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Recommendations for Elephant Transfer - Risk Analysis and Sample Collection North American EEHV Advisory Group Updated August 2022

- The North American EEHV Advisory Group (NAEAG) is comprised of 30 subject matter experts and our mission is to decrease elephant illness and death due to EEHV, while supporting institutions that care for elephants. Many of us have personally experienced the loss of an elephant to EEHV hemorrhagic disease (EEHV HD) and we all want to do our best to prevent our colleagues from having the same experience.
- The NAEAG disseminates knowledge on current best practices related to EEHV monitoring and treatment, provides technical assistance, and facilitates research through collaboration. The NAEAG is not a governing or accrediting body and does not set policy or approve or deny elephant transfers.
- The NAEAG recognizes the ongoing decline in elephant sustainability in North America and that facilitating elephant transfers is an important tool for improving reproduction.
- The information provided below is meant to provide as much up to date knowledge as is available to the elephant care leaders, zoo leaders, and veterinarians who will assess risk and prepare for elephant transfers. As new information is available, this document will be amended and re-shared.
- For more information, contact:
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Risk Analysis: EEHV Serology

1) Rationale:

- a) Recent publications indicate that, as placentally derived antibodies wane, elephants' antibodies towards one or more EEHV species can decrease or become undetectable within a few years.
- b) Repeat serologic (antibody) testing at least every 6 months in at risk elephants (1-15 years of age) is recommended to identify changes in serologic status.
- c) Undetectable or decreasing EEHV levels against one or more subtypes indicates that the elephant is considered to be at a higher risk of disease due to EEHV HD caused by this corresponding EEHV subtype.

- i) Serostatus helps to identify elephants at highest risk for disease or death due to EEHV HD prior to an elephant transfer, and allows elephant institutions to plan accordingly.
 - ii) Refer to treatment/management docs for more information:
<https://eehvinfo.org/eehv-management-documents/> (password required)
- 2) Sample information:
 - a) Sample size of 0.2ml of serum preferable, but 0.1ml is sufficient for testing.
 - b) Banked, frozen serum can be used.
 - c) Two independent samples separated by 6 months and as close to the time of transfer are ideal.
- 3) Pre-transfer recommendations
 - a) EEHV serologic testing should be performed on all elephants in receiving herd, and on elephant(s) to be transferred.
 - b) Pan EEHV, EEHV 1A, and EEHV1B for Asian elephants. EEHV4 is also available; it has been associated with fewer fatal EEHV HD cases and is considered recommended, but optional.
 - i) Serologic test for EEHV5 is not yet available; in progress.
 - c) Pan EEHV, and EEHV3 for African elephants.
 - i) Serologic tests for EEHV2 and EEHV6 are available as an experimental assay.
- 4) Interpretation of pre-transfer serologic results
 - a) Positive EEHV serologic result indicates the elephant has antibodies to that particular EEHV subtype and has therefore been previously infected by the specific EEHV subtype.
 - i) Data so far indicate that exposure to one EEHV species may not confer significant protection to another EEHV species.
 - b) A seronegative elephant may be at greater risk for EEHV HD during a change in herd status or location if exposed to this subtype.
 - i) Refer to eehvinfo.org references for details on treatment/preparation
<https://eehvinfo.org/eehv-management-documents/> (password required)
 - c) Contact EEHV Advisory Group subject matter experts for assistance in interpreting serologic results and implications for EEHV HD risk pre-transfer
- 5) Post-transfer Recommendations
 - a) EEHV serologic testing should be performed on all elephants at risk (see above) in receiving herd within 2 months of transfer.
 - b) Serum samples should be submitted every 6 months for up to 2 years post transfer.
- 6) Interpretation of post-transfer serologic results
 - a) Interpretation is similar to pre-transfer serologic results.
 - b) Contact EEHV Advisory Group subject matter experts for assistance in interpreting serology results and implications for EEHV HD risk post-transfer.
- 7) Recent publications on EEHV serologic testing:
 - i) Asian elephants:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7000966/pdf/JVI.01528-19.pdf>
 - ii) African elephants:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8528115/pdf/spectrum.00983-21.pdf>

Data Collection: Trunk Wash Analysis

1) Rationale

- a) Anecdotal evidence has suggested that a change in herd status, such as introduction of one or more elephants into a new herd, has resulted in a clinical EEHV HD episode within several months of the change (limited data thus far on specific timeline).
- b) Trunk wash (TW) collections from elephants of both herds (incoming and recipient herd) can help establish and track the types and subtypes of EEHV viruses that exist in both herds, how the viruses are shared after the move, and identify the source of any EEHV HD episodes that develop.
- c) This may not impact immediate decision making before, during or after an elephant transfer.
- d) Collection of these valuable samples will add to the bank of knowledge on the behavior of EEHV in elephants and allow for more evidence-based recommendations in the future.

2) Sample Information:

- a) Should be considered if surveillance of either herd has not been performed within the previous 12 months.
- b) Collected with standard trunk collection protocols (see Addendum below for instructions) and shipped on ice packs.

3) Pre-transfer recommendations

- a) 1-2x/trunk washes per week for 12 to 24 weeks on all elephants from both herds prior to transfer, with final samples collected within 2 months of the transfer
- b) Analysis: qPCR for EEHVs 1, 3/4, 5 (Asian elephants); 2, 3/4, 6 (African elephants).
 - i) Should consider testing for all EEHVs if elephants have been housed with elephants from another species in the past.
 - (1) Contact EEHV Advisory Group for additional trunk wash instructions if collecting for all 5 viruses.

