EEHV Advisory Group

Eehvinfo.org

Elephant Endotheliotropic Herpesvirus (EEHV) Monitoring and Diagnostic Testing of "At Risk" Juvenile African Elephants

For detailed information on sample collection and treatments used in Asian elephants refer to http://eehvinfo.org/eehv-management-documents/

Careful preparation for an EEHV case is an essential part of excellent elephant care. The website eehvinfo.org is a valuable resource, and should be consulted for protocols, training recommendations, treatments options, and clinical findings, among other information prior to an elephant becoming ill with EEHV. ***The calf training needed for proper care in monitoring and treating EEHV is lengthy and should be accomplished long before an EEHV viremia occurs.***

Routine monitoring of Asian elephant calves for EEHV by quantitative PCR (qPCR) is proven to detect low levels of EEHV in the blood before clinical signs occur, allowing increased monitoring and early therapeutic intervention if viral level increases (Stanton et al., 2013). Less is known about the kinetics and epidemiology of EEHV in African elephants. Healthy African elephants have been shown to have EEHV2, 3, 6, and 7 in pulmonary and skin nodules, saliva, and trunk secretions and gammaherpesviruses in conjunctival and vaginal secretions, as well as trunk secretions. In the past, there have been two known deaths due to EEHV2, one death and one illness due to EEHV6, and one illness due to EEHV3B in young African elephants (Bronson et al., 2017; Kohngmakee et al., 2015). Recently, there have been three deaths in young African elephants with high levels of EEHV3 present, as well as three young African elephants that survived significant EEHV3 viremias with treatment. Typically, we see EEHV HD in Asian calves between the ages of 1 - 8 years; these recent cases of EEHV Hemorrhagic Disease (EEHV HD) have been in African elephants between the ages of 6 – 13 years.

Routine monitoring of African calves is recommended for two reasons:

1. to monitor for low levels of EEHV before clinical signs occur, as in Asian calves
2. to increase our knowledge of the kinetics and epidemiology of EEHV in this population

The increased sensitivity of qPCR and multiple rounds of cPCR and the ability to quantify whole blood viral levels with qPCR allows for better management of calves with regards to possible EEHV HD development. If qPCR isn't available, multiple rounds of cPCR can be a sufficient, but not ideal, replacement. It is now possible to detect and quantify low levels of EEHV in the blood to distinguish between a subclinical or non-hemorrhagic herpes infection and the much more serious EEHV HD as well as to monitor closely for rapid increases in viral levels. Asian elephants can have low levels of EEHV in the blood with no or minimal clinical signs (Stanton et al., 2013) for up to two months, and possibly for over one year (Bauer et al., 2018). Viral DNA has been detected in blood of Asian elephant calves at low levels (100 – 1,000 vge/ml) for as long as one month before clinical signs occurred and EEHV HD developed. With the increased monitoring that has been done in the US in 2019, detection of transient low levels (100 – 1,000 vge/ml) of

viral DNA in African elephants, both calf/juvenile and adult, has been more frequent than has been seen in Asian elephants in the US. Shedding of viral DNA in TW secretions has also been more commonly seen in African elephants than in Asian elephants. The significance of these findings is unknown at this time.

In Asian elephants trunk wash screening can detect shedding of virus (as DNA, detected by PCR) for several months during convalescence after primary viremic infection or occasionally from reactivation of a latent infection. While there may be some overlap between high levels of viremia (virus in the blood) and shedding in the trunk secretions, viremia is the only parameter that correlates most consistently with disease. High levels of EEHV in blood are typically found in cases of EEVH HD. Based on what we know in Asian elephants, screening trunk wash samples for 2-3 months may allow the determination of the types of EEHV present in the herd, with the caveat that only EEHVs that are being shed in the trunk secretions during the collection period would be detected. Little work on saliva screening in Asian elephants has been done. Studies to determine the usefulness of saliva samples for detection of EEHV DNA in Asian and African elephants are needed and some are in progress. Eleven species and subtypes of EEHV and gammaherpesviruses have been found in skin nodules and saliva of asymptomatic juvenile and adult wild and zoo African elephants (Pearson et al, 2016).

