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

(Update: 9-2-2021)

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 ≥20 µl should be adequate (Optimum; at least 300 µl 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 on page 5). Please always contact the lab before sending your samples!

A rough estimate of viral load is provided if EEHV nucleic acids detected in any of the submitted samples.

Periodic **serum samples** (every 3 months) should be taken to measure the antibody titer (ELISA). This sample can be stored at -20°C and sent (on ice) in batches to:
Utrecht University
Faculty of Veterinary Medicine

Att. to Tabitha E. Hoornweg, PhD
Section Virology/ Section Immunology
Yalelaan 1
3584 CL Utrecht
The Netherlands

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 is 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 a nearby lab (see table on page 5).

  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 required in order to perform CBC, WBC-differentiation and serum for full blood chemistry.
  A serum sample (min 1 ml) should be stored at -20° for measuring the antibody level (ELISA) at the Veterinary Faculty Utrecht.

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 nearby qPCR lab for EEHV investigation and qPCR.

- ❖ Whole blood chemistry should be performed as well as 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 serum 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 initiated by the following participants of the 10th international EEHV-workshop held in Houston (USA), 17-18 February 2015:

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List of European labs that run qPCRs for EEHV. Note that subtypes EEHV1a, 1b, 4 and 5 are specific for Asian elephants and EEHV2, 3a, 3b, 6 and 7 are specific for African elephants.

Name of institute	Universität Berlin, Institut für Virologie	Rotterdam Zoo, Veterinary Department	Copenhagen Zoo	SCG Diagnosztika Kft / UVM Large Animal Diagnostic Centre	Chester Zoo	Irish Equine Centre
Adress	Robert von Ostertag-Str. 7-13, 14163 Berlin, Germany	Postbus 532, 3000AM Rotterdam, the Netherlands	Roskildevej 38, Copenhagen, Denmark	H-2225 Üllő, Dóra major, Hungary	Chester Zoo	Johnstown, Naas, County Kildare, Ireland, W91RH93
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qPCR EEHV subtypes	EEHV-1 and EEHV-2 using specifc primers and probes for each strain.We also have the capacity to run distinguishable test for EEHV-3, EEHV-4, EEHV-5, and EEHV-6 using specific primers and probes. However, we don't have positive control for these strains.	EEHV-1, 2, 3/4, 5 and 6	EEHV1a, 1b, 2, 3, 4, 5	EEHV-1,2,3/4 and 5	EEHV1 & 3/4	EEHV1; EEHV3/4; and EEHV5
Remarks					Not commercially available for routine testing, may be able to help in an emergency	Dublin Zoo but other zoos should contact us if they require regular monitoring.