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Research article

Quantification and risk factor analysis of elephant endotheliotropic herpesvirus-haemorrhagic disease fatalities in Asian elephants *Elephas maximus* in Europe (1985–2017)

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Abstract

Elephant endotheliotropic herpesvirus-haemorrhagic disease (EEHV-HD) is frequently stated to be the most common cause of death in captive Asian elephant *Elephas maximus* populations in Europe and North America. However, the impact on the European population has not been quantified. The aim of this study was to quantify and describe EEHV deaths in Asian elephants in Europe between 1985 and 2017, and to evaluate potential risk factors. Asian elephants born in the study period were tracked for eight years, or until death, depending on which occurred first. Excluding stillborn and perinatal deaths (<1 day old), 44 elephants died within the study period. EEHV-HD accounted for 57% of these cases, and was therefore the most frequent cause of death. While all known Asian elephant endemic EEHV-genotypes were represented, EEHV-1A was responsible for 80% of EEHV deaths. The median age of EEHV fatalities was 2.6 years and there was no difference in risk between males and females. The only significant risk factor for EEHV death, analysed using Cox regression analysis, was an institutional history of a previous EEHV death. Importantly, exposure to new elephants was not associated with an increased risk of EEHV death.

Introduction

Elephant endotheliotropic herpesvirus-haemorrhagic disease (EEHV-HD) is the most common cause of death of juvenile Asian elephants *Elephas maximus* in captivity, and is increasingly recognised in wild and captive populations in range countries (Howard and Schaftenaar 2019). Despite virus-host-adaptation and co-evolution, EEHV-associated severe morbidity and acute mortality have been reported in Asian elephants since 1990 (Ossent et al. 1990; Richman et al. 1999; Richman et al. 2014). The seven identified genotypes (EEHV-1 to -7) cluster within the genus *Proboscivirus*, and healthy adult Asian elephants can be latently infected with EEHV-1, -4 and -5, with periodic shedding in trunk and other body fluids (Stanton et al. 2010; Hardman et al. 2012; Sripiboon et al. 2016; Zachariah et al. 2018). Recent investigations have demonstrated that EEHV fatalities can be associated with waning maternally-derived antibody levels and primary infection in the immunologically naïve calf (Fuery et al. 2020). While adult asymptomatic EEHV infection is endemic, EEHV-HD is a sporadic disease. Herd clustering of deaths has led to speculation that there may be, as yet unidentified, location-, genetic- or husbandry-related risk factors predisposing some herds to higher fatality rates. The aim of this investigation was to quantify and describe EEHV deaths in Asian elephants in Europe between 1985 and 2017. More specifically, this study aimed to calculate EEHV mortality and describe the distribution of fatalities according to sex, age and EEHV genotype. The following hypotheses were tested using Cox proportional hazards regression analysis.

Effect of location

Anecdotally, EEHV deaths appear to cluster in certain locations. Therefore, it was hypothesised that there would be an increased risk of EEHV death at locations where one (or more)

previous EEHV death(s) had occurred. Because genetic factors may play a role in this, it was hypothesised that some dams and/or sires would be associated with an increased risk of EEHV death.

Effect of sex

Male calves eventually leave the herd, whereas female calves will stay with their family unit. Weaning occurs from 2 years of age (EAZA 2020) and therefore coincides with the risk period of EEHV-HD (Nofs et al. 2013). Males were hypothesised to have a higher risk of EEHV death, due to weaning-associated social stress.

Effect of exposure to new elephants

Viral sequencing has identified that there are unique strains of EEHV in geographically separate groups of elephants (Long et al. 2016), so mixing of elephants from different groups will expose individuals to novel strains. Depending on when this occurs, calf exposure may induce EEHV viraemia resulting in EEHV-HD and/or a protective antibody response (Fuery et al. 2020). Transport and introductions of new elephants has been associated with EEHV shedding (Sanchez et al. 2016). Therefore, exposure to new elephants through import of new individuals into the herd, or calf export to a new herd, was hypothesised to affect the risk of EEHV death.

Effect of exposure to calving events

Shedding of EEHV has also been documented during pregnancy and lactation, although there is currently no clear association between reproductive status and shedding (Bennett et al. 2015). Larger herds are expected to have more frequent birth events than smaller herds. Calf exposure to higher numbers of birth events was hypothesised to indirectly reduce the risk of EEHV death due to increased exposure to local EEHV strains (frequent shedding associated with reproduction, and/or more frequent shedding because of the presence of more elephants).