The following protocol has been developed as a guide for the monitoring and testing of any managed elephant and calf training should be a priority to facilitate this.

Below, we provide the following recommendations:

A. routine monitoring of calves with an EDTA whole (WB) sample, with follow-up testing for a positive EEHV PCR test
B. trunk wash screening
C. flow chart to help with sample/assay determination
D. flowchart summarizing the recommendations

A. Routine EDTA whole blood (WB) screening

Recommended testing for calves aged 1-13 years:

Weekly or 2x/weekly testing by qPCR (or two rounds of cPCR)
African elephants—test for EEHV2, EEHV3-4, and EEHV6

Recommendations:

1. Look at your elephants every day for any behavioral changes that deviate from the norm as that could be an early indication of the onset of EEHV.
2. Take HR, RR, fecal bolus temp and indirect blood pressure routinely to develop individual reference intervals.
3. Evaluate weekly CBC (using a consistent lab familiar with elephant blood) to:
    a. determine normal reference intervals for your own calf/calves and to
    b. detect changes on WBC morphology, monocyte: lymphocyte ratio and platelet numbers. Early changes in either of these parameters should trigger a follow up response including repeat of a CBC and/or send a WB sample for PCR.
4. Perform serum biochemistries monthly
5. Bank EDTA WB and serum samples from the rest of the herd weekly for epidemiological investigation, future serology studies, and in case of a positive EEHV PCR result or clinical signs in a member of the herd.

IF AN EDTA WB HAS A PCR (+) RESULT:

In African elephants, EDTA WB samples can often have transient positive results between 100 – 1,000 vge/ml EEHV. Results over 1,000 vge/ml EEHV are more concerning and should be monitored as follows:

Collect EDTA WB samples as often as daily in the first week and closely monitor the viral levels provided by the testing laboratory. Bank whole blood and serum samples as frequently as possible, to facilitate future EEHV research. Initiate recommended anti-viral and supportive therapy based on:

CBC and Platelet count
Observation of clinical signs
Rapidly increasing VGE/ml (e.g. more than a three-fold increase from one sample to the next)

Consult members of the EEHV Advisory Group and the eehvinfo.org professional content subsection for current treatment recommendations and Clinical Findings Associated with EEHV Hemorrhagic Disease in Elephants.

Continue collecting samples up to daily after the first week and use the information on viral load, CBC trends, and clinical observations to determine if testing frequency can be reduced. Continue monitoring the viral load until EEHV is undetectable in EDTA WB. Viral DNA may be detectable for a month or more. Resume recommended weekly monitoring after viremia is undetectable.

Recommendations:

1. Test serum by qPCR in addition to EDTA WB, for possible clues to prognosis. It has been noted that when EEHV is found at high levels (10% or more of the levels in the EDTA WB) in the serum of Asian elephants, the prognosis may be poor (Hayward, pers comm). As we learn more, these numbers may change.
2. Continue banking EDTA WB samples from the rest of the herd weekly or according to the institution's normal husbandry procedures.
3. Consider joining ongoing studies like Brown (BrownJan@si.edu) and/or Ling (PLing@bcm.edu,). Current EEHV studies can be found on eehvinfo.org.
4. If clinical signs in the EEHV-positive calf are observed, collect EDTA WB samples from the rest of herd (at least twice weekly for calves, up to once weekly for adults) for EEHV PCR testing for at least 3-4 weeks. If no animals are found to be positive for EEHV during this period, return to weekly testing for the at risk juveniles as above.
5. If baseline TEG values are available for the calf, do daily TEG testing to look for deviations (prolonged R or K time or low MA), which can be helpful for determining clinical prognosis.

Please consult the EEHV Research and Tissue Protocol *and* the Elephant Necropsy Protocol for samples from EEHV HD cases needed for research purposes.

