

## Elephant Endotheliotropic Herpes Virus (EEHV) and the best care for your elephants: A recommendation of the EAZA elephant TAG (25 August 2022)

Dear vet colleagues,

As you possibly know, I am participating in the EEHV research project at the Veterinary Faculty of the Utrecht University. So far we have been able to develop a couple of reliable ELISAs that can detect antibodies against glycoprotein B (gB), which basically is a general test for all EEHV (sub)species and ELISAs that can detect antibodies against the individual EEHV (sub)species, using gH/gL as discriminating antigens.

### What have we learned so far?

1. All adult elephants are carrier of one or more EEHV (sub)species, with most of them likely carrying multiple subspecies. In the uterus the calf receives EEHV-specific maternal antibodies that remain present at high levels until the age of approximately 12 months. From that point, waning of EEHV-specific maternal antibodies becomes clearly visible until these EEHV-specific maternal antibodies become undetectable at approximately 3-3.5 years. Our recent findings indicate that low to non-detectable levels of antibodies are a clear risk factor for the development of EEHV-Hemorrhagic Disease (HD). Additionally our results strongly suggest that high levels of maternal antibodies provide protection against EEHV-HD. Therefore, the calf needs to be exposed to the virus before or (roughly) around the age of 12 months, when maternal antibodies are still high.
2. We have seen that all the young elephants (n=60) in an orphanage in Sri Lanka had high levels of antibodies, irrespective of age, while waning of maternal antibodies was clearly visible for most young elephants in zoos. This indicates that in the orphanage EEHV-shedding is taking place more regularly than in most zoos, where herds are much smaller and contact with multiple elephants is far more limited (similar to many wild situations in Asian range countries due to habitat fragmentation!). In zoos, we have always tried to limit the stress-inducing situations as much as possible. This may have resulted in a reduction of the EEHV shedding events in our zoo elephant herds. As shedding of several herpesviruses in other mammalian species is induced by stress, we believe that the absence of stressful situations in zoos results in a decrease of shedding events, which is particularly important for calves that **need to be infected** in the short time frame when maternal antibodies are still high.

As we now can detect EEHV-antibodies, we can make an estimation of the risk that a young calf runs on getting EEHV-HD. Monitoring the antibodies every three months from the age of 6 months till the moment the antibody levels start to increase (which means that the calf apparently was infected) or have decreased below the -what we assume so far- as “safety” threshold, can prepare yourself for the best veterinary care you can provide for the young elephant. If the antibody levels remain high until 18 months and beyond, you can be relatively sure that the calf is protected against the EEHV-subspecies for which it has antibodies. If a decline is observed, you are warned for the possibility that a future EEHV-infection is likely to result in EEHV-HD, often followed by the death of the calf. Closely monitoring (weekly qPCR on a blood sample) is the only way to detect early infection, allowing early treatment (viremia precedes clinical signs 10-14 days!).

If voluntary blood collection is not possible, you should **consider** collecting blood using standing sedation at 9, 12 and 15 months. It is also advisable to collect blood from the mother at the same time; when the level of antibodies in the calf is higher than the antibody level in the mother, this indicates that the calf has been infected and is producing its own antibodies. This will probably generate sufficient data to get a reliable impression of the immune status. We understand that this is quite a tough schedule, especially if multiple standing sedations are required. However, we also

believe that it provides the best opportunity to give the optimal veterinary care. And please note: standing sedation in elephants is a very safe procedure. We have learned from so many cases - even in debilitated elephants – that the risks of negative effects are negligible.

**Elephant Standing sedation protocol** (see also: the EAZA elephant-TAG document “Emergency care for elephants clinically ill from Elephant Endotheliotropic Herpes Virus–hemorrhagic disease (EEHV-HD)”):

- Detomidine 0.01-0.022 mg/kg IM (can be reversed by atipamezole at 3 times the dose of detomidine)  
AND
- Butorphanol 0.045-0.075 mg/kg given at same time as detomidine. Butorphanol can be reversed with naltrexone at 2.5-5 times the dose of butorphanol in emergency situations, but reversal is not essential and should preferably not be carried out if the calf is considered to be in pain.

**Please also consider:** if you perform a standing sedation in your calf, take an extra whole blood sample to perform a cross match test with potential plasma donor individuals. Plasma substitution in case of EEHV-HD is believed to be a substantial part of the successful therapy at this stage. Useful in addition is the preservation of buffy coat cells (Annex 3).

If you feel that you can apply the above mentioned method of monitoring EEHV-antibody serology (not for virus detection!), even if only partly, please send your blood samples to (no test costs as long as the project has sufficient funding):

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

Contact data:

Tabitha Hoornweg, PhD  
Email address: t.e.hoornweg@uu.nl  
Telephone number: +31 (0)6-27 88 16 79  
Utrecht University

We encourage you to contact the vet advisors prior to any planned procedure to further discuss the sampling and testing.

Kind regards,

Vet advisors to the EAZA Elephant TAG  
Willem Schaftenaar (coordinator)  
Thomas Hildebrandt  
Michael Fluegger  
Endre Sos  
Imke Wiemann

### Annex 1: Blood collection

To run a full assay of EEHV-antibodies, a minimum volume of 10µL serum or plasma is required, however at least 50µL of whole blood is preferred.

To train young calves (starting from 5 months of age) for desensitizing for the ear touch, a bottle with something nice (enriched milk replacer for example) can be offered. While they are busy sucking, one can manipulate the ear; they are so eager for this bottle, that they do let blood collection happening. They only get the bottle for this occasion obviously and only a small amount, just to give enough time for this procedure.

In case the elephant is not trained for blood collection with a butterfly needle, blood can be collected using a needle prick or a 'Unistick 3 extra' lancet. The tip of the holder must be filed away to allow deep penetration into the elephant ear skin (see white arrow indication in the photo).



Blood droplets can be collected in a glass microcapillary tube or a Mitra<sup>®</sup> cartridge device (<https://www.neoteryx.com/mitra-cartridge-blood-sampling-device-dbs?hsLang=en>)



### Annex 2: Training chute.

Several zoos have developed a training chute for elephant calves, like Chester Zoo, Planckendael Zoo and Dublin Zoo. You can contact one of these zoos for advice.



Zoo Planckendael

### Annex 3: Buffy coat preservation

As an additional action besides the serum/plasma collection in the sedated elephant, recovery for buffy coat preparation for future cell culture should be considered. If the direct culture isn't feasible at that time due to a missing laboratory, the buffy coat should be cryopreserved with 10% DMSO for later use. This additional action point would provide a valuable source for future cellular research

related or unrelated to EEHV. The buffy coat samples can be easily produced by the commercial product (see photo) and can be send to the Institute for Zoo and Wildlife in Berlin overnight so that cell culture can be done or cells will be stored in liquid nitrogen.

Address to send the buffy coat sample:

Leibniz Institute for Zoo and Wildlife  
Research  
Att. Th. Hildebrandt  
Alfred-Kowalke-Strasse 17  
10315 Berlin  
Germany