Materials and methods

Data collection

Studbook data obtained from the Asian Elephant European Association of Zoos and Aquaria (EAZA) ex-situ Programme (EEP) were used for this study. The study period was defined as from 1 January 1985 until 31 December 2017, because the index case of EEHV-HD in Europe was born in 1985 (Ossent et al. 1990). A report of all birth events in the study period was created using a studbook management software programme (SPARKS version 1.6, International Species Information System, Eagan, MN, USA). For each birth event, the following demographic data were extracted and tabulated in Microsoft® Excel® (Microsoft Headquarters, Redmond, WA, 98052, USA): calf sex, calf studbook number, dam studbook number, sire studbook number, calf date of birth and calf institution of birth. Studbook numbers and institutions were anonymised. Based on existing literature, the risk period for EEHV-death was defined as from one day of age until 8 years (2922 days) of age (Howard and Schaftenaar 2019). Therefore, life status changes for each calf were recorded for a period of 8 years from birth, or until the calf died or was exported out of the European population, depending on which occurred first. Life status changes included: calf exposure to a birth event within the herd, calf exposure to new elephants imported into the herd (imports), calf exposure to a new herd via calf export (export), calf export out of the European population (lost to follow-up) and survival or death. Survival was defined as being alive at 8 years of age, or on the 31 December 2017, depending on which occurred first. Death was classified as due to EEHV or due to other causes. In cases where the cause of death was not reported, attempts were made to obtain more information from the institution. If further information was not available, then the death was classified as due to other causes. Case definition for an EEHV death was positive EEHV-specific polymerase chain reaction (PCR) analysis on blood and other tissues, associated with systemic haemorrhage and oedema at the time of death. Because the studbook data do not consistently include the cause of death, EEHV deaths were identified from the published literature, and from a review of deaths reported to the European Taxon Advisory Group veterinary advisors (Schaftenaar et al. 2012; Schaftenaar 2015; Schaftenaar 2017; Schaftenaar 2019) and/or discussion with individual institutions. Institutional EEHV history was defined once EEHV deaths had been identified. Institutions with no history of EEHV deaths were classified as unaffected. Institutions with an EEHV death were classified as affected from the date of the first EEHV death onwards. The date of each life status change (and institutional EEHV status change, if applicable) was recorded, and the age of the elephant on that date was calculated. EEHV genotype was recorded for EEHV deaths and institutions were contacted to establish the weaning status of each calf at the time of EEHV death.

Data analysis

Descriptive statistics for birth events and EEHV deaths were calculated in GraphPad Prism (GraphPad Software, San Diego, CA, 92108, USA). The distribution of ages of male and female EEHV deaths were tested for normality and a Mann-Whitney test used to compare rank ages of males and females. Statistical significance was set at $P<0.05$.

For risk factor analysis, death due to EEHV was defined as the outcome of interest. Calves that were born dead, or did not survive the first 24 hours of life, were excluded from analysis. Competing events were identified and included: death due to causes other than EEHV, export out of the European population (lost to follow-up), and not yet 8 years of age at the end of the study period. These individuals were included in the analysis up until the date of their removal from the study population (non-EEHV death, export or study end date, respectively). Cox proportional hazards regression (Cox 1972) analyses were applied using the statistical program R (R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/), to analyse the following potential risk factors: sex of the calf, import of new elephants, export of the calf, calf exposure to birthing events within the herd, and location history (whether there had been a previous EEHV fatality at that institution). Statistical significance was set at $P<0.05$.

Results

There were 263 birth events in the European Studbook breeding population between 1985 and 2017 (Table 1). Of these, 55 (21%) were born dead or did not survive the first 24 hours of life (perinatal deaths) and were excluded from further analysis. In total 208 elephants were included in the survival analysis. Of these, there were 19 deaths from causes other than EEHV, and 79 calves were not yet 8 years old at the end of the study period. Two elephants were exported out of the European population prior to 8 years of age. A total of 25 calves died of EEHV-HD, of which 23 died of EEHV-1, one of EEHV-5 and one of a co-infection of subtype EEHV-1A and EEHV-4. Furthermore, 20 of the 23 EEHV-1 cases were subtype 1A, constituting 80% of all EEHV deaths. There were two EEHV-1B cases and the remaining EEHV-1 case was not sequenced to identify the subtype. There were 12 females and 13 males. Age of death ranged from 267 to 2786 days (8.8 months to 7.6 years). The median age of EEHV death was 967 days (2.6 years). There was no statistical difference in the age of male and female EEHV deaths (Mann-Whitney rank test $P=0.13$) (Figure 1).

