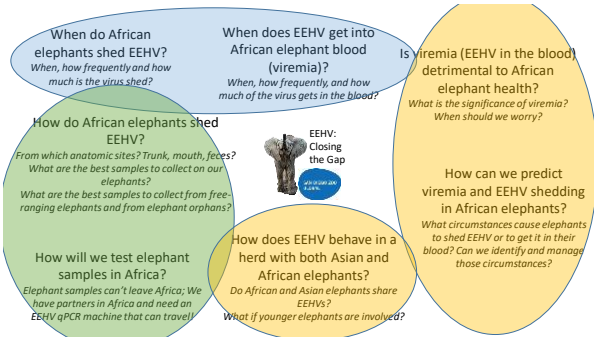
How do African elephants shed EEHV?
From which anatomic sites? Trunk, mouth, feces? What are the best samples to collect on our elephants? What are the best samples to collect from free-ranging elephants and from elephant orphans?

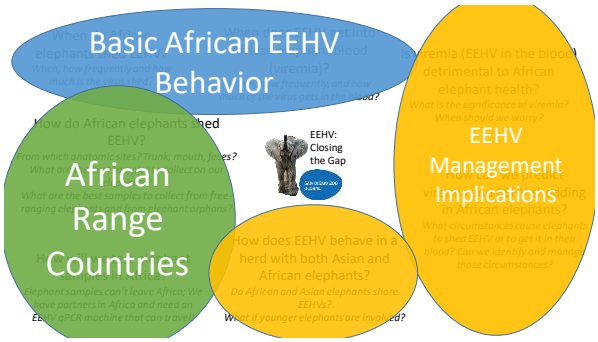
EEHV: Closing the Gap

How can we predict viremia and EEHV shedding in African elephants?
What circumstances cause elephants to shed EEHV or to get it in their blood? Can we identify and manage those circumstances?

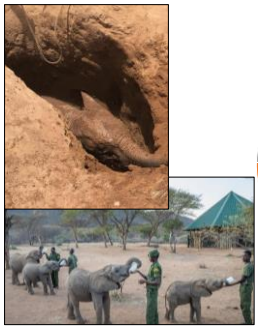
How will we test elephant samples in Africa?
Elephant samples can't leave Africa; We have partners in Africa and need an EEHV qPCR machine that can travel!

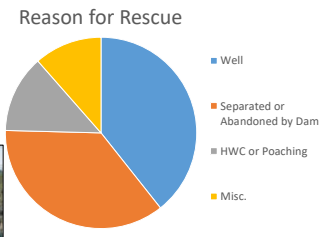
How does EEHV behave in a herd with both Asian and African elephants?
Do African and Asian elephants share EEHVs? What if younger elephants are involved?

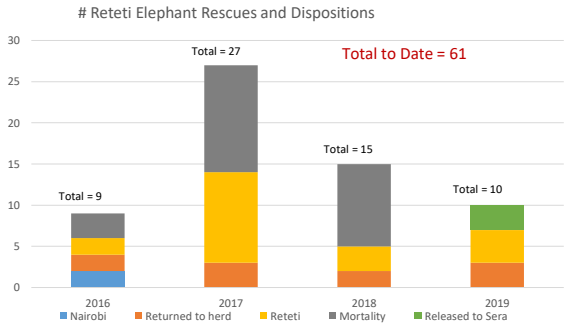




Reteti Elephant Sanctuary / Namunyak Wildlife Conservancy




Reason for Rescue
Well
Separated or Abandoned by Dam
HWC or Poaching
Misc.


Reteti Elephant Rescues and Dispositions
30
25
20
15
10
5
0
Total = 9
Total = 27
Total = 15
Total = 10
Total to Date = 61
2016
2017
2018
2019
Nairobi
Returned to herd
Reteti
Mortality
Released to Sera

Pharmacokinetics of famciclovir and its metabolite penciclovir in African elephants (*Loxodonta africana*)

John A. Griffioen, D.V.M[1]., Melissa A. Fayette, D.V.M.[1], Jeffry S. Proudfoot, D.V.M[1], Mark G. Papich, D.V.M., MS, Dipl. A.C.V.P.[2]

Indianapolis Zoo, Indianapolis, IN 46222 USA; North Carolina State University, Raleigh, NC 27606 USA

