Houston Zoo
Asian Elephant
EEHV Protocol

February 2020

HOUSTON ZOO ASIAN ELEPHANT EEHV PROTOCOL

HOUSTON ZOO EEHV "FAST PLAN"

FAST PLAN: WHEN TO START TREATMENT

Initiate antiviral treatment if one or more of the following criteria occur:

- Clinical signs present
- WBC and/or monocyte and/or platelet count has dropped significantly below elephant's normal range
- 5,000 to 10,000 vge/ml (qPCR at BCM) or greater
- Rapidly increasing vge/ml (qPCR at BCM)
- Animal is viremic for EEHV strain and is known to be seronegative for it

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 present in the remainder of the EEHV protocol and on the website www.eehvinfo.org.

FAST PLAN: INITIAL DIAGNOSTICS AND TREATMENTS

1. Collect baseline information
    - TPR and Blood Pressure
    - Physical Exam
    - Blood collection
        1. **Purple topped tube** (EDTA for whole blood): **2 small tubes initially**
            1. 1st priority is to collect 1 ml of blood for qPCR at Baylor
            2. 2nd priority is to collect 0.5 ml of blood for CBC at HZI
            3. Any extra tubes can be banked
        2. **Red/Grey (tiger) topped tube** (serum separator for serum): **1 tube initially**
            1. 3rd priority at least 1 ml of whole blood for serum biochemistry at HZI
            2. 4th priority at least 0.5 ml of whole blood for serology at BCM
            3. Any extra tubes can be banked
2. Administer rectal fluids (10-20 ml/kg rectal, ~8 min duration from HZI barn hose)
3. Administer 15 mg/kg Famciclovir orally or rectally (mix powder with water to make into a paste)
    a. If given rectally, a minimum of one hour before or after rectal fluids

FAST PLAN: SEDATION AND FURTHER TREATMENTS

4. Standing sedation with Butorphanol 0.045-0.075 mg/kg and Detomidine 0.005 – 0.022 mg/kg IM given at same time
    a. Reverse with 2.5 times Naltrexone dose and 5 times Atipamezole dose

    b. Average doses at HZI are Butorphanol 0.06 mg/kg and Detomidine 0.015 mg/kg IM
    c. Provide supplemental oxygen via nasal cannula when possible
5. Place IV catheter into ear vein (with injection cap)
    a. Place second IV catheter if possible in another ear vein or leg after first is secure
6. Administer elephant plasma either fresh or frozen, and/or whole blood from acceptable donor, between 0.5 - 2 ml/kg IV, not to exceed 10 ml/kg
    a. Give diphenhydramine 0.5 mg/kg IM prior to transfusion
    b. Fresh famciclovir fortified plasma is first choice
    c. Fresh plasma is second choice
    d. Frozen plasma is third choice
    e. Whole blood, which may be famciclovir fortified, is selected if animal is anemic and may be given in combination with plasma
7. Administer IV Normosol or other crystalloid between 0.3 - 4 ml/kg (~3-4 liters in a 1000 kg calf)
8. Administer IV Hetastarch between 0.25 - 0.5 ml/kg (~250-500 ml for 1000 kg calf)
9. Administer rectal fluids after administration of IV fluids
10. Administer ceftiofur sodium (Naxcel) at 1.1 mg/kg IV
11. Administer ceftiofur crystalline free acid (CCFA, Excede) at 6.6 mg/kg IM
12. Administer flunixin meglumine 0.25 to 0.5 mg/kg IV/IM
    a. If there is renal compromise, go to low end or consider meloxicam at 0.03 to 0.06 mg/kg IV/IM
13. Administer B-complex 5 ml IM
14. Administer Vitamin E 10 ml IM
15. Administer Vitamin C 6 mg/kg IV
16. Administer famotidine 0.5 mg/kg IV slow
17. Administer omeprazole 0.7-1.4 mg/kg PO

FAST PLAN: COMMUNICATION PLAN

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

- The Curator of Large Mammals is responsible for communicating with the VP of Animal Operations, General Curator and Veterinary Team
    i. Contact the grounds vet during the day
    ii. Contact the on-call (hospital) vet after hours
- The Elephant Manager is responsible for communicating with the members of the Elephant Team

- The veterinarians are responsible for communicating with members of the Clinic Team and with Baylor College of Medicine (BCM)
    i. The veterinarians are responsible for all communication with Dr. Paul Ling and BCM team members regarding sample coordination and results
    ii. If the veterinarian is aware of a potential EEHV case first, it is that person's responsibility to communicate with the Curator of Large Mammals, Elephant Manager, General Curator, and VP of Animal Operations initially, then responsibilities disseminate as outlined here
- The VP of Animal Operations is responsible for communicating with the Zoo CEO and the Director of Public Relations
- Cell phones should be called first and then home phones if necessary
- Receipt of the message should be confirmed by all parties

GENERAL EEHV INFORMATION

EEHV PATHOGENESIS

Elephant endotheliotropic herpesvirus (EEHV) is the most important viral disease of elephants and is the primary cause of death in Asian elephant calves in human care in North America and Europe. EEHV is also known to cause mortality in Asian elephant range countries, though the greater impact on wild populations remains to be studied. African elephants are also suspectable to EEHV.

There are 7 strains of EEHV: EEHV 1A and 1B, EEHV4, and EEHV 5 which affect Asian elephants and EEHV 2, EEHV 3, EEHV 6, and EEHV 7 which affect African elephants. EEHV is an ancient, natural virus that is normal for both Asian and African elephants and is shed asymptotically with variable frequency. It is not from mixing Asian and African elephants and is not a product of human care. It is currently believed that in small herds, in both the wild and human care, not all calves are exposed to different viral strains while under the protection of maternal antibodies. When maternal antibodies wane and the calf encounters a strain of the virus for the first time (primary infection) without immunocompetency a potentially fatal clinical form of the virus may develop: EEHV hemorrhagic disease (EEHV-HD).

EEHV-HD primarily affects Asian elephant calves between 1-8 years of age (though lethal cases have been reported in elephants greater than 8 years of age) and the virus is detected by qPCR on whole blood. During the primary infection, viremia may lead to systemic disease, damage to the vascular endothelium, internal hemorrhage, and death. Treatment is aimed at supporting the calf through fluid, antiviral, and other adjunctive therapies. Rapid and aggressive treatment may increase chances of survival for the calf.

CLINICAL EEHV INFORMATION

CLINICAL SIGNS OF EEHV-ASSOCIATED DISEASE

Clinical signs are usually the last indicator of EEHV viremia to appear. Changes in the CBC and detectable DNA in blood can be found up to 28 days before the onset of clinical signs (*Stanton et al. 2013*) and will occur prior to the onset of clinical signs. When clinical signs appear, internal damage has already occurred, and treatment needs to be started immediately.

The following clinical signs may indicate a possible EEHV infection.

- Lethargy, dullness, or depression
- Slowness to train

- Decreased appetite or water intake
- Signs of abdominal discomfort (colic)
- Diarrhea or constipation
- Red or purple or cyanotic (blue) oral mucosa
- Scleral injection
- Edema of the head, neck, trunk and/or thoracic limbs (and ventral abdomen)
- Cyanotic, swollen tongue – starts at tip and moves caudally typically
- Bruising or hemorrhage
- Oral ulceration
- Stiff joints with or without discomfort
- Lameness or inability to rise or lay down
- Any changes in behavioral patterns, including changes in sleep
- Change (decrease) in blood pressure
- Tachycardia (rapid heart rate)
- Alteration from normal body temperature (fecal bolus temperature)

Any concerns should be brought to the Elephant Manager, Curator of Large Mammals, and Veterinary Staff immediately.

INITIAL SAMPLE COLLECTION FOR AN EEHV SUSPECT CASE

If an elephant is showing clinical signs consistent with EEHV infection, blood should be collected as soon as possible for diagnostic purposes.

Blood should be collected into:

Purple topped tube (EDTA): **2 small tubes initially**

4. 1st priority is to collect 1 ml of blood for PCR at BCM
5. 2nd priority is to collect 0.5 ml of blood for CBC at HZI
6. Any extra tubes can be banked

Red/Grey (tiger) topped tube (serum separator): **1 tube initially**

4. 3rd priority at least 1 ml of whole blood for serum biochemistry at HZI
5. 4th priority at least 0.5 ml of whole blood for serology at BCM
6. Any extra tubes can be banked

SAMPLE COLLECTION FOR AN EEHV CONFIRMED CASE

Once an elephant is confirmed to have EEHV, there are many diagnostic and research sampling needs. Below is a listing of sample needs, but it is advised to contact individual researchers and the EEHV Advisory Group for the most current sample needs as able.