EEHV was responsible for 57% (n=25/44) of deaths for elephants born alive and surviving more than 24 hours, with an overall EEHV mortality of 12% (n=25/206) of all elephants born alive and surviving more than 24 hours (excluding two animals lost to follow-up). Weaning status was obtained for 19 EEHV deaths; 16 were considered partially weaned and three were completely weaned. In addition, three of the 19 individuals had a history of supplemental bottle feeding.

A total of 41 institutions reported birth events (including stillborn and neonatal deaths), of which 14 (34%) were affected by one or more EEHV deaths. Number of EEHV deaths in each affected institution were 1 (n=9), 2 (n=2), 3 (n=1), 4 (n=1) or 5 (n=1). There were 39 known sires and 104 known dams having birth events. The sire ID was not recorded in the studbook for four calves, including one EEHV death, for which the dam was also not recorded. Excluding the one EEHV death with unknown parentage, 13 sires and 19 dams produced one or more calves that later died from EEHV. Respective sires produced 1 (n=8), 2 (n=3) or 5 (n=2) calves that died from EEHV, and respective dams produced 1 (n=15), 2 (n=3) or 3 (n=1) calves that died from EEHV. Because of the large number of sires and dams, and relatively small sample size of calves, further analysis of the effect of dam and sire on the risk of EEHV death was not possible.

Location history of a previous EEHV death was highly significantly associated with EEHV deaths (P=0.001, hazard ratio 3.8, 95% confidence interval 1.7–8.8), compared with institutions with no history of EEHV deaths. Other potential risk factors (male sex, import of new elephants, export of the calf and calf exposure to birthing events within the herd) were not found to be significantly associated with EEHV death.

Discussion

EEHV-HD was responsible for 57% of deaths of elephants below 8 years of age, which were born alive (and survived more than 24 hours) in Europe between 1985 and 2017. This is similar to reported EEHV mortality in North America (Howard and Schaftenaar 2019). The start of the study period (1985) was chosen to include all known European EEHV fatalities. It is likely that the number of EEHV deaths is underestimated in this study due to lack of EEHV awareness and testing in the beginning of the study period. The index case of EEHV occurred in 1988, but was first reported in 1990 (Ossent et al. 1990). EEHV was then named and described in 1999 (Richman et al. 1999). One case was retrospectively identified using modern EEHV-specific molecular testing, having tested negative with a pan-herpes PCR at the time of death (Seilern-Moy et al. 2016). At least one of the non-EEHV deaths in the present study had a history of sudden death, which could be compatible with EEHV-HD. However, it was not possible to obtain post-mortem reports or tissues for testing. One additional EEHV death was reported in Europe during the study period but was not included, because the individual was born in the USA and therefore did not fulfil the inclusion criteria. This male elephant was transferred to Germany at 10 years of age and succumbed to EEHV-HD 8 months later (Ehlers et al. 2001).

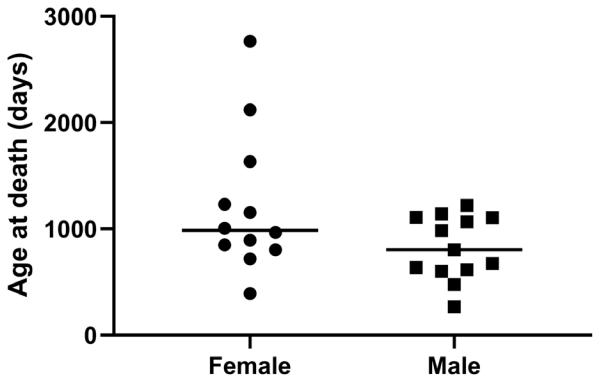

Figure 1. Age distributions of male and female Asian elephant *Elephas maximus* EEHV deaths in Europe between 1985 and 2017.

