Elephant Endotheliotropic Herpesvirus (EEHV) Protocol

April 2021

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EEHV FAST PLAN

Initiate antiviral treatment if:

- Clinical signs consistent with EEHV HD are present
- WBC and/or monocyte and/or platelet count has dropped significantly below elephant's normal range
- EEHV q-PCR result > 5,000 VGE/ml (this is based on Asian elephant results)
- Elephant has a rapidly increasing EEHV VGE/ml (measured via q-PCR)

This plan is intended to be an instruction sheet to get therapy initiated as quickly as possible. Background information, details, and reasoning for these steps are presented later in the EEHV protocol and on the website www.eehvinfo.org.

1. Collect baseline information: Temperature, Pulse, Respiration, blood pressure.
2. Blood collection **minimum** volume (see page 11 for submission details)
    a. Purple topped tube (EDTA for whole blood 3 ml tubes): 2 tubes initially
    b. Royal blue topped tube: at least 4 to 12 ml of blood
3. Administer rectal fluids. A bolus treatment of 10-20 ml/kg is often used. Attending DVM to determine volume goals.
4. Administer 15 mg/kg Famciclovir orally or rectally (grind with mortar and pestle, mix with water to make into a paste)
5. Standing sedation.
    a. In Asian elephants, Butorphanol 0.06 mg/kg and Detomidine 0.015 mg/kg **IM** given at same time has been used. (can reverse with 2.5 X dose Naltrexone and 5 X dose Atipamezole)
    b. In adult African elephants, Butorphanol 0.02 mg/kg and Detomidine 0.02mg/kg **IM** or Butorphanol 0.015mg/kg and Detomidine 0.01mg/kg **IV** given at same time has been used. (can reverse with 10 X dose Naltrexone and 1.5-2 X dose Atipamezole)
6. Provide supplemental oxygen via nasal cannula when possible
7. Place 16 g IV catheter into ear vein (with injection cap) – consider multiple venous catheters.
8. Administer elephant plasma either fresh separated or frozen, between 0.5 and 1 ml/kg IV, not to exceed 10 ml/kg (fresh plasma is preferable). Be sure to have appropriate filter.
9. Administer IV Normosol between 0.3 ml/kg to 1 ml/kg (at least 4 liters in a 1500 kg calf)
10. Administer rectal fluids after administration of IV fluids
11. Administer Naxcel at 1.1 mg/kg IV, administer flunixin meglumine 0.25 to 0.5 mg/kg IV/IM

Critical Steps in Communication

- **Keepers are the first line of defense against EEHV**. It is essential that keepers report **all** abnormal behavior, even if it is **subtle**. It is better to have fifty false alarms and be overly cautious then have one sick elephant go undetected.
- **Any concerns, however minor, MUST be reported to the Supervisor, Manager and Duty Veterinarian immediately.**

Communication Plan

In the event of a suspect EEHV case, the following communication plan should be implemented immediately:

SAN DIEGO ZOO SAFARI PARK

At the first sign of illness, keepers will contact Lauren Coates, Mindy, and the Duty DVM. Mindy will notify Jacob Shanks and elephant staff who are off that day. Jacob will notify Kristi Burtis and Steve Metzler Once the responding veterinarian has confirmed the suspicion of EEHV, this call down list will commence.

Steve Metzler		Steve will call Greg
Kristi Burtis		Kristi will call Lisa
Greg Peccie		
Duty Veterinarian	See on call sheet	Duty DVM to call Lauren Howard, SP Clinical Lab and Molecular Diagnostic Laboratory (MDL) to alert that ASAP Testing is needed.
Nadine Lamberski		
Lauren Howard	713-417-7979	Lauren will call Nadine, Laura Keener, and other veterinarians that are off that day.
Lisa Peterson		Lisa will notify necessary executive staff
Jacob Shanks		Kristi or Jacob will alert the elephant team working that day and all necessary elephant staff who are off that day for assistance
Mindy Albright		Mindy will alert the elephant team working that day and all necessary elephant staff who are off that day for assistance
Christina Simmons		Lauren or Nadine will contact PR

Lianne Heddich		
Josephine Braun Cailin LaClaire Jennifer Burchell Asa Preston Patty Gaffney		Call down list for emergency EEHV Testing through MDL (Beckman) Please text if call is not answered MDL staff to alert Patty of ASAP EEHV testing