The anti-viral drug famciclovir has been used commonly in the treatment of EEHV in both Asian and African elephants. Concentrations of famciclovir and its active metabolite penciclovir have been evaluated in Asian elephants previously. However anatomic and physiologic differences, as well as known differences in metabolism of some drugs between Asian and African elephants, warrants additional research in African elephants. The purpose of this study is to evaluate pharmacokinetic parameters of famciclovir and its metabolite penciclovir in African elephants under managed care. The study aims to include at least 10 African elephants, determined to be clinically healthy via physical exam, complete blood count, serum biochemistry, and EEHV polymerase chain reaction (PCR) screening. Famciclovir will be administered (15 mg/kg) both orally and rectally in a complete crossover study design. Blood will then be collected at preselected sample points and pharmacokinetic parameters evaluated. The pharmacokinetic data obtained may influence dose and frequency of administration of famciclovir in African elephants. This may also further elucidate differences in drug absorption and metabolism between Asian and African elephants. To date, six elephants at the Indianapolis Zoo have each contributed 13 blood samples within a 24 hour period to evaluate the oral administration route with planned collection dates for rectal administration in the near future. Blood collection was possible through operant conditioning with no animals refusing to participate in any of the sampling time points thus far. The authors are seeking additional animals to contribute to the data set and are grateful for institutional collaboration.

High Prevalence of Low-Level Persistent Infections with EEHV3A, 3B, 7A and 7B as well as EEHV2 and EEHV6 Detected in Saliva Swabs from African Elephants in USA Zoos as well as with EEHV3A, EEHV3B, EEHV7A and EEHV7B in both Skin Nodules and Saliva Swabs from Wild Savanna and Forest Elephants in Kenya, Botswana, South African and Gabon.

Virginia R. Pearson, Chimene Nze Nkogue, Stephanie Bourgeois, J-C Zong, Sarah Y. Heaggans and Gary S. Hayward

1

Twenty Known Types of Elephant Herpesviruses

Asian (E. maximus)	African (L. africana)
Deltaherpesvirus-Proboscivirus genus	
EEHV1A, 1B	EEHV6
EEHV5A, 5B	EEHV2
EEHV4A, 4B	EEHV3A, 3B
	EEHV7A, 7B
Elephant Gammaherpesviruses	
EGHV1A	EGHV1B
EGHV2	
EGHV3A	EGHV3B
EGHV4	
EGHV5A	EGHV5B

2

SUMMARY of *Lox africana* EEHV cPCR DNA DETECTION SCREENS: (1A) RESULTS with SALIVA SWABS (SS) COLLECTED from USA ZOO ELEPHANTS

A) At F-1, 49x weekly SS samples over a 13-month time period from each of two healthy female adults (E1J, E2L) gave TER positive for EEHV2 in 7x samples from E1J (early and late episodes), but not in EJ2; TER positive for EEHV6 in 13x E1J (middle and late episodes) and in 8x E2J (middle episode only); TER positive for EEHV3 in 6x E1J (early episode), 6x E2L (early episode) plus 5x E1J (late episode) with a total of five distinct EEHV3 variants involved. POL positive for EEHV2 in 17x samples (two episodes each with the same 2x novel variants). OBP positive for EEHV3 in 18x samples (7x E1J, 11x E2L). Six other adult HM tested over the final 3-month period gave 9x EEHV3 POL positives and 5x EEHV3 OBP positives. Overall, among nine adult LA tested, 6 were positive for EEHV6, 5 for EEHV3B, 4 for EEHV2, 2 for EEHV3A and 1 for EEHV7A.

3

SUMMARY of *Lox africana* EEHV cPCR DNA DETECTION SCREENS: (1B) RESULTS with SALIVA SWABS (SS) COLLECTED from USA ZOO ELEPHANTS

B) At F-2, a surviving 5 yo male (Samson NAP62) with symptomatic EEHV3B viremia was positive for EEHV3 U71/gM PCR locus in 16/18 successive SS collections over 8-month period during recovery phase. One asymptomatic adult HM positive 5x with the same strain. This calf and all three tested adult HM were also positive sporadically for either three or four EGHV gamma herpesviruses, sometimes two or three at the same time (including EGHV1B, 2, 3B and 4 but not 5B). [Bronson et al, 2017]

C) At F-3, SS and TW samples from six asymptomatic adult HM over a 3-month period gave multiple positives for EEHV2 (HEL, TER) and for EEHV6 (TER, total of two strains and three variants) as well as for EEHV3(UDG) in one HM.

