Oklahoma City Zoo
Asian Elephant
EEHV Protocol

6 December 2012

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Oklahoma City Zoo

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 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 40 North American cases, only 9 have survived. All survivors were Asian calves under the age of 2 years that were treated with the anti-herpes drug Famciclovir. The most recent of the surviving calves was treated with Ganciclovir after an initial 2 day treatment of Famciclovir, another animal at the same facility had virus in the blood but had shown no visible clinical signs.

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. This is the only time EEHV can be detected in blood samples. Scientists do not know where elephant herpes viruses hide in their latent phase and there is not yet a direct test to detect elephant herpes viruses in a healthy animal with a latent (hidden) infection. 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. Most elephants that are PCR positive are gravely ill with all or many of the classic clinical signs of EEHV disease.

An immunoassay (ELISA) was developed to detect previous infection/exposure to EEHV. Serologic determination of previous exposure to herpes virus infections is accomplished through screening for antibodies to one or more of the herpes virus antigenic proteins. The assay is now used to provide epidemiologic data on virus transmission patterns within a herd by predicting earlier or a recent exposure

that could result in virus shedding and transmission to non-immune elephants. 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 deceased blood delivery to the other organs of the body.

CLINICAL SIGNS

EEHV has a rapid onset and progression. Animals have died within 1-7 days of the onset of clinical signs. In 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
- Decreased capillary refill time as shock develops
- Difficulty in auscultation of the heart
- Weak, thready pulses
- Unresponsive to commands

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 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.

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).

TREATMENT

Anti-viral therapy needs to be started immediately and often without a confirmed diagnosis of EEHV. If delayed, therapy is unlikely to be efficacious and treatment regimes should be put in place before the time when you may need them. 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.

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 would be administered intravenously after cross-matching donor and recipient blood samples to assure compatibility. Plasma may help the patient by maintaining circulating blood volume as well. With EEHV, blood vessels "leak" fluid into the interstitial space, resulting in low blood pressure and reduced cardiac efficiency. Fluids will be given to help maintain circulatory volume and tissue perfusion. Caution should be exercised to prevent overloading the lungs and causing pulmonary edema.

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-inflamatories 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.

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 (fecal bolus).
- Weekly blood pressure readings on all elephants (more frequently in calves if possible).
- 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 Supervisor and the Mammal Curator, immediately. Veterinarians will be contacted by either the Elephant Supervisor or the Mammal Curator. If there are questionable signs and the Elephant Supervisor 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 then 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 ultrasound can also be used in adults and auscultation in calves.

Blood pressure will be monitored at least weekly for the adults. More frequent monitoring may be requested for calves. The blood pressure monitor is a Cardell model 9401 with various cuff sizes. The cuff (use largest size for adults) 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 the handwritten log and communicated to the Veterinarians any time there is suspicion of abnormal health.

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

ROUTINE SAMPLING AND SURVEILLANCE

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), and trunk washes (for PCR at BCM). Other samples may be included when and if indicated. See below for more specific sampling information.

* 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 monitoring and to help study the spread of EEHV virus and contribute to development of treatment protocols. 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 Appendix I: EEHV Sampling Guidelines Quick Chart for summary of information on sampling. Weekly blood collection for EEHV surveillance should include filling at least:

1 purple topped tube (3 ml capacity, 3 ml)
2 red/grey topped tube (4 ml capacity, 8 ml)

<u>Purple Topped Tubes</u> (EDTA anticoagulant, 3 ml capacity)

- Fill all EDTA tubes at least half way with blood so the blood is not diluted with EDTA.
- National Zoo needs: 1 - 2 ml whole blood for PCR
- Samples stored in the -80 freezer and sent to National Zoo quarterly
- Baylor wants 1 ml whole blood for PCR

Red and Grey Topped Tubes (Serum separator, 4 ml capacity)

- National zoo wants at least 2 ml serum (= 5 ml blood) for ELISA
- Samples stored in the -80 freezer and sent to National Zoo quarterly

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 to the hospital on the day of collection to be processed and stored in the -80 freezer.

