Investigation of the immunological response to elephant endotheliotropic herpesvirus in Asian elephants (*Elephas maximus*) and African elephants (*Loxodonta africana*)

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UCDAVIS VETERINARY MEDICINE Students Training in Advanced Research

Background

Elephant endotheliotropic herpesvirus (EEHV) is the cause of an acute fatal hemorrhagic disease (EEHV-HD), and the leading cause of juvenile captive Asian elephant mortality in elephants 1-8yrs[4]. Up to 65% of elephant deaths in North America, Europe, and Asia have been caused by EEHV-HD since 1980, with an unknown but increasing impact seen in wild populations in range countries[3]. There are seven major subtypes: EEHV1A/B, 4, and 5 in Asian elephants, and EEHV2, 3, 6, and 7 in African elephants[1]. EEHV is characterized by a primary infection, followed by latency and sporadic reactivation and shedding of the virus. Asymptomatic shedding, often by adults, is concern for transmission to at-risk age groups that are seronegative after waning of maternal antibodies at approximately two years of age. Calves within the vulnerable age range are more likely to have a primary infection with increased viral load and rapid onset of EEHV-HD, likely resulting in death within hours to days[1].

Project Significance

- Epidemiology and prevalence of seroreactivity within North American elephants is not well documented and vital for understanding burden of disease and preparing management, monitoring, and treatment strategies.
- Primary infections have not been thoroughly documented in Asian or African elephants to date and are necessary for further understanding EEHV pathogenesis and the role of maternal antibodies in timing of primary infection.

Objectives

Objective 1:

Determine prevalence of seroreactivity in Asian (*Elephas maximus*) and African elephants (*Loxodonta africana*) to specific EEHV species within North American AZA institutions and examine demographic and management related risk factors.

Objective 2:

Investigate the immunological response and clinical presentation in a case of presumed primary EEHV infection in an Asian elephant calf at the Houston Zoo via Luciferase Immunoprecipitation System (LIPS) assays and qPCR.

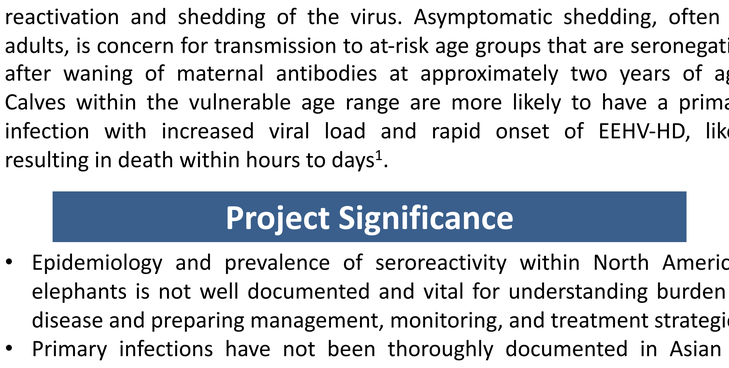

Figure 1. Houston Zoo elephant herd. (photo credit: Kristin Windle)

Methods

Objective 1

Study Population: Asian elephants (n=40) and African elephants (n=37) at 15 AZA institutions in North America (Table 1).

Table 1. Demographics of study populations (n=77).

Species	Age Range	# Female	# Male
Elephas maximus	1 day – 58 yrs 8 mo	26	14
Loxodonta africana	1 yr 10 mo – 51 yrs	28	9

- Serological data was collected from March 2021-June 2022 as part of routine EEHV health screening.

LIPS Serology Assay: Serum was processed using standardized LIPS assays[2] with species specific luciferase tagged EEHV antigens (Table 2).

Table 2. LIPS assay antigens

panEEHV	EEHV1A	EEHV1B	EEHV4	EEHV3
gB	ORF-Q Daizy, Ramen, Kimba	ORF-Q Emelia	E34	E34

Statistical Analysis:

- Prevalence of immunoreactivity to EEHV species by age group was determined to evaluate highest risk age groups for primary EEHV infection (Table 4).
- Associations between seropositive EEHV status and individual risk factors were evaluated by the 2-sided Fisher exact test or the Chi ^{2} test and associations measured by odds ratios (Table 4).
- Mixed effects multivariable logistic regression was used, incorporating fixed and random effects (AZA location) to assess the association of risk factors that were significant on bivariate analysis.

