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Seroprevalence of Equine Herpesviruses 1 and 9 (EHV-1 and EHV-9) in Wild Grévy's Zebra (*Equus grevyi*) in Kenya

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ABSTRACT: Equid herpesviruses types 1 (EHV-1) and 9 (EHV-9) are unusual among herpesviruses in that they lack strong host specificity, and the full extent of their host range remains unclear. The virus establishes latency for long periods and can be reactivated and shed, resulting in clinical disease in susceptible species. A sensitive and specific peptide-based enzyme-linked immunosorbent assay was developed to study the seroprevalence of both viruses in a broad range of species among both wild and captive populations. We used this assay to study the seroprevalences of EHV-1 and EHV-9 in a natural population of the highly endangered Grévy's zebra (*Equus grevyi*) in Kenya, sampled during a 4-yr period (2012–15). The results were compared with those obtained from captive Grévy's zebras from a previous study. The wild population had a significantly higher seroprevalence of EHV-9 compared with the captive population, suggesting that captivity might reduce exposure to this serotype. In contrast, the seroprevalences of EHV-1 between captive and wild groups was not significantly different. The seroprevalence of EHV-9 was not significantly higher than EHV-1 in zebras within the wild Kenyan population.

Key words: Captive, EHV-1, EHV-9, Grévy's zebra, Kenya, seroprevalence, wild.

The family *Herpesviridae* is classified into three subfamilies *Alpha-*, *Beta-*, and *Gammaherpesvirinae*. In equids, nine equid herpesviruses (EHVs) have been classified to date, of which EHV-1, EHV-3, EHV-4, EHV-8, and EHV-9 belong to the genus *Varicellovirus* of the subfamily *Alphaherpesvirinae* (Davison et al. 2009). Highly contagious, EHV-1 is usually transmitted by direct contact, mainly through infected nasal discharge. It can result in respiratory disease, abortion, neonatal death, and equine herpesvirus myeloencephalopathy (Ma et al. 2013). Herpesviruses are generally considered to be species-specific, but EHV-1 and EHV-9 differ in their ability to infect multiple species (Abdelgawad et al. 2016). Nonetheless, EHV-1 is a general pathogen of horses and other equids, whereas EHV-9 is prevalent in wild equids. The EHV-9 isolate shares over 95% sequence similarity with EHV-1, making the viruses difficult to distinguish, particularly serologically (Rebelo et al. 2015).

The prevalence of antibodies against EHV-1 and EHV-4 in wild zebras was first described by Barnard and coworkers in Burchell's zebras (*Equus burchellii*) in Kruger National Park (Barnard and Paweska 1993). A year later, antibodies against EHV-1, EHV-2, and EHV-4 were described in wild mountain zebras (*Equus zebra*) in Namibia (Borchers and Frölich 1997). In 2005, the first report of seroprevalence of EHV-9 in wild zebras was described in a study of a migratory group of the same zebra species in Serengeti National Park, Tanzania (Borchers et al. 2005). The prevalence of EHV-9 antibodies was high and the species was therefore suggested to be a natural host.

Equid herpesvirus 9 is a relatively recently discovered herpesvirus previously called gazelle herpesvirus 1 because of its initial detection in 1993 in a herd of Thomson's gazelles (*Eudorcas thomsonii*) presenting with encephalitis in a zoological garden in Japan (Fukushi et al. 1997). Further analysis of the nucleotide sequences showed a genetic simi-

larity to EHV-1, and it was later recognized as a new equine herpesvirus, EHV-9 (Yanai et al. 1998). The Thomson's gazelles involved in the outbreak in Japan shared pasture and a water source with zebras that were considered a possible source of the virus. Originally, the EHV-9 virus is thought to have evolved in Burchell's zebra (Greenwood et al. 2012), and EHV-9 glycoprotein B (gB)–specific sequences were found in the trigeminal ganglia of a Burchell's zebra from the Serengeti ecosystem (Borchers et al. 2008). The virus has a tropism for neuronal and respiratory tissue during experimental infections (Donovan et al. 2009). Virus experimentally inoculated intranasally in horses (*Equus caballus*) in Japan caused subclinical nonsuppurative encephalitis and pneumonia (Taniguchi et al. 1999). The virus has the ability to cause disease in at least eight additional mammalian taxa and is also believed to be a zoonotic pathogen (Schrenzel et al. 2008). A fatal meningoencephalitis, confirmed as being EHV-9–induced, occurred in a polar bear (*Ursus maritimus*) in San Diego, with zebras housed nearby implicated as possible reservoirs (Donovan et al. 2009).