All blood and other samples should be brought to the HZI clinic laboratory for processing. Based on the situation, the attending Veterinarian, the Curator of Large Mammals, and the Elephant Manager will determine how best to distribute the samples, if quantity is limited. Samples may be collected for research purposes as approved by the HZI Investigative Review Committee, Curator of Large Mammals, and attending Veterinarian.

Samples should be collected at least once daily during an active EEHV case or more frequently as indicated. Priority is assigned to samples that will have immediate clinical impact, followed by research samples.

<u>Blood</u>

- **CBC**
  - 1 ml whole blood in EDTA
  - Run in-house at HZI
- **qPCR**
  - 1 ml whole blood in EDTA
  - Transfer to Baylor College of Medicine
- **Chemistry + SDMA**
  - 1 ml serum from serum separator tube
  - Run in-house at HZI
    - If not available, send to IDEXX with "Stat" on processing
  - SDMA significance is unknown, but is under investigation
- **iSTAT**
  - 0.2 ml whole blood in heparin
  - Run in-house at HZI patient side
  - Chem8+ and CG4+ clips
- **Lactate**
  - 0.2 ml whole blood in heparin for CG4+ clip (lactate included)
  - 1 drop whole blood for lactate meter strip
  - Run in-house at HZI
- **Crossmatching**
  - 1 ml whole blood in EDTA from donors and recipient
  - Run in-house at HZI (ideally prior to clinical case)
  - Re-crossmatch donors and recipient if more than 3 days of blood or plasma transfusions
- **Blood culture**

    - 1 ml whole blood in culture isolator tube (yellow top), aseptically collected
    - Transfer to MSI with local pick up
    - Consider culture at onset of illness to detect secondary infection and repeat as needed if there are signs of fever, phlebitis, or sepsis; and/or at the end of treatment
- **EEHV gB, 1A, 1B, 4 Serology**
    - 1 ml serum from serum separator tube
    - Transfer to Baylor College of Medicine
- **T-Cell Assay**
    - 20 ml whole blood in heparin
    - Transfer to Baylor College of Medicine
- **Acute phase proteins**
    - 1 ml serum from serum separator tube
    - Ship to University of Miami
    - These samples may be banked and batch sent at the end of treatment
- **Thromboelastography (TEG)**
    - Two 1.8 ml citrate (light blue) tubes, filled precisely to the fill line on the tube (very important), labeled with date and time collected
    - Leave 1 tube as whole citrated blood, refrigerate
    - Spin down 1 tube and decant citrated plasma into cryovial, freeze
    - Ship refrigerated whole citrated blood chilled and ship citrated plasma frozen on dry ice overnight
    - Ship to the University of Georgia
        - Contact Dr. Benjamin Brainard (brainard@uga.edu)
- **Whole blood banking**
    - Any extra whole blood in EDTA should be banked frozen at HZI
- **Serum banking**
    - Any extra serum should be banked frozen at HZI

Urine

- **Urinalysis**
    - 5-10 ml urine in sterile container
    - Run in-house at HZI
    - Culture is not necessary as sample will not be sterile
    - Urine specific gravity will not be helpful, but the sediment looking for crystals (normal) and blood or casts (abnormal) may be helpful (*Wiedner et al. 2009*)
- **Urine banking**

- Urine should be spun down like a trunk wash, excess urine decanted, and the pellet with a little urine should be banked frozen in a cryovial at HZI for future qPCR
- Urine banking is more of a priority in an azotemic animal and does not need to be saved for every case
- Immediate qPCR is not necessary

Feces

- **Fecal enteric pathogen culture**
  - Aliquot of feces in enteric media
  - Prepare in-house at HZI
  - Transfer to MSI with local pick up
  - Rules out secondary infections like Salmonella or Clostridium
- **Fecal occult blood**
  - Aliquot of feces
  - Run in-house at HZI
  - The rectal tissue of an elephant is very absorptive and therefore the fecal occult blood tests may be false negative; however, given the ease and inexpensive of in-house testing, it is still recommended. A positive test should be considered significant.
- **Fecal cytology**
  - Aliquot of feces
  - Run in-house at HZI
  - A fecal cytology for bacterial population or signs of hemorrhage should be performed at least once during the course of illness
- **Feces banking research**
  - 2 grams of fresh feces in whirl pack bank frozen immediately for PCR
  - 2 grams of fresh feces in whirl pack bank frozen immediately for glucocorticoid extraction
  - 2 grams of dry feces (leave out for 2 weeks to dry), then put in whirl pack and bank frozen for glucocorticoid extraction
    - Contact: Dr. Tierra Smiley-Evans

Trunk Wash

- **qPCR**
  - Follow trunk wash protocol
  - If running for clinical monitoring, transfer unspun trunk wash fluid to Baylor College of Medicine
  - If banking for research, spin, decant extra fluid, and retain pellet in cryovial frozen at HZI

        - Contact: Dr. Tierra Smiley-Evans

Other

- **Chewed hay**
    - Small amount of chewed hay may be placed in viral transport media (Thermo Scientific Micro Test Tubes Remel M6 B) and banked frozen
        - Contact: Dr. Tierra Smiley-Evans
- **Oral swab**
    - Dacron oral swab coated in saliva may be placed in a cryovial with 0.5 ml viral transport media fluid (Thermo Scientific Micro Test Tubes Remel M6 B) and banked frozen
        - Contact: Dr. Tierra Smiley-Evans

TREATMENT FOR SUSPECTED OR CONFIRMED EEHV CASE

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. Alternatively, lidocaine gel (lidocaine 2% mixed in with water-based lubricant at and approximate 1:3 ratio [1 ml lidocaine to 3 ml lube]) may be applied to the rectal mucosal and inner anal sphincter 15 minutes prior to rectal fluid administration may be helpful. 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.

The hose in the HZI elephant barn next to the treatment stall delivers ~3 gallons per minute and fluid is delivered for ~8 minutes for an adult cow, for example, then reevaluated if the fluids are being absorbed or just expelled to either continue or end treatment.

Antiviral Therapy

Antiviral medications are recommended in most suspect or confirmed EEHV cases to reduce 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 of when to decrease the frequency of antiviral treatment should be made based upon dialogue between veterinary and husbandry staff. If calf compliance is poor for TID or BID, SID administration is preferred to not receiving any famciclovir at the 15 mg/kg dosage.

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.

Famciclovir (1 g/g) is stored as raw powder in 500 gm tubs in the pharmacy. Each elephant dose is weighed out individually and dispensed in individual bags. Famciclovir may also be kept in the pharmacy in 500 mg tablets, 30 tablets/bottle as part of expired stock.

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.

Ganciclovir is stored in sterile vials contain 500 mg of powder, 25 vials/box in the HZI pharmacy.

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.

Houston Zoo does not stock this medication.

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
        i. Houston Zoo typically starts low at 2.5 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.

The sedated adult may remain close to the calf either within the same stall or in an adjacent stall. Human safety should always be considered and people need to be out of trunk reach. This is done at the discretion of the Curator of Large Mammals and the Elephant Manger.

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
        i. Houston Zoo usually starts low at 1.5 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 at 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. In severe cases or in late stages of EEHV-HD, the vessels will need to be held off more firmly and for longer durations of time than would be considered normal. A minimum of 5 minutes is suggested, as clotting is expected to be abnormal.

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 a high antibody titer 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 after the end of EEHV treatment and/or after 3 days of transfusions if further transfusions are anticipated.

Ideally, a calf will be crossmatched to donors prior to an EEHV illness to save precious time.

At the Houston Zoo, crossmatching is not performed at birth, but rather is performed opportunistically as samples are collected from the calf. Generally, the calf is not sedated for the sole purpose of obtaining blood for a crossmatch when the animal is still in venipuncture training.

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.

Elephants do have blood types, but this is under research and does not seem to be a significant clinical factor when considering a blood transfusion. No blood typing or considerations are considered necessary at this time.

<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

Crossmatching

Minor crossmatch: used to assess the compatibility of a donor's serum/plasma with the red cells of a recipient. Used in elephants when recipient is getting plasma from another elephant.

***Major crossmatch*:** used to assess the compatibility of a donor's red blood cells with recipient's plasma. Typically, not used with elephants unless the recipient is getting whole blood or packed red blood cells.