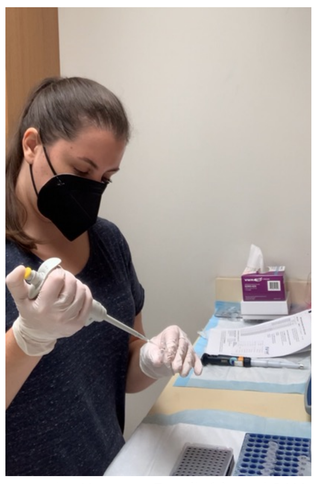

Figure 2. Serum collection of an adult Asian elephant at the Houston Zoo (left), and DNA extraction of elephant trunk wash (right). (photo credit: Michelle Cowell)

Methods

Objective 2

Table 3. Signalment and presentation of elephant calf at the Houston Zoo

Age	Sex	Presenting Clinical Signs	CBC Results	Start Date of EEHV1A	Start Date of EEHV5	Treatments
1 Yr 3 Mo	Male	Alert, Active, Eating	Monocytosis, Leukocytosis	9/1/21	9/27/21	Rectal Fluids, Dormosedan Gel, Lidocaine 20%

Sample Collection:

- Whole blood (WB) and serum were collected weekly from May 2020 to June 2022.
- Trunk wash (TW) was collected after viremia onset, from October 2021 to January 2022 once calf was trained.

DNA Extraction and Quantitative Polymerase Chain Reaction (qPCR):

- DNA was extracted from whole blood and trunk wash using DNeasy™ blood and tissue kits (Qiagen Inc).
- qPCR was performed on extracted DNA targeting genes for EEHV1A, EEHV1B, EEHV4, and EEHV5. Parallel qPCR reactions were performed using elephant tumor necrosis factor α (TNFα) as a control for DNA quality.

LIPS Serology Assay:

- LIPS assays were performed across 14 time points: birth to June 2022 (post EEHV1A and EEHV5 viremia).
- Antibody status was assessed for EEHV1A, EEHV1B, and EEHV4 (Table 2).
- An additional antigen (E52), proven reactive to the Houston Zoo herd, was used to assess antibody status to EEHV1A.

Figure 3. One month old Asian elephant calf at the Houston Zoo. (photo credit: Kristin Windle)

Results

Objective 1

Table 4. Prevalence (P), odds ratios (OR) and p-values (*p*) of risk factors associated with EEHV species.

Risk Factor	EEHV1A					EEHV1B					EEHV4				
	No. Pos	No. Neg	P	OR	*p*	No. Pos	No. Neg	P	OR	*p*	No. Pos	No. Neg	P	OR	*p*
Age Group (Years)															
<1	2	0	0.05	NC	NC	1	1	0.03	0.56	0.69	2	0	0.05	NC	NC
1-4	0	0	0.00	NC	NC	0	0	0.00	NC	NC	0	0	0.00	NC	NC
5-10	4	0	0.10	NC	NC	3	1	0.08	1.83	0.62	1	3	0.03	0.09	0.05
11-50	23	1	0.58	NC	NC	14	10	0.35	0.25	0.07	16	8	0.40	0.49	0.36
>50	10	0	0.25	NC	NC	9	1	0.23	7.41	0.07	10	0	0.25	NC	NC
# Seroreactive EEHV Antigens															
1	6	1	0.15	NC	NC	3	4	0.08	0.26	0.10	2	5	0.05	0.13	0.02
2	13	0	0.33	NC	NC	7	6	0.18	0.55	0.39	10	3	0.25	1.33	0.71
3	20	0	0.5	NC	NC	16	4	0.40	4.4	0.04	17	3	0.43	3.49	0.11
Captive vs. Wild															
Captive	24	1	0.60	1.6	0.75	15	10	0.38	0.68	0.57	14	11	0.35	NC	NC
Wild	15	0	0.38	Ref	0.75	11	4	0.28	Ref	0.57	15	0	0.38	NC	NC
Region															
West	5	0	0.13	NC	NC	3	2	0.08	0.85	0.87	2	3	0.05	0.19	0.10
Midwest	15	1	0.38	0.62	0.75	13	3	0.33	4.00	0.07	13	3	0.33	2.04	0.36
Northeast	5	0	0.13	NC	NC	1	4	0.03	0.11	0.06	4	1	0.10	1.54	0.72
South	14	0	0.35	0.56	0.69	9	5	0.23	0.79	0.73	11	4	0.28	1.01	0.99
# Historical Locations															
1-3	22	1	0.55	1.29	0.86	15	8	0.38	1.19	0.79	13	10	0.33	0.08	0.02
4-6	13	0	0.33	0.50	0.63	9	4	0.23	1.06	0.93	12	1	0.30	7.65	0.07
7-10	4	0	0.10	NC	NC	2	2	0.05	0.54	0.65	4	0	0.10	NC	NC
Total	39	1	0.98	NC	NC	26	14	0.65	NC	NC	29	11	0.73	NC	NC