Initial assumptions that Asian elephant EEHV's originated from African elephants *Loxodonta africana* have been refuted, and there is only one report of an African elephant endemic EEHV genotype (EEHV-3) causing disease in one Asian elephant (Garner et al. 2009). Herpesvirus infection does not necessarily result in disease, and latent infections with EEHV-1, -4 and -5 are endemic in adult Asian elephants worldwide (Schaftenaar et al. 2010). In fact, EEHV DNA sequencing has demonstrated that viral strains observed in captive elephants in Europe and North America originated from their recent wild ancestors in range countries (Zachariah et al. 2018). Disease severity is determined by the complex relationship between virus pathogenicity (virulence) and host susceptibility (MacLachlan and Dubovi 2017). Of the EEHV genotypes (species), EEHV-1 is by far most frequently associated with EEHV-HD in Asian elephants, with subtype EEHV-1A causing 80% of EEHV deaths in Europe. Analysis of genotype 'strain' variability has shown that no two institutions anywhere in the world have the same distinct viral strains, and most institutions have multiple strains present and circulating in the herd (Long et al. 2016). Sporadic outbreaks of EEHV-HD are strain-specific, and only elephants housed in the same institution will have identical strains. While most cases of EEHV-HD are associated with EEHV-1A, it is currently unknown if this is due to greater pathogenicity or more frequent exposure. Existing data about latent infection, assessed via trunk washes, are limited. EEHV-1A was most commonly associated with EEHV deaths in Thailand; however, fatality rates for EEHV-1A and EEHV-4 were both 67% (Boonprasert et al. 2019). Virus pathogenicity may be a factor in the development of EEHV-HD; however, exposure to EEHV-1 appears to be widespread (Hardman et al. 2012; Azab et al. 2018; Sripiboon et al. 2020), so other factors are likely to be more important.

This study found that calves born at European institutions with a history of an EEHV death had a 3.8 times greater risk of dying from EEHV-HD, compared to calves at an institution without a history of an EEHV fatality (Table 1). Currently there is understandable concern about the potential to introduce new viral strains when transferring an elephant from an institution with a history of EEHV-HD to an 'unaffected' institution. This study found no effect of new elephants being imported into the herd, nor of calves being exported to new herds, on the risk of EEHV death. Thus, there is currently no evidence to suggest that transfers should not occur between affected and unaffected herds.

Host (calf) immune competence will also determine whether EEHV infection results in EEHV-HD. Recent investigations have shown that Asian elephants receive EEHV-genotype specific antibodies from their dam in utero (Fuery et al. 2020). These maternal antibodies wane over the first 36 months of life, and EEHV-HD and death is associated with nadir levels (Fuery et al. 2020). By adulthood, seroconversion had occurred and all adult elephants tested were strongly seropositive. The median age of

Table 1. Descriptive statistics for Asian elephant *Elephas maximus* births and outcomes (over an 8 year period) in Europe between 1985 and 2017. EEHV; elephant endotheliotropic herpesvirus, NA; not applicable.

Outcome	Excluded	EEHV deaths	Non-EEHV deaths	Lost to follow-up	Alive <8 years	Alive >8 years
Total number	55	25	19	2	79	83
Male	34	13	10	2	42	42
Female	20	12	9	0	37	41
Unknown sex	1	0	0	0	0	0
Total at risk days	NA	25,231	5,839	2,968	100,454	242,526
Calf exposure to herd birthing event(s)						
0	NA	12	13	0	23	10
1	NA	7	2	0	20	8
2	NA	3	1	0	10	11
3+	NA	3	3	0	26	54
Total number of birth events	NA	23	15	0	163	317
Average per individual	NA	0.9	0.8	0	2.0	3.8
Average per risk day	NA	0.0009	0.003	0	0.002	0.001
Calf exposure to imported elephant(s)						
0	NA	15	16	1	64	33
1	NA	8	0	1	10	23
2	NA	1	2	0	4	12
3+	NA	1	1	0	1	15
Total number of import events	NA	14	7	1	21	113
Average per individual	NA	0.6	0.4	0.5	0.3	1.4
Average per risk day	NA	0.0006	0.001	0.0003	0.0002	0.0005
Calf export to new herd						
0	NA	24	16	0	63	34
1	NA	1	2	1	13	42
2	NA	0	1	1	3	7
3+	NA	0	0	0	0	0
Total number of export events	NA	1	4	3	19	56
Average per individual	NA	0.04	0.2	1.5	0.2	0.7
Average per risk day	NA	4.0E-05	0.0007	0.001	0.0002	0.0002
Location history of EEHV						
Yes (risk days)	6	14,523	1,600	1,012	22,884	27,444
No (risk days)	49	10,708	4,239	1,956	77,570	215,082

EEHV death in Europe was 32 months, which is similar to EEHV deaths in Thailand (Boonprasert et al. 2019) and corresponds well to this period of humoral susceptibility. Age of death ranged from 8.8 months to 7.6 years and likely reflects the dynamic relationship between virus exposure, waning maternal antibody levels and subsequent host seroconversion. There was no significant difference in the ages of male and female EEHV deaths, suggesting that factors determining susceptibility to EEHV are similar in both sexes.