PLASMA

Large volume blood collections will be attempted on the adult 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, ½, 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 OKC Zoo clinic -80 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 OKC Zoo clinic -80 freezer for storage, and for potential future therapeutic use.

CLINICAL CASE SUSPECT

The following clinical signs may indicate a possible EEHV infection. Any concerns should be brought to the Elephant Supervisor, Mammal Curator 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

Because of the rapid onset and progression of the disease, treatment needs to be initiated based on clinical impression rather than absolute diagnosis. Confirmation by PCR may take 2-3 days. The decision whether or not to start an elephant on an antiviral medication will be made by the Elephant Supervisor, Mammal Curator 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. NOTE: Famciclovir is considered in the group B pregnancy class, meaning there is no evidence that the drug causes adverse effects on embryo fetal development in pregnant animals but specific studies have not been performed. NOTE: 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.

Once an animal is started on an antiviral medication, the medication must be continued until follow-up whole blood PCR is negative.

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. 1st priority is to collect 1 ml of blood for PCR at Baylor and 2 ml blood for National EEHV laboratory.
   b. 2nd priority is to collect 0.5 ml of blood for CBC at OKC Zoo
   c. 3rd priority is to collect 10 to 30 ml of whole blood for Cornell to try viral culture
   d. 4th 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 OKC Zoo
   b. At least 6 ml of whole blood for serum ELISA at National Zoo
   c. 20 to 50 ml whole blood for Dr. Hayward at Hopkins for research.

Samples should be brought to the OKC Zoo clinic laboratory and processed and distributed 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 in Appendix II.

TREATMENT FOR SUSPECTED OR CONFIRMED EEHV

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

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 Supervisor and/or the Mammal Curator.

No non-essential staff will be present in the elephant area during the therapy and treatment process unless approved by the Mammal Curator, Elephant Supervisor, Assistant Zoo Director or the Zoo Director.

Antiviral therapy (See Appendix VI for additional drug dosages)
Famciclovir: 8-15 mg/kg PO or per rectum TID
Ganciclovir: 5 mg/kg IV BID (give slowly over 1 hour in NaCl)

- Recipe for oral suspension (5 mg/kg PO BID) – based on recommendation from Roche chemists
    - Use 14 vials ganciclovir injectable (500 mg / vial) per dose.
    - Mix each vial with 3 ml sterile water.
    - Withdraw dissolved drug from each vial, place together in one clean beaker.
    - Add 140 ml oral sweetener solution (such as simple syrup, or Orasweet, OTC syrup at any drug store)
    - Add 2.8 ml 3% H2O2 (=hydrogen peroxide)
    - Mix well, add sweetener to total volume of 280 ml
    - End Product: 25 mg/ml suspension
    - 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
    - Oral bioavailability of ganciclovir suspension has not been determined

INTENSIVE CARE OF THE EEHV PATIENT

Antiviral medications are recommended in any 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.

Sedation (See Appendix VI for additional drug dosages)

- Butorphanol 0.045 – 0.075 mg/kg 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

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). 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 0 PDS.

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.

Maintenance fluid therapy requirements have not been determined for elephants but are assumed to be similar to other mammals:

- 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

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. 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.

Blood Tansfusion

If HCT falls below 14%, blood transfusion should be considered. 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 (*will most likely be sent out):

- 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).

Antibiotics (see Appendix VI for drug dosages)

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 antibiosis should be instigated immediately.

Analgesia (see Appendix VI for drug dosages)

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-steriodal 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. 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.