Materials needed:

1. EDTA (preferred) or serum tube (without the separator gel) from donor and recipient animals (all animals involved)
2. Centrifuge

3. Small tubes (glass preferred) for separating the plasma and for testing (estimate minimum 3 tubes/animal)
4. Physiologic saline (0.9% saline without preservatives)
5. Droppers or pipettes.
6. Incubator 35-37°C
7. Markers for labeling tubes
8. Paper for recording results

Step one: Prepare a 3-5% red cell suspension.
1. Collect blood from both donor and recipient in EDTA.
2. Centrifuge the tube and separate the plasma from the red cells. Save both.
3. Place 1 drop of recipient red cells into a small (2-5 ml) clean test tube.
4. Add approx. 1-2 ml of normal saline to the tube with the red cells.
(or 1 drop RBC to 40 drops saline)
5. Centrifuge at 2500 RPM for 20 seconds.
6. Remove the supernatant, leaving the red cell button on the bottom.
7. Repeat steps 4-6 three times (for a total of 4 washes).
8. Add 1 drop of newly washed recipient red cells to a new test tube.
9. Add approximately 20-40 drops of saline and mix to suspend the red cells. This should be an approximate 3-5% cell suspension to work with. 2

Step two: Minor crossmatch.
1. Add 1 drop of the recipient's 3-5% red cell suspension to a labeled test tube. Add 1 drop of the recipient's 3-5% red cell suspension to another labeled test tube to be used as a control.
2. Add 2 drops of donor plasma or serum to the test tube.
3. Add 2 drops of saline to the control tube.
4. Incubate these tubes at 37°C for 15 minutes.
5. Centrifuge the tubes for 20 seconds at 2500 RPM.
6. Observe the supernatant for signs of hemolysis. If present in the crossmatch tube and not the control tube, the match is not compatible. If present in both, start again with a new cell suspension.
7. If no hemolysis, then gently rock the test tube back and forth to re-suspend the cell button. Observe the cell button while rocking the tube and grade for the presence of agglutination. Grade on a 0-4 scale where 0 is no agglutination and 4 is heavy clumping. Record your results.

Step three: Major crossmatch.
1. Add 1 drop of the donor's 3-5% red cell suspension to a labeled test tube. Add 1 drop of the donor's 3-5% red cell suspension to another labeled test tube to be used as a control.

2. Add 2 drops of recipient's plasma or serum to the test tube.
3. Add 2 drops of saline to the control tube.
4. Incubate these tubes at 35-37°C for 15 minutes.
5. Centrifuge the tubes for 20 seconds at 2500 RPM.
6. Observe the supernatant for signs of hemolysis. If present in the crossmatch tube and not the control tube, the match is not compatible. If present in both, start again with a new cell suspension.
7. If no hemolysis, then gently rock the test tube back and forth to re-suspend the cell button. Observe the cell button while rocking the tube and grade for the presence of agglutination. Grade on a 0-4 scale where 0 is no agglutination and 4 is heavy clumping. Record your results.

HZI Elephant Minor Crossmatching (updated March 2017), example.

Elephant Minor Crossmatch Worksheet

Donor / Recipient	Methai 1425	Thai 1426	Shanti 19269	Tucker 24328	Tess 24329	Baylor 25332	Tupelo 25695	Duncan 28665
Methai	XXX	1+	2+	1+	1+	1+	1+	
Thai	1+	XXX	1+	1+	3+	1+	1+	
Shanti	1+	1+	XXX	1+	1+	1+	1+	
Tucker	1+	1+	3+	XXX	1+	1+	1+	
Tess	1+	1+	1+	1+	XXX	1+	1+	
Baylor	1+	1+	1+	1+	1+	XXX	1+	
Tupelo	1+	1+	1+	1+	1+	1+	XXX	
Duncan								XXX

<u>Whole Blood and Plasma Collection from Donor</u>

Whole blood and plasma may be collected from a donor elephant provided that the donor meets the following criteria:

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

Collection bags come in multiple sizes and in multiple bags connected together, but a standard unit is 450 ml. Generally, standard 450 ml units work well in ear veins and larger units should be reserved for larger vessels in the rear limbs. Citrate Phosphate Dextrose Adenine (CPDA-1), Citrate Dextrose Sodium (ACD) anti-coagulant, and others, have been used successfully in elephants. Collection bags are available through multiple veterinary suppliers, but Houston Zoo purchases through Jorgensen Veterinary Supply.

To collect a standard 450 ml unit, identify and aseptically prepare an ear vein from the donor. Alternating chlorhexidine and alcohol should be used with a final wipe of alcohol. Asepsis is critical to prevent contamination of blood products. Tare the empty plasma collection bag on a scale. Ensure that the appropriate seals are broken and lines clamped for collection (collection bag dependent). Insert the needle (generally 19 ga) into the vein and establish blood flow. Fill the bag to the labeled 450 ml volume using the scale and equating 1 ml blood is equivalent to 1 gram blood. Do not overfill the bag. Gently rock the bag back and forth during collection to prevent clots. Once the bag is filled or if blood flow ceases, remove the needle and hold off the vessel to prevent hematoma formation. Clamp or tie off the blood collection line, label the bag (animal name, species, identification number, date, and any medications currently taking/sedated with at the time of collection), and refrigerate until further processing.

Collecting blood or plasma from a leg vein or using a multi-unit collection set up will be similar to the description above, but the particular set up should be evaluated on an individual basis by the attending veterinarian, Curator of Large Mammals, and Elephant Manager.

Off-site Whole Blood and Plasma Donation

Occasionally, plasma may be donated from elephants outside of the Houston Zoo and the HZI Off-site Elephant Blood Donor Protocol should be followed (see separate document).

Whole Blood and Plasma Processing

If whole blood is to be used emergency, it may be refrigerated with no further processing.

If plasma is to be used, the most efficient way to separate it from the red blood cell component is to spin the bag in the designated plasma centrifuge located in the Houston Zoo's Veterinary Services Building.

The Gulf Coast Regional Blood Center (www.giveblood.org) has donated the blood bag centrifuge to the Houston Zoo. The GCRBC provides maintenance for this machine. The veterinary technicians maintain a standard operating protocol for plasma processing, and this is stored on the P-drive in the lab folder. The main GCRBC contact for centrifuge maintenance is Tarel Washington (twashington@giveblood.org, 713-791-6328).

If the in-house centrifuge is not available, spinning the bag at the Gulf Coast Regional Blood Center may be possible (see contact information above, if Tarel is not available, call GCRBC main number).

Alternatively, the bag may be hung in the refrigerator overnight with the ports up to allow gravity to separate the blood components; however, this is considered a less complete separation method.

Once the blood components are separated in the whole blood collection bag, the plasma may be transferred into the plasma collection bag. The plasma collection bag may be attached already or may need to be attached to an available port on the whole blood collection bag. Weigh the plasma collection bag prior to starting the transfer process. Using the plastic plasma transfer device, gently and smoothly squeeze the whole blood collection bag so that the plasma moves through the plasma collection line into the plasma collection bag. Take care to stop the transfer before any red cells enter the plasma collection line. Strip any residual plasma from the line into the bag. Clamp off the line and prepare the bag for use or storage. Ensure the plasma bag is labeled similarly to the whole blood bag.

Whole Blood and Plasma Storage

Storage information is from Schalm, 6th Edition, Veterinary Hematology.

Whole blood: will last up to 35 days at 1-6 C (refrigerator temperature), but labile clotting factors reduce after 8 hours.

Non-frozen plasma: will last up to 48 hours at 1-6 C (refrigerator temperature), but labile clotting factors reduce after 8 hours, so it is ideally used or spun within 8 hours of collection. It is only considered "fresh" plasma if used or spun and frozen within 8 hours.

Frozen plasma: will last 1 year at -18C (regular freezer temperature) and 7 years at -65-80C or below (large scientific freezer temperature).

Plasma Collection and Storage Schedule

Plasma should be collected from Houston Zoo donor elephants on a rotating basis to ensure an adequate supply of frozen plasma for at risk calves. The Curator of Large Mammals and

Elephant Manager will work with the attending veterinarian to schedule collections. Ideally, at least 4 bags of frozen plasma from each eligible donor should be in the plasma bank at any time to provide for at least 1 treatment for an at-risk calf.

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 sequelae 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
- 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*).

