

European recommendations for monitoring exposure to Elephant Endotheliotropic Herpes Virus (EEHV) in young elephants.

Routine monitoring of both Asian and African elephant calves for EEHV viral loads is now a tool that can be used in the management of captive elephants. Recent developments have made it possible to detect low levels of EEHV in the blood before clinical signs occur, allowing increased monitoring and early treatment when viral levels increase (Stanton, 2013). The increased sensitivity of qPCR and multiple rounds of cPCR as well as the ability to quantify whole blood viral levels with qPCR allow for better management of calves with regard to possible EEHV Hemorrhagic Disease (EEHV HD). It is now possible to pick up low levels of EEHV in the blood and monitor closely for rapid increases in viral levels to distinguish between a calf's "normal" primary herpes infection and the much more serious EEHV HD. Elephants can have low levels of EEHV in the blood and show no or minimal clinical signs (Stanton, 2013), for up to two months, but possibly for as long as one year. In order to prevent severe and often fatal disease caused by EEHV, young elephants between the ages of 1-8 years (optimal 1-13 years) must be monitored on a weekly basis by performing a PCR on a whole blood sample. If weekly sampling is not feasible, sampling could be done once every 14 days. However, it should be kept in mind that there is less time to act in case of the detection of viraemia. The level of VGE/ml considered significant may vary between different EEHV strains but has been established as 5000 VGE/ml or greater in EEHV-1 cases. Further research is required to reliably establish low/base levels in cases of EEHV-3, EEHV-4 and EEHV-5 involvement. Routine monitoring will allow each collection to further increase their understanding of base levels present in their animals. Until then, treatment is recommended in all cases with viral loads of 5000 VGE/ml or greater.

The first signs of a clinical EEHV-viraemia are reflected in a sudden drop of white blood cells (predominantly monocytes) and thrombocytes. Therefore these parameters should also be monitored in young elephants.

Elephants trained for blood collection

Elephants that are trained for blood collection should be bled on a **weekly** basis.

- For real time PCR, EDTA-whole blood from any volume of $\geq 20 \mu\text{l}$ should be adequate (Optimum; at least $300 \mu\text{l}$ to allow for real time PCR repeat in case of failure).
- In addition, a complete blood count can be carried out on the EDTA whole blood, including a platelets count and cell differentiation.

For real time PCR, the **EDTA-whole blood sample** should be sent on the day of collection for next day delivery to one of the following laboratories (see table below). Please note that samples from African elephant must be sent to Berlin. For Asian elephants you can choose between Weybridge and Berlin, depending on the best air carrier connections. Please contact the lab before sending your samples!

Asian elephant samples ONLY	African and Asian elephant samples
Animal and Plant Health Agency (APHA) Mammalian Virus Investigation Unit Department of Virology, Att. Mr. Akbar Dastjerdi PhD, New Haw, Addlestone Surrey, KT15 3NB, United Kingdom Phone: Office: +44 1932 357 509; Lab: +44 1932 357 474 E-mail: Akbar.Dastjerdi@apha.gsi.gov.uk	Armando Damiani / Sebastian Bischofberger FU Berlin, FB Veterinärmedizin Zentrum für Infektionsmedizin Institut für Virologie Raum 260 Robert-von-Ostertag-Str. 7-13 14163 Berlin, Germany Tel: +49-30-838 51842/51958 Email: adamiani@zedat.fu-berlin.de Email: bischofberger.s@fu-berlin.de

No ice is required during express shipment.

Diagnostic EEHV real time PCR at the APHA is currently performed on **Wednesdays/Thursdays**. This means that samples should arrive at the APHA on or before Wednesdays. A rough estimate of viral load is provided if Asian elephant EEHV nucleic acids detected in any of the submitted samples.

