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ANTIBODIES AGAINST EQUINE HERPESVIRUSES AND EQUINE ARTERITIS VIRUS IN BURCHELL'S ZEBRAS (*EQUUS BURCHELLI*) FROM THE SERENGETI ECOSYSTEM

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ABSTRACT: A total of 51 sera from a migratory population of Burchell's zebras (*Equus burchelli*) were collected in the Serengeti National Park (Tanzania) between 1999 and 2001 to assess levels of exposure to equine herpesvirus types 1, 2, 4, 9 (EHV-1, −2, −4, −9), EHV-1 zebra isolate T965, and equine arteritis virus (EAV). Using virus-specific neutralizing antibody tests, seroprevalence was high for EHV-9 (60% of 45), moderate for EAV (24% of 51), and lower for the EHV-1-related zebra isolate (17% of 41), EHV-1 (14% of 49), and EHV-4 (2% of 50). No evidence for exposure to EHV-2 was found (0% of 51). The high level of exposure to EHV-9 is interesting because evidence of infection with this virus has not been previously described in any wild equine population. Although the epidemiology of EHV-9 in Burchell's zebras is presently unknown, our results suggest that in East Africa, this species may be a natural host of EHV-9, a neuropathogenic virus that was only recently isolated from captive Thomson's gazelles (*Gazella thomsoni*) in Japan. There is currently no evidence that EHV-9 induced mortality in Burchell's zebras in the Serengeti, but because of the reported virulence of this virus for more susceptible species such as Thomson's gazelles, viral transmission from infected zebras to ungulates may result in mortality.

Key words: Equine arteritis virus, equine herpesviruses, *Equus burchelli*, Serengeti ecosystem, serologic survey.

INTRODUCTION

Equids are host to at least 11 herpesviruses (EHV), including six genera of the subfamily *Alphaherpesvirinae* (EHV-1, −3, −4, and −9; asinine herpesvirus 1 and 3) and five of the *Gammaherpesvirinae* (EHV-2 and −5; asinine herpesvirus 2, 4, and 5). Alphaherpesviruses infect a relatively wide range of hosts, have quick replication rates, and induce lysis of cells in culture. In general, they are neurotropic and establish latency in sensory ganglia. In contrast, the gammaherpesviruses grow slowly in vitro, are restricted in host tropism, and establish latency in the lymphoreticular system.

Equine herpesvirus 1 and 4 are widespread in the domestic horse population worldwide (Crabb and Studdert, 1995). In domestic horses, EHV-4 mostly causes respiratory disease (Studdert, 1974), whereas EHV-1 typically induces abortion and encephalitis (Allen and Bryans, 1986). Transmission of EHV-1 and EHV-4 occurs by direct or indirect contact with infectious nasal discharges, aborted fetuses, and placentas. Equine herpesvirus 1 infection has been diagnosed in various equine species in captivity (Przewalski's horse, *Equus przewalskii*; Burchell's zebra, *E. burchelli*; Grevy's zebra, *E. grevyi*; Persian wild ass (*E. hemionus onager)* (Montali et al., 1985; Wolff et al., 1986; Kahrmann et al., 1993). Restriction enzyme profiles of EHV isolates from captive zebras and onagers indicate that these isolates are related to EHV-1 but have patterns that differ from EHV-1 isolates from domestic horses (Wolff et al., 1986) and in this publication will be referred to as the EHV-1 zebra strain. Antibodies against EHV-1 have also been found in captive Przewalski's horses and mountain zebras (*E. zebra*) (Borchers et al., 1999). Rare cases of EHV-1 infection have occurred in a wide range of nonequine species in captivity, including most notably Thomson's gazelles (*Gazella thomsoni*) (Thorsen et al., 1977; Chowdhury et al., 1988; Rebhun et al., 1988; Kennedy et al., 1996), a species that is sympatric with Burchell's zebras.

Equine herpesvirus 9 was identified after an outbreak of acute encephalitis in captive Thomson's gazelles in Japan (Fukushi et al., 1997) and currently its natural host is unknown. Because acute encephalitis can be induced by experimental EHV-9 infection in cats, dogs, goats, pigs, and mice but not in domestic horses (Taniguchi et al., 2000a, b; Narita et al. 2000, 2001; Yanai et al., 2003a, b), it has been suggested that the domestic horse may serve as a host for this virus (Yanai et al., 2003b). Although there are genetic similarities between EHV-9 and the EHV-1 isolates from domestic horses and zebras, there is sufficient difference to consider EHV-1 and EHV-9 to be genetically distinct (Borchers et al., unpubl. data).