- NC = not calculated (data with perfect prediction or highly collinear, or no data available)
- Odds ratios were calculated for each category using all other categories for that risk factor as the reference.

Risk factors "age group" and "# seroreactive EEHV antigens" were found to perfectly predict EEHV1A. Risk factors "# vulnerable aged individuals" were found to perfectly predict EEHV1A and EEHV3.

On mixed effects multivariable logistic regression, "# seroreactive EEHV antigens" and "age" were significant risk factors for EEHV1B and EEHV4 respectively when adjusting for zoo location as the random variable (p = 0.0012, p = 0.00).

Objective 2

Whole Blood (WB) and Trunk Wash (TW) qPCR:

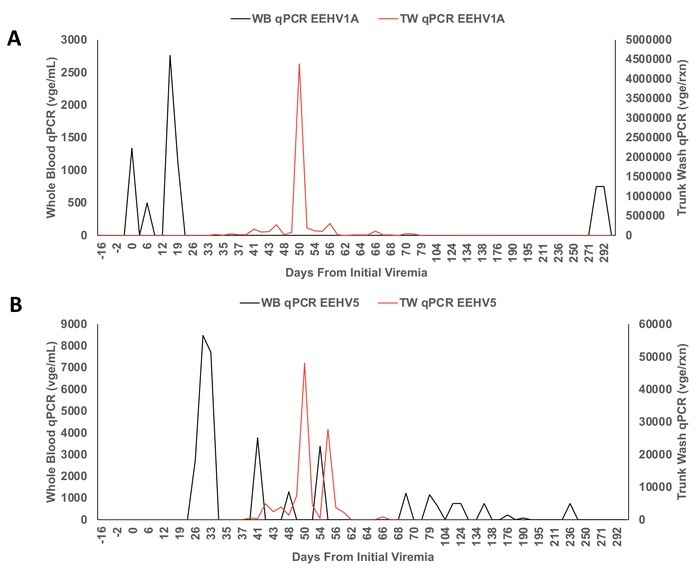

Figure 4. WB and TW qPCR for EEHV1A (**A**) and EEHV5 (**B**). X-axis = days relative to initial detection of EEHV1A. Y-axis = viral genome equivalents/mL WB (left) and per test reaction in TW (right).