- A **heparin plasma sample** should be taken for measurement of the antibody titer (ELISA). This sample can be stored at -20°C and sent (on ice) in batches (for example once a year) to:
Artemis-One Health institute
Androclus building 2nd floor, Att. Mr. Byron Martina PhD
Yalelaan 1
3584 CL Utrecht, The Netherlands;
Phone: +31-30-6355444
E-mail: info@artemisononehealth.com)

Elephants not trained for blood collection

If the elephant calf is not yet trained for blood collection, alternative methods to collect a few blood droplets can be used. One is to apply a disposable lancet over an ear vein e.g. Unistick 3 neonatal 18 G lancet (Owen Mumford Inc) used in human diabetics for glucose level monitoring. Chester Zoo has successfully used a modified Unistick 3 neonatal to increase the penetration depth by filing down the tip (fig 1). Other brands may work as well. The procedure can be repeated safely and with minimal reaction from the calf, until sufficient blood is obtained. The blood droplets should be collected on a plain swab for transport to the testing laboratory.

It is worth noting that, although useful to monitor untrained elephants, this method has a lower sensitivity (10 fold reduction) than whole EDTA blood in Vacutainer tubes. This means that it will be less efficient in picking up infection and all efforts should be made to get the animals trained for venipuncture as soon as possible..

If possible, an extra blood droplet should be collected on a glass slide for a blood smear. After staining a differentiation of the white blood cells should also be performed. Although not providing total numbers of WBC's, weekly WBC-differentiation alone can increase chances of detecting a viraemia in time.



Figure 1. 'Unistick 3 extra' lancets. The arrow indicates the area that has been filed to increase penetration depth.

NB: decisions concerning elephants that are not trained for routine blood collection should always be taken in the light of the need for future cooperation between the animal and their care takers (blood collection, treatment, etc.). Early training of elephant calves should have a high priority in each institution that breeds elephants.

Actions to be taken after receiving the PCR result:

- If the **real time PCR result is negative** and the elephant shows **no signs of clinical disease**: no action is required.
- If the **real time PCR result is positive** and the elephant shows **no signs of clinical disease**: contact APHA and make arrangements for submitting more blood samples, preferably on a daily basis. Telephone number: Office: +44 1932 357 509; Lab: +44 1932 357 474).

A quantitative PCR (qPCR) will be performed if the initial real time PCR is positive.

- If the qPCR reveals a high viral load [5000 viral genome copies (vgc) /ml or greater], treatment of the affected elephant should be considered even in the absence of clinical symptoms.
- If the viral load is considered low, no treatment is required at this stage, but the elephant should be observed closely and sampling rate for virus level should be increased.
- If the viral load is not clear, because of the small sample size (droplet method), antiviral treatment should be considered as well as monitoring viraemia every 24-48 hours. If viraemia persists despite of treatment, clinical examination and blood collection under sedation is recommended.
- If the **real time PCR result is positive** and the elephant shows **signs of clinical disease**: immediate EEHV-treatment should be considered. A whole blood sample is also required in order to perform CBC, WBC-differentiation and full blood chemistry.

Optional: Heparin plasma should be stored at -20° for measuring the antibody level (ELISA) at a later stage.

If at any time point an elephant (independent of its age!) shows clinical signs suggestive of EEHV-disease;

- ❖ An EDTA whole blood sample or blood swab should be sent instantly to the APHA for EEHV investigation and qPCR.
- ❖ Whole blood chemistry should be performed including CBC and WBC-differentiation. A sudden drop in total white blood cells (predominantly monocytes) and/or a drop in thrombocytes are suggestive of clinical EEHV and hence necessity for emergency treatment, including administration of fluids, an antiviral drug, antibiotics to protect against toxemia/septicemia originating from the gut flora and other supportive drugs (see annex 1).

Always consider anti-viral treatment if:

- *Clinical signs present, including drop of monocytes and platelets**
- *5000 VGE/ml or greater**
- *Rapidly increasing VGE/ml**

Additional general recommendations:

- A. Bank EDTA whole blood samples from the rest of the herd weekly for testing if indicated, e.g. if elephants show clinical signs, calf tests EEHV-positive (-20°C).
- B. Bank heparin plasma samples from the rest of the herd weekly for retrospective study of antibody responses to EEHV exposure (-20°C).
- C. Bank trunk wash samples (only the pellets after centrifugation) weekly for identifying EEHV-shedders and identification of the EEHV-strains in the elephant herd (-20°C).