The weaning period coincides with the EEHV risk period, potentially increasing calf and dam stress levels. Stress is associated with reactivation of human alpha- and beta-herpesviruses (Prösch et al. 2000; Sainz et al. 2001), but the relationship between stress and EEHV reactivation is unclear. Increased faecal cortisol was predictive of asymptomatic EEHV viraemia in a single calf (Kalirathinam et al. 2019). However, no direct relationship was observed between urinary or serum cortisol concentrations and EEHV shedding in juvenile and adult Asian elephants, measured during the introduction of a new bull elephant (Sanchez et al. 2016). Male calves may experience more stress as they are weaned, but this hypothesis was not supported as there was no evidence of a sex predisposition for EEHV death in the European population in this study, or in previous studies in Thailand (Sripiboon et al. 2016; Boonprasert et al. 2019). Pregnancy can reactivate latent herpesvirus infections, and EEHV shedding during pregnancy and lactation has been observed in Asian elephants (Bennett et al. 2015). However, exposure to birthing events did not affect the risk of EEHV death in the current study.

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Host genetics can also influence disease severity, and 13 of the 39 sires and 19 of the 104 dams produced at least one calf that succumbed to EEHV-HD. Unfortunately, the large number of individual sires and dams, and (statistically speaking) relatively few EEHV deaths, meant that parentage could not be analysed as a potential risk factor in the current study. While most EEHV fatalities occur prior to the opportunity to reproduce, the oldest reported EEHV death was a 7.6 year old female with a 21 month old calf (Schaftenaar 2017). This calf developed EEHV-HD shortly after the dam's death, but survived with antiviral and supportive treatment. Not included in the study population was an 11 year old male elephant, born in the USA which died of EEHV 8 months after transfer to a German collection in 1998 (Ehlers et al. 2001). Interestingly, the next EEHV fatality at this institution was the 8.8 month old male offspring from this individual in 2000 (Ochs et al. 2001). Familial clustering of cases has led to hypotheses that there may be genetic factors that predispose some herds to EEHV-HD cases. However, familial groups also share the same viral strains, husbandry conditions and medical care. Investigations into the relationship between strain variability, viral shedding, calf antibody status and development (or not) of EEHV-HD should be prioritised to enable evidence-based management decisions in the future.

Conclusions

EEHV-HD was responsible for a staggering 57% of deaths in elephants from one day to 8 years of age, and thus represents the main cause of death in this age group in the European Asian elephant population. Median age at death was 2.6 years, coinciding with weaning. The most significant risk factor for EEHV death in Europe is a history of a previous EEHV death in the institution of birth. Further research is needed to elucidate which factors are influential in protecting calves from EEHV-HD, as avoiding exposure to EEHV is not possible. Importantly, this study did not support hypotheses that calf transfer or import of new elephants increased the risk of EEHV death. Further research is sorely needed, and all zoos, regardless of their EEHV history, should establish frequent PCR screening for EEHV viraemia in elephants until at least 8 years of age to enable early identification and treatment of EEHV-HD.

References

Azab W., Damiani A.M., Ochs A., Osterrieder N. (2018) Subclinical infection of a young captive Asian elephant with elephant endotheliotropic herpesvirus 1. *Archives of Virology* 163(2): 495–500.

Bennett L., Dunham S., Yon L., Chapman S., Kenaghan M., Purdie L., Tarlinton R. (2015) Longitudinal study of Asian elephants, *Elephas maximus*, indicates intermittent shedding of elephant endotheliotropic herpesvirus 1 during pregnancy. *Veterinary Record Open* 2(1): e000088.

Boonprasert K., Punyapornwithaya V., Tankaew P., Angkawanish T., Sriphiboon S., Titharam C., Brown J.L., Somgird C. (2019) Survival analysis of confirmed elephant endotheliotropic herpes virus cases in Thailand from 2006 - 2018. *PLoS ONE* 14(7): e0219288

Cox D.R. (1972) Regression models and life-tables. *Journal of the Royal Statistical Society: Series B (Methodological)* 34(2): 187–202.

