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SHORT COMMUNICATION

Young elephants in a large herd maintain high levels of elephant endotheliotropic herpesvirus-specific antibodies and do not succumb to fatal haemorrhagic disease

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Abstract

Elephant endotheliotropic herpesviruses (EEHVs) have co-existed with elephants for millions of years, yet may cause fatal haemorrhagic disease (EEHV-HD), typically in elephants between 1 and 10 years of age. EEHV is omnipresent in (sub)adult elephants, and young elephants with low EEHV-specific antibody levels are at risk for EEHV-HD, suggesting that fatal disease may occur due to an insufficiently controlled primary infection. To further address this hypothesis, sera of three large elephant cohorts were subjected to a multiple EEHV species ELISA: (I) 96 Asian elephants between 0 and 57 years, including 13 EEHV-HD fatalities, from European zoo herds typically sized five to six elephants, (II) a herd of 64 orphaned elephants aged 0–15 years at the Elephant Transit Home in Sri Lanka and (III) 31 elephants aged 8–63 years, part of a large herd of 93 elephants at Pinnawala Elephant Orphanage, Sri Lanka. All Sri Lankan elephants showed high EEHV-specific antibody levels regardless of their age. While antibody levels of most European zoo elephants were comparable to those of Sri Lankan elephants, the average antibody level of the European juveniles (1–5 years of age) was significantly lower than those of age-matched Sri Lankan individuals. Moreover, the European juveniles showed a gradual decrease between 1 and 4 years of age, to be attributed to waning maternal antibodies. Maintenance of high levels of antibodies in spite of waning maternal antibodies in young Sri Lankan elephants is likely due to the larger herd size that increases the likelihood of contact with EEHV-shedding elephants. Together with the observation that low levels of EEHV-specific antibodies correlate with increased numbers of EEHV-HD fatalities, these results suggest that infection in presence of high maternal antibody levels may protect calves from developing EEHV-HD, while at the same time activating an immune response protective in future encounters with this virus.

KEYWORDS
Asian elephant, EEHV, gB, herd size, serology

1 | INTRODUCTION

Elephant endotheliotropic herpesviruses (EEHVs) are a group of elephant-specific herpesviruses associated with an acute, highly lethal disease in elephants, known as EEHV-haemorrhagic disease (EEHV-HD). Currently, eight EEHV (sub)species are discerned: EEHV1A, −1B, −4 and −5 naturally infect Asian elephants (*Elephas maximus*) while EEHV2, −3, −6 and −7 are carried by African elephants (*Loxodonta* species). While healthy adult elephants may be (latently) infected with one or multiple EEHV (sub)species without clinical signs, EEHV-HD primarily occurs in young elephants, commonly between 1 and 10 years of age, and is the single greatest cause of mortality of young Asian elephants (Long et al., 2016). Over the last 35 years, the disease was responsible for 50%–60% of all young Asian elephant deaths in Western zoos, killing 12%–17% of the Asian elephant calves born during this period (Howard & Schaftenaar, 2018; Jesus et al., 2021; Perrin et al., 2021). The disease also affects both (semi-) captive and free-ranging elephants in Asian elephant range countries (Bouchard et al., 2014; Lee et al., 2021; Long et al., 2016; Oo et al., 2020; Prompiram et al., 2021; Reid et al., 2006; Zachariah et al., 2018), yet exact mortality rates in these populations are unknown.

Using novel EEHV-specific ELISAs applying mammalian-cell expressed glycoproteins, it was recently shown that all (sub)adult elephants, living both in European zoos and Laos, one of the Asian elephant range countries, were EEHV seropositive (Hoornweg et al., 2021). Of note, other studies found much lower levels of seropositive animals (Angkawanish et al., 2019; Prompiram et al., 2021; van den Doel et al., 2015) probably due to reduced sensitivity resulting from the use of bacterially expressed antigens or peptides. Importantly, multiple recent studies (Fuery et al., 2020; Hoornweg et al., 2021; Pursell et al., 2021) indicate that low levels of EEHV-specific antibodies are a risk factor for EEHV-HD, suggesting that fatal cases mainly occur in animals experiencing a primary EEHV infection.