4) Interpretation of pre-transfer TW qPCR results:

- a) It is expected that all elephants may sporadically shed EEHV in their nasal secretions, and the presence of a positive TW qPCR is NOT an indication of impending EEHV HD.
- b) The presence of a positive TW qPCR during the sampling period is an indication that the elephant has been infected with this specific EEHV previously.
- c) Negative TW qPCR results during the sampling period may indicate:
 - i) The elephant has been infected by this specific EEHV before but is not currently shedding it (shedding is intermittent, especially in Asian elephants).
 - ii) The elephant has not been infected by this specific EEHV before and would not have mounted an immune response to it.
 - iii) This elephant may be at greater risk to EEHV HD during a change in herd status or location if exposed (and does not have a known history of EEHV viremia with a particular EEHV).
 - (1) Measurement of serologic status is a more reliable indicator of previous EEHV exposure and immune status

- d) Contact EEHV Advisory Group subject matter experts for assistance in interpreting trunk wash results and implications for EEHV HD risk pre-transfer.
- 5) Post-transfer recommendations –
- a) 1-2x/trunk washes per week for 12 to 24 weeks on all elephants in receiving herd.
- b) TW DNA should be saved at -80° C to allow for sequencing of positive strains should an EEHV HD event emerge.
- i) Analysis: qPCR for EEHVs 1, 3/4, 5 (Asian elephants); 2, 3/4, 6 (African elephants).
- ii) Should consider testing for all EEHVs if elephants have been housed with elephants from another species in the past.
- (1) Contact EEHV Advisory Group for additional trunk wash instructions if collecting for all 5 viruses.
- 6) Interpretation of post-transfer TW qPCR results
- a) The purpose of post transfer TW is to bank TW DNA for epidemiologic investigation if an EEHV HD event occurs. This is to increase knowledge to create better evidence-based recommendations in the future.
- b) If resources are available, TW qPCR testing could be run in real time, on a weekly basis, to determine if EEHV shedding within the herd is increasing.
- i) In theory, this can help alert elephant managers and veterinarians that there is an increased risk of exposure to EEHV for elephants considered at risk due to age or immune status.
- ii) For an elephant with negative or decreasing serologic result:
1. Weekly TW qPCR testing of the herd, run in real time with results available the same week, could determine if EEHV shedding within the herd is increasing, and help to identify higher risk times for the development of EEHV HD.
2. If times of higher risk for the development of EEHV HD are identified, increasing the frequency of blood collection for monitoring for early EEHV Viremia and early CBC changes should be discussed.

Where can I get these tests done?

- *qPCR analysis for Trunk Washes - primary laboratory*
Erin Latimer
Smithsonian's National Zoo and Conservation Biology Institute
Department of Pathology, 3001 Connecticut Ave, NW, Washington, DC 20008

Estimated cost of trunk wash PCR: \$75/trunk wash
Please contact before collecting trunk washes to discuss best practices.

Office: 202-633-4252
Cell: 703-855-9611
Fax: 202-633-8717
Email latimere@si.edu

- *qPCR analysis for Trunk Washes – back up/ secondary laboratory*
 Dr. Paul D. Ling
 Baylor College of Medicine
 Department of Molecular Virology And Microbiology. One Baylor Plaza, Houston,
 Texas 77030
 Office: 713-798-8474
 Cell: 281-460-1696
 Fax: 713-798-3170
- *Serology*
 Dr. Paul D. Ling
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 Cell: 281-460-1696
 Fax: 713-798-3170
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ADDENDUM: Trunk Wash Collection Procedure for EEHV Screens:

Generally follow standard trunk wash collection protocol for *M. tuberculosis* culture:

- 1) Instill 50mL sterile saline solution into the nares. Try to divide the 50mL evenly between the nostrils.
- 2) Elevate the trunk 2-3ft for 30-60 seconds.
- 3) Have elephant blow into a clean container – use new ziplock/plastic bags for each sample to reduce the chance of cross contamination.
- 4) Transfer sample to a 50mL conical tube – the more mucous and exfoliated respiratory epithelial cells the better (SNOT IS GOOD).
- 5) Chill the sample on ice until it can be centrifuged.
- 6) Centrifuge at 1500Xg for 10min @ 4°C or room temperature.
- 7) Gently pour off and discard the supernatant. **Allow as much fluid to drain from the pellet as possible.**
- 8) Ideally the cell pellet is stored in the 50mL conical tube @ -80°C and shipped on dry ice, however it is acceptable to store cell pellets @ -20°C for up to several days and ship on ice.

Notes:

- If samples are being collected and tested each week (not banked), freshly collected trunk washes may be centrifuged, refrigerated @ 4°C (not frozen) and shipped overnight (on ice packs) on the day of collection.

Please note handling recommendations:

- **Best**--centrifuge onsite (elephant-holding facility), pour off supernatant, ship on ice packs each collection day or freeze the pellets at -80 C and ship entire set of pellets to the NEHL on dry ice or ice packs that have been at -80 C

- **OK**--centrifuge onsite, pour off supernatant, freeze the pellets at -20 C and ship frozen pellets on ice packs each week
- **Least preferable**--ship uncentrifuged/not frozen tubes each collection day on ice packs (if centrifuge is not available)
- Please contact prior to collection/shipment to clarify instructions and so the shipment is expected: Erin Latimer -- latimere@si.edu