DAILY MONITORING OF AN EEHV CASE

Monitoring of Suspect/Confirmed Positive Elephant

If EEHV is suspected but the elephant is not clinically ill, a veterinary physical examination will be performed twice daily during the first week of treatment and should coincide with the treatment schedule. If EEHV is diagnosed via PCR and the elephant is placed on intravenous catheter 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. 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 5 ml daily) – CBC (0.5 ml), EEHV PCR at BCM (2 ml) and National EEHV lab (2 ml).
- Serum (red/grey top, total 17 ml daily) – serum biochemistry (2 ml), EEHV ELISA at National EEHV Lab (5 ml), and banked serum for BCM (6-10 ml)
- 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
    - Ganciclovir levels lab – collect blood into a plain red top tube (no serum separating gel) and promptly centrifuge and separate serum or plasma into a plastic screw-capped vial using approved guidelines (requires 2 ml serum or plasma) – http://www.nmslab.com/SearchResults.aspx?code=2154SP
- 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) – OKC Zoo 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 an elephant is confirmed 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 (4-5 ml total from each elephant) – BCM PCR (1 ml), National EEHV Lab PCR (2 ml), OKC Zoo CBC (0.5 ml)
- Serum (7 ml whole blood from each elephant) – National EEHV Lab ELISA (5 ml), OKC Zoo serum biochemistry (2 ml)
- Urine (30 ml total from each elephant) – OKC Zoo 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)

RESOURCE CONTACT LIST

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APPENDIX I. SAMPLE GUIDELINES – QUICK CHART (IN ORDER OF PRIORITY)

	Routine Weekly Monitoring	**Clinical Suspect or Sick Elephant**	**Herdmates of Sick Elephant**	**Daily Monitoring of Sick Elephant**
Purple Top Tubes (whole blood)	Total 3 ml blood	Total 6-40 ml blood	Total 4-5 ml blood	Total 5 ml blood
3 ml tubes	1-2 ml WB National for PCR	2 ml National for PCR	1-2 ml National for PCR	0.5 ml OKC Zoo for CBC
	1 ml WB BCM for PCR	1 ml BCM for PCR	1 ml BCM for PCR	2 ml BCM for PCR
		0.5 ml OKC Zoo for CBC	0.5 ml OKC Zoo for CBC	2 ml National for PCR
	2-5 ml BCM for gene sequencing	10-30 ml Cornell for culture		
		2-5 ml BCM for gene sequencing		6-10 ml OKC Zoo (banking)
Red/Grey Top Tubes (Serum)	Total 7 ml blood	Total 8-58 ml blood	Total 7 ml blood	Total 17 ml blood
4 ml tubes	2 ml serum National (ELISA)	2 ml OKC Zoo (chem)	5 ml National (ELISA)	2 ml OKC Zoo (chem)
		6 ml National (ELISA)	2 ml OKC Zoo (chem)	5 ml National (ELISA)
		20-50 ml Johns Hopkins (research)		6-10 ml OKC Zoo (banking)
Urine		Total 60 ml	Total 30 ml	Total 30 ml
50 ml conical vials		5-10 ml OKC Zoo (urinalysis)	5-10 ml OKC Zoo (urinalysis)	5-10 ml OKC Zoo (urinalysis)
		20-30 ml BCM for PCR	20-30 ml BCM for PCR	20-30 ml BCM for PCR
		10-20 ml Johns Hopkins (research)		
Trunk Wash	Total 30 ml	Total 70 ml	Total 70 ml	
50 ml conical vials	30-50 ml BCM for PCR	30-50 ml BCM for PCR	30-50 ml BCM for PCR	
		10-20 ml Johns Hopkins (research)	20-30 ml National for PCR	
Swabs				
		Oral swabs Johns Hopkins (research)	Ocular swabs National for PCR	Ocular swabs Oral swabs
Plain Red Tubes (no separators)				5 ml blood for Ganciclovir levels
Royal Blue Top (minerals)				6 ml for mineral levels
Fecal Samples				Fecal sample

