

# HOUSTON ZOO ASIAN ELEPHANT EEHV PROTOCOL

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ASIAN ELEPHANT EEHV PROTOCOL  
HOUSTON ZOO, INC.

## EEHV “Fast Plan”

Initiate treatment if :

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

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

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

## COMMUNICATION PLAN AND CONTACT LIST

In the event of a suspect EEHV case, the following communication plan shall apply. The Elephant Manager will communicate with the members of the Elephant Team. This may take the form of assigning one Keeper the responsibility of contacting the balance of the team. The Curator of Large Mammals will notify the Director of Veterinary Services and the VP of Animal Operations. The Director of Veterinary Services will be responsible for communicating with the rest of the Veterinary Team. The VP of Animal Operations will be responsible for communicating with the Zoo Director and the Director of Public Relations. Cell phones should be called first and then home phones if necessary. A call back number should be provided so that staff members can confirm receipt of the message. *Phone numbers were removed from this edition- Please contact the Houston Zoo staff if you need contact information- MLM.*

## EEHV – DEFINITION

Elephant Endotheliotropic Herpes Virus (EEHV) is a rapidly fatal disease affecting mainly Asian elephants and is caused by similar, but genetically distinct novel herpes viruses. The onset of EEHV is sudden and death can occur as early as hours after the first clinical signs are observed, even without clinical signs, or the elephant may exhibit clinical signs for a week prior to death. Reproductive failures and young elephant deaths in North America and Europe have been attributed to EEHV. This is a particularly devastating disease for elephant managers and conservationists as it is young elephants that are most vulnerable. EEHV is a serious threat to all populations of Asian elephants.

## INCIDENCE OF EEHV

The disease was first described in 1995 in an Asian elephant at the National Zoo. Since then, dozens of cases have been identified in North American zoos dating back to the 1970s (through banked tissue samples). While most cases involve Asian elephants, there are 2 mortalities that have been documented in African elephants. In Europe (Germany, Switzerland, Netherlands), 18 cases of similar herpes virus infections have been identified in Asian elephants with two additional cases from Israel. Infection has developed in elephants ranging from 1-42 yrs of age, although most have become infected under age 7 yrs. Of these younger cases, most have occurred around the time of weaning (16-24 months of age).

Of the 37 North American cases, only 9 have survived. 8 of the survivors were Asian calves and 1 was an African calf. The survivors were under the age of 2 years that were treated with the anti-herpes drug Famciclovir. A few of the most recent of the surviving calves were treated with Ganciclovir after an initial treatment of Famciclovir. Recently, Baylor College of Medicine has been able to detect varying levels of virus in the blood and trunk washes of healthy animals.

## POTENTIAL EFFECT ON CAPTIVE POPULATIONS

Without treatment, all clinically apparent cases of EEHV viremia in elephants have led to fatalities. Prior to successful treatment of an Asian calf in 1997, EEHV was responsible for the deaths of approximately 18% of all Asian elephants born in North America since 1983. While the impact on African elephants has been less (one calf and one adult), the potential for marked mortality exists with this species as well.

## ETIOLOGIC AGENTS (THE VIRUSES)

For reasons not completely understood, herpes viruses can come out of latency and circulate through the bloodstream, going to other organs and causing disease. There is no cure for herpes viruses in animals or humans. Drugs can only suppress the growth of the virus.

Polymerase Chain Reaction (PCR) on DNA extracted from whole blood confirms active (viremic) cases of EEHV. This whole blood PCR test developed specifically for EEHV of both African and Asian elephants is, to date, the definitive test to diagnose the disease. Until recently, all elephants that tested PCR positive were gravely ill with all or many of the classic clinical signs of EEHV disease. With a new, more sensitive RT-PCR assay, Baylor College of Medicine has detected PCR positive elephants that never became clinical cases of EEHV.

It is thought that an elephant naive to EEHV will, at some point, be exposed to an elephant shedding EEHV and either seroconvert with unapparent/mild illness or develop disseminated EEHV disease. An elephant that makes antibodies to EEHV 1 has probably been exposed to the virus at some point during its life and is probably protected from getting the acute form of the disease with the same subtype of EEHV.

## PATHOGENESIS

The word “tropism” comes from the Greek word tropos (turn) and refers to the affinity or predilection that one object (usually animate) has for another (animate or inanimate). In the case of herpes viruses, most are epitheliotropic or have a predilection for epithelial cells. Target organs for these herpes viruses usually include skin, oral/urogenital mucosa, liver, adrenal glands, and the brain. In contrast, the viruses causing EEHV are endotheliotropic with a predilection for the capillary (smallest blood vessel) endothelial cells (cells that line the vessel wall) of the heart, liver, and tongue.

As mentioned, the mode of transmission is uncertain at this time. However, based on lesions and the course of the disease, the proposed pathogenesis is as follows: once the elephant becomes viremic (circulating virus in the blood stream), ensuing viral replication occurs in the heart and leads to endothelial cell damage with resultant capillary leakage and severe myocardial hemorrhage and edema. This damage can lead to cardiac failure due to disruption of the electrical conduction system of the heart, alterations in heart function due to increased swelling of cardiac muscle, myocardial ischemia (compromised delivery of oxygenated blood to tissue) with necrosis, and/or metabolic (e.g. potassium, calcium, ATP) derangement. The tongue cyanosis (“blue tongue”) often noted might actually be the result of cardiac insufficiency and decreased blood delivery to the other organs of the body.

## CLINICAL SIGNS

EEHV has a rapid onset and progression. Animals have died within 10 hours of the onset of clinical signs. With at least three of the animals that survived with treatment, clinical signs generally worsened for 1-2 days after the initiation of therapy and slowly dissipated over the course of 10-15 days. In most cases the first sign is an acute onset of lethargy. Decreased appetite and water consumption, and mild signs of colic may or may not be present. In one of the successfully treated cases, decreased food and water consumption coincided with an increased sensitivity to touch in the area of the tusks. Consequently, the abnormal behavior was initially attributed to discomfort associated with tusk eruption.

Historically, a great deal of significance has been placed on the development of a swollen and cyanotic “blue” tongue (cyanosis tends to progress from the tip caudally). However, this has not been seen in all cases or has developed several days after the onset of other clinical signs. Another clinical sign is the development of ulcers in the oral and pharyngeal cavity. While the examination of the tongue is somewhat easy, visualization of the hard palate, gum lines, and back of the throat can be more difficult in a calf, particularly an animal that is not yet clinically ill. Due to the rapid progression and onset of EEHV, it is imperative to evaluate the oral/pharyngeal cavity twice daily.