This document was produced by the following participants of the 10th international EEHV-workshop held in Houston (USA), 17-18 February 2015:

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Annex 1: Treatment protocol as published by the EEHV advisory group on <http://www.eehvinfo.org/> (member domain)

Treatment of Elephants with Elephant Endotheliotropic Herpesvirus Hemorrhagic Disease

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General Introduction

Treatment protocols for EEHV Hemorrhagic Disease (EEHV HD) are derived from therapies used for the treatment of human hemorrhagic diseases. Like EEHV, these human diseases, which include Ebola and Marburg, present initially with non-specific and often subtle signs. Also like EEHV, these diseases progress rapidly with death likely resulting from shock, hypovolemia, and possible disseminated intravascular coagulation (DIC), but not from blood loss, per se, although anemia and thrombocytopenia can be substantial.

The goal of therapy with human hemorrhagic diseases is to provide supportive care and to maintain tissue and organ perfusion. This is also the goal of EEHV treatment. Circulatory support and maintenance of blood volume require aggressive fluid therapy using crystalloids, colloids and sometimes both, in conjunction with an intensive care approach to monitoring the patient and symptomatic treatment. Although antiviral drugs are used for EEHV, their actual role in fighting disease is controversial and as yet unconfirmed.

Logistics of providing medical care to sick young animals

Elephants need to be trained to accept medical procedures. Calf training should be started immediately after birth with the youngster being taught to stand still and tolerate being touched, and over time, progressing to having blood drawn, allowing intramuscular and intravenous injections, swallowing oral medication, and receiving rectal fluids. If the calf has not been trained, then sedation will be needed so that medical care can be given. Not providing medical care to a sick elephant because of political ideologies that preclude contact with the animal or for financial reasons is unacceptable. Facilities that are breeding should make calf training a priority, but any facility with elephants should accustom their animals to routine medical care so that sedation, always tricky, but riskier in sick animals, isn't definitively necessary.

Courses on training elephants are available in the United States and Europe through various organizations. Calf training is discussed here: [Minimum standards of elephant care](#).

Strategic planning is needed for the eventuality of an EEHV-HD case. Some facilities in the United States perform preparatory drills where the supplies are checked and confirmed (adequate amounts, not expired, properly stored, etc), the elephants' training levels are reassessed, the phone tree numbers are checked and so on. A team approach is critical for these cases; thus the head of the facility, the public relations coordinator, and even neighboring institutions that could lend moral or physical support should be included in the preparation

Because elephants with EEHV usually need round-the-clock care, facilities should determine how to provide such staffing well in advance of an actual emergency. Facilities should also have a

budget for medications and equipment, and should keep adequate amounts of supplies on hand, as well as basic laboratory equipment that can be kept near or in the elephant barn. Because care for EEHV-HD affected elephants is performed in the barn – not in a hospital -- facilities need to be able to turn the barn into an intensive care unit (ICU). Recommended supplies for an elephant ICU are listed in **Table 1**.

Table 1: Supplies for the Elephant Barn

Easily cleanable table for storing supplies
Sharps container
Red top and purple top vacutainer tubes
Styrofoam containers
Microhematocrit tubes and clay
Microhematocrit tube centrifuge
Microhematocrit PCV chart
Oxygen tank (E-tank) on a portable stand with wheels and tubing to provide intranasal oxygen
Ophthalmoscope
Stethoscope
Thermometer
Glucometer and glucose sticks
Urinalysis strips
Refractometer
Light Microscope
Glass microscope slides
Needles and syringes
Small refrigerator for medication and blood tube storage
Record keeping supplies (notebook and paper, computer access etc)
Blood pressure cuff
Good quality flashlight
Soft rubber tubing for administering rectal fluids
Large equine dosing syringe to attach to tubing
Equine stomach pump and a large metal bucket for administering fluids
Handwashing supplies
Gloves for personnel

