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## Elephant Endotheliotropic Herpesvirus (EEHV) Monitoring and Diagnostic Testing of “At Risk” Juvenile Asian Elephants

Careful preparation for an EEHV case is an essential part of excellent elephant care. The website [eehvinfo.org](http://eehvinfo.org) is a valuable resource, and should be consulted for protocols, training recommendations, recommended treatments, and clinical findings, among other information prior to an elephant becoming ill with EEHV. ***The calf training needed for proper care in monitoring for 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). 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 regard to possible EEHV Hemorrhagic Disease (EEHV HD) development. If qPCR is not 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 and to monitor closely for rapid increases in viral levels. 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 at low levels (100 – 1,000 vge/ml) for as much as one month before clinical signs occurred and EEHV HD developed.

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, viremia is the only parameter that correlates most consistently with disease. High levels of EEHV in blood are typically found in cases of EEHV HD. 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 are needed and some are in progress.

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. A similar document has been developed and approved by the European Elephant TAG.

Prior planning is important for the proper care of elephant calves. EEHV protocols from a few US zoos can be found on the [eehvinfo.org](http://eehvinfo.org) website; these can be used as a basis to developing a plan for your own facility. Important features of an EEHV protocol include calf monitoring, calf training, treatment, equipment and drugs to have on hand, drug dosages, and staff drills. Information on many of these topics can be found on the [eehvinfo.org](http://eehvinfo.org) website; EEHV Advisory Group members are available to give advice and answer questions.

Below, we provide the following recommendations:

- A. routine monitoring of calves, 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 1-8 years:

- Weekly EDTA WB testing by qPCR (or two rounds of cPCR)
- Asian elephants—test for EEHV1, EEHV3-4 or 4, and EEHV5

Suggestions:

1. Look at your elephants every day for any behavior changes that deviate from the norm as that could be an early indication of the onset of EEHV
2. Collect weekly CBC to have normal values for calves and to quickly detect changes in values.
3. Collect weekly TEG long enough (four to five months is recommended as a minimum) to get baseline normal values for calves to be able to detect variations during a viremia. Variations can be helpful for determining clinical prognosis.
4. Perform serum biochemistries monthly
5. Bank EDTA WB and serum samples from the rest of the herd weekly for epidemiological investigation 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:**

Collect EDTA WB samples as often as daily and closely monitor the viral levels provided by the testing laboratory. Initiate recommended anti-viral and supportive therapy based on:

- CBC and Platelet count
- Observation of clinical signs
- Viral load of 5,000 viral genome (VGE)/ml or greater
- Rapidly increasing VGE/ml

**N.B.** If the viral type is EEHV1, don't wait for the observation of clinical signs before treating; immediate treatment is recommended if CBC or platelets are off, or if viral load is >5,000 vge/ml or rapidly rising, due to the possible severity of EEHV HD.

Consult members of the [EEHV Advisory Group](#) and the [eehinfo.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, viral 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.

Suggestions:

1. If baseline TEG values are available for the calf, do daily TEG testing to look for variations, which can be helpful for determining clinical prognosis.
2. 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 in the serum, the prognosis is poor (Hayward, pers comm).
3. Continue banking EDTA WB samples from the rest of herd weekly or according to the institution's normal husbandry procedures.
4. Consider joining ongoing studies like Brown/Edwards ([BrownJan@si.edu](mailto:BrownJan@si.edu), [EdwardsKL@si.edu](mailto:EdwardsKL@si.edu)) and/or Ling/Fuery ([PLing@bcm.edu](mailto:PLing@bcm.edu), [Angela.Fuery@bcm.edu](mailto:Angela.Fuery@bcm.edu)). Current EEHV studies can be found on [eehinfo.org](#).
5. 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.

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](#)**

Annually, collect trunk washes once/week on all herd mates, for a duration of 2 months (minimum) or 3 months (optimal); test for EEHV1, EEHV3-4 or EEHV4, and EEHV5


Consider collecting saliva swabs on same days as TWs for comparison of efficacy of EEHV detection in the two samples.

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

If the trunk wash sample is EEHV (+) at high enough levels, sequence appropriate genes to determine the subtype for epidemiology purposes.

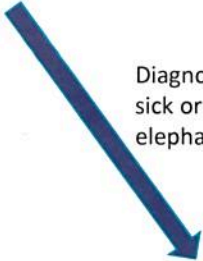
### C. What is the purpose of your testing?