A more obvious indicator that EEHV is present is the development of subcutaneous edema. This edema is usually bilateral in distribution and often affects the head (especially the face and proboscis), neck, thoracic limbs, and flanks. In addition, edema and discoloration may be noted in the ocular conjunctiva. As edema/effusion can also develop in the respiratory system, regular measurements (2-3 times daily) of the respiratory rate should be made starting on day of birth, with special attention being paid to trends. The normal respiration rate in a one week old calf is about 20 breaths/minute; the adult rate is approximately 4-6 breaths/minute. Any marked change in rate or signs of increased respiratory effort should be reported.

Other vital signs should also be monitored daily because increases in heart rate and body temperature have been reported in cases of EEHV. The normal heart rate during week one of life is 115 bpm; 50-56 bpm in a 16 month old animal; and 25-35 bpm in an adult. Normal body temperature, taken by inserting a thermometer into a freshly passed fecal bolus is 36-37 °C. (97.5-99.0 °F.) with temperatures greater than 38 °C. (100 °F.) considered to be elevated.

Defecation should be closely monitored including frequency, stool quantity, and stool consistency as colitis/enteritis have developed in at least one Asian calf during the course of the disease, presumably due to damage to endothelial cells of capillaries in the gastrointestinal system. However, colitis/enteritis secondary to medical treatment can not be ruled out. Special attention should also be paid to body weight (measured daily or as frequently as possible), nursing behavior, urination, and overall activity/attitude. It is important to note that early behavioral changes may be subtle. It is also important to note that the signs listed below can occur in any order. The following clinical signs are associated with EEHV infections:

- Sudden death
- Lethargy
- Dullness
- Anorexia
- Mild colic
- Edema of the head, neck, trunk and thoracic limbs and ventral abdomen
- Cyanotic, swollen tongue: starts at tip and moves caudally typically
- Oral ulceration

- Stiff joints with no apparent discomfort, limping or lameness

Other clinical signs sometimes seen as the disease progresses include:

- Dribbling due to the swollen tongue
- Reduced trunk movement
- Ataxia
- Recumbency
- Difficulty in auscultation of the heart
- Weak, thready pulses
- Unresponsive to commands
- Sand eating
- Slight aggression towards keepers
- Lameness
- Discharge from trunk
- Decrease in frequency of urination
- Less playful
- Increased sleeping
- Changes in food preferences

#### DIAGNOSTIC TESTS

##### Complete Blood Count (CBC)

On presentation, affected animals often have an elevated white blood cell count (leukocytosis) with an absolute decrease in lymphocytes (lymphopenia). Occasional absolute monocytosis has been observed. Thrombocytopenia (decreased platelets) is usually present and anemia (decreased hematocrit, hemoglobin, and red blood cell count) is sometimes noted to varying degrees. As with clinical signs, the CBC profile may worsen for a few days even after the initiation of therapy. A follow-up CBC is important in tracking recovery or decline of the animal's condition.

##### Serum Biochemical Analysis (SBA)

Some elephants with EEHV demonstrate hypoproteinemia although it is uncertain if this is due to decreased production due to hepatic (liver) compromise, increased loss due to increased capillary permeability, or a combination of factors. Other SBA abnormalities noted in some, but not all cases, include elevations in fibrinogen, triglycerides, liver enzymes (LDH, AST, total bilirubin), and CPK due to injury/insult to the liver and muscle tissue, respectively. In addition, azotemia (elevated BUN and creatinine) has been seen in association with dehydration in one animal that clinically demonstrated decreased water consumption.

##### Urinalysis

Urine specific gravity is not an accurate indicator of hydration status in elephants. In a previous EEHV case, bilirubin was detected in the urine. Evaluation of the urine for RBCs or for protein may help to identify early renal compromise.

##### Polymerase Chain Reaction (PCR) Testing

This test is run on whole blood collected preferably in ethylenediaminetetraacetic acid, or EDTA (purple topped tubes) and is used to detect herpesvirus viremia. The blood sample is analyzed for evidence of any of the known strains of the viruses that cause EEHV. In addition to diagnosis, PCR can be used to monitor response to treatment as the test will move from strong positive to weak positive and finally to negative as the viremia is cleared. A shift from a positive to negative test may take between 8-14 weeks (data limited at this time).

#### CLINICAL CASE SUSPECT

The following clinical signs may indicate a possible EEHV infection. Any concerns should be brought to the Elephant Manager, Curator of Large Mammals and Veterinary Staff immediately.

- Lethargy
- Dullness
- Anorexia
- Mild colic
- Edema of the head, neck, trunk and thoracic limbs (and ventral abdomen)
- Cyanotic, swollen tongue: starts at tip and moves caudally typically
- Oral ulceration
- Stiff joints with no apparent discomfort
- Any changes in behavioral patterns

#### SAMPLE COLLECTION FOR A CLINICAL SUSPECT

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:

1. **Purple topped tube** (EDTA for whole blood 3 ml tubes): **2 tubes initially**
  - a. 1<sup>st</sup> priority is to collect 1 ml of blood for PCR at Baylor and 2 ml blood for National EEHV laboratory.
  - b. 2<sup>nd</sup> priority is to collect 0.5 ml of blood for CBC at HZI
  - c. 3<sup>rd</sup> priority is to collect 10 to 30 ml of whole blood for Cornell to try viral culture
  - d. 4<sup>th</sup> priority is to collect 2-5 ml of whole blood for BCM for sequencing
2. **Red/Grey topped tube** (serum separator for serum): **at least 8 to 58 ml of blood**
  - a. At least 2 ml of whole blood for serum biochemistry at HZI
  - b. At least 6 ml of whole blood for ELISA at National Zoo
  - c. 20 to 50 ml whole blood for Dr. Hayward at Hopkins for research.

**All blood samples should be brought to the HZI clinic laboratory for processing.** Based on the situation, the attending veterinarian will determine how best to distribute the blood. It will be processed in house for CBC and chemistry, and samples will be sent to Baylor College of Medicine, the National EEHV Laboratory in Washington DC, and Cornell College of Veterinary Medicine. Shipment guidelines and instructions can be found on Page 24.