Fluid Therapy for Confirmed EEHV cases

The first line of treatment is rectal fluids. Plain water administered through a garden hose or through rubber tubing via an equine gastric pump can be lifesaving for these animals. This is fortunate for the clinician because administering copious quantities of rectal fluids is straightforward and appears to be very safe. The elephant colon is extremely absorptive, and elephants will absorb what they need while excreting the rest. To administer rectal fluids, the animal should be restrained in a chute. The hind legs may need to be tethered. Manual evacuation of manure is done expediently; it is important not to irritate the colon which will increase peristaltic activity and cause the fluid to be expelled before it is absorbed. Next, the hose (with all metal parts removed) or soft rubber tubing (such as an equine nasogastric tube) should be lightly lubricated and pushed into the rectum to the length of the clinician elbow. The clinician should then withdraw the arm and push the tubing or hose in another meter if possible.

Lukewarm rectal fluids should be administered a slow steady rate. Too fast, and the fluid will stimulate peristaltic contractions, which is not desirable. At the end of fluid administration, the

tubing should be swiftly withdrawn and the elephant's tail held down for about a minute. If the animals start to strain at any point, the fluid should be turned off but the hose left in place until the straining stops. The fluid should then be started at a somewhat slower rate.

Fluid rates and doses are empirical. A bolus technique of 10 to 20 ml/kg dose is often used. Most elephants will show immediate improvement after receiving fluids. Fluids often need to be given hourly or every two hours. However, it is important to be gentle because it is possible to damage rectal mucosa. If the clinician's glove comes away with blood on it, this indicates rectal irritation. Fluids should be continued, but additional lubrication and more care should be used.

If the elephant doesn't show improvement with rectal fluids, or blood work and physical exam indicate the animal's condition is deteriorating, i.e. tachycardia, tachypnea, increased depression and lethargy, intravenous fluids can be added to the regime. Asian elephants have very low serum osmolarity and are hyponatremic and hypochloremic compared to other species. African elephants are thought to have similarly low serum osmolarity. Thus, commercial fluids such as Plasmalyte, Norm-R and similar, are actually hypertonic for elephants. The author uses these fluids in elephants as she would use actual hypertonic solutions in other species; in other words, very small amounts (1 liter per 450 kg) are given through an IV catheter and followed afterwards with large amounts of rectal fluids.

Colloids may be needed as well, and small amounts can be extremely helpful. Plasma or blood can be banked and stored or collected fresh from another elephant. [Fresh plasma is better than frozen.](#) [Dose: 0.25-0.5ml/kg \(cross match prior to use\).](#) [Frozen plasma can be kept at -80C for up to seven years.](#) It is important to make sure that the donor elephant is not viraemic and is in good health. In addition, because elephants have preformed blood group antigens, cross-matching in advance of a transfusion is essential. Blood and plasma should be administered through an appropriate blood filter to prevent clots. Very small amounts of plasma, 2-4 liters per elephant, have been quite beneficial for sick elephants.

Colloids must be administered through an intravenous catheter. Catheters can be placed in an ear or saphenous vein. Aseptic technique should be used to place the catheter. After fluid administration is completed, the catheter should be pulled and adequate pressure placed on the insertion site to prevent hematoma formation. Leaving the catheter in doesn't work in elephants because they will remove or damage it.

Antiviral Therapy

EEHV is such a peculiar virus that it is unclear at this time what drug is best to combat it. Fanciclovir is most commonly used and can be administered orally or rectally. Acyclovir, which can be given orally and gancyclovir which requires intravenous administration, have also been used. The author prefers to use fanciclovir rectally because compliance is straight-forward and no side effects are reported with its use. Gancyclovir is associated with severe side effects in other species including bone marrow suppression and development of Fanconi's syndrome.