SAN DIEGO ZOO At the first sign of illness, keepers will call Ann and Robbie and then the duty DVM. Once the responding veterinarian has confirmed the suspicion of EEHV, this call down list will commence.		
Robbie Clark		Robbie and Ann will contact Matt and Curby
Ann Alfama		Robbie and Ann will contact Matt and Curby
Matt Akel		Matt or Curby will contact Dwight
Curby Simerson		Curby or Matt will contact Dwight
Director of Wildlife Care		
Dwight Scott		Dwight will contact necessary executive staff
Duty Veterinarian		Duty DVM to call Meg, SDZ Clinical lab and MDL to alert that STAT Testing is needed.
Meg Sutherland-Smith		Meg will call Nadine, Lauren, Laura Keener and other veterinarians.
Nadine Lamberski		
Lauren Howard	713-417-7979	
Deena Brenner		
Christina Simmons Lianne Heddich		Meg or Nadine will contact PR
Josephine Braun Cailin LaClaire Jennifer Burchell		Call down list for emergency EEHV Testing through MDL (Beckman) Please text if call is not answered

Asa Preston Patty Gaffney		MDL staff to alert Patty of ASAP EEHV testing

Up to date information on EEHV background, history, diagnostics, monitoring and treatment can be found at www.eehvinfo.org. Individuals must get their own sign on and password ahead of time to access the professional content (recommendations, protocols, images, etc.)

EEHV and EEHV HD

Our research has shown that most elephants are likely to carry and/or shed EEHV. We consider EEHV ubiquitous in elephant herds. Presence of the EEHV virus in an elephant's secretions or blood does not equal disease. EEHV Hemorrhagic Disease, or EEHV HD, is the presence of the virus in association with changes in the CBC and/or changes in the elephant's clinical findings, and is a serious disease/health concern. Suspicion of EEHV HD is cause for immediate action as outlined in this protocol

Interpreting Routine EEHV qPCR results

Because EEHV is ubiquitous in elephant herds, routine whole blood samples on the Safari Park elephants may occasionally reveal a "low level" EEHV qPCR result, such as a vge/ml result that is below 1,000 vge/ml (in 1 or 2 wells), as reported by NEHL. The attending DVM should follow the steps below when a non-negative EEHV qPCR result is received:

1. Routine NEHL EEHV qPCR results are sent via email to: SDZ and SDZSP veterinarians, clinical laboratory staff, and elephant supervisor, elephant manager, and Director of Wildlife Care at both SDZ and SDZSP.
2. Look over the attached excel sheet from Erin Latimer to determine if this elephant has documented viremia of this specific EEHV virus (2, 3 or 6) previously.
3. Evaluate the elephant's CBC: is the WBC, monocyte % and absolute #, and platelets, consistent with the previous 3 to 5 CBCs? (see Page 9 for additional interpretation)
    a. If the CBC is abnormal in any of those 3 parameters, recheck blood NEXT DAY
4. Double check in with the elephant team to ensure no clinical concerns were observed recently.
5. Make a plan with the elephant lead/supervisor and clinical laboratory team to recheck blood (both CBC and qPCR) within 2 to 5 days, keeping the following in mind:
    a. Shorter recheck interval for first time documented viremias
    b. Next day recheck interval for ANY CBC abnormality
    c. NEHL prefers not to receive samples on Saturdays, and can't receive samples on Sunday. This makes Thursday a good day for recheck blood going into the weekend, and Mondays a good recheck day post weekend
    d. ICR/MDL is available for clinical suspect/emergency cases
6. Attending DVM to write a clinical note in the elephant's record, and put the recheck blood day on the Google/Outlook Calendar, with an assigned DVM.
7. NOTE: if EEHV qPCR climbs quickly, or rises above 1000 vge/ml, see Fast Plan.
    a. In our Safari Park herd, we have not experienced EEHV vge/ml results between 2,000 and 5,000 vge/ml yet.

b. If any questions, particularly in a novel situation, it is always okay to call Lauren (713) 417 7979.