#### TREATMENT FOR SUSPECTED OR CONFIRMED EEHV

A suspect/confirmed elephant may be locked in the barn. Calves and/or subordinate animals may be accompanied by other herd mates for companionship. Once treatment starts the suspect animal will be separated from contact with the other elephants and may be restrained with the use of leg restraints. Herd management during treatment will be based on the decision of the Elephant Manager and/or the Curator of Large Mammals.

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, VP of Animal Operations or the Zoo Director.

#### Anti-viral therapy

Anti-viral therapy needs to be started immediately on an elephant that is clinically ill with EEHV signs, has a VGE of 5000 or more, or has rapidly increasing viral loads. Treatment may often start without a confirmed diagnosis of EEHV, as confirmation by PCR may take several hours. If delayed, therapy is unlikely to be efficacious. Treatment needs to be aggressive from the beginning and may involve management changes, antiviral and supportive therapy. Treatment will be directed at the causative virus, supportive care for the animal, and controlling secondary infections that could arise.

Anti-viral drugs work to inhibit viral replication, but by themselves, do not correct the damage done by the virus to the animal's cells. It is important to treat the virus as soon as possible in an infection to prevent further cellular and tissue damage. The two drugs that have been used to treat EEHV are Famciclovir and Ganciclovir. Famciclovir has been used in more cases and it has the convenience of being administered via oral or rectal routes. The disadvantages are that it may not be the most effective drug to use against EEHV. It is used in human medicine to treat alpha herpes infections, and is less effective in cases of disease caused by beta herpes viruses. Absorption in healthy animals is good through oral and rectal routes, however, animals clinically affected by EEHV may not have effective absorption and distribution of the drug due to the cardiovascular effects of the disease. Ganciclovir is a drug used in human medicine to treat beta herpes virus infections. It is administered twice a day, intravenously over the period of 1 hour to achieve adequate blood levels. It is thought to be a better choice in treatment of EEHV, but pharmacokinetic studies have not been performed in elephants, so dosages have been extrapolated from human patients.

The decision whether or not to start an elephant on an antiviral medication will be made by the Elephant Manager, Curator of Large Mammals and Veterinarians. If an elephant is suspected or confirmed pregnant, this condition should be taken into consideration when deciding to treat with anti-viral medication. Discussion on whether to treat with Famciclovir or Ganciclovir should include Zoo Director.

The decision of whether to treat a suspect case with oral or intravenous anti-viral medication will be based on the animal's clinical condition and on confirmation of infection via positive PCR test. In most cases antiviral medication will be started orally but if a patient that is clinically ill is confirmed as PCR positive, or if clinical signs and/or viral load in the blood (as measured by RT-PCR at BCM) progress despite oral treatment, then intravenous anti-viral medication (Ganciclovir) is recommended.

Once an animal is started on an antiviral medication, the medication must be continued until follow-up viral load (as measured by RT-PCR at BCM) is negative, if it drops and remains below 5,000 vge/ml, or if attending veterinarians and elephant care managers elect to discontinue treatment based the situation or on other new information.

**Antiviral Drugs:**

Famciclovir: Although only 9 cases have survived to date, the use of Famciclovir (Famvir, SmithKline Beecham Pharmaceuticals, Philadelphia, Pennsylvania 19101, USA) was used in six of the nine cases as the primary antiviral treatment. Researchers are still not sure if Famciclovir is effective against the EEHV virus. Famciclovir is an orally administered pro-drug of the anti-herpes agent penciclovir to which it is quickly biotransformed upon consumption by the patient. One pharmacokinetic study performed in 2003 by Isaza recommended a dose of 8-15 mg/kg PO or rectally TID in Asian elephants. Famciclovir is a Class B pregnancy Drug (Class B: Animal studies have not yet demonstrated a risk to the fetus, but there are no adequate studies in pregnant women). There have been no effects on embryo-fetal development in studies on rats and rabbits at 2 to 10 times the human dose. Famciclovir has caused mammary tumors in female rats and testicular toxicity in male rats when given very large dosages for 10 weeks or more.

There are multiple dosage recommendations for Famciclovir treatment in elephants.

- Famciclovir 12 mg/kg QID first day then BID for 3 weeks, PO or rectally – from EEHV Protocol (Cracknell, et al, 2005)
- Famciclovir 8-15 mg/kg TID PO or rectally – from pharmacokinetic study (Isaza, et al, Proc AAZV, 2003)
- Famciclovir loading dose of 12.8 mg/kg PO, then Famciclovir 6.4 mg/kg PO TID for first 4 days, then decrease to BID (from case report, Schmitt, et al, JZWM, 2000)
- Famciclovir 10.6 mg/kg BID per rectum as a paste for first three days, then 6.7 mg/kg BID (from case report, Schmitt, et al, JZWM, 2000)
- Formulation: Famciclovir, Famvir 500 mg tablets, 30 tablets/bottle, kept in the pharmacy

The Famciclovir Dosage for HZI elephants:

Famciclovir 15 mg/kg QID initially PO or per rectum.

May decrease to 15 mg/kg TID after an improvement in clinical signs or viral load is seen.

- See chart below for quick dosing for HZI elephants – Removed weights –MLM

Famciclovir Dosing for HZI elephants, 15 mg/kg PO QID initial dose			
Elephant	Body Wt (7/7/10)	Drug dose (mg)	# tablets/tx, (# bottles/day)

Ganciclovir: Ganciclovir (Cytovene – IV, Roche Laboratories Inc. Nutley, New Jersey, 07110) is an antiviral medication used to treat humans for cytomegalovirus, which is a beta-herpesvirus (similar to EEHV). It has been used three times in active EEHV cases and no pharmacokinetic studies have been done in elephants. It was used to treat a 2 year old hand raised Asian elephant calf in 2009. She was treated with 5 mg/kg Q 12 hrs (this is the recommended human dosage), initially intravenously and then orally. Ganciclovir has poor bioavailability orally, however the manufacturer has recommended a recipe to mix the intravenous powder into a form that can be given orally (see recipe in treatment section below). The calves that were treated with Ganciclovir did survive infection. In these elephants, post treatment Ganciclovir levels have not been detected and absorption of PO ganciclovir has not been confirmed.

