Published in final edited form as:

Vet Rec. 2012 February 25; 170(8): 202–203. doi:10.1136/vr.e1212.

Conservation: clarifying the risk from herpesvirus to captive Asian elephants

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Blood and necropsy tissue from Asian elephant (*Elephas maximus*) calves that die suddenly from acute haemorrhagic disease contain extremely high levels of DNA from an unusual type of herpesvirus known as elephant endotheliotropic herpesvirus (EEHV) (Richman and others 1999). Eight different types or species of EEHV, all belonging to the *Proboscivirus* genus, are now known that appear to be unique to, and have co-evolved with, the ancestors of modern Asian and African elephant (*Loxodonta* species) hosts (Garner and others 2009, Latimer and others 2011). Evidently, for as yet unexplained reasons, primary infections with two chimeric variants of the EEHV-1 species (ie, EEHV-1A and EEHV-1B), and rarely with other species, such as EEHV-2, EEHV-3 and EEHV-4, lead to rapid systemic spread and vascular endothelial cell damage associated with uncontrolled virus replication (Richman and others 2000).

This disease primarily affects calves between one and four years of age and has been lethal in at least 80 per cent of all animals with a positive DNA blood test. Only 10 survivors of symptomatic disease are known, which were all treated aggressively with human antiherpes drugs, although such treatments were not effective in many other cases. Overall, there have been 32 confirmed cases of EEHV disease in North America, with at least 25 suspected cases in Europe, and up to two dozen calf deaths with the same disease pathology have been recorded recently in wild and orphan calves in several Asian countries. Up to 24 per cent of Asian elephant calves born in North America and Europe within the past 20 years have been affected by this disease and it has been responsible for nearly 60 per cent of all deaths in monitored Asian elephant calves between eight months and 15 years of age over the past 20 years.

Until recently it was not clear whether Asian elephants were the natural hosts in which EEHV-1A and EEHV-1B have evolved and adapted and where infections would be expected to be universal and predominantly asymptomatic (or latent), or whether the unexpected severity of this disease could be explained by, for example, an unusual cross-species infection from African elephants.

In a paper summarised on p 205 of this week's *Veterinary Record*, Hardman and others (2011) use a Taqman PCR assay to add evidence to the idea that asymptomatic infections with EEHV-1 may indeed be common and possibly nearly universal in adult Asian elephants in a UK zoo herd. Schaftenaar and others (2010) also reported the presence of EEHV-1 DNA in oral lesions of several otherwise healthy adults in a continental European zoo. Stanton and others (2010) reported that during routine long-term monitoring of weekly trunk wash secretions by a sensitive real-time PCR assay, low levels of EEHV-1 DNA could be detected sporadically in the majority of the healthy adults in a small herd in a USA facility that had a long history of EEHV disease. In fact, in some younger animals very high levels of viral DNA were detected transiently in trunk washes, especially following a brief spike in levels in the blood apparently associated with a primary infection. In the study by Stanton

and others (2010), several animals were found to be secreting exactly the same strain of EEHV-1A as had killed a calf in the herd two years earlier, whereas another herdmate was secreting an EEHV-1B strain. However, two other calf deaths over the previous eight years each involved other EEHV-1A strains. That work has subsequently been extended (Stanton and others, unpublished observations) to track multiple primary infections of several different strains of both EEHV-1A and EEHV-1B (as well as EEHV-5) in three USA Asian elephant breeding facilities, suggesting that at least some calves can undergo and survive mild primary EEHV-1 and EEHV-5 infections without drug treatments.

Although Hardman and others (2011) did not carry out the added level of DNA sequence analysis necessary to convincingly show that they did not have a PCR contamination problem, and did not investigate whether the strains being detected were the same as or different from the EEHV-1B strain that killed a calf at their UK facility in 2006 or the single EEHV-1A strain that killed two calves there in 2009, the overall body of evidence now clearly indicates that it is likely that the majority of asymptomatic adult Asian elephants carry and occasionally reactivate latent EEHV-1 infections. Furthermore, there are a very large number of widely diverged strains of genetically stable EEHV-1 distributed across both Europe and North America (and also in Asia) and it is quite clear that there has been no direct chain of transmission of EEHV-1 strains between cases of this disease at different elephant housing facilities.

Accumulating evidence shows that wild African elephants carry four other viruses – EEHV-2, EEHV-3, EEHV-6 and EEHV-7 – in benign lung nodules in adults, as well as in skin nodules in juveniles. These new results, when combined with the absence of any further evidence to confirm a single early report of EEHV-1 in skin lesions from African calves in Florida (Richman and others 1999), make clear that another explanation other than crossing between mixed African and Asian elephant host species in zoos needs to be sought for why Asian, but apparently not African, elephant calves are so susceptible to this devastatingly severe disease, both in the wild and in captivity.

The ability to carry out strain/subtype fingerprinting by PCR DNA sequence analysis that can discriminate between all known species of EEHV and all distinct strains of EEHV-1A and EEHV-1B that are not closely and directly epidemiologically connected, combined now with access to very sensitive and quantitative real-time PCR assays to identify positive clinical samples suitable for strain analysis, has greatly expanded the ability of wildlife veterinarians and virologists to evaluate and monitor EEHV infections worldwide. Because these viruses have not yet been propagated in cell culture, obtaining the complete genome sequence of EEHV-1 has been a major challenge. Ehlers and others (2006) showed that EEHV-1 is distantly related to *Roseolovirus* and *Cytomegalovirus* and contains many of the core genes present in all herpesviruses, but over half of the EEHV-1 genome encodes some 60 novel genes not present in any other herpesviruses. Therefore, the molecular biology and mechanisms of latency and of pathogenesis are expected to be very different than in any other herpesvirus subfamilies.

Obviously EEHV disease is a major added threat to the long-term survival of endangered Asian elephant populations everywhere, and new resources and funding to maintain the recent high level of progress are urgently needed. Much more needs to be done, especially with regard to answering whether these viruses are truly sensitive to the very expensive antiviral drug treatments that have been used in all survivors of symptomatic disease so far, and to address whether EEHV-4 and EEHV-5, both of which may also be endogenous viruses of Asian elephants, are less pathogenic or just far less prevalent than EEHV-1A and EEHV-1B, and whether prior infections with them can lead to immunological responses that might be capable of providing protection against subsequent EEHV-1 disease. It may also

now be quite plausible and desirable to determine the EEHV-1 positive or negative status of all Asian elephant calves under human care. Collection and some analysis of trunk washes in Asian camp elephants has been accomplished, but that is a somewhat difficult process; the suggestion here by Hardman and colleagues that EEHV-1 can be found also directly in conjunctival swabs might potentially make such studies to evaluate the extent of asymptomatic EEHV infections in a range of countries more feasible.

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Figure 1.

Acute haemorrhagic disease, caused by elephant endotheliotropic herpesvirus, has been responsible for nearly 60 per cent of all deaths in monitored Asian elephant calves over the past 20 years. Now it appears that elephants can also be asymptomatic carriers of the virus