B. Basic TW screening to determine herd EEHV prevalence

When initially evaluating a herd for EEHV, collect TWs once/week on all herdmates for a duration of 2 months (minimum) or 3 months (optimal); test for EEHV2, EEHV3-4, and EEHV6. This should be done at least once, but may be done as often as annually. These samples can be frozen and then shipped for testing at a later date.

Consider collecting saliva swabs and feces on same days as TWs for comparison of efficacy of EEHV detection in the two samples. Contact Erin Latimer (latimere@si.edu) or Lauren Howard (lhoward@sandiegozoo.org) for saliva and fecal collection protocols.

Only EEHVs that are being shed in the trunk secretions during the collection period will be detected.

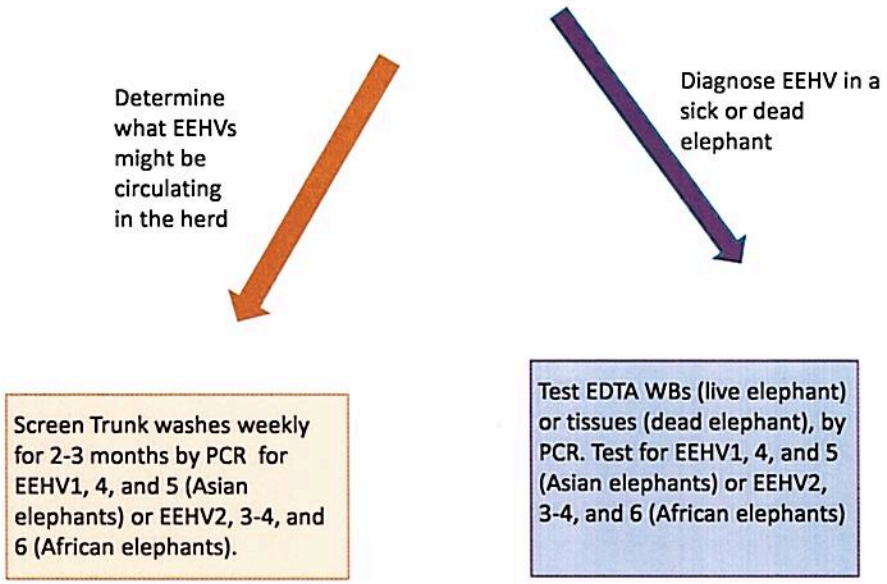
If the TW sample is EEHV (+) at high enough levels, request that the lab performs sequencing of appropriate genes to determine the subtype for epidemiological purposes.

C. Sample collection of untrained or partially trained calves

We encourage to start training the calf as young as possible and have the calf trained for different behaviors by 1 year of age. However, there are cases where the calf is only partially trained or untrained.

Although sedation can be achieved using IM or IV injections, some calves will react negatively to this, impacting training further. A sedative gel administered under the tongue (Detomidine hydrochloride; Dormosedan gel; Orion Pharma) has been successfully used in elephant calves for sample collection and training sessions. Doses between 20-50mcg/kg (0.020-0.050mg/kg) administered sublingually will achieve a maximum effect in about 30-45mins with initial effects occurring between 15-20 mins (Sanchez C., Saiers R., pers. Comm.). The low dose has been very effective for relatively non-invasive procedures such as blood draw in an ERD. The drug can be reversed with injectable atipamezole at 5:1 the detomidine dose or alternatively at 0.1mg/kg, both IM if sedation needs to be reversed, although some institutions are not administering reversals.

C. What is the purpose of your testing?



D. EEHV Calf monitoring and testing

Immediate action

Sign up for professional access to eehvinfo.org BEFORE you have an EEHV HD case

and

Develop an institutional plan for EEHV (examples available on eehvinfo.org)

and

Check eehvinfo.org for information about ongoing studies that might request sample collection

Make sure your calves are trained for behavioral observations, venipuncture, treatment, etc.