HERD AND BARN MANAGEMENT FOR SUSPECTED OR CONFIRMED EEHV CASE

If a calf has been diagnosed with EEHV, it is not necessary to isolate them from other herd members, unless there are other calves who are known to be susceptible to the EEHV strain that is likely being shed. Susceptibility is determined by clinical history, shedding history, and serology.

However, once treatment starts the EEHV suspect or confirmed EEHV positive animal may be separated from contact with the other elephants and may be restrained with the use of leg restraints and/or sedated to facilitate treatments as needed. Other herd mates may accompany calves and/or subordinate animals for companionship purposes if necessary and may be restrained with the use of leg restraints and/or sedated.

It is especially important to keep a young calf with the mother as much as possible during EEHV illness to provide comfort, decrease stress, and give opportunities for nursing or transfaunation.

Herd management during treatment will be based on the decision of the Elephant Manager and/or the Curator of Large Mammals in conjunction with the attending Veterinarian, General Curator, and VP of Animal Operations.

No non-essential staff will be present in the elephant area during the therapy and treatment process unless approved by the Curator of Large Mammals, Elephant Manager, General Curator, VP of Animal Operations, or the CEO.

It is the responsibility of the Curator of Large Mammals, Elephant Manager, General Curator and VP of Animal Operations to communicate with Rangers to block off any public pathways or elephant barn viewing sites as needed, at their discretion.

Any overnight elephant keeper staff staying in the barn will be designated by the Curator of Large Mammals and Elephant Manager. Any overnight veterinary staff will be designated by the attending veterinarian in conjunction with the veterinary technician supervisor and

may stay in the clinic or barn as indicated. If overnight staff are necessary, the General Curator and VP of Animal Operations will be informed.

EEHV CASE RESOLUTION

Survivor

Signs of EEHV case resolution and survivorship include, but are not limited to the following signs:

- Increasing WBC (can be >20,000/uL)
- Increasing monocyte (can be >70%)
- Increasing platelets (can be >1,000,000/uL)
- Decreasing whole blood qPCR viral load (vge/ml)
- Increasing trunk wash qPCR viral load (vge/ml)
- Improved clinical signs

Rectal fluids and antiviral medications should be continued until the viral load is zero. Other supportive care may be continued as indicated by the clinical case.

Monitoring bloodwork during the recovery period is highly recommended until complete resolution has occurred, which includes normalization of the CBC and whole blood qPCR results stabilizing at zero.

It is important to note that during illness and especially in the recovery period, the animal will start to shed massive amounts of viral particles and this may be detected in trunk washes. Performing qPCR on trunk washes is therefore a useful adjunctive diagnostic test during the recovery period. Trunk wash qPCR is not a substitute for whole blood qPCR.

If there are other vulnerable calves in the herd, continued weekly or increased (twice weekly) monitoring of the CBC and whole blood qPCR is recommended during the period of known massive EEHV shedding. There are multiple institutions that have experienced back to back cases of EEHV-HD. Continued vigilance is critical.

Fatality

EEHV-HD carries a nearly 80% fatality rate and preparations for animal death should be considered.

For a spontaneous death, the animal should be visually monitored for signs of respiration and movement. The animal should only be approached when it is safe to do so. Safety is determined by the Curator of Large Mammals, Elephant Manager, and attending veterinarian. Death should be confirmed by the attending veterinarian.

Euthanasia should be considered when treatment options have been exhausted, the animal is suffering, and has a grave prognosis. The animal may or may not need general anesthesia to lay down or heavy sedation prior to euthanasia. Euthanasia should be accomplished with euthanasia solution delivered IV (1 ml/10 lbs IV) with or without potassium chloride (75-150 mg/kg) overdose. Death should be confirmed by the attending veterinarian.

The American Association of Zoo Veterinarians published *Guidelines for the Euthanasia of Nondomestic Animals* and this reference may be consulted.

A balance between social needs of the herd and necropsy expediency will be decided upon by the Curator of Large Mammals, Elephant Manager, and attending veterinarian.

COMMUNICATION PLAN FOR EEHV CONFIRMED CASE

In the event of a confirmed EEHV case, clear and transparent communication is essential, both internally and externally.

Internal Communication Plan (same as "Fast Plan")

- The Curator of Large Mammals is responsible for communicating with the VP of Animal Operations, General Curator and Veterinary Team
    i. Contact the grounds vet during the day
    ii. Contact the on-call (hospital) vet after hours
- The Elephant Manager is responsible for communicating with the members of the Elephant Team
- The veterinarians are responsible for communicating with members of the Clinic Team and with Baylor College of Medicine (BCM)
    i. The veterinarians are responsible for all communication with Dr. Paul Ling and BCM team members regarding sample coordination and results
    ii. If the veterinarian is aware of a potential EEHV case first, it is that person's responsibility to communicate with the Elephant Manager, Large Mammal Curator, General Curator, and VP of Animal Operations initially, then responsibilities disseminate as outlined here
- The VP of Animal Operations is responsible for communicating with the Zoo CEO and the Director of Public Relations
- Cell phones should be called first and then home phones if necessary
- Receipt of the message should be confirmed by all parties

External Communication Plan

- Communication with Zoo Staff, Volunteers, and the General Public
  a. Communication with Zoo Staff and Volunteers is at the discretion of the VP of Animal Operations, Zoo CEO, and Director of Public Relations
     i. Input from the General Curator, Curator of Large Mammals, Elephant Manager, and attending Veterinarian will be necessary
  b. The EEHV Advisory Group Public Relations and Education Subcommittee is available for support
     i. Subcommittee Chairperson: Jill Allread (jallread@pcipr.com)
  c. In the past, the Houston Zoo has elected the following strategy (which may be changed as indicated)
     i. Non-life threatening or low-level viremias – no formal internal announcement or press release
     ii. Life-threatening viremia or EEHV-HD survivor – formal internal announcement via email and intranet and press release after the end of the case
     iii. Life-threatening viremia or EEHV-HD fatality – formal internal announcement via email and intranet, press release, and press conference after the end of the case

ROUTINE HERD TRAINING, MONITORING, AND SURVEILLANCE

The Houston Zoo elephant herd undergoes daily observation and vital sign monitoring and weekly sample collection as part of HZI's routine EEHV surveillance program. Routine daily monitoring and weekly sample collection is performed by the elephant keeper team.

Routine Herd Training

Successful diagnosis and treatment will depend on the ability to access the animal for visualization, sample collection, and treatment, including oral, rectal, intramuscular injections, and intravenous catheter placement. Intensive care therapy may require isolation from the herd for potentially extended periods of time.

By one year 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 (ear or leg vein)
- Urine collection
- Body temperature measurement (fecal bolus, rectal)
- Blood pressure measurement (cuff on base of tail)
- Oral exam
- Accept oral and rectal medications
- Auscultation of heart w/stethoscope
- Ultrasound of heart

Training schedule, priorities, and techniques will be determined by the Curator of Large Mammals and Elephant Manager.

Routine Herd Training: Detomidine Gel Sedation and Topical Lidocaine

Occasionally, calves may need very light sedation to facilitate blood collection training. Anecdotally, Detomidine gel (Dormosedan Gel; 7.6 mg/ml; Zoetis) has been used sublingually with success. Dosages between 20 – 50 mcg/kg (0.020-0.50 mg/kg) administered sublingually have achieved maximum effect in 30-45 minutes. This may be reversed with injectable atipamezole at 5x the Detomidine dose IM or left to wear off (*Dr. Sanchez, Saiers, personal communication*). This product should not be mixed with any sticky food item and will not have an effect if swallowed. Keepers should wear gloves to administer this medication.

Detomidine gel also seems to have effects when given rectally. At the Houston Zoo, in a calf that had poor sublingual compliance, Detomidine gel was administered per rectum following clean out at dosages between 20 – 31 mcg/kg (0.020 - 0.031 mg/kg), with consistent and notable effects seen at the higher end of this range of 31 mcg/kg (0.031 mg/kg). Peak effects also seem to be faster when given per rectum at 20-30 minutes post administration, compared to sublingual administration. Keepers should wear gloves when administering this medication.

Topical lidocaine may also be useful in calf venipuncture training. Commercially available lidocaine cream (EMLA cream, 2%) may be used; however, the Houston Zoo and others have seen more notable effect with a compounded lidocaine gel formula (lidocaine 20%, tetracaine 4%, and phenylephrine 2%; Taylor's Pharmacy; contact Shelby Owens 321-274-9014). Note that some elephants may find the numbing sensation more irritating than helpful, as manifested by swatting the ears and touching the site that is numb. Peak effect is at 1.5-2.5 hours. Keeper staff applying this ointment should wear gloves.