With almost 6000 Asian elephants, Sri Lanka holds almost 13% of the remaining free-living Asian elephant population. Human-elephant conflict is intense and leads to the death of approximately 150 elephants each year, which orphans 10–15 elephant calves. The Pinnawala Elephant Orphanage (PEO) and the Elephant Transit Home (ETH) were founded to provide care for orphaned free-living elephant calves. Additionally, a breeding program was initiated at the PEO, which currently contains a single large herd of >90 elephants. The ETH, located at the edge of Udawalawe National Park, rehabilitates orphaned elephant calves, aiming at release back to the wild. The ETH holds a large herd of young Asian elephants at the risk ages for developing EEHV-HD that may interact freely with free-ranging elephants in the National Park (B. V. Perera et al., 2018).

The aim of the present study was to screen Asian elephants from two large herds in Sri Lanka, one consisting of relatively young Asian elephants at the Elephant Transit Home (ETH) and a large mixed-age herd at Pinnawala Elephant Orphanage (PEO), to identify animals potentially at risk of developing EEHV-HD. To this end, a recently developed EEHV1A gB ELISA, with which antibodies elicited against multiple EEHV (sub)species can be detected (Hoornweg et al., 2021), was used. The results were compared with those obtained from a cohort of 96 Asian elephants from European zoos, partly reported previously (Hoornweg et al., 2021).

2 | MATERIALS AND METHODS

2.1 | Serum samples

Seventy-two serum samples from 64 elephants, aged 2 months to 15 years, living at the ETH in Udawalawe, Sri Lanka and 31 serum samples from 31 individual elephants aged 8 years to 63 years, living at the PEO in Rambukkana, Sri Lanka, were used for this study. Additionally, 193 serum samples of 96 individual elephants, aged 0 days to 57 years, from 18 European zoological collections (all available samples) were included. All blood samples were taken aseptically from ear or leg veins by veterinary staff at the ETH, PEO and European zoos, and sera were transported at 4°C to our respective institutes for diagnostic purposes. Sera were stored at −20°C until use. For each of the three cohorts, elephants were divided into different age groups according to published classification criteria (Arivazhagan & Sukumar, 2008). Specifics of the cohorts are summarized in Table 1.

2.2 | ELISA

ELISA plates were coated with 5 ng recombinant EEHV1A gB per well, washed and blocked, as described previously (Hoornweg et al., 2021). Pre-coated plates were frozen and stored at −20°C until use. Freezing of plates was shown not to affect assay performance (data not shown). To test the Sri Lankan cohorts pre-coated plates were shipped frozen to the University of Peradeniya in Sri Lanka. To allow comparison of the cohorts, ΔOD values were normalized relative to the average ΔOD value measured for adult elephants within each cohort. In view of the high conservation of gB among EEHV (sub)species and as EEHV1A gB was shown to be recognized by antibodies elicited towards gB of other EEHV (sub)species than EEHV1A (Fuery et al., 2020; Hoornweg et al., 2021), the gB ELISA used in this study is considered a multiple EEHV species ELISA. The exact breadth of the antibody responses detected remains, however, to be determined.