In people, treatment with Ganciclovir is associated with side effects such as anemia, low platelet counts, neutropenia, and renal impairment (elevated BUN and Creatinine). Ganciclovir is a Category C drug for pregnancy (Class C: Animal studies have shown an adverse effect on the fetus, but there are no adequate studies, or there are no animal reproduction studies). It has been shown to be embryotoxic in rabbits and mice following IV administration and teratogenic in rabbits. It may be teratogenic or embryotoxic at dose levels recommended for human use.

The Ganciclovir Dosage for HZI Elephants:

- 5 mg/kg IV Q 12 hrs to be given slowly over 1 hour IV in 1 to 2 liters of NaCl
- Should not be given intra-arterially
- Formulation: Ganciclovir, Cytovene IV, sterile vials contain 500 mg powder, 25 vials/box, kept in the pharmacy on the left hand side.

Ganciclovir Dosing for HZI elephants, 5 mg/kg IV BID			
Elephant	Body Wt (7/7/10)	Drug dose (mg)	# tablets/tx, (# bottles/day)

Ganciclovir PO Oral Suspension Recipe\*

- PO dose: 5 mg/kg of home made suspension PO Q 12 hrs – it has not been confirmed that Ganciclovir has bioavailability when given orally.
- Oral Dose: 5 mg/kg of home-made Ganciclovir suspension, PO Q 12 hours
- Example: Recipe for a 1675kg elephant
  1. Ganciclovir Oral Suspension (based on recommendation from Roche chemists)
  2. Use 17 vials ganciclovir injectable (500 mg / vial) per dose.
  3. Mix each vial with 3 ml sterile water.
  4. Withdraw dissolved drug from each vial, place together in one clean beaker.
  5. Add 170 ml oral sweetener solution (such as simple syrup, or Orasweet, OTC syrup at any drug store)
  6. Add 3.4 ml 3% H<sub>2</sub>O<sub>2</sub> (=hydrogen peroxide)
  7. Mix well, add sweetener to total volume of 340 ml
  8. End Product: 25 mg/ml suspension
  9. Dose: 340 ml = 8,500 mg PO twice daily

Since we don't know the shelf life of the suspension, the suspension should be mixed up fresh prior to each treatment, anything left over should be discarded

**\*there is no pharmacokinetic data to support the suggestion that this suspension is absorbed orally in elephants**

INTENSIVE CARE OF THE EEHV PATIENT

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

Sedatives may be administered to facilitate treatment and to manage pain. Low doses have been safely used in clinical cases. Opioids are preferred to the use of non-steroidal anti-inflammatories due to the latter's effects on the urinary system. Antibiotics have no effect on viral infections, but will be given to affected animals to prevent secondary infections with bacterial organisms. Initial doses will be administered intravenously. Once the animal is removed from IVs, a change to intramuscular or oral products will be made if appropriate.

Rectal Fluid Therapy

Fluids can be administered rectally to an elephant that is dehydrated but ambulatory or to partially rehydrate an elephant prior to anesthesia and placement of an intravenous catheter. Rectal fluids can be given in boluses of up to 20 ml/kg, with evaluation of patient vital signs and attitude after each bolus. Retention is aided by pausing if the elephant starts to strain and by holding the tail down firmly following administration.

Sedation

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



## Intravenous Catheter Placement

An IV catheter can be placed in an ear vein, a larger bore IV catheter can be placed in a cephalic vein (proximal medial forelimb) or a saphenous vein (lower medial aspect of hindlimb). Veins may be hard to visualize in a recumbent, anesthetized/weak elephant, the Sonosyte US machine may help to visualize vessels. A 14 g 2 inch catheter can be placed in the cephalic vein but may require a surgical approach. It can be sutured in with o PDS. Bupivacaine can be infused around the catheter placement site to provide local anesthesia. Once the catheter is placed, it must be thoroughly secured to prevent removal by the elephant. Consider placing sponges on either side of the the catheter hub and duct taping in place to provide a cushion or buffer for the catheter itself.

Recently, an elephant in an intensive care environment died due to complications from MRSA infection. Attention to hygiene and biosecurity is very important in elephants being treated for EEHV, particularly due to their immunocompromised status. Frequent handwashing, prompt removal of waste products, and regular sanitizing of equipment are recommended. Any handling of the intravenous catheter or associated fluid lines should be done with gloves.

## **Intravenous Fluid Therapy**

Intravenous fluids are recommended to support circulation and hydration. Physiologic crystalloids such as lactated Ringer's or Normosol can be used for rapid rehydration or for maintenance fluids. Sodium chloride should be used if the elephant is hyponatremic or hypochloremic, and/or if the elephant is on diuretics. It is important to remember that rapid infusion of large volumes of crystalloids in patients that have "leaky" capillaries due to viremia may result in moving fluids out of vessels and worsening edema.

An initial 20 ml/kg bolus of IV fluids (or 10 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.

Maintenance fluid therapy requirements have not been determined for elephants but are assumed to be similar to other mammals (Mikota, 2006):

- Maintenance (adult) = 2ml/kg/hour = 2 liters/1000kg/ hour
- Maintenance (calf) = 4ml/kg/hour = 4 liters/1000kg/hour
- Surgical rate = 10ml/kg/hour = 10 liters/1000kg/hour
- Shock rate = 90ml/kg/hour = 90 liters/1000kg/hour
- Volume replacement fluid (litres) = Body weight (kg) x percentage dehydration

If the elephant is ambulatory, it will be necessary for staff to hold IV poles and follow the elephant around the stall. Five liter IV bags can very heavy. Flagpole holders can be used by staff to help keep IV poles upright and mobile.

## **Plasma Transfusion**

Colloids such as fresh or frozen plasma, or hetastarch, are often more effective than crystalloid fluids for volume expansion in viremic or seriously ill animals. The larger molecules in these fluids do not leak out of capillaries as easily, and increase plasma volume. Additionally, animals with active infection are not expected to have antibody to the virus. If it is available, a plasma transfusion from a donor with a high antibody titer may help bind up virus particles in the patient.

Plasma should only be administered intravenously after cross-matching donor and recipient blood samples to assure compatibility. Based on equine recommendations, plasma should be administered at an average rate of 10 ml/kg/hr. The first 100 ml should be given slowly, and heart rate, respiratory rate, and temperature should be monitored. Possible transfusion reactions would include fever, rash, or anaphylaxis. Mild signs can be treated with antipyretics or antihistamines and 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 to 15-25 ml/kg/hr.

## **Blood Tansfusion**

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

There are no known blood types in elephants; cross matching is recommended prior to transfusion, any agglutination or lysis indicates an unacceptable match.