Routine Herd Monitoring: Vital Signs and Physical Condition

Routine monitoring of physiologic parameters such as body temperature, respiratory rate, heart rate, and indirect blood pressure will help to establish normal values for each individual elephant and yield important information for assessing any elephant that may be suspect for EEHV or other disease problems.

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

- Body weight (should be obtained by keepers prior routinely, but is not done daily)
- TPR and blood pressure weekly
- Evaluation of mucous membranes (tongue, oral mucosa, palpebrae, sclera, vulva)
- Evaluation of head, limbs, ventrum for edema
- Evaluation for any other abnormalities or potential clinical signs
- Evaluation of fecal quality and quantity
- Evaluation of appetite, food consumption and nursing
- Evaluation of the animal's responsiveness to cues, stimuli and training
- Evaluation of sleep and rest patterns
- Evaluation of overall mentation, attitude and appearance (including respiration, locomotion, coordination)

Data collected will be recorded and shared between the elephant and veterinary teams (see Vital Signs Monitoring below).

The portable ultrasound can be used to visualize the heart and to count a heart rate as

indicated.

Blood pressure is monitored weekly, measured with an off-the-shelf blood pressure cuff (Walgreens: HoMedics Deluxe Automatic Blood Pressure Monitor with a standard 9-13 inch arm cuff). The cuff is placed on the tail at approximately the level of the animal's heart. Consistent placement is critical to the precision and accuracy of the readings. Readings are recorded on both a handwritten chart kept in the barn and logged into a spreadsheet on P:\Large Mammals\Elephants\OLD P Drive\Elephant vitals and communicated to the Veterinarians any time there is suspicion of abnormal health. Average values for each elephant can be seen in the chart below.

Body temperatures are monitored and recorded on all elephants using measurement of a fresh fecal bolus. Temperatures in excess of 100° F should be considered elevated.

Elephant Vital Signs Data (updated January 10, 2019)

Parameter	Thai	Methai	Shanti	Tess	Tucker	Baylor	Tupelo	Duncan
	Average Values (updated 1/10/2019)							
Blood Pressure (mmHg)	186.5	184.2	176.9	159.7	140.6	158.8	136.3	143.9
	127.8	123.2	122.1	116.4	101.8	98.9	87.4	93.4
Heart Rate (bpm)	47.5	66.1	40.4	44.2	48	66.4	51.1	49.2
Bolus Temperature	91.6	92.7	91.4	94.7	96	97.5	97.6	97.7

Any concerns, however minor, MUST be immediately reported to the Elephant Manager and the Curator of Large Mammals. The Elephant Manager or Curator of Large Mammals, or a keeper in their absence will notify the attending Veterinarian.

Keepers are the first line of defense against EEHV. Observing signs of EEHV early is what may save an elephant's life.

ROUTINE HERD SURVEILLANCE: SAMPLE COLLECTION

In an effort to discover subtle changes that might indicate early signs of infection, biological samples are collected, and analyzed regularly.

A weekly sample rounds list is generated to help keep samples organized. The list includes all EEHV and non-EEHV related samples. It is the responsibility of the veterinary staff to maintain and share this list via email at least the night before sample collection day. This document is located on the P-drive: P:\Animal Programs Manager\Elephant weekly sample rounds list.

An example of the monthly schedule is shown below (updated June 2019).

Monthly General Schedule (Rev. 6/19)	1425 Methai	1426 Thai	19269 Shanti	24329 Tess	24328 Tucker	25332 Baylor	25695 Tupelo	28665 Duncan	31712 Joy	32816 Tilly
1st Tuesday	Progesterone CBC Chemistry PCR Serum bank for BCM serology	CBC Chemistry PCR Serum bank for BCM serology	Progesterone CBC Chemistry PCR Serum bank for BCM serology	Progesterone CBC Chemistry PCR Serum bank for BCM serology	CBC Chemistry PCR Serum bank for BCM serology	CBC Chemistry PCR Serum bank for BCM serology	Progesterone CBC Chemistry PCR Serum bank for BCM serology	CBC Chemistry PCR Serum bank for BCM serology	CBC Chemistry PCR Serum bank for BCM serology	CBC Chemistry PCR Serum bank for BCM serology
2nd Tuesday	Progesterone		Progesterone	Progesterone	CBC PCR	CBC PCR	Progesterone CBC PCR	CBC PCR	CBC PCR	CBC PCR
3rd Tuesday	Progesterone PCR	PCR	Progesterone PCR	Progesterone PCR	CBC PCR	CBC PCR	Progesterone CBC PCR	CBC PCR	CBC PCR	CBC PCR
4th Tuesday	Progesterone		Progesterone	Progesterone	CBC PCR	CBC PCR	Progesterone CBC PCR	CBC PCR	CBC PCR	CBC PCR
5th Tuesday	Progesterone PCR	PCR	Progesterone PCR	Progesterone PCR	CBC PCR	CBC PCR	Progesterone CBC PCR	CBC PCR	CBC PCR	CBC PCR

Blood Samples

Blood samples are typically collected from an ear vein using a butterfly catheter and syringe or vacutainer following aseptic preparation. Blood samples may also be collected from a leg vein using similar technique.

The general schedule for blood sampling of elephants at Houston Zoo is:

- EEHV1, 3/4, and 5 qPCR at Baylor College of Medicine
    - Weekly on calves/juveniles (Tilly, Joy, Duncan, Baylor, Tupelo, Tucker)
    - Every other week on the first, third and fifth week of the month on adult elephants (Thai, Methai, Tess, Shanti).

- CBC in-house at HZI
    - Weekly on calves/juveniles (Tilly, Joy, Duncan, Tupelo, Baylor, Tucker)

    - Monthly on adult elephants (Thai, Methai, Tess, Shanti)

- Serum biochemistries in-house at HZI
    - Monthly on calves/juveniles (Tilly, Joy, Duncan, Baylor, Tupelo, Tucker)
    - Monthly on adult elephants (Thai, Methai, Tess, Shanti)

- Serology at Baylor College of Medicine
    - Monthly on all elephants (Tilly, Joy, Duncan, Tupelo, Baylor, Tucker, Thai, Methai, Tess and Shanti) on the first of the month
        - Ideally, serum (1-2 ml) should be collected and banked for this work
        - The absolute minimum volume for serology is 0.2 ml serum for the test

- Changes to the frequency of testing may be performed as needed

Other samples may be submitted when and if indicated.

The transition between the calf/juvenile elephant testing schedule and the adult testing schedule will be determined by the attending veterinarians, Curator of Large Mammals and Elephant Manager. As of 2020, the oldest animal on weekly testing is 15 years of age.

Blood samples are obtained weekly for routine health monitoring. Samples should be taken to the clinic for processing within an hour of collection to ensure accuracy of results. Samples will be shared with the Baylor College of Medicine.

See the P-drive for the Elephant blood tube quick guide and further examples of the elephant sample list.

Trunk Wash Samples

Trunk wash samples are collected as circumstances dictate, but not performed routinely for EEHV surveillance at this time.

Trunk wash technique

- Trunk washes (minimum 30 ml of fluid recovered) are collected using 60 ml sterile saline infused into the trunk, then collected into clean plastic bags, and stored in 50 ml conical vials.
- Samples are taken to the BCM laboratory of the day of collection.
- If samples are not taken to BCM same day, the trunk wash may be spun down in the centrifuge and the pellet saved frozen in a cryovial at -80 F for future analysis.

Any additional samples to be collected should be approved by the HZI Investigative Review Committee, Curator of Large Mammals, and attending Veterinarian.

SPECIAL CONSIDERATIONS FOR OBTAINING DIAGNOSTICS ON CALVES:

Although the goal is to have a calf trained for venipuncture by one year of age, this may prove challenging. Keepers understand the importance of whole blood sampling and have a routine training schedule to build foundations for a solid blood collection behavior. It is important to balance the need for whole blood sampling and preservation of behavior.

At the Houston Zoo, there have been several cases in young calves less than 2 years of age that have been non-clinical, EEHV naive, but weak positive on whole blood PCR or are shedding in trunk washes. In these cases, blood volume has been small and CBC results are often not available. There are many variables that should be accounted for in these types of situations and a verbal discussion between the veterinarians, Curator of Large Mammals, Elephant Manager, General Curator, and VP of Animal Operations should occur to determine the next step, which could range from monitoring clinical signs to sedating for obtaining a diagnostic sample. Factors to discuss include serostatus, shedding, clinical history, age, current herd status, clinical signs, and other diagnostic sampling needs.