A key property of the alphaherpesviruses is their ability to establish life-long latency in natural hosts, making elimination of herpesviruses almost impossible. Latency can be established in the neurons of the sensory ganglia or in lymphoid cells, giving the viruses an important epidemiologic advantage and allowing for evasion of the host immune system during the latent phase and establishment of persistent infection. After multiple types of stimulation, the virus can reactivate and be transmitted to susceptible hosts, which may occur with or without clinical disease (Abdelgawad et al. 2016).

Grévy's zebras (*Equus grevyi*) live in arid and semiarid grasslands and shrublands in Kenya and Ethiopia. The Grévy's zebra population has been reduced from an estimated population size of 5,800 individuals in the 1980s to a current population of approximately 2,680 individuals in 2016, with the major part of the population in Kenya. Today the species is listed as endangered under criterion A2acd in the International Union for Conservation of Nature Red List (Rubenstein et al. 2016).

Here, we report the seroprevalence of EHV-1 and EHV-9 in wild Grévy's zebras in Kenya and compare this with a recent comprehensive serologic study of 17 captive specimens of the same species (Abdelgawad et al. 2015).

Blood was collected from 32 wild Grévy's zebras (see Supplementary Material Table 1) in northern Kenya over a 4-yr period (2012–15). Eight free-ranging donkeys (*Equus africanus asinus*) sharing pasture with the zebras were also sampled for comparison. The research authorization committee at Kenya Wildlife Service (KWS) Department of Veterinary and Capture Services approved the studies related to this publication. We followed KWS guidelines on wildlife veterinary practice (from 2006). And all KWS veterinarians complied with the *Veterinary Surgeons and Veterinary Para-Professionals Act* regulating veterinary practice in Kenya (Kenya Veterinary Board 2011).

A previously described custom peptide-based enzyme-linked immunosorbent assay using EHV-1-E (1E) and EHV-9-G (9G) peptides (Abdelgawad et al. 2015) was applied to detect and differentiate between EHV-1– and EHV-9–specific antibodies in serum samples obtained from different animal species. All statistical analyses were conducted with GraphPad Prism version 5.0a software (GraphPad Software, San Diego, California, USA). Specific EHV-1-E (1E) and EHV-9-G (9G) peptides were used to differentiate between EHV-1 and EHV-9 antibodies in zebra and donkey serum samples. We used the same cutoff value used for zebra population in the Abdelgawad et al. (2015) study. Samples were considered positive when the optical density value was greater than 0.2 and 0.14 for EHV-1 and EHV-9, respectively. A total of 84% (27/32) of the Grévy's zebras we tested were positive for EHV-1 and 91% (29/32) were positive for EHV-9. All eight donkeys were positive to both EHV-1 and EHV-9 (see Supplementary Material Fig. 1a, b), showing possible co-adaptation to the virus. All Grévy's zebra specimens (WZ-20–WZ-24) seronegative in total were sampled in the same