Herd Surveillance: Biological Sampling

Blood Samples

General schedule for blood sampling of African elephants at the San Diego Zoo Safari Park and African and Asian elephants at the San Diego Zoo:

- CBC on calves/juveniles 1-15 years of age every 1 - 2 weeks to establish baseline values
- EEHV 2, 3 and 6 qPCR every 1-2 weeks on calves/juveniles 1-15 years of age (whenever CBC is collected), sent to the NEHL in Washington, DC.
    - ICR will be back up for NEHL but not likely the primary lab for blood PCR until 2022
- Serum banking on calves/juveniles 1-15 years of age every 2 - 4 weeks for future study
- Serum biochemistries every 3 months on calves/juveniles 1-15 years of age
- CBC and serum biochemistries every 3-6 months on adult elephants >15 years old

Trunk Wash Samples

Trunk washes are collected as circumstances dictate, but not routinely at this time.

- Collect trunk wash using minimum 60 ml of saline in each nare and blown back out
- Trunk wash is collected into Ziploc bag, transfer wash into 2 conical tubes (divided equally between them) and close lids
- Label tubes with elephant number and collection date
- Submit to HVMC or SDZ Clinical Laboratory for processing and storage
- HVMC or SDZ Clinical Laboratory will process same day as received
    - Centrifuge at 1500Xg for 10 min @ room temperature
    - Gently poor off and discard the supernatant.
    - Allow as much fluid to drain from the pellet as possible
    - Store pellets in 50 ml conical tubes at -80 C (or at -20 C if necessary)
    - Trunk wash pellets will be analyzed via qPCR by ICR Molecular Diagnostic Laboratory

Herd Surveillance: Behavioral Assessments and Physiologic Monitoring

Possible observation and vital sign monitoring:

Two types of assessments are completed and tracked (Training records and Electronic Red Book) to assess overall health of the elephants.

- **BA (Behavioral Assessment)**-*visual observations* (keepers have none or little direct contact with the elephant)...locomotion, eating, moving with the herd normally, body condition, looks bright and alert, normal fecal output (if observed)
- **FBA (Full Behavioral Assessment)**-*Under stimulus control*. Look at overall body condition (check for new cuts or swelling or signs of trauma), see that they are alert,

attentive, food motivated, examine feet, mouth, teeth and tusks, evaluate ability to move and or flex their muscles by presenting feet, stretching or lying down.

Daily

- General assessment of the elephants' overall attitude and appearance (respiration rate, locomotion, coordination, etc.)
- Visual inspection of the elephants, looking for swelling or abnormalities in the animals' overall appearance
- Assessment of the animals' appetite
- Assessment of the animals' responsiveness to cues and stimuli, training

Weekly Monitoring

- Indirect blood pressure (cuff around tail) readings on all elephants, results to be kept in spreadsheet for monitoring trends
- Body temperature readings on all elephants <15years of age, results to be kept in spreadsheet for monitoring trends
- Visual inspection of mouth, tongue, palate for ulcers, lesions, discoloration, or visual changes

Behavioral Training

Successful diagnosis and treatment for EEHV associated hemorrhagic disease will depend on the ability to access the animal for close visualization, blood collection, and treatment, including oral and rectal treatments, intramuscular injections, and intravenous catheter placement. This may also include the ability to provide standing sedation/restraint for more intensive treatment

- By three years of age the following behaviors should be part of routine daily husbandry:
    - Isolation from dam/other elephants
    - Leg restraints
    - Lay down
    - Injections (IM and SQ)
    - Blood collection
    - Urine collection
    - Body temperature measurement (fecal bolus, rectal, life chip)
    - Blood pressure measurement (cuff on base of tail)
    - Oral exam
    - Accept oral and rectal medications
    - Accept rectal fluids
    - Auscultation of heart w/stethoscope
    - Ultrasound of heart
    - Trunk wash

Clinical Findings Associated with EEHV HD

It is possible for an elephant with early EEHV hemorrhagic disease (EEHV HD) to have no observable signs of illness, which highlights the importance of regular blood collection for EEHV testing.

The following clinical signs may also be associated with a case of EEHV HD. Any concerns should be brought **immediately** to the Elephant Lead, Supervisor, Manager, and Veterinary Staff:

- Lethargy or dullness
- Decreased appetite or water intake
- Changes in response to training, shifting, etc.
- Red oral mucosa
- Red or injected sclera (whites of the eyes)
- Stiff joints with no apparent discomfort
- Lameness
- Any changes in behavioral patterns, including changes in sleep patterns
- Change (drop) of blood pressure
- Tachycardia (rapid heart rate)
- Alteration from (individual's) normal body temperature
- Abnormal fecal output, including diarrhea or reduced amount of feces
- Signs of abdominal discomfort (colic)
- Edema of the head, temporal glands, neck, trunk and/or thoracic limbs*
- Cyanotic, swollen tongue – starts at tip and moves caudally typically*
    - **these findings are often associated with very late stage EEHV HD*