4

(2). SUMMARY OF RESULTS OF EEHV PCR DNA DETECTION SCREENS IN BOTH AFRICAN SAVANNA ELEPHANTS (*Loxodonta africana*) and AFRICAN FOREST ELEPHANTS (*Loxodonta cyclotis*):

Almost all tested Saliva Swab and Skin Nodule Biopsy samples collected from wild *Lox. africana* adults and juveniles with skin nodules from Kenya (13x), Botswana (14x) and South Africa (5x) were found to be low level positive by nested cPCR sequencing for multiple EEHV species and a wide variety of subtypes or strains (including EEHV2, EEHV3A, EEHV3B, EEHV6, EEHV7A and EEHV7B). SS from some mother/calf pairs were identical, but more often different. Similarly, half of 25x SS samples from *Lox. cyclotis* in Gabon were positive for at least one EEHV3 or EEHV7 strain, but many of these were also present as unusual rare strains in *Lox. africana.*

We interpret that these assays detect evidence of previous persistent infections rather than necessarily currently active systemic infections or shedding as would be detected at high levels in blood or trunk wash samples.

5

SUMMARY of *Lox africana* EEHV DNA DETECTION SCREENS: (3) RESULTS with SKIN NODULE BIOPSIES from WILD KENYA, BOTSWANA, RSA ELEPHANTS:

Skin nodules were biopsied from five immobilized juveniles from Samburu Park in Kenya and eight other wild Elephants from Botswana or South Africa. These were examined at up to 12 PCR primer sets that detected known selected high GC-branch EEHV3 or EEHV7 loci as well as several more generic PCR primer sets that also detected EEHV2 or EEHV6.

All were positive and mostly with multiple viruses. Overall, these assays identified four examples of EEHV2, six of EEHV3A, six of EEHV3B, seven of EEHV7A and two of EEHV7B. Nine samples also contained EGHV1B, four EGHV2, two EGHV3B, one EGHV4 and two EGHV5B. EEHV6 (as well as EEHV2, EEHV3A, EEHV7A and two EGHVs) were also detected in random necropsy lung tissue from a wild Kenyan adult.

Saliva swab samples from four of the same Kenya juveniles also detected between one and three types of EEHV3 or EEHV7 and an EGHV each, some of which matched the strains found in the skin nodules but mostly did not.

6

SUMMARY of *Lox africana EEHV* cPCR DNA DETECTION SCREENING: (4) RESULTS with SALIVA SWABS from KENYA, BOTSWANA, RSA ELEPHANTS.

Saliva swabs collected from 26 wild adult and juvenile ***Lox. africana*** from Botswana, Kenya and South Africa were tested at up to seven selected GC-rich branch cPCR loci (POL, HEL, U71/gM, OBP, TK, vGCNT1 and UDG) yielding 73 positives with unambiguous DNA sequences plus another 18 that were unresolvable mixtures. Overall 21/26 samples gave at least one positive locus, with 6x having just one, 4x 2, 4x 3, 3x 4, 1x 5, 1x 6 and 1x 7 positive loci. Among the positives there were 36x EEHV3A, 15x EEHV3B, 3x EEHV3C, 2x EEHV3D, 11x EEHV7A and 3x EEHV7B, as well as 3x EEHV2 (EEHV6 not tested).

In terms of number of distinct virus strains in individual samples, five were negative, 8x had just one strain, 8x had two strains, 3x had three strains, 1x had four strains and 1x had five different strains present. These results closely resemble previous published findings in lung nodules and necropsy lung tissue (Zong et al, 2015).

There were also at least 12x EGHV-positive samples found (7x EGHV1B, 1x EGHV2, 5x EGHV3B and 1x EGHV5B), although not all samples were tested.

7

(5). SUMMARY of *Lox. cyclotis* EEHV cPCR DNA DETECTION SCREENING RESULTS with SALIVA SWABS COLLECTED from IMMOBILIZED WILD GABON ELEPHANTS

Saliva swabs were collected from 25x immobilized ***Lox. cyclotis*** forest elephants in Gabon and screened by cPCR DNA sequencing for GC-rich branch EEHVs at six selected loci. Overall, there were 25 positive results from 13/25 DNA samples (all as single strains).

POL = 4x EEHV3B-like and 1x indeterminate EEHV3 subtype.
HEL = 2x EEHV3B-like.
TER = 1x EEHV3A, 3x indeterminate EEHV3 subtype and 1x EEHV7-like.
UDG = 2x EEHV3C, 1x EEHV3D and 1x EEHV7-like.
TK = 1x EEHV3D, 3x EEHV3F and 1x EEHV7A-like.
OBP = 1x EEHV7B.