EAZA. (2020) Reproduction. In: *EAZA Best Practice Guidelines for Elephants* (2nd ed) 20–24. Retrieved from https://www.eaza.net/assets/Uploads/CCC/BPG-2020/Elephant-TAG-BPG-2020.pdf

Ehlers B., Burkhardt S., Goltz M., Bergmann V., Ochs A., Weiler H., Hentschke J. (2001) Genetic and ultrastructural characterization of a European isolate of the fatal endotheliotropic elephant herpesvirus. *Journal of General Virology* 82(3): 475–482.

Fuery A., Pursell T., Tan J., Peng R., Burbelo P.D., Hayward G.S., Ling P.D. (2020) Lethal hemorrhagic disease and clinical illness associated with elephant endotheliotropic herpesvirus 1 are caused by primary infection: Implications for the detection of diagnostic proteins. *Journal of Virology* 94(3): e01528-19.

Garner M.M., Helmick K., Ochsenreiter J., Richman L. K., Latimer E., Wise A.G., Maes R.K., Kiupel M., Nordhausen R.W., Zong J.C., Hayward, G.S. (2009) Clinico-pathologic features of fatal disease attributed to new variants of endotheliotropic herpesviruses in two Asian elephants (*Elephas maximus*). *Veterinary Pathology* 46(1): 97–104.

Hardman K., Dastjerdi A., Gurrala R., Routh A., Banks M., Steinbach F., Bouts T. (2012) Detection of elephant endotheliotropic herpesvirus type 1 in asymptomatic elephants using TaqMan real-time PCR. *Veterinary Record* 170(8): 205.

Howard L.L., Schaftenaar W. (2019) Elephant endotheliotropic herpesviruses. In: Miller R.E., Lamberski N., and Calle P (eds.), *Fowler's zoo and wild animal medicine*. Current therapy Vol. IX. St. Louis, Missouri: Elsevier Inc., 672-679.

Kalirathinam U.K., Elangkovan S., Kawi J., Cabana F. (2019) Sleep monitoring of an Asian elephant *Elephas maximu*s calf at Night Safari, Singapore: testing whether sleep time is a significant predictor of cortisol or the onset of positive elephant endotheliotropic herpesvirus viraemia. *International Zoo Yearbook* 53(1): 128–137.

Long S.Y., Latimer E.M., Hayward G.S. (2016) Review of elephant endotheliotropic herpesviruses and acute hemorrhagic disease. *ILAR Journal* 56(3): 283–296.

MacLachlan N.J., Dubovi E.J. (2017) Pathogenesis of viral infections and diseases. In: MacLachlan N.J. and Dubovi E.J. (eds.), *Fenner's Veterinary Virology* (5th ed) St. Louis, Missouri: Elsevier Inc., 47-78.

Nofs S.A., Atmar R.L., Keitel W.A., Hanlon C., Stanton J.J., Tan J., Flanagan J.P., Howard L., Ling P.D. (2013) Prenatal passive transfer of maternal immunity in Asian elephants (*Elephas maximus*). *Veterinary Immunology and Immunopathology* 153(3–4): 308–311.

Ochs A., Hildebrandt T.B., Hentschke J., Lange A. (2001) Birth and hand rearing of an Asian elephant (*Elephas maximus*) at Berlin Zoo - veterinary experiences. *Vehr. Ber. Erkrg. Zootiere* 40: 147–156.

Ossent P., Guscetti F., Metzler A.E., Lang E.M., Rübel A., Hauser B. (1990) Acute and fatal herpesvirus infection in a young Asian elephant (*Elephas maximus*). *Veterinary Pathology* 27(2): 131–133.

Prösch S., Wendt C.E.C., Reinke P., Priemer C., Oppert M., Krüger D.H., Hans-Dieter V., Döcke W.D. (2000) A novel link between stress and human cytomegalovirus (HCMV) infection: Sympathetic hyperactivity stimulates HCMV activation. *Virology* 272(2): 357–365.

Richman L.K, Zong J.C., Latimer E.M., Lock J., Fleischer R.C., Heaggans S.Y., Hayward G. (2014) Elephant endotheliotropic herpesviruses EEHV1A, EEHV1B, and EEHV2 from cases of hemorrhagic disease are highly diverged from other mammalian herpesviruses and may form a new subfamily. *Journal of Virology* 88(23): 13523–13546.