The following abnormalities in laboratory results may be associated with a case of EEHV HD. In general, reduction of cell amounts is of much higher concern than increase in cell amounts. **It is important to compare an elephant's current CBC to the elephants' own historic CBC results and NOT to compare to ZIMS "expected results" or non-specific elephant blood ranges from the literature, these are not specific enough to highlight subtle, but important, changes or trends.**

- Reduction in total white blood cell count
- Reduction in total monocyte count
- Reduction in percentage of monocytes
- Reduction in the total number of platelets
- Reduction in HCT
- Increase in percentage of heterophils/neutrophils or lymphocytes

Sample Collection for an EEHV HD Suspect

If an elephant is showing clinical signs consistent with EEHV HD associated illness, blood should be collected as soon as possible for diagnostic purposes. If the opportunity arises, in this instance, the elephant team is authorized to collect blood prior to getting approval from attending DVM. **In an immediate, clinical concern, Disease Investigations/MDL should be the first option for whole blood EEHV qPCR testing, for rapid turnaround of results.** Duplicate samples should be overnighted to NEHL for confirmation EEHV qPCR. Ultimately, whether to send blood to DI/MDL or NEHL or both for EEHV qPCR will be dependent on the day, time, situation, staff availability, and clinician discretion.

Blood should be collected into:
1. Purple topped tube (EDTA for whole blood 3 ml tubes): 2 tubes initially
a. 1st priority is to collect 1 ml of blood for EEHV qPCR at Disease Investigations/MDL
b. 2nd priority is to collect 0.5 ml of blood for CBC at HVMC or SDZ Clinical Laboratory
c. 3rd priority is to collect 1 to 2 ml blood for EEHV qPCR at NEHL
d. 4th priority is to collect 2-5 ml of whole blood for EEHV for sequencing

2. Royal blue topped or serum separator tube: at least 4 to 12 ml of blood
a. At least 2 ml of whole blood for serum biochemistry at HVMC or SDZ Clinical Laboratory
b. 2 to 10 ml of whole blood for serum banking.

All blood samples should be brought to the HVMC or SDZ clinic laboratory for processing. Based on the situation, the attending veterinarian will determine how best to distribute the blood. It will be processed in-house for CBC and chemistry and samples will be sent to Disease Investigations Molecular Diagnostic Laboratory (MDL) and NEHL EEHV Laboratory in Washington, DC for immediate EEHV PCR.

Contact Information

<u>National Zoo EEHV Lab:</u>
Shipping:
Erin Latimer
National Zoo
3001 Connecticut Ave, NW
Washington, DC 20008
Cell: 703-855-9611

NEHL CAN receive samples on Saturday if it is urgent, there is an extra shipment fee associated with Saturday delivery.

NEHL does NOT receive samples on Sundays.

Treatment for Confirmed or Suspected EEHV HD

This protocol is intended to provide the attending veterinary, laboratory, and elephant care staff with all the resources needed to make optimal treatment and monitoring decisions for an elephant suspected/confirmed to have EEHV-HD.

This protocol is not intended to provide a day to day treatment plan for an EEHV HD case, as each situation varies and there is no magic formula.

Once an elephant starts treatment for EEHV HD, attending veterinarians and elephant leaders <u>should meet twice daily</u>:

- Morning meeting; update on how treatments/elephant did overnight, make plan for the day, distribution of responsibilities for the day
- Afternoon meeting: update on how treatments/elephant did during the day, review new lab work, make plan for next 24-48 hours, identify overnight and on call staff

<u>General expectations for treatment of an EEHV HD Case are</u>:

- Daily blood collection for CBC, serum chemistry, and EEHV qPCR. Potential twice daily CBC if elephant is compliant, particularly in first 7 days.
- Famciclovir and rectal fluids treatment three times/day (Q8 hours) for at least 2 weeks
- Standing sedation regularly for administration of plasma, stem cell, and other treatments.
    - Standing sedation may be performed typically every other day, but may be done as often as once daily or even twice in one day if clinically indicated.
    - No elephant treated for HD has been described as worse off after standing sedation/treatment
- An "all hands on deck" approach to sourcing treatments, collecting and preparing elephant plasma, and ensuring all necessary supplies are available
- Wildlife Care leaders should anticipate a reduction in clinical productivity during an EEHV HD case, as veterinary services team is focused on the sick elephant.