Most of these strains were also found as rare subtypes in ***Lox africana*** SS or skin nodules.

8

Total Numbers and Strain Designations of DNA-Sequenced cPCR-Amplified EEHV3 and EEHV7 Samples from Skin Nodules and Saliva Swabs Collected from Savanna Elephants in Africa (Kenya, Botswana and South Africa):

Gene Locus	*EEHV3A*	*EEHV3B*	*EEHV3C-F*	*EEHV7A*	*EEHV7B*	*Total*
U48.5(TK)	16x	4x	10x	3x	-	33x
U73(OBP)	10x	13x	6x	2x	1x	32x
U81(UDG-N)	14x	3x	3x	5x	2x	27x
U77(HEL)	10x	9x	-	8x	3x	30x
U71/gM	9x	2x	-	5x	3x	19x
E4(vGCNT1)	14x	3x	-	2x	-	19x

9

10

11

12

Genome Analysis of Four Prototype EEHV3 Strains from African Elephants with Viremia or HD in the USA - 1

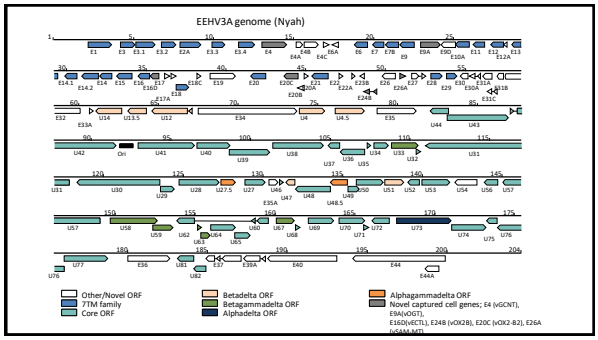
Results of collaborative studies from the Baylor College of Medicine (Paul Ling group), the Houston Genome Center (Xiang Qin group), Johns Hopkins University (Gary Hayward group), Fox Chase Cancer Center, Philadelphia (Virginia Pearson), the Smithsonian National Zoo (Erin Latimer), Indianapolis Zoo and Fresno Zoo.

Comparative data are based on:

(1) The recent compilation and annotation of the complete prototype 205-kb genome of EEHV3A(Nyah, NAP97) encoding 120 genes.

(2) Additional new Sanger PCR data obtained for EEHV3B(MB3, NAP96), EEHV3B(Samson, NAP62) and EEHV3A(Hansa, NAP27) at 18 x conserved and variable gene PCR loci, including POL, TER, HEL, U71/gM, U39(gB), U46(gN), U47(gO), U48.5(TK), U51(vGPCR1), U81(UDG), U82(gL), E4(vGCNT1), E6B, E9B/C, E16D(vECTL1), E20C(vOX2-N), E23B/E24B(vOX2-B) and E26A.

13



14

Major Features of Four Prototype EEHV3 Strains from African Elephants with Viremia or HD in the USA - 2

A) EEHV3A(Nyah) is 204,633-bp in size and has gained two new genes including E20C(vOX2-N) (= equivalent to E54(vOX2-1) in EEHV1), as well as E26A but lost one gene E6B compared to the Asian elephant viruses EEHV4A(NAP22) and EEHV4B(Baylor) its closest relatives within the GC-rich branch of the *Proboscivirus*es.

B) EEHV3 strains fall into two major chimeric subtype clusters similar to those described previously as EEHV1A/B, EEHV4A/B and EEHV5A/B. These differ greatly (by between 15 to 45%) in several linked but non-adjacent chimeric domains (CD-I, CD-II, CD-III and CD-V) totalling 15 to 20-kb in size, but by just 2 to 3% everywhere else. In EEHV3A strains only, parts of the CDs including vECTL, gB, gO, TK, vGPCR1, gL and UDG can instead have several other alternative highly diverged but unlinked subtype clusters (designated 3C, 3D, 3E and 3F).