Appendix II: Shipment Guidelines for EEHV Samples

National EEHV Laboratory

1. In a case where EEHV is clinically suspected:
    a. Whole blood in EDTA (0.5 to 2 ml) for EEHV-1, 2, 3, and 4 PCR, sent on ice packs
    b. Serum (> 2 ml) for EEHV ELISA, sent frozen on dry ice
2. Necropsy samples should be collected as described in Appendix VII and sent on dry ice.
3. Send samples to:
    a. Erin Latimer, Research Assistant
        Department of Pathology, Smithsonian's National Zoo
        3001 Connecticut Ave, NW
        Washington, DC 20008
        202-633-4252 (office phone)
        202-633-8717 (fax)
        703-855-9611 (cell)
    b. Email Erin Latimer (latimere@si.edu) and Laura Richman (RichmanL@MedImmune.com) to let them know sample is coming.
        1. Do not write the word "herpes" in the subject line.
    c. Call the phone numbers above to alert the lab that a clinical or post mortem sample will be coming, have tracking number available for Erin.
    d. If it is after hours or on Saturday or Sunday, call home numbers for Erin or Laura to arrange for shipment and sample testing. Sample may have to be sent to Erin's house.
        1. Erin 703-471-2168 (Home)
        2. Laura 301-253-8723 (Home)

Baylor College of Medicine

1. In a case where EEHV is clinically suspected: whole blood EDTA (1 ml) for EEHV-1a and 1b PCR
2. Keep sample cold in refrigerator until it can be taken to lab at BCM
3. Send samples to:
    Dr. Jeff Stanton
    Department of Molecular Virology and Microbiology
    Baylor College of Medicine
    Mail Stop BCM-385
    One Baylor Plaza
    Houston, TX 77030
    (713) 253-9282
4. Dr. Jeff Stanton should be contacted when a clinical sample is collected (Cell: 713-253-9282).
5. Dr. Paul Ling is back up to Dr. Stanton. He can be reached at:
    a. Cell phone (281) 460 1696
    b. Office phone (713) 798 8474
    c. Lab phone (713) 798 8475
6. Dr. Alan Herron can be reached as a back- up contact: office (713) 798 3417

Cornell College of Veterinary Medicine

1. In a case where EEHV is clinically suspected:
    a. Whole blood EDTA (10 to 30 ml) to try to culture EEHV virus from blood
        i. If possible sample should be collected BEFORE Famciclovir treatment

   b. Samples collected every 6-8 hours if possible
2. Necropsy samples should be collected as described in Appendix VII
3. Blood samples and necropsy samples should be shipped on ice, overnight delivery, to:
   a. Mary Beth Matychak – Cornell University, College of Veterinary Medicine, Ithaca, NY 14853 (607-253-3493)
   b. FedEx Account #1834-4049-7
   c. If samples are shipped on a Friday (for Saturday delivery) – use Dr. Abou-Madi's home address: 123 E. King Road, Apt. C1, Ithaca, NY 14850 (Phone 607-227-1238)
4. Contact Mary Beth or Dr. Abou-Madi by phone or email prior to sending samples (na24@cornell.edu)
5. Do not delay shipping of initial sample if additional samples are pending – please send samples as soon as collected.

APPENDIX III: 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
- Ultrasound lubricant
- 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:

Drug administration/supportive care:

- Famciclovir 500 mg tablets, 30 tablets/bottle: 15,000 mg / bottle
    - Minimum 3 day supply for Asha, which is 30,000 mg PO TID, 6 bottles/day = 18 bottles
- Ganciclovir 500 mg vials, 25 vials / box: 12,500 mg/ box
    - Minimum 3 day supply for Asha: 12,500 mg IV BID, 2 boxes/day = 6 boxes
- Other drugs (see drug list appendix V)
- Mortar and pestle (to grind in case rectal administration is necessary)
- Ultrasound gel (for mixing with famciclovir 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)
- 1 liter sterile water to mix dose of ganciclovir
- 10-14 GA catheters (long)
- 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)
- Alcohol
- Scrub
- Stainless steel bucket (medical grade)
- Rolled cotton (3 rolls)
- 4X4 gauze (6 packages)
- Bandage scissors
- Heparin
- Large animal surgery pack
- Scalpel blades: 10, 15

- Sterile Gloves (6 1/2, 7)
- Lidocaine
- Drapes
- Sharps container
- Suture (0, 1, 2 prolene or similar with cutting needle)
- Tissue glue
- 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)
- Injection caps
- T port
- 5 liter fluids (all in stock)
- IV pump
- IV pole
- Ropes/wire to hang bags
- Extension cord
- Flashlights/head lamps
- Portable surgery lights
- Plasma (stored from herd, keep on ice)
- Plasma administration filter
- Hetastarch

