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EEHV Monitoring and Diagnostic Testing of “At Risk” Juvenile African Elephants

Careful preparation for an eventual EEHV case is an essential part of excellent elephant care. The website eehvinfo.org is a valuable resource, and should be consulted for protocols for planning for an EEHV case, training recommendations, recommended treatments, and clinical findings, among other information.

Routine monitoring of Asian elephant calves for Endotheliotropic Herpesvirus (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 and saliva and trunk secretions and gammaherpesviruses in conjunctival and vaginal secretions, as well as trunk secretions. 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, submitted, Kohngmakee et al, 2015).

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 regard to possible EEHV Hemorrhagic Disease (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 calf's subclinical or non-hemorrhagic herpes infection and the much more serious EEHV HD and 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, but possibly for as long as one year. Viral DNA has been detected in blood of Asian elephant calves 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 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 EEVH 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 in Asian 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 juvenile and adult wild and zoo African elephants (Pearson et al, 2016).

This document has been developed as a guide for the monitoring and testing of any managed elephant; calf training should be a priority to facilitate this. A similar document has been developed and approved by the European Elephant TAG.

Below, we provide recommendations for:

- A. routine monitoring of calves, with follow up testing for a positive EEHV PCR test
- B. trunk wash screening,

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)

African elephants—test for EEHV2, EEHV3-4, and EEHV6

Suggestion: 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 and THERE ARE NO CLINICAL FINDINGS INDICATIVE OF EEHV HD:

Collect EDTA WB samples 2-3 times in the first week and closely monitor the viral levels provided by the testing laboratory. Consider initiating 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

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 1-2 times per week after the first week and use 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.

Suggestion: Bank sera from affected elephants weekly. Continue banking EDTA WB samples from the rest of herd weekly or according to the institution's normal husbandry procedures.

IF AN EDTA WB HAS A PCR (+) RESULT and THERE ARE CLINICAL FINDINGS INDICATIVE OF EEHV HD:

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](#).

In addition to considering treatment of the affected animal:

Immediately submit serum and EDTA WB from affected elephant for EEHV qPCR for diagnosis and prognosis. Continue testing EDTA WB 2-3 times per week until EEHV is undetectable. It has been noted that when EEHV is found in the serum in Asian elephants, the prognosis is not good (Hayward, pers comm). Data is lacking on viral levels in serum of African elephants.

Please consult the [EEHV Research and Tissue Protocol and the Elephant Necropsy Protocol](#) for samples from EEHV HD cases needed for research purposes.

Collect EDTA WB samples from the rest of herd (twice weekly for calves, once weekly for adults) for EEHV PCR testing for at least 3-4 weeks; if none are positive for EEHV during this period, return to weekly testing for the at risk juveniles as above.

Suggestion: Bank serum from herd.

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 EEHV2, EEHV3-4, and EEHV6

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 EEHV (+), sequence appropriate genes to determine the subtype for epidemiology purposes.

References

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