Richman L.K., Montali R.J., Garber R.L., Kennedy M.A., Lehnhardt J., Hildebrandt T., Schmitt D., Hardy D., Alcendor D.J., Hayward G.S. (1999) Novel endotheliotropic herpesviruses fatal for Asian and African elephants. *Science* 283(5405): 1171–1176.

Sainz B., Loutsch J.M., Marquart M.E., Hill J.M. (2001) Stress-associated immunomodulation and herpes simplex virus infections. *Medical Hypotheses* 56(3): 348–356.

Sanchez C.R., Wagener T., Nevitt D., Latimer E., Brown J. (2016) Correlation between serum and urinary cortisol levels and shedding of elephant endotheliotropic herpesvirus (EEHV) 1, 3, 4 and 5 in calves and adult Asian elephants (*Elephas maximus*) pre- and post-arrival of a new bull elephant. *Proceedings of the Joint AAZV / EAZWV / IZW Conference*, 43–44. Atlanta, Georgia.

Schaftenaar W., Martina B., Osterhaus A. (2012) Elephant endotheliotropic herpes virus: an update on recent developments. *Proceedings International Conference Diseases Zoo and Wild Animals*, 70–72. Bussolengo, Italy.

Schaftenaar W., Reid C., Martina B., Fickel J., Osterhaus A.D.M.E. (2010) Nonfatal clinical presentation of elephant endotheliotropic herpes virus discovered in a group of captive Asian elephants (*Elephas maximus*). *Journal of Zoo and Wildlife Medicine* 41(4): 626–632.

Schaftenaar W. (2015) Summary of EEHV-related events in Europe between February 2013 and February 2015. *Proceedings of the 10th International Elephant Endotheliotropic Herpesvirus Workshop*, 18–19. Houston, Texas, USA.

Schaftenaar W. (2017) Elephant endotheliotropic herpesvirus (EEHV): An update from Europe February 2015 - May 2017. *Proceedings of the Eleventh International Elephant Endotheliotropic Herpesvirus (EEHV) Workshop*, 11–12. London, UK.

Schaftenaar W. (2019) An update on elephant endotheliotropic herpes virus hemorrhagic disease cases in Europe in 2018-2019. *Proceedings of the North American EEHV Workshop*, 23. Houston, Texas, USA.

Seilern-Moy K., Bertelsen M.F., Leifsson P.S., Perrin K.L., Haycock J., Dastjerdi A. (2016) Fatal elephant endotheliotropic herpesvirus-1

and 4 co-infection in a juvenile Asian elephant in Europe. *JMM Case Reports* 3(2): e005005.

Sripiboon S., Ditcham W., Vaughan-Higgins R., Jackson B., Robertson I., Thitaram C., Angkawanish T., Phatthanakunanan S, Lertwatcharasarakul P., Warren K. (2020) Subclinical infection of captive Asian elephants (*Elephas maximus*) in Thailand with elephant endotheliotropic herpesvirus. *Archives of Virology* 165(2): 397–401.

Sripiboon S., Jackson B., Ditcham W., Holyoake C., Robertson I., Thitaram C., Tankaew P., Lertwatcharasarakul P., Warren K. (2016) Molecular characterisation and genetic variation of Elephant Endotheliotropic Herpesvirus infection in captive young Asian elephants in Thailand. *Infection, Genetics and Evolution* 44: 487–494.

Stanton J.J., Zong J.C., Latimer E., Tan J., Herron A., Hayward G.S., Ling P.D. (2010) Detection of pathogenic elephant endotheliotropic herpesvirus in routine trunk washes from healthy adult Asian elephants (*Elephas maximus*) by use of a real-time quantitative polymerase chain reaction assay. *American Journal of Veterinary Research* 71(8): 925–933.

Zachariah A., Sajesh P.K., Santhosh S., Bathrachalam C., Megha M., Pandiyan J., Jishnu M., Kobragade R.S., Long S.Y., Zong J.C., Latimer E.M., Heaggans S.Y., Hayward G. S. (2018) Extended genotypic evaluation and comparison of twenty-two cases of lethal EEHV1 hemorrhagic disease in wild and captive Asian elephants in India. *PLoS ONE* 13(8): e0202438.