TABLE 1 Specifics of the Asian elephant cohorts included in this study

Age category (years)	Asian elephants living at ETH, Sri Lanka			Asian elephants living at PEO, Sri Lanka			Asian elephants living in European zoos			Statistical difference in number of fatal HD-cases‡
	# of samples	# of animals	# of HD fatalities	# of samples	# of animals	# of HD fatalities	# of samples	# of animals	# of HD fatalities	
Baby (<1)	3	3	0	NA	NA	NA	9	6	0	$p > .9999^a$ / NA[b]
Juvenile (1–5)	26	26	0	NA	NA	NA	59	29	12	$p = .0001^a$ / NA[b]
Subadult (5–15)	40	32	0	11	11	0	37	26	1	$p = .4483^a$ / $p > .9999^b$
Adult (15+)	3	3	0	20	20	0	88	44	0	$p > .9999^a$ / $p > .9999^b$
Total	72	64	0	31	31	0	193	96†	13	$p = .0018^a$ / $p = .0370^b$

†The sum of individual animals per age category exceeds the total numbers of individual animals in this cohort, as paired samples from multiple animals fall within different age categories.
‡Statistical difference in number of fatal HD cases was tested between both Sri Lankan cohorts and the European zoo cohort using the Fishers exact test in GraphPad Prism.
[a]ETH cohort compared to the European zoo cohort.
[b]PEO cohort compared to the European zoo cohort.

3 | RESULTS

3.1 | Elephant cohort characteristics

Neither in the current cohort study nor historically have any cases of EEHV-HD been observed at the ETH and PEO. In the European zoo cohort, 13 of the 96 elephants (13.5%) succumbed to EEHV-HD, predominantly between 1 and 5 years of age (Table 1). Within this age group, the number of EEHV-HD cases differed significantly between the European zoo and the ETH cohort ($p = .0001$; Fishers exact test). Serum samples of animals below 5 years of age were not available in the PEO cohort.

3.2 | EEHV-specific antibody levels detected in the different elephant cohorts

All elephants living at the ETH in Sri Lanka were found to be seropositive for EEHV (Figure 1). Although mean antibody levels increased slightly with age, ranging from normalized ΔOD values of 0.83 for animals less than 1 year old to 1.00 for adult animals (15+ years old), the values did not differ significantly between either of the age categories and the adult elephants (Figure 1). For the PEO only subadult (5–15 years old) and adult elephants could be tested, which were all seropositive for EEHV with no significant difference in ΔOD values between both age categories (mean normalized ΔOD values of 1.11 and 1.00, respectively; Figure 1). In contrast, the juvenile elephants (1–5 years old) in the European zoo cohort had a significantly lower average antibody level (normalized ΔOD value of 0.62) than all other age groups within the European zoo cohort (normalized ΔOD values between 0.98 and 1.00), both age groups at the PEO and the juvenile and subadult elephants at the ETH (Figure 1; $p < .0001$ for all comparisons).

3.3 | EEHV-specific antibody levels in elephants in the ETH and European zoo cohorts from birth to 5 years of age

To explore the differences in antibody levels within the youngest two age groups in the cohorts (less than 1 year old and 1–5 years old), normalized ΔOD levels were plotted according to age at sampling. EEHV-specific antibody levels of ETH elephants did not change considerably with increasing age (Figure 2a), while the EEHV-specific antibody levels of Asian elephants living in European zoos displayed marked variation (Figure 2b). In the latter group, a clear decline of EEHV-specific antibodies from one to approximately 4 years of age was observed, whereas a number of sera from elephants beyond 2 years of age (29 sera of 16 elephants) showed high antibody levels. From 2–3 years of age animals were found to be either highly seropositive or (close to) seronegative (Figure 2b). The apparent decline of antibody levels plotted separately revealed a half-life of 1.7 years, with antibody levels tending to become undetectable after 3.5 years (Figure 2c).