Determine what EEHVs might be circulating in the herd



Screen Trunk washes weekly for 2-3 months by PCR for EEHV1, 4, and 5 (Asian elephants) or EEHV2, 3-4, and 6 (African elephants).

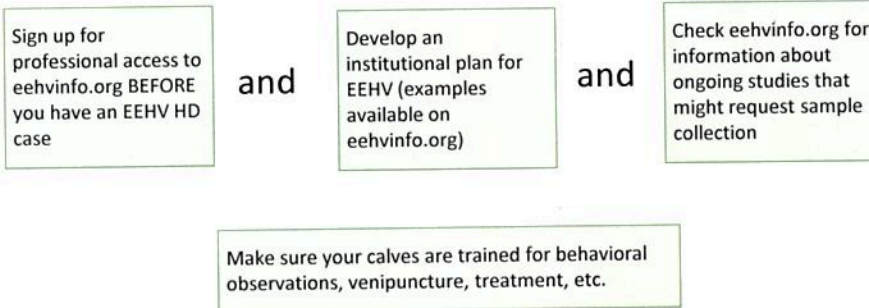
Diagnose EEHV in a sick or dead elephant



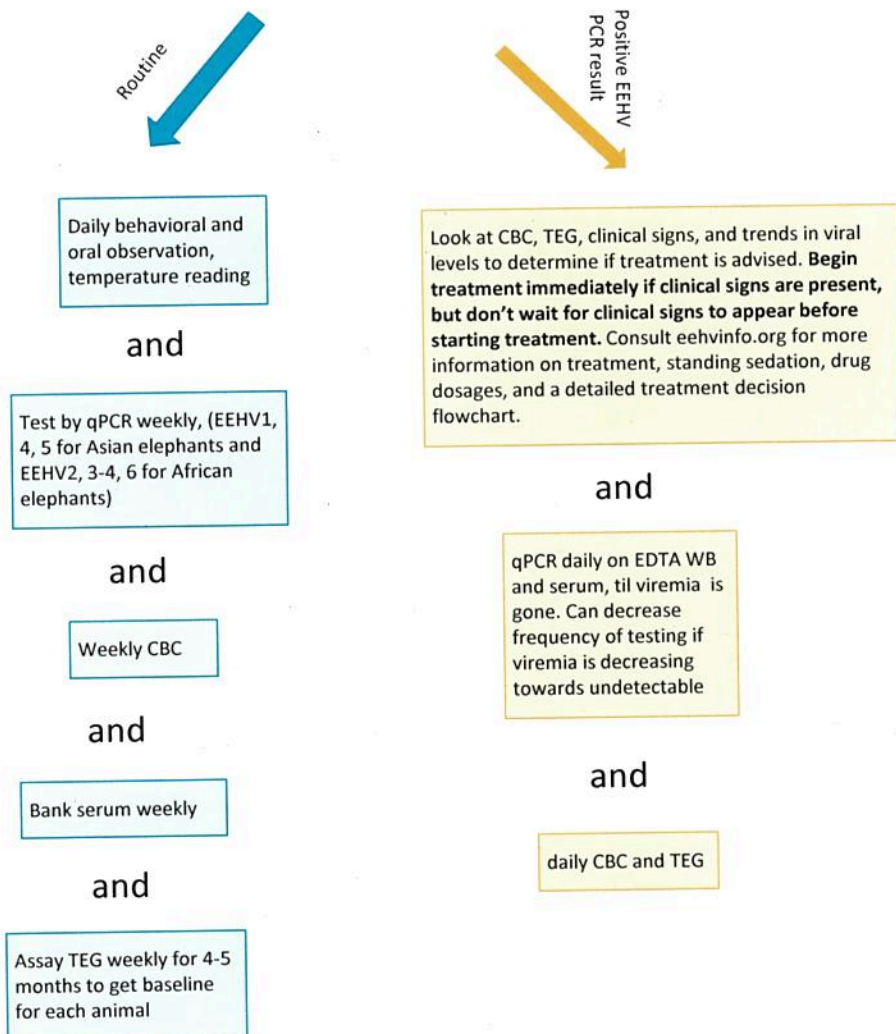
Test EDTA WBs (live elephant) or tissues (dead elephant), by PCR. Test for EEHV1, 4, and 5 (Asian elephants) or EEHV2, 3-4, and 6 (African elephants)

## D. EEHV Calf monitoring and testing

### Immediate action



### When monitoring



### 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

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*, accepted for publication