Having direct, open discussion about risks and benefits with all stakeholders is critically important to decision making for calf care.

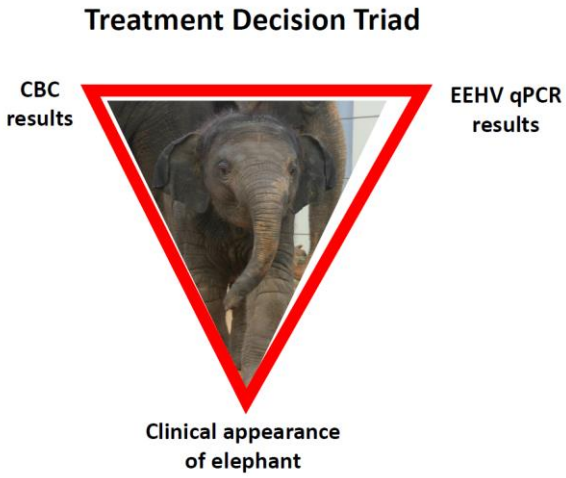
INTERPRETATION OF EEHV PCR AND CBC RESULTS

Three factors are considered when determining whether to institute rectal fluid therapy, antiviral therapy, and other treatments in a viremic elephant:

1. CBC results
2. Whole blood qPCR results
3. Clinical status of the elephant

In the face of a viremic elephant, these factors are evaluated daily by the veterinary staff, Curator of Large Mammals, and Elephant Manager. A group decision on how to proceed be should made.

Any concerns from any party should be shared with the entire decision-making group right away, so no treatment or non-treatment decision is made in isolation.



CBC Results

CBC results are critical to determining the health status of the elephant and are generally the first laboratory test that indicates EEHV viremia, as this sample is run in-house at HZI.

It is important to know each individual elephant's normal CBC values to be able to detect and interpret changes effectively. HZI establishes a normal reference interval for all CBC values individually for each HZI elephant once blood can be collected regularly. This is important to do for each individual elephant based on the technology used to general the values. A general elephant species reference range is not an equal substitute for individual elephant ranges.

HZI maintains individual elephant blood charts on the P drive: P:\Vet Clinic\Vet Files\Elephant protocols. This is accessible to the veterinary staff, Curator of Large Mammals, Elephant Manager, General Curator and VP of Animal Operations.

In HZI elephants, a drop in the total WBC and/or a drop in monocyte counts have been observed in association with increasing or peaking EEHV1, EEHV4, and EEHV5 viremia. A decrease in platelets has also been observed. In cases of EEHV-HD, anemia has been noted by a low total RBC, HCT, and PCV. A concurrent decrease in total protein is consistent with frank internal hemorrhage.

While continued decreases in WBC, monocytes, and platelets can be seen during initial antiviral and supportive treatment, we have seen these counts eventually increase in coincidence with decreasing viral loads and improved clinical condition of the elephant during recovery. Often, once viremia is resolving, there should be a rebound increase in WBC, monocyte, and platelet counts. The rebound may be marked with the WBC increasing to >20,000/uL, monocyte increasing to >70%, and platelets increasing to nearly 1,000,000/uL.

There may be a cut-off value for absolute platelet count in young Asian elephants, below which is a very poor prognosis for survival. In three fatal EEHV cases that had platelet counts available pre-mortem, total platelet counts ranged from 50,000 to 70,000. An absolute platelet value below 100,000 indicates significant disease may be present and is cause for immediate and aggressive treatment, this is also an indicator of poor overall prognosis (*Dr. Ling, personal communication*).

Key initial CBC Changes in EEHV viremia:

- Decreased WBC (leukopenia)
- Decreased monocytes (monocytopenia)
- Decreased platelets (thrombocytopenia)

Other CBC Changes in EEHV-HD:

- Decreased RBC, HCT, and PCV (anemia)
- Decreased total protein (hypoproteinemia)

CBC results are visible in the Houston Zoo's electronic medical records keeping system to the veterinary staff, Curator of Large Mammals, Elephant Manager, General Curator and VP of Animal Operations. If results are not entered into the medical record same day, it is the responsibility of the attending veterinarian to communicate any abnormalities seen to the group listed above.

PCR Results

Research by BCM regarding EEHV epidemiology and diagnosis is ongoing and will be continually adding to the base of knowledge regarding this disease.

Weak positives

Houston Zoo elephants that are clinically normal have been detected as "weak positive" or "1 of 2 wells positive" on the BCM EEHV qPCR test, indicating a low level of viremia. Low level persistent viremias have also been reported in other institutions (*Bauer et al 2018*). Previous to this discovery, any positive PCR result on whole blood sample was considered indicative of active EEHV infection. Evaluation of any low-level viremias should be made in relation to the animal's CBC results, clinical signs, serostatus, and history. In general, BCM will re-run "1 of 2 wells positive" samples during the next batch of PCR to confirm the result, but this should also be specifically requested as needed. Alternatively, a fresh sample may be collected from the elephant and submitted as a recheck PCR to confirm the result.

It should be kept in mind that several clinically normal, weak positive viremic elephants have been documented to shed large amounts of virus from their trunks following the onset of viremia. This may have implications for other members of the herd.

Positives

If a clinically normal elephant with a normal CBC has a positive EEHV PCR result on a routine whole blood sample for the first time, blood samples should be collected again for several days within one week to establish the course of viremia. If enough blood is available, CBC minimally should also be run and chemistries as volume allows. If the viral load levels decline or stabilize at a low level and the animal is clinically normal with a normal CBC, then frequency of testing may be reduced. History and serostatus should also be considered. Treatment may not be needed in this case.

If an elephant has a viral load that reaches 5,000 to 10,000 vge/ml, treatment should be initiated. If the viral load is rapidly climbing, treatment could be initiated prior to reaching this number. If the EEHV strain detected is suspected to be the animal's primary infection with that strain based on serology and history, greater consideration for early treatment should be given. Moreover, it is known that EEHV1A is the most likely strain to be fatal and detection of this strain, especially in a primary infection, should be treated aggressively as soon as possible. The remaining order for EEHV fatalities is as follows: EEHV1B, EEHV4, and EEHV5.

Any elephant with an abnormal CBC and viremia, regardless of vge/ml, should be started on an antiviral and supportive care regime immediately. Clinical signs do not need to be present to start treatment in this situation.

Any elephant with clinical signs of EEHV-HD, regardless of vge/ml, should be started on an antiviral and supportive care regime immediately. Follow up with CBC and whole blood qPCR. Do not wait to start treatment if clinical suspicion is high.

Evaluation of whole blood viral loads in elephant calves that were clinically ill but survived infection has revealed some trends regarding viral loads. In these survivors, clinical signs were seen when whole blood viral loads approached 10,000 vge/ml and viral loads peaked 2-13 days after the onset of clinical signs. To date, there are no similar flow charts from non-surviving elephants, so it is unknown if the course of disease is similar in fatal cases. It is also important to note the trajectory of the viral load numbers as well as the absolute number of vge/ml; a rapid increase in numbers may indicate impending onset of clinical disease. A viral load of > 1,000,000 vge/ml carries an extremely poor prognosis (*Dr. Ling, personal communication*).

Therapy decisions and implementation will be made in concert with the attending Veterinarian, Curator of Large Mammals, Elephant Manager, General Curator and VP of Animal Operations.

Key qPCR Changes in EEHV Viremia:

- Increased viral load (vge/ml) in 2 of 2 wells
- EEHV strain (Lethality: EEVH1A > EEHV1B > EEHV4 > EEHV5)

SUMMARY: WHEN TO START TREATMENT

Initiate rectal fluids, antiviral, and supportive treatments if one or more of the following criteria occur:

- Clinical signs present
- WBC and/or monocyte and/or platelet count has dropped significantly below elephant's normal range
- 5,000 to 10,000 vge/ml (qPCR at BCM) or greater
- Rapidly increasing vge/ml (qPCR at BCM)
- Animal is viremic for EEHV strain and is known to be seronegative for it

BIRTH SAMPLE COLLECTION

There is tremendous need for elephant samples surrounding the birth of a calf for EEHV clinical and research purposes.