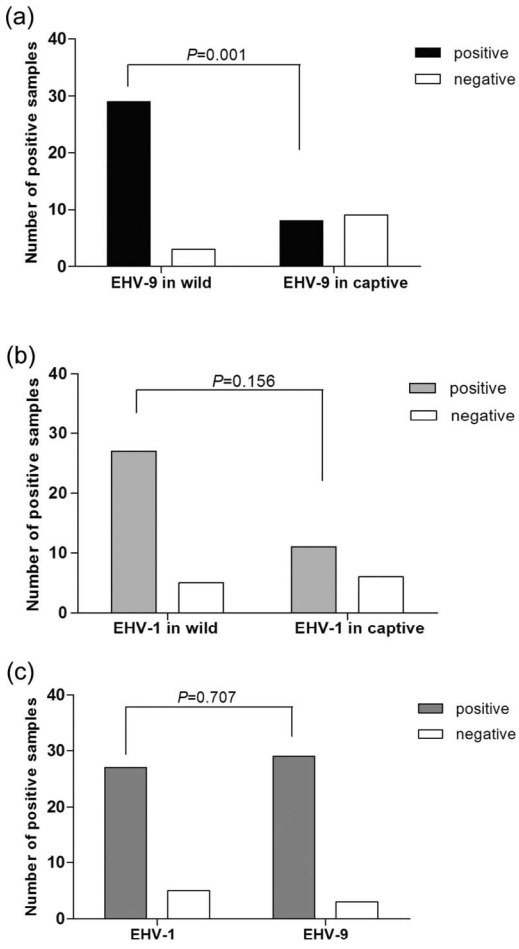

FIGURE 1. Comparative seroprevalences of equid herpesviruses types 1 (EHV-1) and 9 (EHV-9) in wild and captive Grévy's zebras (*Equus grevyi*), Kenya, sampled during a 4-yr period (2012–15) and tested with a peptide-based enzyme-linked immunosorbent assay. (a) The seroprevalence of EHV-9 antibodies in wild Grévy's zebras was significantly higher (P=0.001, Fisher's exact test) than in captive Grévy's zebras. (b) The seroprevalence of EHV-1 antibodies was not significantly higher (P=0.156, Fisher's exact test) in wild than in captive Grévy's zebras. (c) The seroprevalence of EHV-9 antibodies is not significantly higher (P=0.707, Fisher's exact test) than EHV-1 antibodies in wild Grévy's zebras.

geographic region within Ol Pejeta Conservancy (0°0′15″S, 36°57′49″E), Kenya. However, two animals from this region were seropositive (WZ-20 and WZ-21) to EHV-9. Further sampling in this region would be required to determine whether it has an unusually low EHV prevalence. None of the sampled individuals showed any clinical sign of herpesvirus-related disease at the time of sampling.

We compared the prevalence of EHV-1 and EHV-9 antibodies among the wild Grévy's zebras in the current study and captive Grévy's zebras tested in a previous study (Abdelgawad et al. 2015). The analysis showed a significant difference (P=0.001, Fisher's exact test) in the prevalence of EHV-9 antibodies, with a higher prevalence in the wild Grévy's zebras compared with the captive zebras (Fig. 1a). By contrast, EHV-1 antibody seroprevalence between the wild and captive Grévy's zebras was not significantly different (P=0.156, Fisher's exact test; Fig. 1b). Within the Kenyan wild Grévy's zebra population tested in this study, analysis by Fisher's exact tests showed that the antibody prevalence against EHV-1 was not significantly different from that of EHV-9 antibodies (P=0.707; Fig. 1c). Although we could discriminate EHV-9–positive serum samples from EHV-1–positive samples, serologic analysis is limited to antibody detection and does not provide viral sequence information or information regarding the existence of possible recombination between the two viruses. Further molecular analysis will be needed to provide more details about viral strain diversity and possible recombination events.

In Kenya, the national conservation strategies for the Grévy's zebra involve monitoring species distribution, behavioral ecology, and diseases. Our serologic analysis was done to determine the prevalence of EHV-1 and EHV-9 in the highly endangered Grévy's zebra population in Kenya and to compare the results with captive specimens of the same species. Both EHV-1 and EHV-9 were highly prevalent in the wild population, with a significantly higher prevalence of EHV-9 in the wild population relative to the captive study group. This observation suggests that both groups have been exposed to both viruses, but to varying degrees. Possible explanations for the higher seroprevalence in wild animals include longer latency in the captive animals and more frequent shedding of virus in the wild ones. Stressors in the wild that cause herpesvirus to exit latency possibly are absent or reduced in captivity (e.g., predation stress).

Previous studies have shown high seroprevalence in wild Burchell's and mountain zebras, the former often sharing pasture with Grévy's zebras. The results suggest all zebra species were either exposed to herpesvirus in a common ancestor or that cross-species transmission among equids occurred after they evolved from a common ancestor.

Disease is an underestimated threat to the survival of wildlife populations. Especially in small remaining populations of endangered species like the Grévy's zebra, susceptibility to disease could be a serious threat and should be monitored with regular surveillance. The presence of a viral antigen or nucleic acid was not tested in this study, and further studies to define levels of viral load should be performed in future analyses.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2018-01-003.

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