<u>Antiviral Therapy</u>

The typical Famciclovir dosage for Asian elephants is **15 mg/kg TID PO or per rectum**, and will be considered the dose for African elephants until research proves otherwise. This means given **every 8 hours**, not three doses within an 8 to 10 hour work day. This dosage may decrease to 15 mg/kg BID after improvement in clinical signs or viral load has been observed. The decision to decrease the frequency of antiviral treatment will be made based upon dialogue between veterinary and animal care staff.
Famciclovir is kept in the HVMC pharmacy in 250 mg and 500 mg tablets, 30 tablets/bottle. Our policy at the SDZSP is to keep enough drug in stock to treat our two largest at-risk elephants (in 2020 this is Kami and Khosi) for 3 days. In 2020 our minimum amount to keep is 675 gm (this is $819 in 2020).

The SDZ pharmacy will stock a 3 day supply each for the two young African bull elephants, equaling approximately 655 gm of FCV.

INTENSIVE CARE OF THE EEHV PATIENT

Antiviral medications are recommended in most suspect or confirmed EEHV cases to reduce or eliminate viral replication and thus reduce the viral load on the patient. However, the antiviral medications do not reverse the damage the virus has already done to internal organs. Aggressive supportive therapy and close monitoring of the patient is recommended as an adjunct to antiviral medication. Temporary placement of an intravenous catheter in a large, peripheral vein is recommended for fluid and colloidal support and administration of other medications. If placement and maintenance of an IV catheter is not possible under training or manual restraint, sedation may be required.

Sedatives may be administered to facilitate treatment and to manage pain. Low doses have been safely used in clinical cases. Opioids are preferred to the use of non-steroidal anti-inflammatories in order to avoid effects on the urinary system. Antibiotics have no effect on viral infections, but may be given to affected animals to prevent secondary infections with bacterial organisms. Initial doses should be administered intravenously. Following cessation of intravenous treatment, a change to intramuscular or oral products will be made if appropriate.

Rectal Fluid Therapy

- Fluids can be administered rectally to an elephant that is dehydrated but ambulatory or to partially rehydrate an elephant prior to anesthesia and placement of an intravenous catheter.
- Rectal fluids should also be given after the administration of IV fluids 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.

Standing Sedation of Clinical Suspect

Young Asian elephants

- Butorphanol 0.045–0.075 mg/kg IM – reverse with Naltrexone 2.5–5 X Butorphanol dose
- Detomidine 0.011–0.022 mg/kg IM – reverse with Atipamezole 5 X Detomidine dose
- This results in a good standing sedation “sawhorse stance” which allows placement of catheters in the ear or front and rear leg
- Initial dose lasts about 2 hours, then supplemented as needed
- Higher doses of Detomidine may result in lateral recumbency
- Reversal w/Naltrexone and Atipamezole should be complete

Young African Elephants

- Detomidine and Butorphanol 0.017 to 0.02 mg/kg (both) IM at same time
- This dose was used several times at FCZoo in a 13 year old female
- Reversals as above

Adult African elephants

- Butorphanol 0.02 mg/kg and Detomidine 0.02mg/kg **IM**
- or Butorphanol 0.015mg/kg and Detomidine 0.01mg/kg **IV** given at same time
- This dose has been used at SDZ
- Reversal with 10 X dose Naltrexone and and 1.5-2 X dose Atipamezole

Light Sedation in Adult Elephant

- It may be necessary to sedate the dam or other adult herd mates so they are not stressed during manipulations of a calf
- Houston zoo has given three adult female Asian elephants Butorphanol 20 mg (0.006 mg/kg) and Detomidine 10 mg (0.0026 mg/kg) intramuscularly
- Calming/light sedative effect occurred within 10 to 15 minutes and lasted 1-2 hours
- No adverse side effects were seen
- Sedation can be reversed as described above but is not necessary
- Azaperone has been used to facilitate medical procedures in sick female Asian elephants at SDZ (0.3 mg/kg/ 87.5 mg IM; effect at 30 min; leg ropes on at 60 min; used 100 mg on another occasion with good results)