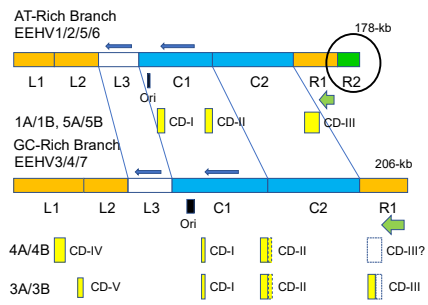
15

Major Features of Four Prototype EEHV3 Strains from African Elephants with Viremia or HD in the USA - 3

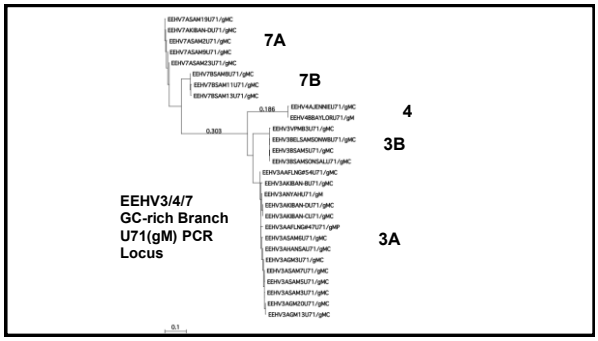
C) EEHV3A(Nyah, NAP97) has typical distinctive features of a prototype EEHV3A strain at 15 out of 16 PCR loci tested, and EEHV3A(Hansa) displays almost exactly the same EEHV3A patterns at 13/16 loci, but is a different novel subtype (3E) across all three loci (gO, TK and vGPCR1) within the CD-II region.

D) EEHV3B(MB3, NAP96) has typical distinctive features of a prototype EEHV3B strain at 15 out of 16 PCR loci tested (total >10,000-bp analyzed), and EEHV3B(Samson) displays almost exactly the same EEHV3B patterns at 14 out of 16 loci tested, with just the two most left-hand side loci (including vGCNT1) having an EEHV3A-like pattern instead.

16



17



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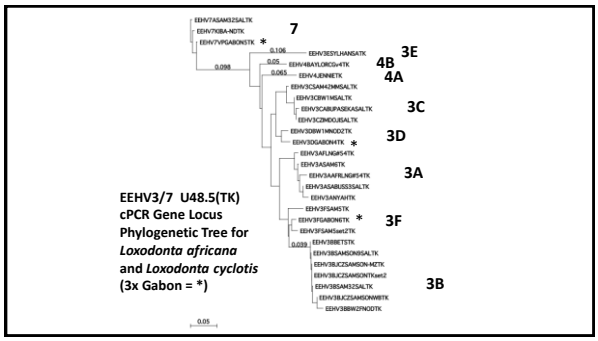

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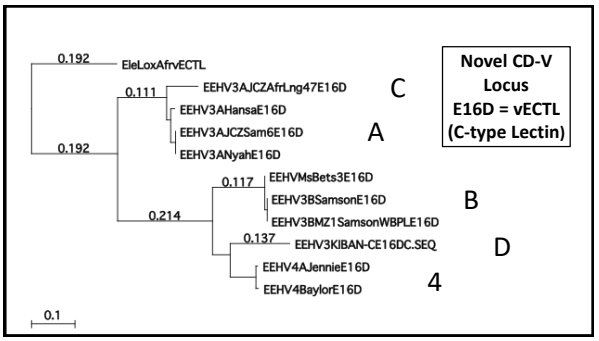

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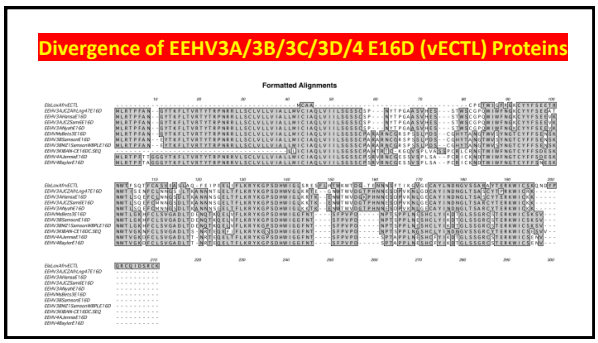

21

Highest Protein Level Subtype Differences Among EEHV3 Strains:

Locus	Gene Name	Amino Acid Differences (%) Subtype Designation				
		MB3 Nyah B vs A	SAM6/7 Nyah C vs A	KIBA-N Nyah D vs A	Hansa Nyah E vs A	AfrLng47 Nyah C vs A
CD-V	E16D/vECTL	50/185(27)		72/147(47)		12/185(6.5)
CD-V	E17	15/88(17)	6/83(7)			
CD-II LHS	U47/gO	30/137(22)	54/247(22)			
CD-II Center	U48./TK	18/248(7.2)			29/248(12)	27/248(11)
CD-II RHS	U51/vGPCR1	5/127(4)			8/127(6.3)	
CD-III LHS	U81/UDG	61/170(36)	71/178(41)			
CD-III LHS	U82/gL	14/63(22)	28/67(42)			

22

23

24