4 | DISCUSSION

In the current cross-sectional study, all elephants within the two Sri Lankan cohorts, irrespective of their age, displayed high antibody levels against EEHV. In contrast, a clear decline in antibody levels was observed for juvenile Asian elephants within the European zoo cohort, with antibodies becoming undetectable at 3.5 years of age. This decline likely reflected waning of maternal antibodies in line with a previous report describing that maternal antibodies in elephant calves ($n = 2$) remained detectable for approximately 3 years (Fuery et al., 2020). The fact that no decline in antibody levels could be observed for the animals living at the ETH suggests that these animals were all infected

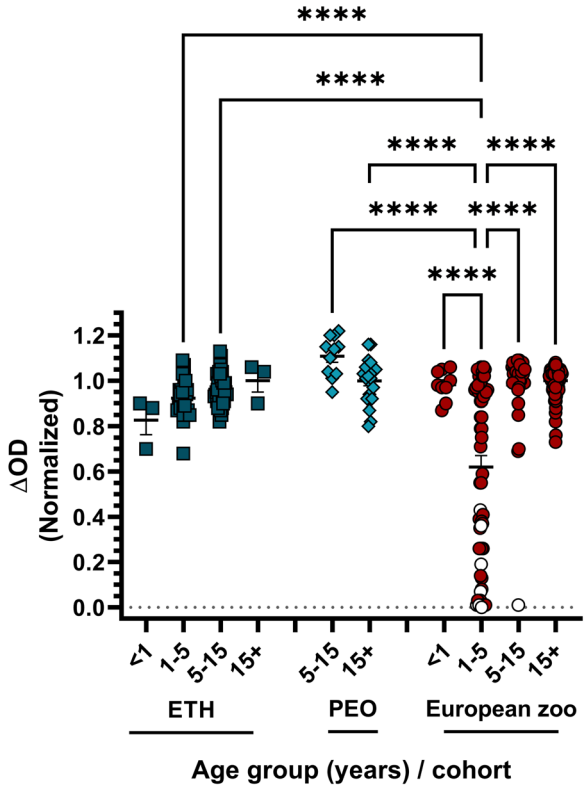

FIGURE 1 Anti-EEHV antibody levels in two Asian elephant cohorts. ELISA results for 72 serum samples from 64 individual elephants from ETH, Sri Lanka (dark teal squares), 31 serum samples from 31 individual elephants from PEO, Sri Lanka (light teal diamonds) and 193 elephant sera from 96 individual elephants from European zoos (red circles). All samples were tested at 1:100 dilution and ΔOD values were obtained by subtraction of serum-specific background signals from gB-specific signals. Subsequently, values were normalized as described in Section 2. Samples are grouped per age category, showing individual values ± standard error of the mean (SEM). For fatal EEHV-HD cases only the last sample before death is shown in white. Statistical significance was tested by ANOVA using GraphPad Prism: $^{****}p < .0001$. OD = optical density

with one or multiple EEHV (sub)species at an early stage of life, resulting in maintenance of high EEHV-specific antibody levels in spite of waning maternal antibodies. Due to the ELISA format chosen for this study, the EEHV (sub)species responsible for the early infections in the ETH cohort remain elusive and will be subject to further study. The decrease of antibody levels noted for a large proportion of juvenile elephants born in European zoos suggests that these animals were not yet infected when maternal antibodies started to wane. In view of EEHV being omnipresent in (sub-)adult elephants (Hoornweg et al., 2021), zoo elephants will thus likely get infected at a later stage of life than their congeners at the ETH.