Intravenous Catheter Placement

- Placement of a temporary IV catheter in the ear during standing sedation is described below.
- Elephant plasma is hypo-osmotic relative to standard crystalloid intravenous fluids, hence, standard intravenous fluids given to elephants work like hyperosmotic saline used in large animal medicine.
- A relatively small volume of fluids can make a difference to the elephant's response. Minimally one liter/1,000 lbs seems to give visible results.
- Venous access is normally achieved using the vasculature on the caudal aspect of the ear.
- The skin should be prepped with chlorhexidine (nolvasan) scrub using standard aseptic procedures. A 14-16 ga, 3" intravenous catheter should be placed and stabilized with tape and skin staples (possible in an animal in standing sedation).
- Fluids can be given through a 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 administration line and the catheter to facilitate changing fluids and administration of medications along with the IV fluids.
- 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.
- Elephants in an intensive care environment can be subject to secondary infections, such as MRSA. 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 gloves.

Intravenous Fluid Therapy

Intravenous fluids are recommended to support circulation and hydration. An initial bolus of 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.

Asian elephants have very low serum osmolarity and are hyponatremic and hypochloremic compared to other species. African elephants are thought to have similarly low serum osmolarity. The normal osmolality of Asian elephants ranges from 252-270 mOsm/L.

Crystalloid fluids have an osmolality as listed:
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

Thus, commercial fluids such as Plasmalyte or Norm-R are actually hypertonic for elephants. Dr. Wiedner recommends to using fluids in elephants as you would use actual hypertonic solutions in other species; in other words, very small amounts (one liter per 1,000 lbs; one liter per 450 kg) are given through an IV catheter and followed afterwards with large amounts of rectal fluids.

Plasma Transfusion

Colloids, such as fresh or frozen plasma or hetastarch, are often more effective than crystalloid fluids for volume expansion in viremic or seriously ill animals. The larger molecules in these fluids do not leak out of capillaries as easily, and increase plasma volume. Additionally, animals with active infection are not expected to have antibody to the virus. Hetastarch has been used in ill Asian calves at 0.25-0.5 ml/kg as an IV bolus, followed by rectal fluids.

Plasma should only be administered intravenously after cross-matching donor and recipient blood samples to assure compatibility. Additionally, the donor animal's blood should be PCR tested to ensure the donor does not have a high EEHV viremia.

At several US Zoos, fresh elephant plasma (collected the day before and separated overnight) and frozen elephant plasma has been administered IV. 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) were used successfully as filters in several elephant plasma transfusions in 2014 at the Houston Zoo. The first 100 ml should be given slowly, and heart rate, respiratory rate, and temperature should be monitored. 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. A dosage of 0.5 to 2 ml/kg has been used successfully in ill Asian calves, and higher doses have been given without ill effect.

Shelf life of fresh frozen plasma is one year from collection if frozen at -18C and seven years if frozen at or below -65 C (Schalm, 6th Edition, Veterinary Hematology, p. 736). A delay of more than 8 hours from collection to freezing of plasma can result in reduction of labile clotting factors.

Penciclovir Fortified Plasma

In response to EEHV HD, several elephant care institutions have administered famciclovir (15-30 mg/kg) PO to an adult donor elephant, and collected plasma about 1 hour later. This plasma is spun down immediately and fresh separated plasma, fortified with penciclovir (the active metabolite of famciclovir). It can be administered to the ill elephant as per plasma administration recommendations above.

African Elephant 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 (Allogeneic). 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.

SDZWA, in conjunction with Vet Stem, has a bank of African elephant mesenchymal stem cells (MSC). These MSC were umbilical derived from a healthy calf and dam and are available for immediate use (locally: pick up in Poway, for outside institutions: FedEx overnight). Important information for MSC use in elephants is detailed below;

- VetStem Biopharma – contact information
  - Physical address – 12860 Danielson Court, Suite B, Poway, CA 92064
  - Bob Harman, Founder and CEO – bharman@vetstem.com
  - Sue Harman, Director of Clinical Development – sharman@vetstem.com
  - Kristi Hauta (Vet Stem office: office phone number: (858) 218 8660)
- The following must be provided to VetStem – elephant name, sex, birth date, weight, identification, and shipping addresses
- # of MSC's, volume, shipping logistics, costs, and administration protocols will be determined by VetStem representatives. Generally, MSC are administered as an IV bolus infusion, through an IV catheter

Blood Transfusion

If HCT falls below 14%, blood transfusion should be considered (Mikota pp 330). The amount of blood needed to transfuse in elephants is unknown. In a report of a hemorrhaging adult elephant with a HCT of 13%, a whole blood transfusion of 8 liters, produced tremendous clinical improvement, although this dose of blood is very low compared to transfusion recommendations for other species.