To perform a cross match:

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

### **Oxygen Therapy**

Supplemental oxygen therapy should be administered, when possible, to all patients undergoing treatment for EEHV. Oxygen can be administered at 2 to 4 l/hr via a flexible plastic tube passed into one side of the trunk. If the elephant will not tolerate oxygen therapy while awake, consider sneaking the tube into the trunk while the elephant is sleeping.

### **Antibiotics**

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

See Drug dosing chart for specific information.

### **Analgesia**

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

Administration of omeprazole for gastrointestinal protection during nSAID treatment should be considered. The equine dose is 0.7 - 1.4 mg/kg PO once daily.

Opioids are also a useful adjunct to providing relief and in some cases mild sedation to assist in the management of animals being treated. Be aware that there is the possibility with behavioral changes in the elephant when using opioids and that animals should be treated with extra care, as trained behaviors may well be lost or less responsive.

See Drug dosing chart for specific information?

Vitamin E has been suggested as a supportive measure. The equine dose is: 8000-9000 IU per day for adjunctive therapy for EPM. IM Injections can cause transient muscle soreness, most doses are listed as PO SID.

### **DAILY MONITORING OF AN EEHV CASE**

#### **Monitoring of Suspect/Confirmed Positive Elephant**

If the elephant is not critically ill or on intravenous therapy, a veterinary physical examination will be performed twice daily during the first week of treatment. If the elephant is placed on intravenous therapy and Ganciclovir treatment, 24 hr monitoring by veterinary and elephant staff will be instituted. Regular measurements of vital signs, including respiratory rate, heart rate, blood pressure, and body temperature are to be made starting Q1 to 6 hrs on the first day that EEHV is

suspected. (Refer to P: drive document for each animal's normal values). Body weight should be taken daily (if possible) to evaluate hydration status. A daily ultrasound of the heart will be performed to evaluate heart rate and contractility and also monitor for the development of pericardial effusion. Elephant care staff will monitor behavioral parameters.

Daily blood samples for first week of treatment (listed in order of priority):

- Whole blood (total 6-8 ml daily) – CBC (0.5 ml), EEHV PCR at BCM (3-6 ml) and National EEHV lab (2 ml).
  - PCV/TP should be monitored TWICE daily
- Serum (red/grey top, total 8-20 ml daily) – serum biochemistry (2 ml), EEHV ELISA at National EEHV Lab (5 ml)
  - BCM would like 10-100 ml in serum topped tubes once, as early in disease course as possible
- Serum (royal blue top tubes, 6 ml weekly) – mineral panel for baseline
- Serum (plain red top/non-serum separator, 5 ml daily)
  - Ganciclovir levels (2 ml serum)\*\* possibly twice daily after treatment
- If Ganciclovir is used, close monitoring of CBC, Creat/BUN, HCT, and urine production is recommended.
- If Famciclovir is used, penciclovir levels may need to be monitored.

Urine samples: (50 ml conical vials, total 30 ml daily) – HZI urinalysis (5 – 10 ml), EEHV PCR at BCM (20- 30 ml).

Fecal sample: daily fecal sample collected and frozen for future analysis.

Upon request, ocular swabs and oral swabs may need to be provided.

#### Surveillance of Herd Mates

If a clinically ill elephant is confirmed EEHV PCR positive, then samples should be collected from the rest of the elephant herd for EEHV testing at the Baylor College of Medicine and the National Zoo EEHV Lab.

- Whole blood (6-8 ml total from each elephant) – BCM PCR (3-6 ml twice weekly), National EEHV Lab PCR (2 ml), HZI CBC (0.5 ml)
- Serum (12 ml whole blood from each elephant) – National EEHV Lab ELISA (5 ml), HZI serum biochemistry (2 ml), BCM 5 ml once weekly
- Urine (30 ml total from each elephant) – HZI urinalysis (5-10 ml), BCM PCR (20-30 ml)
- Trunk washes (70 ml total from each elephant) – BCM PCR (30-50 ml), National Zoo EEHV Lab PCR (20-30 ml)
- Ocular swabs – one from each eye to National Zoo EEHV Lab PCR
  - 1 from each eye to National Zoo EEHV lab (PCR)

#### ROUTINE SURVEILLANCE AND SAMPLING

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.

#### DAILY HERD MANAGEMENT

- Visual inspection of mouth, tongue, palate for ulcers, lesions, discoloration, or visual changes.
- Visual inspection of the elephants. Looking for swelling or abnormalities in the animals overall appearance.
- Assessment of the animals' appetite.
- Assessment of the animals' responsiveness to cues and stimuli.
- General assessment of the elephants overall attitude and appearance (respiration rate, locomotion, coordination, etc.).
- Daily temperature readings on all elephants (bolus, Life Chip-study).
- Twice weekly blood pressure readings on all elephants.
- Daily Blood pressure readings on calves.
- Data collected will be recorded and shared between the elephant and veterinary teams (see Vital Signs Monitoring below).

#### BEHAVIORAL TRAINING

Successful diagnosis and treatment will depend on the ability to access the animal for visualization, sample collection, and treatment, including oral, rectal, and 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
- Urine collection
- Body temperature measurement (fecal bolus, rectal, life chip)
- Blood pressure measurement (cuff on base of tail)
- Oral exam
- Accept oral and rectal medications
- Auscultation of heart w/stethoscope
- Ultrasound of heart

**\* Any concerns, however minor, MUST be reported to the Elephant Manager and the Curator of Large Mammals, immediately. Veterinarians will be contacted by either the Elephant Manager or the Curator of Large Mammals. If there are questionable signs and the Elephant Manager or Curator is not available, the on-duty Veterinarian will be notified as soon as possible.**

**\* Keepers are the first line of defense against EEHV. Observing signs of EEHV early is what will save an elephant's life. Keepers should not make excuses about why signs are occurring and just observe and report that the elephant is off. It is better to have fifty false alarms and be overly cautious than have one sick elephant go undetected.**

**This protocol is to be adhered to without exception.**

#### VITAL SIGNS MONITORING

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 give us important information for assessing any elephant that may be suspect for EEHV or other disease problems. Respiratory rates: baseline respiratory rates will be established for all elephants. Heart rates: the indirect blood pressure monitor gives this, however the portable Sonosite ultrasound can be also used to visualize Tucker's heart and a heart rate can be counted off the screen.

