Long-term, intermittent, low-level elephant endotheliotropic herpesvirus 1a viremia in a captive Asian elephant calf

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Running head: EEHV1A viremia in Asian elephant calf
Abstract. A 2-y-old male Asian elephant (*Elephas maximus*), with an elevated platelet count 
(1,100 × 10⁹/L [1,100 × 10³/mm³]), tested positive for elephant endotheliotropic herpesvirus 1A 
(EEHV-1A) on conventional PCR (cPCR) of EDTA whole blood. No clinical signs were ever 
reported and no treatment was administered, but low-level viremia persisted for 2.5 y based on 
results of cPCR and/or real-time PCR (rtPCR). Sequencing confirmed that the EEHV-1A 
detected was identical at the beginning through the end of the time period. No other elephants in 
the herd tested positive for EEHV-1 during this time period. Platelet counts remained elevated 
throughout the viremia and throughout the animal’s life, and direct correlation between the 
elevated platelet counts and EEHV-1A viremia could not be confirmed. We document long-term, 
intermittent, low-level viremia of EEHV-1A and provide additional information to consider 
when determining if treatment is warranted in a case of EEHV infection.

Key words: Elephants; *Herpesviridae*; platelet count; PCR; viremia.
Elephant endotheliotropic herpesviruses (EEHVs; type species *Elephantid betaherpesvirus 1*) are implicated in cases of fatal hemorrhagic disease in Asian elephants (*Elephas maximus*) and African elephants (*Loxodonta africana*). EEHVs are members of genus *Proboscivirus* classified as of 2017 in subfamily *Betaherpesvirinae*; there is a proposal to create a new subfamily, *Deltaherpesvirinae*, and reclassify the viruses accordingly. To date, 12 virus subtypes have been identified (EEHV-1A, -1B, -2, -3A, -3B, -4A, -4B, -5A, -5B, -6, -7A, -7B), and deaths have been associated with EEHV-1A, -1B, -2, -3A, -4A, -5A, and -6. Evidence suggests that EEHV-1A, -1B, -4A, -4B, -5A, and -5B are endogenous in Asian elephants worldwide.

In mid-May 2011, a 2-y-old male Asian elephant at a North American zoo was noted to have increased platelets, and a manual platelet count confirmed an elevated value (1,100 × 10⁹/L; [1,100 × 10³/mm³]; reference interval: 142–914 × 10⁹/L). Due to the abnormal platelet count, blood was submitted for EEHV testing. Blood was collected from auricular veins into EDTA anticoagulant tubes, refrigerated, and sent to the testing laboratory on wet ice overnight. Banked recent routine samples from late April–mid-May 2011 stored at –80°C were also submitted. The calf tested positive for EEHV-1A on conventional PCR (cPCR) on both current and retrospective samples, with the earliest positive sample recorded on April 28, 2011. Additional blood samples were collected approximately weekly, banked at –80°C, and sent for EEHV testing approximately once per month during 2011 and periodically during 2012–2013.

At the testing laboratory, 200 µL of whole blood was processed for DNA as described previously and also per the protocol for column prep (Generation capture column kit, cat. 159916, Qiagen, Valencia, CA). Conventional PCR was completed as described previously (Table 1). Real-time PCR (rtPCR) was performed with previously described methods. All DNA sequencing was carried out by direct cycle sequencing on both strands of purified PCR...
DNA products from 2 rounds of nested or semi-nested PCR amplification. The correctly sized PCR products were purified after agarose gel electrophoresis (QIAquick gel extraction kit, Qiagen). Sequencing reactions were carried out and analyzed (ABI PRISM BigDye terminator v.3.1 cycle sequencing kit, ABI 310 DNA sequencer, Applied Biosystems, Foster City, CA) or sequenced at Macrogen (Rockville, MD) and Genewiz (South Plainfield, NJ). All DNA sequence editing, analysis, and manipulation was performed using Sequencher (Gene Codes, Ann Arbor, MI) and Clustal-Omega Multiple Sequence Alignment (EMBL-EBI, Wellcome Genome Campus, Hinxton, Cambridgeshire, UK) together with BLASTn nucleotide comparison program (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Thirty-seven blood samples were collected over a period of ~2.5 y, and 22 of the samples were positive for EEHV-1A based on cPCR. Real-time PCR was performed on all of the samples to quantify the level of viremia (Fig. 1). Several genes were sequenced periodically throughout the 2.5 y; a total of 35 sequencing runs were conducted for all of these genes. All sequences were identical at 5 loci tested over the 2.5 y, which is consistent with the low mutation rates of other herpesviruses, calculated to be $1.8 \times 10^{-8}$ mutations per nucleotide, per genomic replication. However, variation in nucleotides in parts of the genome not included in the 5 loci tested cannot be ruled out.