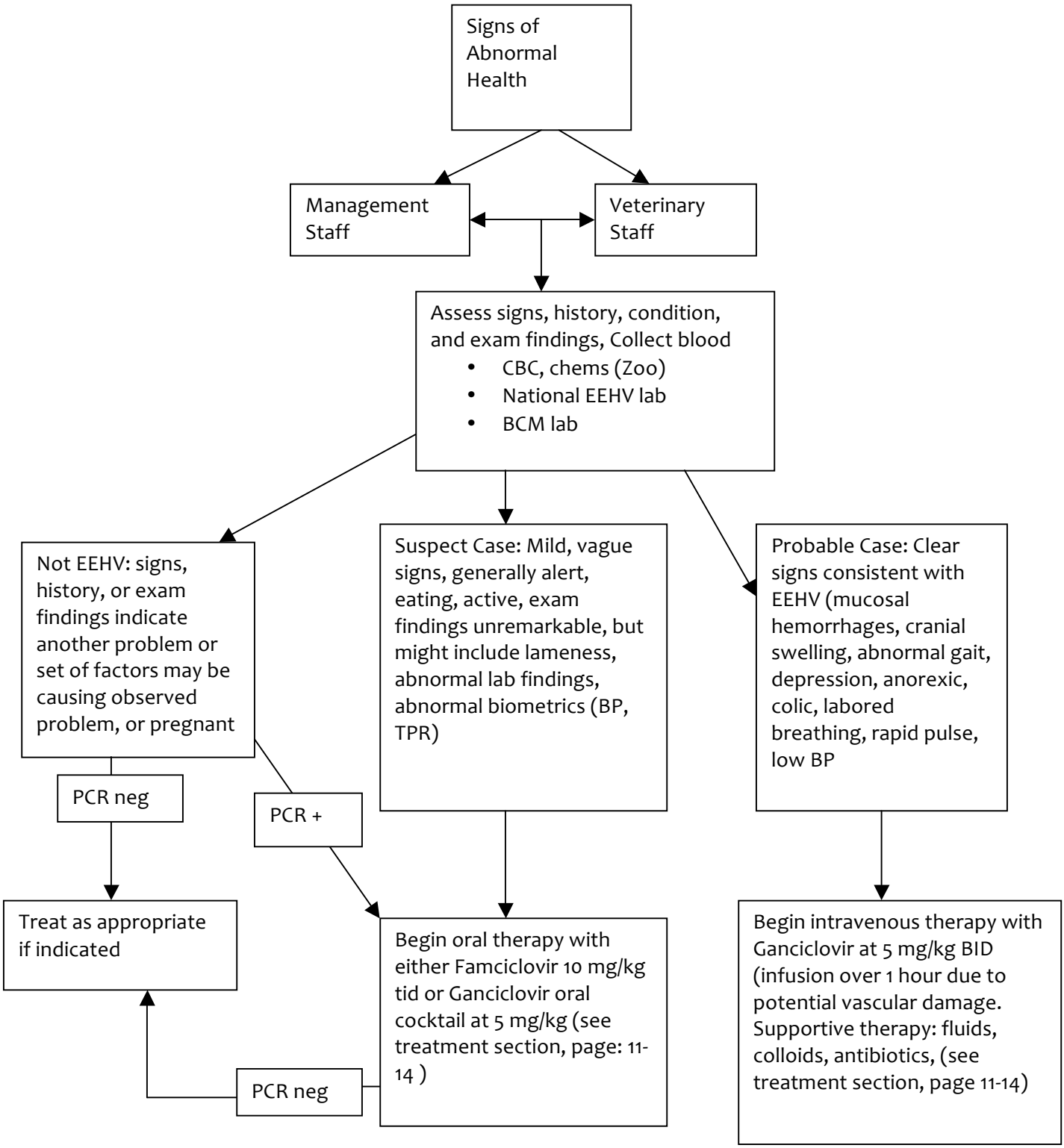
Sedation/anesthesia:

- 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)
- Emergency drug box
- Emergency bag
- 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)
- Oxygen tanks
- Portable anesthesia machine
- ET tubes (24, 22, 20)
- Catheter type stylets for intubation
- Laryngoscope w/long blade
- Y piece (nasal administration)
- Ropes (open mouth)
- Blocks (open mouth)
- Pulse oximeter
- I-stat
- Stethoscope
- Thermometer
- Endoscope and associated equipment (intubation)

Miscellaneous:

- ICU flow sheet, pens, clipboard, watch
- Sonosite
- Doppler
- Blood pressure cuff
- ECG (cerclage wire contacts vs. sticky pads?)
- Digital camera
- Video camera
- Opthalmoscope (1)
- Opthalmoscope extra battery
- Culturettes
- Pericardiocentesis
    - 60 cc regular tipped syringes
    - 3 way stop cocks (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

APPENDIX IV. EEHV FLOW CHART



APPENDIX V. EEHV "FAST PLAN"

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 flow chart on previous page)
   - TPR
   - BP
   - Blood collection (12 ml in purple tops 30 ml in tiger tops)
2. Administer 10 mg/kg Famciclovir
   - Orally or
   - Rectally (grind with mortar and pestle, mix with Ultrasound gel)
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)
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

APPENDIX VI. DRUG DOSAGES

	Drug	Concentration	Dosage range	Comments
Sedation	Butorphanol	10 mg/ml	0.045-0.075 mg/kg IM	Reverse with Naltrexone
	Naltrexone reversal	50 mg/ml	2.5-5 x Butorphanol mg	
	Detomidine	10 mg/ml	0.005-0.011-0.022 mg/kg IM	Give 15-20 min after Butorphanol, reverse with Atipamazole
	Atipmazole reversal	5 mg/ml	5 x detomidine mg	No notes regarding using as a Detomidine reversal
	Azaparone		0.024-0.038 mg/kg IM	
Treatments	Famciclovir	500 mg/tab	8-15 mg/kg TID	PO or rectally
	Ganciclovir IV	500 mg/bottle	5 mg/kg BID	Give IV 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
Fluids	Maintenance adult		2 ml/kg/hour IV	
	Maintenance calf		4 ml/kg/hour IV	
	Surgical rate		10 ml/kg/hour IV	
	Shock rate		90 ml/kg/hour IV	
Analgesics	Buprinorphine	0.3 mg/ml	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	10 mg/ml	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	20 mg/ml	0.003-0.005 mg/kg IM	Possible sedation
Analgesics	Flunixin meglumine	50 mg/ml	0.8 mg/kg S-BID IM	
	Meloxicam		0.6 mg/kg SID IV-PO	

	Drug	Concentration	Dosage range	Comments
	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
	Aspirin		10 mg/kg S-BID PO	Equine dose
	Ibuprofen		6 mg/kg BID PO	
	Methadone		0.03-0.06 mg/kg IM	Anecdotal?
Antibiotics	Amikacin	250 mg/ml	3-5 mg/kg SID IM/IV	
	Amoxicillin		11 mg/kg SID IM	
	Ampicillin		8 mg/kg B-TID PO	Amp sodium = IV, Amp trihydrate = PO
	Ceftiofur (Naxcel)		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	
	Flofenicol		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	
Emergency	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
	Doxapram HCL	20 mg/ml	0.4 mg/kg IV/IM	
Emergency	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

	Drug	Concentration	Dosage range	Comments
	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	
Other	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	

APPENDIX VII: NECROPSY AND POST MORTEM SAMPLE COLLECTION

Necropsy Procedures (from EEHV research and tissue protocol 2010)

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.

Two 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 Northwest ZooPath, for diagnostic purposes
2. 1 set to be shared with the Elephant SSP Pathologist (follow SSP necropsy protocol)

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 in Appendix II
  - 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 in Appendix II

Tissue Collection

The following fresh 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 in Appendix II.

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 in Appendix II.

Set #3: To be kept at the Oklahoma City 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
- Samples should be stored in -80 freezer at Oklahoma City Zoo until needed

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