Blood pressure will be monitored daily on young elephants to twice weekly in adults. The blood pressure cuff is an off-the-shelf item from 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 will be recorded on both the handwritten document and logged into a spreadsheet 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 will be monitored and recorded daily on all elephants using either temperature measurement of a fresh fecal bolus or the use of handheld Pocket Reader to read the Life Chip implants. Temperatures in excess of 100° F. should be considered elevated. HZI is currently evaluating the relationship between bolus temperature and the temperature read by Life Chip implants in the hope that we can ultimately rely on the reading from the implant.

#### Elephant Vital Sign Data

#### SAMPLE COLLECTION

In an effort to discover subtle changes which may indicate early signs of infection, or detect viral shedding in apparently healthy animals, biological samples will be collected and analyzed regularly. These samples will include blood (for PCR at BCM, PCR and ELISA at National EEHV lab, and in-house CBC and serum chemistry), urine (for urinalysis and PCR at BCM), and trunk washes (for PCR at BCM). Other samples may be included when and if indicated. See below for more specific sampling information. Samples collected will be recorded (see Appendix: Elephant Specimen Collections).

\* If any of the above assessments or diagnostics are off or differ from the norm, the animal will be immediately considered to be suspect for EEHV.

Blood samples will be obtained weekly for routine health monitoring and to help study the spread of EEHV virus and contribute to development of treatment protocols. 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 (BCM) and the National EEHV Laboratory at the National Zoo for EEHV PCR and ELISA testing.

See *EEHV Sampling Guidelines Quick Chart* in excel for summary of information on sampling. Weekly blood collection for EEHV surveillance should include filling at least:

- 2 purple topped tubes (3 ml capacity each, total 4-6 ml)
- 1 red/grey topped tube (12.5 ml capacity)

#### Purple Topped Tubes (EDTA anticoagulant, 3 ml capacity)

- Fill all EDTA tubes at least half way with blood so the blood is not diluted with EDTA.
- If less than 1.5 ml of whole blood is collected, place sample into small 0.5 ml plastic purple topped microtainer tubes, to prevent sample dilution
- HZI laboratory needs 0.5 ml whole blood for CBC on calves less than 6 years old weekly
- National Zoo needs: 1 - 2 ml whole blood for EEHV PCR
- Samples stored in the -80 freezer and sent to National Zoo quarterly
- Baylor CM wants 1 ml whole blood for EEHV PCR
- Samples taken over to BCM laboratory the day of collection, on ice in a cooler

#### Red and Grey Topped Tubes (Serum separator, 12.5 ml capacity)

- HZI Laboratory needs 1 ml serum (= 2 ml whole blood) for serum biochemistry
- National zoo wants at least 2 ml serum (= 5 ml blood) for EEHV ELISA
- Samples stored in the -80 freezer and sent to National Zoo quarterly

Urine samples will be collected weekly from all elephants for diagnostic testing for EEHV at BCM.

- Urine (minimum 30 ml) will be collected mid-stream and stored in plastic 50 ml conical vials
- A complete urinalysis will be performed on urine samples at the HZI lab on the day of collection
- Samples will be split by the Elephant team. Approximately 5 ml will be submitted to the clinic and the remainder of the sample will be taken over to the BCM laboratory on ice in a cooler. Samples should be stored in the refrigerator until delivery to BCM.

Trunk wash samples will be collected weekly from all elephants for diagnostic testing for EEHV at BCM.

- Trunk washes (minimum 30 ml of fluid recovered) are collected using 60 ml sterile saline infused into the trunk, then collected into clean buckets and stored in 50 ml conical vials.
- Samples will be taken over to BCM laboratory on the day of collection.

#### PLASMA

Large volume blood collections are performed frequently. Plasma is collected and stored for potential future treatment for critically ill elephants. Whole blood is collected into a sterile closed-system 450 ml collection bag containing Citrate phosphate dextrose adenine solution (CPDA-1) USP as an anticoagulant. The bag should be labeled as to how full it is (1/4, 1/3, 1/2, etc), as well as the date, time and elephant's ID number. If a bag is less than 1/3 full, the blood should be banked in the HZI clinic -70 freezer for use as possible future research samples. If a bag is more than 1/3 full, it should be hung under refrigeration for 24 hrs to allow gravity sedimentation. After that time, the plasma will be separated off and placed in the HZI clinic -70 freezer for storage, and for potential future therapeutic use.

#### CLINICALLY HEALTHY ANIMALS WITH POSITIVE RESULTS

Research by BCM regarding EEHV epidemiology and diagnosis is ongoing and will be continually adding to the base of knowledge regarding this disease. Houston Zoo elephants that are clinically normal have been detected as “weak positive” on the BCM EEHV RT-PCR test, indicating a low level of viremia. Previous to this discovery, any positive PCR result on whole blood sample was indicative of active EEHV infection.

Evaluation of whole blood viral loads in 3 elephants 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 3-13 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 number may indicate impending onset of clinical disease. HZI elephants have had whole blood EEHV PCR results up to 588 vge/ml on routine samples without evidence of clinical disease. It is important to remain flexible and interpret new information as it is available, rather than adhering to a rigid or outdated protocol.

With all of the information available to date, **HZI has established 5,000 vge/ml as a whole blood viral load at which treatment would be initiated**, even if the elephant is clinically normal. If the viral load is rapidly climbing, treatment could be initiated prior to reaching this number.

If a clinically normal elephant has a positive PCR result on a routine whole blood sample for the first time, blood samples should be collected daily for 1 week to establish the course of viremia. If enough blood is available, CBC and chemistries should also be run. At that point, based on the viral load levels and trend, clinical condition, and other conditions within the herd, the frequency of testing may be reduced to twice weekly.

A clinically normal elephant (including pregnant female) with a positive PCR result that approaches 5,000 vge/ml or has a viral load that is rapidly increasing will be started on anti-viral treatment. Treatment options include oral famciclovir or intravenous ganciclovir (recommended for clinically ill animals or ones with rapidly increasing viral loads).

It should be kept in mind that several clinically normal, weak positive viremic elephants have been documented to shed large amounts of virus in their trunks following the onset of viremia. This may have implications for other members of the herd and may result in some management decisions such as separating elephants out for a period of time.

Therapy decisions and implementation will be made in concert with veterinary, animal care, management, and consultant input.