Previous studies indicate that elephants with low EEHV-specific antibody levels are at risk of developing EEHV-HD (Fuery et al., 2020; Hoornweg et al., 2021; Pursell et al., 2021). Results of the present study largely corroborate these findings. While all juvenile elephants at the ETH, a cohort with no previously reported EEHV-HD cases, showed high EEHV-specific antibody levels, considerably lower EEHV-specific antibody levels were observed for juvenile elephants within the European zoo cohort, which included a total of 13 fatal EEHV-HD cases. Ten of these fatal EEHV-HD cases had low to non-detectable EEHV-specific antibody levels in the last serum sample taken before death. Three animals developed EEHV-HD despite high levels of EEHV-specific antibodies. The EEHV1A gB ELISA used in this study is assumed to detect antibodies elicited towards multiple EEHV (sub)species, likely due to the fact that gB is highly conserved between the different EEHV (sub)species (Fuery et al., 2020; Hoornweg et al., 2021; Pursell et al., 2021). This assumption is supported by the findings that (i) African elephants, which are infected with EEHV species other than EEHV1A, display similar reactivity towards EEHV1A gB as Asian elephants (Hoornweg et al., 2021) and (ii) multiple animals with clear responses towards (EEHV1A) gB lacked antibodies in the EEHV1A-specific ORF-Q assays (Fuery et al., 2020). Nevertheless, the exact breadth of gB-specific cross-reactive antibody responses remains to be determined. As responses towards multiple EEHV species are detected using the currently used ELISA, it is likely that the high levels of antibodies detected in three fatal EEHV-HD cases were elicited towards an EEHV (sub)species different from the (sub)species to which the animal eventually succumbed, as reported previously (Fuery et al., 2020). Overall, the current results suggest that while low gB-specific antibody levels correlate with an increased risk of developing EEHV-HD, high levels of gB-specific antibodies do not always correlate with protection against EEHV-HD. In this respect, it must be noted that it is currently unknown whether gB-specific antibodies may directly contribute to protection against EEHV-HD or solely serve as markers of the protective immunity mounted in response to previous EEHV infections.

Based on the current knowledge on EEHV(-HD), we hypothesize that elephants that are infected at an early stage of life, in the presence of high levels of maternal antibodies, manage to control the infection without developing EEHV-HD, and subsequently mount a lasting immune response. Being infected in the presence of EEHV-specific maternal antibodies above an as yet unknown threshold will likely prevent uncontrolled replication of the virus, hence abrogating induction of a deleterious cascade that might lead to EEHV-HD. Under the protection of maternal antibodies, the immune system of young elephants is enabled to recognize and control the virus, resulting in lifelong protection against EEHV-HD, a phenomenon in line with the notion that maternal antibodies protect against severe symptoms caused by herpes simplex virus infections in human neonates (Patel et al., 2019).

One of the factors that may contribute to the age at which elephants first get infected by EEHV is the size of the herd they live in. The average breeding herd size in European zoos consists of five to six elephants (pers. comm. Jeroen Kappelhof, European Associations of Zoos and Aquaria (EAZA), Asian elephant EAZA Ex situ Programme), whereas the number of young elephants that live in the ETH herd varies between 60 and 70 and the mixed age herd at the PEO consists of >90 individuals. It is known that essentially all elephants get infected with EEHV at some point of life (Hoornweg et al., 2021) and EEHV is regularly shed by its carriers (Ackermann et al., 2017; Atkins et al., 2013; Hardman et al., 2012; Stanton et al., 2010; Stanton et al., 2014).