It is recommended that individual researchers and institutions be contacted at least 6 months prior to an expected birth to identify research sample needs and gather necessary materials.

At the Houston Zoo, a separate birth protocol and EEHV sample collection sheets are maintained. These documents are located on the P drive: P:\Vet Clinic\Vet Files\Elephant protocols\Elephant Birth Sample Collection Protocols.

Historically, the following clinical samples collected at a calf birth for EEHV treatment

- Umbilical cord for stem cell preservation, Ingeneron Inc.
- Blood for CBC, chemistry, qPCR, banking for HZI internal use
    - Blood for crossmatching is not collected at the time of birth, but rather will be done opportunistically prior to an EEHV illness, as able

Historically, the following researchers and institutions have requested samples collected at a calf birth for EEHV Research:

- Dr. Paul Ling, Baylor College of Medicine
- Ms. Erin Latimer, National EEHV Laboratory
- Dr. Virginia Pearson, Fox Chase Cancer Center and Drexel University
- Dr. Josh Schiffman, Huntsman Cancer Institute
- Dr. Valerie Johnson, Colorado State University

The EEHV Advisory Group Research Subcommittee may also be contacted for any additional research needs. Subcommittee chairperson: Dr. Priya Bapodra (Priya.Bapodra@columbuszoo.org).

The Elephant TAG-SSP may also be contacted for any additional research needs. Contact person: Dr. Jaime Landolfi (jaimeland@gmail.com).

NECROPSY AND POST-MORTEM SAMPLE COLLECTION

It is important to perform the necropsy as soon after death as possible, to increase the chance of recovering viable virus from post-mortem tissues. Timely collection of tissues samples and submission to EEHV laboratories are paramount to facilitate viral culture. However, based on the unique social requirements of elephants and need to grieve and accept the death of a herd mate, it is unlikely we will be able to remove the body immediately. The compromise between elephant social needs and need for samples will be reached by collecting the samples listed below (whole heart blood and tongue tissue) and sending them for culture immediately, then performing the complete necropsy and sample collection when herd mates are ready.

Necropsy References

Prior to a necropsy, please reference the following for specific post-mortem sample needs:

Elephant TAG/SSP Research and Necropsy Protocol

- 2019 version saved as a PDF on the P-drive
    - P:\Vet Clinic\Vet Files\Elephant EEHV Information and Protocols\EEHV Protocol
    - P:\Animal Programs Manager\EEHV Protocols
- Contact SSP pathologist to inquire about more updated version

EEHV Research Necropsy Protocol Supplement

- 2019 version saved as PDF on the P-drive
    - P:\Vet Clinic\Vet Files\Elephant EEHV Information and Protocols\EEHV Protocol
    - P:\Animal Programs Manager\EEHV Protocols
- Contact EEHV Advisory Group to inquire about more updated version
- Complete TAG/SSP consent form
    - Consult with Houston Zoo Investigative Research Committee if biomaterials request form is also needed

EEHV Advisory Group Recommendations

- eehvinfo.org
    - Professional Content tab
    - Pathology and necropsy information available here

Necropsy Research Contacts

Prior to a necropsy, please contact the following people for specific post-mortem sample needs:

Dr. Jaime Landolfi for current EEHV Advisory Group and Elephant TAG-SSP sample needs

Dr. Jennifer (Jaime) Landolfi, DVM, PhD, DACVP
University of Illinois
Zoological Pathology Program
jaimeland@gmail.com

Dr. Paul Ling, PhD for current Baylor College of Medicine sample needs

Dr. Paul Ling, PhD
Baylor College of Medicine
(713)798-8474
pling@bcm.edu

Dr. Gary Hayward, PhD for current Johns Hopkins sample needs

Dr. Gary Hayward, PhD
Johns Hopkins University
(410)955-8686
gary.s.hayward@gmail.com

Dr. Valerie Johnson, DVM, MS for current sample needs

Colorado State University
(603)443-2099
Valerie.Johnson@colostate.edu

Erin Latimer, Laboratory Manager for current sample needs

Department of Pathology, Smithsonian's National Zoo
202-633-4252 (office phone)
202-633-8717 (fax)
703-855-9611 (cell)
latimere@si.edu

Dr. Priya Bapodra, BVetMed (Hons) MSc, Dipl. ACZM, MRCVS for current sample needs

EEHV Advisory Group Research Subcommittee Chairperson
614-724-3643 (office phone)
740-255-4504 (cell)
Priya.Bapodra@columbuszoo.org

Necropsy Assistance

Dr. Raquel Rech from the Texas A&M College of Veterinary Medicine Department of Pathology has offered her help with any elephant necropsy. If she is available, she will come to the Zoo to help with necropsy, even after hours or on week-ends. If the elephant is small enough to be transported, we are also welcome to bring it to his facility at TAMU.

Contact information:
Dr. Raquel Rech, DVM, MS, PhD, DACVP
Clinical Assistant Professor
Department of Veterinary Pathobiology
Texas A&M University
Ph: 979-220-8570
Fax: 979-845-4052
Email: rrech@cvm.tamu.edu

Gross Lesions

The lesions of EEHV are similar in both Asian and African elephants. Gross findings typically include hydropericardium (free fluid in membranous sac around heart), along with extensive petechial (small) and ecchymotic (large) hemorrhages within all layers of the heart. In addition, petechial hemorrhages associated with mesenteric and serosal (external surface of organs) surfaces are diffusely scattered throughout the peritoneal cavity. Cyanosis of the tongue is sometimes present as is hepatomegaly (enlargement of the liver) and ulceration of the oropharynx and large intestine. Histology correlates well with the gross findings and also demonstrates the presence of intranuclear viral inclusion bodies within the capillary endothelial cells of the heart, tongue, liver, and to a lesser extent, the intestinal tract. Electron microscopy readily demonstrates the presence of herpes virus.

Photographs should be taken of all gross lesions.

Known Samples to Collect

Fluid Collection

- The following fluids should be collected during post-mortem examination:
  - Ascites (20 to 60 ml)
  - Whole heart blood (40 to 100 ml)
    - Collect **immediatel**y post-mortem
  - Pericardial fluid (20 to 60 ml)
  - Cerebral Spinal Fluid (20 to 60 ml)
- All fluids should be placed into EDTA tubes (purple topped tubes)
  - 10 to 30 ml of each should be saved and distributed to the National Zoo EEHV Laboratory.

- 40 to 100 ml of whole heart blood should be collected and sent to Dr. Gary Hayward. Samples should be kept cool, but not frozen, and shipped immediately on ice packs for overnight delivery.

Tissue Collection

At a minimum five complete sets of tissue samples should be collected from each organ.

Formalin at a 10:1 ratio

1. 1 set to go to Dr. John Edwards, TAMU pathologist, for diagnostic purposes
    a. Paraffin blocks should be retained at the Houston Zoo after histopathology has been performed
2. 1 set to be kept at the Houston Zoo
3. 1 set to be shared with the Elephant SSP Pathologist (Necropsy protocol updated in 2018, available online at www.AAZV.com)

Frozen in -80 F

4. 1 set should be frozen at the Houston Zoo
    - Large amounts of each tissue should be collected for future diagnostics, research and testing
        - A piece of each tissue the size of an 8 X 11 inch piece of paper, approx. 1 inch thick, should be collected
        - Each piece should be cut into smaller 1-2 inch square samples so later samples can be harvested without thawing the entire piece
    - Tissues should be placed in separate whirl pack bags (one organ/ bag)
- Bags should be labeled with type of tissue, date, elephant name and ISIS number
    - Use a freezer safe marker on the bag that won't rub off
    - (or labels can be made by the HZI lab tech)
- Samples should be stored in -80 freezer at Houston Zoo until needed

5. 1 set should be sent to the National Zoo EEHV Laboratory
    - If a complete frozen set is not possible, collect the following tissues at a minimum: liver, heart, lung, kidney, spleen, tongue, skeletal muscle, brain, lymph node, and any grossly abnormal tissues or tissues with significant hemorrhages
    - Ship to National Zoo EEHV Laboratory
        - Each tissue should be cut in <u>samples measuring 2-3 cm X 2-3 cm</u>

- Each should be placed in a separate sterile whirl pack bag
- Bags should be labeled with tissue, date, and elephant name and closed tightly, then double bagged in a second whirl pack bag.
- Samples should be sent as early as possible on dry ice per shipment instructions below.