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## NECROPSY AND POST MORTEM SAMPLE COLLECTION

### Necropsy Procedures

Whole heart blood should be collected into EDTA tubes **immediately** post mortem.

- 10-30 ml should be sent immediately to Cornell on ice packs
- Additional 30 to 60 ml of whole heart blood should be collected and stored in anticipation of sending to Dr. Gary Hayward or for other diagnostic purposes

A 4 cm X 4 cm piece of tongue should be collected **immediately** post mortem – see Cornell information below for storage and shipment of this sample.

The lesions of EEHV are nearly identical 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.

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 above (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.

Photographs should be taken of all gross lesions.

Three complete sets of tissue samples should be collected from each organ and placed into formalin at a 10:1 ratio.

1. 1 set to go to Dr. John Edwards, TAMU pathologist, for diagnostic purposes
2. 1 set to be kept at the Houston Zoo
3. 1 set to be shared with the Elephant SSP Pathologist (new SSP necropsy protocol pending)

In addition to collecting samples for histopathology, collection of tissues for diagnostic and research purposes is very important to furthering our understanding of EEHV.

### Fluid Collection

- The following fluids should be collected during post mortem examination:
  - Ascites (20 to 60 ml)
  - Whole heart blood (20 to 60 ml)
  - 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 sent to Cornell for possible viral culture
  - Shipment information on page 24
  - Cornell will be sending us a “Post Mortem Kit” including EDTA tubes
- 10 to 30 ml of each should be sent to the National Zoo EEHV Laboratory for diagnostics and evaluation (they will share their samples with Dr. Gary Hayward)
  - Shipment information on page 24

### Tissue Collection

The following tissues should be collected in addition to the formalin samples: liver, heart, lung, kidney, spleen, tongue, skeletal muscle, brain, and any grossly abnormal tissues or tissues with significant hemorrhages.

Three sets of each tissue listed above should be collected:

Set #1: To Cornell

- Each tissue should be cut in samples measuring 2-3 cm X 2-3 cm
- Each should be placed in a separate sterile vial with viral transport media
- Cornell will be sending us a “Post Mortem Kit” with tubes and media
- Tubes should be labeled with tissue and elephant name and closed tightly
- Containers should be wrapped with absorbent diaper and sealed, then placed in two plastic bags
- Samples should be sent as early as possible on ice packs per shipment instructions on Page 24.

Set #2: To National Zoo EEHV Laboratory

- Each tissue should be cut in samples measuring 2-3 cm X 2-3 cm
- 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 on Page 24.

Set #3: To be kept 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



## EQUIPMENT AND SUPPLIES

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

### Elephant Barn supplies:

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

### Clinic Supplies:

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

- Famciclovir 500 mg tablets, 30 tablets/bottle: 15,000 mg / bottle
  - Minimum 3 day supply for Thai, which is 30,000 mg PO TID, 6 bottles/day = 18 bottles
- Mortar and pestle (to grind in case rectal administration is necessary)
- Ultrasound gel (for mixing with famvir for rectal administration)
- OB sleeves and lube
- Duct tape
- Hydrogen peroxide (minimum 1 bottle)
- Orasweet OTC syrup (minimum on hand 4,500 ml)
- Exam gloves (all sizes)
- Towels (10-12)

#### 2) Rectal Fluids

- To be administered if elephant was NOT given famciclovir rectally
- To be administered by elephant team via warm water hose
  - (initial bolus 20 ml/kg)

#### 3) Standing sedation

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

#### 4) Oxygen-portable anesthesia machine

- Portable oxygen tanks
  - Plastic tubing for nasal oxygen administration
- ET tubes (40, 30, 24, 22, 20)
- Catheter type stylets for intubation
- Laryngoscope w/long blade
- Y piece (nasal administration)
- Ropes (open mouth)
- Blocks (open mouth)
- Pulse oximeter and capnograph
- I-stat
- Stethoscope
- Thermometer
- Head lamp
- Flash light
- Endoscope and associated equipment (intubation)

#### 5) Placement of IV Catheter

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

#### 6) IV Ganciclovir (Cytovene)

- Ganciclovir 500 mg vials, 25 vials / box: 12,500 mg/ box
  - Minimum 3 day supply for Thai: 29,000 mg PO BID 5 boxes/day = 15 boxes
- 100 ml bottle of sterile water to mix with ganciclovir powder
- 2 liters NaCl to administer during ganciclovir
- Large syringes (20-60 ml) for mixing up ganciclovir

#### 6) Monitoring

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

- Ophthalmoscope extra battery
- Culturettes

#### 7) Drugs (see chart)

#### 8) Equipment for pericardiocentesis:

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

#### SHIPMENT GUIDELINES FOR EEHV SAMPLES

**National EEHV Laboratory ; Baylor College of Medicine; Cornell College of Veterinary Medicine Johns Hopkins University / Dr. Gary Hayward**

*Please contact the Houston Zoo staff if you need contact information- MLM.*

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## DRUG DOSAGES

	Drug	Concentration	Dosage range	Comments
<b>Sedation</b>	Butorphanol IM	10 mg/ml	0.045-0.075 mg/kg	Reverse with Naltrexone
	Naltrexone reversal	50 mg/ml	2.5-5 x Butorphanol mg	
	Detomidine IM	10 mg/ml	0.005-0.011-0.022 mg/kg	Give 15-20 min after Butorphanol, reverse with Atipamezole
	Atipamezole reversal	5 mg/ml	5 x detomidine mg	No notes regarding using as a Detomidine reversal
	Azaperone		0.024-0.038 mg/kg IM	
	Yohimbine reversal		0.125 mg/kg IV	
<b>Treatments</b>				
	Famciclovir	500 mg/tab	8-15 mg/kg QID	
	Ganciclovir IV	500 mg/bottle	5 mg/kg BID	Give with NaCl slowly over 1 hour, mix each vial with 3 ml sterile water
	Ganciclovir oral	500 mg/bottle	5 mg/kg BID	See recipe for mixing up oral dose
<b>Fluids</b>				
	Maintenance adult		2 ml/kg/hour	
	Maintenance calf		4 ml/kg/hour	
	Surgical rate		10 ml/kg/hour	
	Shock rate		90 ml/kg/hour	
<b>Analgesics</b>				
	Buprenorphine		0.004 mg/kg IV	Equine dose, given with Acepromazine or Xylazine
	Meperidine		0.75-1.5 mg/kg Q6hr	
	Morphine analgesia		0.03-0.06 mg/kg QID IM	
	Morphine sedation		0.06-0.2 mg/kg IM	
	Butorphanol		0.015 mg/kg IV/IM	
	Xylazine	100 mg/ml	0.04-0.08 mg/kg IM/IV	May cause sedation at this dose, caution in doses >400 mg
	Medetomidine		0.003-0.005 mg/kg IM	Possible sedation
<b>Analgesics</b>				
	Flunixin meglumine	50 mg/ml	0.8 mg/kg S-BID IM	
	Meloxicam		0.6 mg/kg SID IV-PO	
	Carprofen		0.7 mg/kg SID IV/PO	Anecdotal
	Ketoprofen		1-2 mg/kg Q24-48hr PO/IV	
	Phenylbutazone		2.2-4.4 mg/kg SID PO	Do not use in ear veins, Equine dose, Max 5 days