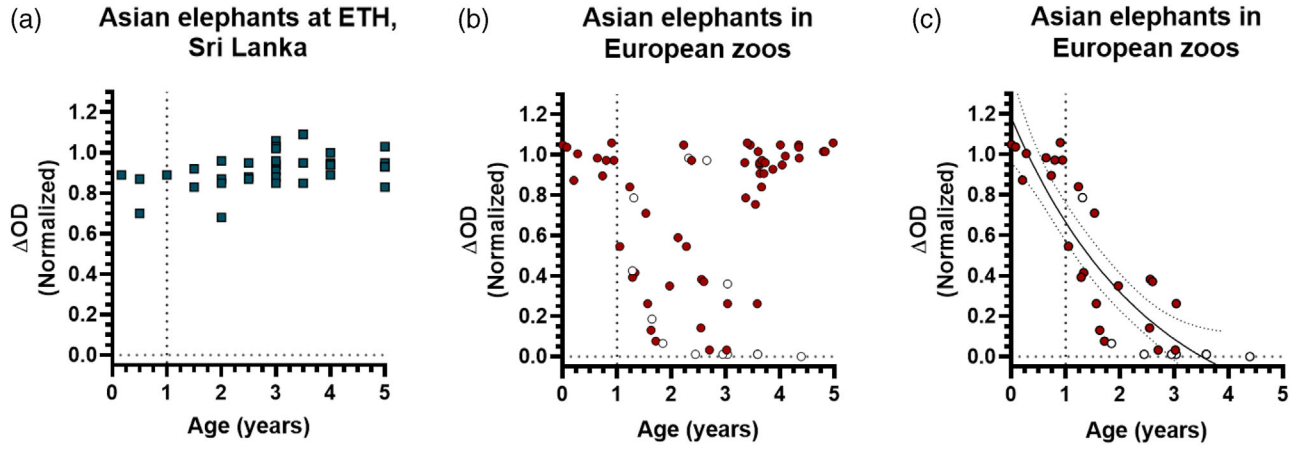

FIGURE 2 EEHV-specific antibody levels according to age of sampling. ELISA results for all animals ≤ 5 years of age from (a) the ETH cohort and (b) the European zoo cohort according to age of sampling. Samples were tested and data was processed as described in Figure 1. (c) One phase decay curve fitting the decline of antibodies observed for Asian elephants ≤5 years of age from European zoos as in b. For curve fitting, all highly positive ΔOD values from animals above 2 years of age as well as longitudinal samples showing an increase in ΔOD values compared to a previous paired sample were removed. The black line depicts the best fitted curve; dashed lines indicate the 95% confidence interval. A vertical dashed line is included in all panels to separate the two age categories depicted in the graphs (<1 and 1–5 years old). OD = optical density

Moreover, in larger herds stress-induced EEHV reactivation appears to increase as a result of increased interactions between elephants (D'Agostino et al., 2022; Titus et al., 2022). Consequently, it is considered likely that living in a large herd will increase the chances an elephant gets infected by EEHV at an early age.

To date, the vast majority of fatal EEHV-HD cases reported from range countries were described in elephants under human care (Boonprasert et al., 2019; Bouchard et al., 2014; Lee et al., 2021; Oo et al., 2020; Reid et al., 2006). For most of these fatal cases, the size of the herds they lived in is not reported, although at least some lived in herds similar in size to those in European zoos (Reid et al., 2006). EEHV-HD fatalities of free-ranging elephants have been reported from India (Zachariah et al., 2018; Zachariah et al., 2013), Thailand (Prompiram et al., 2021) and Sri Lanka (B. V. P. Perera et al., 2018). From the few studies performed, it appears that median herd sizes of free-living elephants are larger than zoo herds, with median sizes of 13–14 elephants reported in Thailand and Sri Lanka (Fernando & Lande, 2000; Htet et al., 2021) and a herd with an estimated size of 40–60 animals reported on Sumatra (Sitompul et al., 2013). Reported herd sizes varied, however, from 7 to over 40 individuals per herd. Thus, although other factors contributing to the development of EEHV-HD in these animals cannot be excluded, it may well be possible that the free-ranging elephants that succumbed to EEHV-HD were part of relatively small herds and were infected with EEHV after maternal antibodies had waned.

In conclusion, in relatively large herds, where young elephants may be in contact with many other conspecifics, the chance of being infected during an early stage of life when maternal antibodies are still present at high levels is likely to be much higher than in relatively small and closed herds. Chances that a free-ranging elephant calf will get infected within this critical period will decrease with numbers of free-ranging Asian elephants diminishing further and herds getting smaller and more isolated from each other, resulting in increasing risk to develop EEHV-HD. Consequently, to prevent EEHV-HD from becoming another prominent threat to free-ranging Asian elephants, it is crucial to preserve and where possible restore natural habitat and halt further fragmentation of free-living elephant herds.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL STATEMENT

Ethical approval was not sought as the present study only made use of archival elephant serum samples taken (for diagnostic purposes) under routine veterinary care by (zoo or orphanage) veterinary staff and which were subsequently shared with our institutes for EEHV research purposes.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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