If fanciclovir is administered rectally, it should not be given at the same time as rectal fluids. Ideally, fanciclovir would be administered first, and then at least an hour allowed to pass before rectal fluids are given. As with administration of fluids, retention of rectal medication requires not

overly stimulating the rectum and causing the drug's expulsion. Famciclovir tablets can be dissolved in water and the solution placed in a dosing syringe. The syringe can be attached to a soft rubber tube (equine nasogastric tube), and the tube inserted into the rectum as for fluid, following removal of manure by hand.

While there is no clear evidence that famciclovir is an ideal drug to fight EEHV-HD, its safety and the possibility that it does help suggest that clinicians should continue to use it. Suggested doses can be found in **Table 2**.

Table 2: Drug doses for elephants with EEHV-HD

Drug	Dose	Frequency	Route	Notes
Butorphanol	0.01-0.03 mg/kg	Q 4 hours	SQ, IM or IV	Can be given more frequently if necessary; an appropriate dose should not make the elephant overly sleepy
Ceftiofur	1.1 mg/kg	IM or IV	Twice daily; Once daily if IV	Can cause abscess formation given IM
Flunixin meglumine	0.2 – 0.5 mg/kg	IV	Once a day	Animal must be adequately hydrated first. Intramuscular administration is extremely necrotizing to tissue
Famciclovir	16 mg/kg for one day then 12 mg/kg	Oral or per rectum	Four times/day	
Sulfa-trimethoprim	22 mg/kg	Oral	Twice daily	
Vitamin E	2.2 IU/kg	Oral	Once daily	Used as an antioxidant
Butorphanol plus detomidine	B: 0.02 – 0.03 mg/kg D: 0.02-0.03 mg/kg	IM	Given in one syringe for sedation	Reverse Butorphanol with naltrexone at 5 mg per 1 mg opioid (optional) Reverse Detomidine w atipamzeole at 0.1 – 0.16 mg/kg

Other therapy

Prophylactic antibiotics, anti-inflammatory drugs, H-2 blockers and other drugs are often used in elephants with EEHV-HD. Doses can be found in **Table 2**.

Monitoring of Confirmed EEHV cases

Monitoring should be done frequently; in some cases hourly as critical parameters change. Watching the hematocrit for progressing anemia and thrombocytopenia is very important. This can be done using a microhematocrit tube so that minimal blood is required, and results can be obtained stallside. A light microscope and H&E stain can be used to evaluate the blood smear. The increase or decrease in reactive white blood cells, band heterophils and/or cellular toxicity in the cells can be prognostic. Temperature, pulse, and respiration (both rate and effort) should be measured. Assessing mentation and somnolence are important because changes can indicate electrolyte disturbances, hypoxia or brain bleeds. Decreased urine output and scant feces can indicate an animal that requires more fluids. Pain can be a component of the disease, evidenced as colic, oral ulcerations or lameness, and requires treatment. Sick juvenile elephants should be weighed daily.

Measuring oxygen saturation of hemoglobin with a pulse oximeter on the tongue is important in more severe cases. The heart should be auscultated in elephants weighing less than 2000 kg by placing the stethoscope just caudal to the elbow, and then as far under the elbow as possible. The presence of tachycardia or heart murmurs can indicate progressing disease. Difficulty hearing the heart raises concern about pericardial effusion; this can be confirmed with a gel-covered ultrasound placed over skin that has been thoroughly soaked first with water. Changes in the color of the mucus membranes, particularly a cyanotic color, are concerning. These animals may benefit from nasal oxygen supplementation.

Some elephants show evidence of hepatopathy by ultrasound, development of lipemic serum, or liver enzyme changes in the SBC. Adding Vitamin E, an antioxidant, may be helpful.

Recumbent elephants should have adequate padding under them, and should be turned regularly. Prognosis declines for animals too weak or sick to get up without assistance.

This document was reviewed by Lauren Howard, DVM, Dipl. ACZM, Houston Zoo.

Recommended references:

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The preferred sites for intravenous cannulation in the elephant: (a) shows the location of the right cephalic vein (red), and (b) shows an intravenous cannula in place in the left medial saphenous in a recumbent elephant. Copious amount of duck tape was sufficient to maintain this cannula but suturing combined with local anaesthesia is an alternative.