Whole blood can be collected in standard blood collection bags. Whole blood stored at 33.8-42.8 F (1-6 C) will last at least 35 days. If not used, plasma can be collected from unused blood and stored up to five years as frozen plasma. There are preformed blood group antigens in elephants; cross matching is recommended prior to transfusion; any agglutination or lysis indicates an unacceptable match.

In addition to cross-matching, it is recommended to submit a whole blood sample from any potential donor elephants for EEHV PCR testing to ensure that donor blood is not collected from an EEHV viremic elephant.

One way to perform a cross match is listed below, techniques may differ between labs:

- Collect blood from both the recipient and donor into red top tubes
- Separate the serum from the clot, and re-suspend the red cells in saline to wash
- For a major cross match: mix 2 drops of donor RBCs with 2 drops of recipient serum
- For a minor cross match: mix 2 drops of donor serum with 2 drops of recipient RBCs
- Mix then centrifuge
- Examine supernatant for hemolysis – hemolysis indicates incompatibility
- Tap to re-suspend cells to look for visible agglutination
- Then transfer a small amount to a slide and examine under 10X power for agglutination – agglutination indicates incompatibility (Pratt, 1985, Laboratory Procedures For Animal Health Technicians)

Please note: cross-matching should only be done with fresh (not frozen/thawed) serum

Antibiotics

Although antibiotics have no effect in treating EEHV, the animal's immune system will be severely compromised and the clinical situation could be complicated by secondary opportunistic infections and therefore antibiotic therapy should be considered.

Analgesia

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 or other nSAIDS should be administered to patients that appear hydrated or are receiving rectal or IV fluids. An endotoxemic dose of flunixin (0.15-0.5 mg/kg SID to BID), or a dose of meloxicam (0.03-0.06 mg/kg IM, PO or SQ SID) have both been used in Asian calves. Administration of omeprazole for gastrointestinal protection during nSAID treatment should be considered. The equine dose is 0.7 - 1.4 mg/kg PO once daily.

Opioids are also a useful adjunct to providing relief and in some cases mild sedation to assist in the management of animals being treated. There is the possibility of behavioral changes in the elephant when using opioids and trained behaviors may well be lost or less responsive. A dose of 0.005-0.015 mg/kg butorphanol IV or IM has been used in Asian calves.

Clotting Aids

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 cross matching.

Additional Treatments recommended by Houston Zoo Dec 2020:
Vitamin C 6 mg/kg IV SID (250 mg/ml)
Vitamin E 10 ml IM Q 48 hours (300 IU/ml)
Furosemide 1 mg/kg SID IM (50 mg/ml)
Famotidine 0.5 mg/kg IV slow (10 mg/ml)

Euthanasia will be considered as needed based on severity of disease and prognosis for recovery (e.g. pericardial effusion, etc).

Elephant Calf Training Goals Timeline for EEHV

0 - 4 Months:

- Scale weights
- Informal (opportunistic) training (trunk target/ distance separation from Mom, etc.) using secondary reinforcements
- Offering primary reinforcement

4 – 12 Months:

- Trunk target with emphasis on duration
- Separation distance training
- Target pole training
- Lean-ins with emphasis on duration and criteria
- Tactile desensitization
- Body exams desensitization
- Ear manipulation desensitization
- Front foot presents
- Overall attention span duration
- Opportunistic concept of a time –out

12 – 24 Months:

- Separation training (under control and short duration)
- Secondary Trainer desensitization (presence, tactile, exams)
- Front foot presents with duration and tactile desensitization
- Turn
- Hind quarters desensitization – duration and tactile (including tail)
- Rear foot presents
- Front foot presents with emphasis on "work" desensitization
- Ear presents during lean-ins with secondary Trainer desensitization
- Blood draw preparation desensitization (alcohol and novalsan swabs, etc.)
- Body exam hand injection preparation desensitization (same idea)
- Body measurements
- Secondary reinforcement training
- Mouth open
- Mouth exams
- Liquid oral meds/ supplements swallowing
- Eye exams
- Trunk wash
- Trunk up
- Trunk back