When monitoring

Routine

Daily behavioral and oral observation, temperature reading

and

Test by qPCR weekly, (EEHV1, 4, 5 for Asian elephants and EEHV2, 3-4, 6 for African elephants)

and

Weekly CBC

and

Bank serum weekly

Positive EEHV PCR result

Look at CBC, TEG, clinical signs, and trends in viral levels to determine if treatment is advised. **Begin treatment immediately if clinical signs are present, but don't wait for clinical signs to appear before starting treatment.** Consult eehvinfo.org for more information on treatment, standing sedation, drug dosages, and a detailed treatment decision flowchart.

and

qPCR daily on EDTA WB and serum, til viremia is gone. Can decrease frequency of testing if viremia is decreasing towards undetectable

and

daily CBC and TEG If available

References

Stanton, J.J., Zong, J.C., Eng, C., Howard, L., Flanagan, J., Stevens, M., Schmitt, D., Wiedner, E., Graham, D., Junge, R.E., Weber, M.A., Fischer, M., Mejia, A., Tan, J., Latimer, E., Herron, A., Hayward, G.S., & Ling, P.D. (2013) Kinetics of viral loads and genotypic analysis of elephant Endotheliotropic herpesvirus-1 infection in captive Asian elephants (Elephas maximus), Journal of Zoo and Wildlife Medicine 44(1), 42-54.

Bronson, E., McClure, M., Sohl, J., Wiedner, E., Cox, S., Latimer, E.M., Pearson, V. R., Hayward, G. S., Fuery, A., and Ling, P. D. Epidemiologic Evaluation of Elephant Endotheliotropic Herpesvirus 3b (Eehv3b) Infection in an African Elephant (*Loxodonta Africana*). J Zoo Wild Med. 2017 Jun;48(2):335-343. doi: 10.1638/2016-0063R.1

Kohngmakee, P., Suttiyaporn, S., Changptech, W., Kongkham, W., Mongkolphan, C., Tonchiangsai, K., Lertwatcharasarakul, P., Sripiboon, S., Siriaroonrat, B., and Banlunara, W.Elephant Endotheliotropic herpesvirus type 6 infection in a captive African elephant (Loxodonta africana) in Thailand. EEHV conference proceedings. 2015. p.41.

Bauer, KL., Latimer, E., & Finnegan, M. (2018) Long-term, intermittent, low-level elephant endotheliotropic herpesvirus-1A viremia in a captive Asian elephant calf. Journal of Veterinary Diagnostic Investigation,

Latimer, E., Zong, J-C.,Heaggans, S.Y., Richman, L.K., and Hayward, G.S. Detection and evaluation of novel herpesviruses in routine pathological samples from Asian and African elephants: Identification of two new probosciviruses (EEHV5 and EEHV6) and two new gammaherpesviruses (EGHV3B and EGHV5). Vet. Microbiol. 2011. 147(1-2):28-41.

Zong, J-C.,Latimer, E.M., Long, S.Y., Richman, L.k., Heaggans, S.Y., Hayward, G.S. Comparative genome analysis of four elephant endotheliotropic herpesviruses, EEHV3, EEHV4, EEHV5, and EEHV6, from cases of hemorrhagic disease or viremia. J.Virol.2014. 88:23. p.13547-13569.

Pearson VR, Zong JC, Heaggans SY, Long SY & Hayward GS. Genetic Characterization of EEHV and Gammaherpesviruses in Lung or Skin Nodules and Saliva from Zoo and Wild African Elephants. 2016, IEF/IRF Research Symposium, Singapore.

Perrin KL, Krogh AK, Kjelgaard-Hansen M, Howard L, Bochsen L, Kiso WK, Schmitt D, Kristensen AT, and Bertelsen MF. Thromboelastography in the Healthy Asian Elephant (Elephas maximus): Reference Intervals and Effects of Storage. J Zoo Wild Med. 2018;49(1):54-63