	Drug	Concentration	Dosage range	Comments
	Aspirin		10 mg/kg S-BID PO	Equine dose
	Ibuprofen		6 mg/kg BID PO	
	Methadone		0.03-0.06 mg/kg IM	Anecdotal?
<b>Antibiotics</b>	Amikacin XXX	250 mg/ml	3-5 mg/kg SID IM/IV	
	Amoxicillin		11 mg/kg SID IM	Amp sodium = IV, Amp trihydrate = PO
	Ampicillin		8 mg/kg B-TID PO	
	Ceftiofur (Naxcel) XXX		1.1 mg/kg B-TID IM	1.1 mg/kg SID IV (PD)
	Ceftiofur-CFA (Exceed)	100 mg/ml	5 mg/kg Q7d IM	
	Doxycycline		10 mg/kg BID PO	No elephant or equine doses available
	Enrofloxacin		2.5-5 mg/kg SID PO	
	Florfenicol		20 mg/kg IM Q48hr	Cattle dose
	Marbofloxacin		2 mg/kg SID IV/SQ/PO	
	Oxytetracycline		20 mg/kg Q48-72 hr IM	
	Penicillin-Dual	300000 IU/ml	2275-4545 IU/kg Q48hr IM	
	SMZ-TMP		20 mg/kg B-QID PO	
<b>Emergency</b>	Aminophylline	25 mg/ml	11 mg/kg BID PO/IV	IV dose should be diluted in 100 ml D5W or Saline
	Atropine (LA)	15 mg/ml	0.015-0.05 mg/kg IM/IV/SQ	
	Calcium gluconate 23%	1 mEq Ca/ml	0.7 mEq/kg IV	Give IV slowly, to effect
<b>Emergency</b>	Doxapram HCL	20 mg/ml	0.4 mg/kg IV/IM	
	Epinephrine 1:1000	1 mg/ml	0.02-0.2 mg/kg IV/IC/IT	
	Lidocaine 2%	20 mg/ml	0.05-0.5 mg/kg IV	To correct heart block
	Dexamethasone	2 mg/ml	0.2-2.0 mg/kg IV/IM	Equine dose
	Sodium Bicarbonate 8.4%	1mEq/ml	0.5-1 mEq/kg IV	Equine dose
	Solu-Delta Cortef	20 mg/ml	0.25-1.0 mg/kg IV/IM/SQ	
<b>Other</b>	B-complex		5 ml SID IM	
	Furosemide x 5 days	50 mg/ml	0.8 mg/kg BID IM/PO	
			0.67 mg/kg SID IM	

## Sample Guidelines Quick Chart

	Routine Weekly Survey	Clinical Suspect (sick elephant)	Herdmates (of sick elephant)	Daily Monitoring (of sick elephant)
<b>Purple Top Tubes</b>  (whole blood)  3 ml tubes  or  1.5 ml microtainers	TOTAL: 4-5 ml blood  1 ml blood BCM (PCR)  1-2 ml blood National (PCR)  0.5 ml HZI (CBC)	TOTAL: 6 to 40 ml blood  1 ml blood BCM (PCR)  0.5 ml blood HZI (CBC)  2 ml blood National (PCR) 10-30 ml blood Cornell (culture) 2-5 ml blood BCM sequencing	TOTAL: 6-8 ml blood  0.5 ml blood HZI (CBC) 3-6 ml blood BCM (PCR) twice weekly 1-2 ml blood National (PCR)	TOTAL: 6-8 ml blood 0.5 ml blood HZI (CBC) 3-6 ml blood BCM (PCR) 2 ml blood National (PCR)
<b>Red/Grey Top Tubes</b>  (amount of whole blood to collect) to be spun down for serum  12.5 ml tubes	TOTAL: 7 ml blood  2 ml blood HZI (chem)  5 ml blood National (ELISA)	TOTAL: 8 to 58 ml blood  2 ml blood HZI (chem) 6 ml blood National (ELISA) 20-50 ml Hopkins (research)	TOTAL: 12 ml blood  2 ml blood HZI (chem) 5 ml blood National (ELISA) 5 ml blood BCM once weekly	TOTAL: 8-20 ml blood 2 ml blood HZI (chem) 5 ml blood National (ELISA) 10-100 ml blood BCM
<b>Urine</b>  50 ml conical vials	TOTAL: 30 ml  5-10 ml urine HZI (urinalysis)  20-30 ml BCM (PCR)	TOTAL: 60 ml  5-10 ml urine HZI (urinalysis)  20-30 ml BCM (PCR) 10-20 ml Hopkins (research)	TOTAL: 30 ml  5-10 ml urine HZI (urinalysis)  20-30 ml BCM (PCR)	TOTAL: 30 ml  5-10 ml urine HZI (urinalysis) 20-30 ml BCM (PCR)
<b>Trunk Wash</b>  50 ml conical vials	TOTAL: 30 ml  30-50 ml BCM (PCR)	TOTAL: 70 ml  30-50 ml BCM (PCR) 10-20 ml Hopkins (research)	TOTAL: 70 ml  30-50 ml BCM (PCR) 20-30 ml National (PCR)	
<b>Swabs</b>		Oral swabs Hopkins (research)	ocular swabs National (PCR)	Ocular swabs Oral swabs
<b>Plain Red Tubes</b> (no separator)				5 ml blood (ganciclovir levels)
<b>Royal Blue Top</b> (minerals)				6 ml blood (mineral levels)
<b>Fecal Samples</b>				fecal sample