Chilled Fresh Tissue Requests

- A 4 cm X 4 cm piece of tongue should be collected **immediately** post-mortem for Dr. Gary Hayward, sample to be kept cool but not frozen and to be shipped immediately on ice packs for overnight delivery.

- One cm square pieces of lung, spleen and lymph node should be collected and placed into whirlpack bags. Samples to be kept cool (not frozen) and shipped on ice overnight to Dr. Gary Hayward at JHU.

EQUIPMENT AND SUPPLIES

The following equipment and supplies will need to be on hand for support during therapy. One staff member will be designated to move these supplies in an organized manner into the hay room of the barn or the keeper office. Supplies used on a daily basis will be cleaned and restocked.

Elephant Barn Supplies:

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

Clinic Supplies:

1) Oral or rectal administration of Famciclovir
   - Famciclovir powder (1 g/g)
     - Have on hand a minimum 3 day supply for all at risk calves at same time, which is at least 500 gm of stock famciclovir powder
     - Bring 2 doses to elephant barn for standing sedation procedure if prescription is not already dispensed
   - Water soluble gel (for mixing with famciclovir for rectal administration)
   - OB sleeves and lube
   - Hydrogen peroxide (minimum 1 bottle)
   - Exam gloves (all sizes)
   - Towels (10-12)

2) Rectal Fluids
   - Staff should wait a minimum of one hour before or after fluids to administer Famciclovir.
   - Excess water in the rectum should be raked out before the rectal administration of meds
   - To be administered by elephant staff via warm water hose
     - Adult elephants at HZI receive ~3 gallons of water per minute
     - Rectal fluid treatments average eight minutes (time is dependent on warm water availability)
     - Methai averaged 25.2ml/kg per treatment in 2011

- Initial bolus of 20 ml/kg recommended

3) Standing sedation
- Print out medication dosage chart from P drive (**print from Printer 50 – legal size**)
- Veterinarians will grab from safe:
  - Butorphanol (minimum 2 bottles, 5 ml/bottle, 50 mg/ml)
  - Detomidine (Minimum 2 bottles 5 ml/bottles, 10 mg/ml)
  - Naltrexone (minimum 2 bottles, 50 mg/ml, 30 ml/bottles)
  - Atipamezole (minimum 2 bottles, 25 mg/ml, 50 ml/bottle)
- Drugs (see dosage chart for amounts):
  - Injectables (in general first line drugs):
    - Ceftiofur sodium (Naxcel) 4 g
    - Sterile water (for reconstitution of Naxcel)
    - Ceftiofur crystalline free acid (Excede, CCFA) 200 mg/ml
    - Diphenydramine (Benadryl) 50 mg/ml
    - Aminocaproic Acid 250 mg/ml
    - Famotidine 10 mg/ml
    - Meloxicam 5 mg/ml
    - Flunixin meglumine (Banamine) 50 mg/ml
    - Vitamin C
    - Vitamin E
    - Vitamin B complex
    - Enrofloxacin 100 mg/ml
    - Penicillin (PPG, Dual Pen) 300,000 IU/ml
    - Normosol or LRS
    - Saline 0.9%
    - Hetastarch
  - Orals (in general first line drugs):
    - Omeprazole paste 370 mg/ml
    - Enrofloxacin paste 300 mg/ml
    - Famciclovir 1 g/g
- Emergency box (make sure there is enough of the following drugs):
  - Large animal atropine
  - Epinephrine
  - Lidocaine 2%
  - Sodium bicarbonate 8.4%
  - Calcium gluconate 23%
  - Furosemide 50 mg/ml
  - Doxapram 20 mg/ml
- Anesthesia clip board

- Calculator
- Pole Syringe
- Syringes (Box each of 60, 35, 20, 12, 6, 1 ml sizes)
- Needles (14g, 16g, 18g, 20g, 22g 1.5", 23g, 25g; one box each)
- Butterfly catheters 19g, 21 ga. (1 box each)
- Supplies to make up Heparin flush
    - 500 ml or 1L bag of NaCL
    - 1mL of 1,000 IU/mL Heparin added to 500 ml of NaCl for 2 IU/mL
        - Or 2mL of 1,000 IU/mL heparin added to 1L of NaCl

4) Oxygen-portable large animal anesthesia machine
- Portable oxygen tanks E tanks (x 3)
    - Plastic tubing for nasal oxygen administration
    - Y piece (nasal administration)
- ET tubes (30, 24, 22, 20 mm or larger)
- Laryngoscope w/long blade
- Ropes (open mouth)
- Blocks (open mouth)
- Pulse oximeter and capnograph + SurgiVet + extension cord
- I-stat with Chem8 and CG4 Cartidges (minimum 4 each)
- Stethoscope
- Thermometer
- Head lamp
- Flash light

5) Placement of IV Catheter
- EVO or Mindray ultrasound machine
- Large animal surgery pack
- Clear instrument pack
- 14-22 ga catheters
- Injection caps, T ports
- Large Animal IV (bungee type) line (3 complete sets)
- Standard IV administration set (3 complete sets)
- Large Animal IV extension set (3 complete sets)
- Standard Extension set (3 complete sets)
- Scalpel blades: 22, 10, 15
- Surgical prep: chlorhexidine scrub and alcohol
- Sterile Gloves (6 1/2, 7, 7.5, 8, 8 ½)
- Lidocaine topical ointment (elephants has this at barn)

- Tourniquet (bungee cords)
- Drapes
- Sharps container
- Suture (0, 1, 2 prolene or similar with cutting needle)
- Skin stapler
- Tissue glue
- Duct tape (3-4 rolls)
- White tape (1 inch, 2 inch, 4 inch; 5 rolls each)
- Vetwrap (2-6", multiple rolls each size)
- Elasticon (2-4", multiple rolls each size)
- Rolled cotton (3 rolls)
- 4 X 4 guaze (6 packages)
- 5 liter LRS fluids (4 bags)
- 1 liter LRS fluids (6 bags)
- 1 liter Normosol fluids (6 bags)
- 1 liter Saline 0.9% (6 bags)
- 500 ml Hetastarch (4 bags)
    - Push bags if bringing 1L bags of fluids
- IV pumps and IV pole
- Carabiners to help hold fluid bags
- Ropes/wire to hang bags
- Extension cord
- Towels
- Flashlights/head lamps
- Plasma (placed in elephant office freezer, keep frozen till needed)
- Plasma administration filters (4 sets minimum)
- Plasma administration lines (4 sets minimum)

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

6) Monitoring
- ICU flow sheet (at end of this protocol and on P-drive), pens, clipboard, watch
- Evo or Mindray U/S Machine
- Doppler

- Blood pressure cuff (Walgreens brand cuff, elephants has this)
- ECG (cerclage wire contacts or sticky pads)
- Ophthalmoscope
- Ophthalmoscope extra handle (charged in wall)

7) Diagnostic Supplies
    - Culturettes for rectal culture (3 sets)
    - Blood tubes
        - EDTA tubes (purple)
        - Heparin tubes (green)
        - Serum separator tubes (tiger top)
        - Blood culture tubes (yellow)
        - Sodium citrate tubes (light blue)
        - No additive tubes (plain red top)
    - Glass slides (1 box)
    - 50 ml conical tubes
    - Syringes (see above)
    - Needles (see above)
    - Butterfly catheters (see above)

8) Equipment for pericardiocentesis:
- Evo or Mindray U/S machine
- Scrub and alcohol
- 60 cc regular tipped syringes
- Three-way stop cocks (2)
- Extension sets (2)
- 5 ¼ " IV catheter, smallest gauge available
- 100 mm dart needles (2) – in darting alcove cabinet
- Sterile urine cup to save for culture
- 50 ml conical vials for storage of fluid

EEHV Resources and Preparedness

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).

Protocol Review

The Houston Zoo's EEHV protocol should be reviewed every 1-2 years by both the veterinary (veterinarians and technicians) and husbandry teams.

Moreover, there should be a minimum of a once per year tabletop talk through the protocol and barn tour to make sure that all staff feel confident with EEHV response. Spontaneous drills requiring equipment may also be performed.

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EEHV Treatment sheet - BLANK Elephant _____ Date _____ **Day** __

		12am	2am	4am	6am	8am	10am	12pm	2pm	4pm	6pm	8pm	10pm
Behavioral	Attitude												
	Ate/ Drank												
	Defecated / urinated												
Visual	Mouth / neck												
Parameters	HR												
	Temp												
	BP												
Treatments													
Iv line													
Other tests	Echo, etc.												
Lab results													