Complete blood count and serum biochemistry was evaluated in concert with all samples that were tested for EEHV. No abnormalities were detected other than elevated platelet counts, which persisted throughout the period of EEHV-1A viremia and after EEHV-1A was no longer detected (mean: $879 \times 10^9$/L [$879 \times 10^3$/mm$^3$], range: $339–2,040 \times 10^9$/L [$339–2040 \times 10^3$/mm$^3$], $n = 168$). Clinical signs are usually not seen with viremias <10$^4$ virus genomic equivalents (vge)/mL.$^{12}$ The highest level seen in this animal was $\sim2 \times 10^3$ vge/mL.
At the time of the positive EEHV-1A result, the elephant herd consisted of 3 adult bulls and 3 adult cows in addition to the male calf. The male calf was born at the same institution where the viremia was detected, and there were no new elephants introduced to the herd or outside contacts from the time of the male calf’s birth to the time of EEHV viremia detection. No animals exhibited any clinical signs of disease, and no treatment was administered. Also, no other animals ever tested positive for EEHV-1, including a female calf born in November 2012, during the period of viremia. In addition, no EEHV testing was completed on the male calf for any dates prior to April 28, 2011. As of fall 2018, the elephant in our case is a 9-y-old healthy bull elephant residing at the same zoo with no evidence of EEHV viremia on routine rtPCR testing.

It is not known if the elevated platelet counts in our case were related to the EEHV viremia. Typically, EEHV clinical infections are associated with decreased platelet counts. However, there have been reports of platelet count rebounding to higher-than-normal values following resolution of clinical signs associated with EEHV. It is possible that a decreased platelet count occurred in this individual at the time of initial infection with EEHV-1A, with a subsequent transient increase to higher-than-normal platelet counts. However, no manual platelet counts were completed at the time of the earliest EEHV viremia detection, and the timing of initial infection with EEHV is unknown. An incidental finding or normal variation in platelet counts for this individual cannot be ruled out, especially given that juvenile animals of other species have been reported to have increased platelet values in comparison to adults. Also, elevated platelet counts have been associated with other clinical diseases in elephants including *Mycobacterium tuberculosis* (Harr et al. Clinicopathological findings in *Mycobacterium*...
tuberculosis culture-positive elephants (*Elephas maximus*) in comparison to clinically normal elephants. Proc Am Assoc Zoo Vet; Sept 2001; Orlando, FL).

Although there have been previous reports of low-level EEHV-1 shedding in trunk samples, our report documents of long-term, intermittent, low-level EEHV viremia. To date, latent forms of EEHV have not been detected by PCR from whole blood, and the level detected in this male calf is suspected to similarly represent continuous reactivation or persistence rather than latency. As has been recommended previously, treatment decisions for EEHV-positive animals should involve the evaluation of multiple factors including the leukogram, the level of viremia, and the clinical signs of the elephant. Platelet counts should additionally be evaluated to continue to add to the knowledge base of this important viral disease.

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**References**


Table 1. Primers used for detection and sequencing of elephant endotheliotropic herpesvirus 1A in a juvenile male Asian elephant (*Elephas maximus*).

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene target</th>
<th>Orientation</th>
<th>Sequence</th>
<th>GenBank accession</th>
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<td>6710</td>
<td>pol-pan herpes</td>
<td>L1/R1 = 530 bp; L1/R2 = 250 bp</td>
<td>L1: ACAAACACGCTGTCRGGTRTCYCCRTA</td>
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<tr>
<td>6711</td>
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<td>R1</td>
<td>GTATTTGATTTTYGCNAGYYTGTAYCC</td>
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<td>R2</td>
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<td>hel</td>
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Figure 1. PCR of elephant endotheliotropic herpesvirus 1A in a juvenile male Asian elephant (*Elephas maximus*) in 2011–2013. The upper y-axis indicates viral load detected via real-time PCR (rtPCR). The presence of a bar on the lower graph indicates a positive conventional PCR (cPCR) result corresponding to that date.