Results

Objective 2

Leukocyte Parameters:

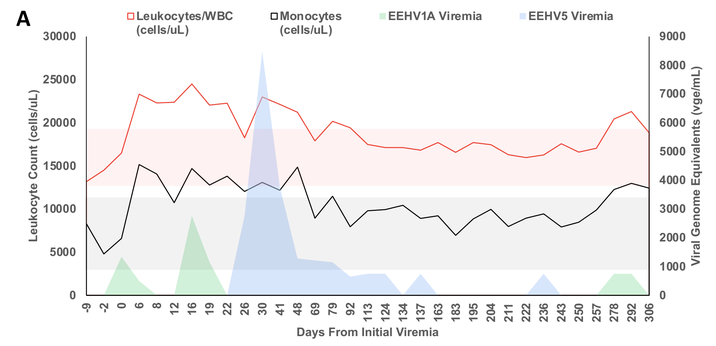

Figure 5. CBC data before, during, and after EEHV1A and EEHV5. X-axis = days relative to initial detection of EEHV1A. Y-axis = # cells/uL WB (left) and # viral genome equivalents/mL WB (right), demonstrated by green and blue peaks. **A.** Total leukocytes (red) and monocytes (black) before, during, and after initial EEHV1A. Individual reference intervals (RI) for leukocytes and monocytes are denoted by red and black horizontal bars respectively. **B.** Platelet counts before, during, and after initial EEHV1A. Individual RI for platelets is denoted by black horizontal bar.

LIPS Assay Serology:

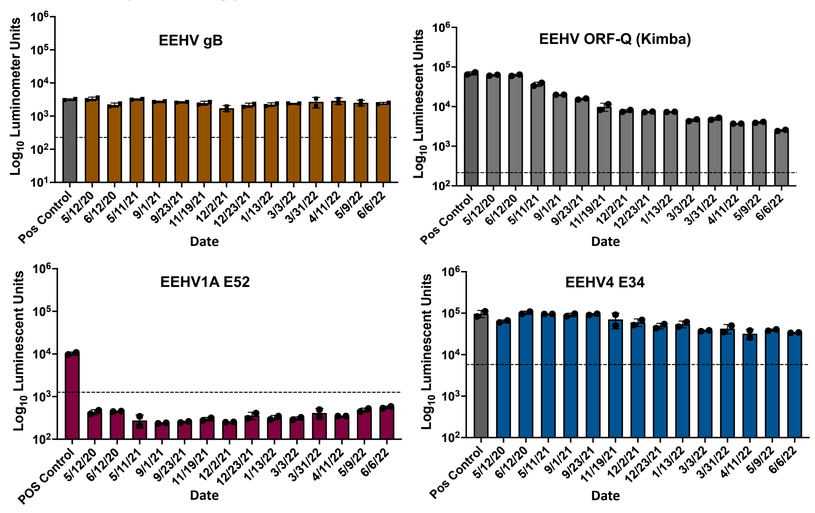

Figure 6. Anti-EEHV antibody levels detected by LIPS assays over time. X-axis indicates 14 time points from birth to post EEHV viremia (June 2022). Y-axis = antibody levels plotted on a $\log_{10}$ scale. Dashed lines represent cutoff values for sensitivity and specificity of each antigen calculated as average antibody titer of the uninfected samples +/- 5 SD.

Conclusion

Figure 7. Six month old Asian elephant calf at the Houston Zoo. (photo credit: Kristin Windle)

Objective 1

1. The majority of Asian elephants (98%) were seropositive for EEHV1A compared to EEHV1B (65%) and EEHV4 (73%).
2. Consistent with previous literature, we found elephants within the 1-4 age group seronegative for EEHV1A, EEHV1B and EEHV4, leaving them at greater risk for primary infection and EEHV-HD. Elephants >5yrs of age demonstrated protective antibodies. This suggests that this age window may be a risk factor for EEHV viremia and surveillance of this age group is critically important.

Objective 2

1. We demonstrated an atypical EEHV1A pattern in a 1yr 3mo elephant, suggesting a reactivated latent virus rather than primary infection.
2. The presence of sustained EEHV4 antibodies similarly suggested the possibility of previous viremia and waning maternal antibodies.
3. We also demonstrated an atypical CBC pattern, whereby there was an increase in CBC parameters during viremia, typically seen in the late recovery phase of infection. This suggests that indicators historically used may not detect all EEHV infections, and an increased level of vigilance may be required to detect low level non-clinical viremias.
4. Serology testing will continue over the following six months to further elucidate these patterns and assist in developing further understanding of the timing of maternal antibody waning.

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