- Separations
- Front foot work
- Rear foot presents with emphasis on duration and tactile desensitization
- Blood draw
- Blood draw maintenance and retraining if there's regression
- Front foot anklet training with emphasis on duration
- Rectal enema training (desensitization and removal of feces)
- Stretch
- Retrieval
- Pill swallowing
- Hand injection desensitization continued

24-36 Months:

- Rear foot work
- Rear foot anklet training
- Front foot tether desensitization
- Rectal palpation
- Rectal fluids desensitization
- Hand injection
- Lay down

EEHV Desensitization Training

- Desensitization training will be scheduled 2-4 times per year. The goal is for keepers to adjust schedule to simulate care of an EEHV animal. Tasks to be completed: separate elephants, gating, perform EEHV related behaviors and collections (6:00 am, 2:00 pm, 10:00pm Rx)

EEHV Emergency Drills

- **To ensure swift and accurate treatment, we will conduct 1 to 2 EEHV drills for elephant care staff per year (Live or round table discussion to include all park partners).**
- **The Manager, Curator or Associate Curator will notify the Veterinarian team ahead of time.**
- **During a drill or an actual emergency, assistance will be required from multiple teams. While assigned tasks and responsibilities may vary depending on staffing, the following are general guidelines for Animal Department assignments:**

SDZ SP Roles:

- **Elephant Keepers:** Treat and manage elephant collection. Collect necessary blood samples.
- **Curator/Associate Curator:** Implement call down list, help assign and manage staffing needs, assist elephant team as needed
- **Other Animal Managers/Supervisors:** Assist with other duties as assigned.
- **Elephant Team Keepers:** Report to barn for assignments. This may include monitoring safety in the barn, transporting samples, or assisting elephant keepers.
- **Mammal Keepers:** Assist as needed.
- **Elephant Keepers:** Close and secure elephant buildings. Transport blood sample to lab. Assist with other duties as assigned.
- **Safety Monitor:** At least one person (likely an Elephant Team member) will be assigned to monitor safety in the elephant barn (communicating location of elephants to staff, watching for safety concerns, and so forth). This person will help veterinary staff showing them where supplies can be placed.
- **Responding Veterinarian:** Verbal/visual assessment of elephant, communication with clinical laboratory and molecular laboratory about sample handling, prescribe initial medications, evaluate need to escalation of treatment, detailed medical records
- **Veterinary Technician:** dispense medications, prepare equipment for potential sedation/treatment, ensure adequate supplies for ongoing treatment needs

SDZ Roles:

- **Elephant Keepers:** Treat and manage elephant collection. Collect necessary blood and trunk wash samples. Close and secure elephant buildings. Transport blood sample to lab. Assist with other duties as assigned.
- **Animal Care Manager/ Supervisor:** Implement call down list, help assign and manage staffing needs, assist elephant team as needed, contact Operations to close area if needed, contact Education to cancel tours

- **Curator / Animal Care Manager:** Contact Zoo Director, assist with management of Elephant team
- **Mammal Keepers:** Assist as needed.
- **Safety Monitor:** At least one person (likely an Elephant Team member) will be assigned by the supervisor or manager to monitor safety in the elephant barn (communicating location of elephants to staff, watching for safety concerns, and so forth). This person will help veterinary staff showing them where supplies can be placed.
- **Duty or Responding Veterinarian:** Verbal/visual assessment of elephant, communication with Director of Veterinary Services, SDZ Clinical Laboratory and MDL about sample handling, prescribe initial medications, evaluate need for escalation of treatment, detailed medical records.
- **Director of Veterinary Services:** Contact Chief Conservation & Wildlife Health Officer, SDZ SP Director Veterinary Services, and other veterinarians.
- **Veterinary Technician:** dispense medications, prepare equipment for potential sedation/treatment, ensure adequate supplies for ongoing treatment needs

Debrief:

- A debrief will be conducted and areas of concern or suggestions for improvement will be implemented.

EEHV qPCR Results Distribution List

Routine and clinical suspect EEHV qPCR whole blood results from NEHL and DI/MDL will be emailed to the following SDZWA elephant stakeholders at SDZ and at SDZSP:

Elephant lead, Elephant Supervisor, Elephant Manager, Curator, and Director of Wildlife Care

All veterinarians, including residents and fellows

All clinical laboratory staff, including Laura Keener

DI: Director of DI, and MDL Laboratory Senior Scientist

Date Created/Updated	Responsible Party	Title
4/21/21	LL Howard	SDZWA EEHV Protocol 2021