Equine herpesvirus 2 has been isolated from domestic horses with various clinical signs and from clinically healthy horses. Its involvement in keratoconjunctivitis and respiratory disease, mainly in foals, has been reported (Collinson et al., 1994; Borchers et al., 1997; Kershaw et al., 2001). Exposure to EHV-2 was detected in a number of captive wild equine species (Borchers et al., 1999) and detection of a high neutralizing antibody titer in one of 21 free-ranging mountain zebras from Namibia (Borchers and Frölich, 1997) indicate that EHV-2 infections are not restricted to domestic horses.

Equine arteritis virus (EAV) is a member of the *Arteriviridae* and infects domestic horses worldwide. Clinical signs include edema, respiratory syndrome, and abortion (Del Piero, 2000). Stallions can also develop latent infections and shed virus in their semen. Pregnant mares infected with EAV may abort. Viral transmission occurs via the respiratory tract and venereally. Only one serotype of EAV has been demonstrated in horses.

Currently, little is known about EHV and EAV exposure or infection among free-living equines in Africa. Exposure to EHV-1 and EHV-4 has been demonstrated in Burchell's zebra populations in Kruger National Park, South Africa (Barnard and Paweska, 1993). Moreover, exposure to EHV-1, EHV-2, and EHV-4 has been demonstrated in free-ranging mountain zebras, on farmland in Namibia (Borchers and Frölich, 1997). To our knowledge, no free-living equine population in Africa has been surveyed for exposure to EHV-9. Antibodies against EAV have been found in horses, donkeys, and mules in South Africa (Moraillon and Moraillon, 1978; Paweska and Barnard, 1993; Paweska et al., 1997a), but not in free-ranging or captive zebra species in southern Africa (Barnard and Paweska, 1993; Paweska et al., 1997b).

The size of the Burchell's zebra population in the Tanzanian sector of the Serengeti ecosystem is approximately 200,000 individuals (Campbell and Borner, 1995). Serengeti zebras follow an annual migration, together with other migratory species, mostly wildebeest (*Connochaetes taurinus*) and Thomson's gazelle. These migratory herds move between their dry season refuges in the north and west of the ecosystem and their wet season grazing areas in the south of the ecosystem. Zebras share pastures with Masai livestock, including domestic donkeys when in the south and east of the ecosystem, but rarely if ever encounter domestic horses. The objective of this study was to assess levels of exposure to EHV-1, −2, −4, −9, EHV-1 zebra strain, and EAV in this large migratory population of Burchell's zebras. Seroprevalence was assessed during 3 yr and within different age classes to establish basic patterns of exposure within the Serengeti zebra population.

MATERIALS AND METHODS

We collected 51 blood samples from Burchell's zebras in the Serengeti National Park between 1999 and 2001, including 44 sera from apparently healthy individuals, four sera from individuals immobilized to remove wire snares set by illegal game-meat hunters (Hofer et al., 1996), and three sera from freshly dead zebra carcasses. Blood was collected in vacutainers, allowed to clot, and spun at 1,000 × G for 14 min. Serum was decanted and stored and transported at between −20 C and −70 C. Serum-sampled individuals included 20 females and 31

males. Juveniles included foals and young animals estimated on the basis of body size and tooth wear to be less than 12 mo of age. Adults included all animals estimated to be older than 12 mo of age. Serum-sampled animals included 15 juveniles and 36 adults.

To detect neutralizing antibodies, serial twofold dilutions of complement-inactivated serum were incubated with 100 plaque-forming units (PFU) of EHV/100 μl for 1 hr at 37 C. After adding 1×10^5 equine dermal (ED) cells/200 μl per well the incubation was continued in Dulbecco's modified Eagle medium (EDM) with 5% newborn calf serum (NCS) (Life Technologie GmbH, Berlin, Germany) for another hour and finally overlayed with 1.6% carboxymethylcellulose and 2% NCS in EDM. The reaction was stopped in the case of EHV-1, EHV-4, and EHV-9 after 2 days and in the case of EHV-2 after 1 wk with 4% formalin and the plaques were stained with Giemsa. The neutralization titer was calculated and expressed as the reciprocal of the dilution that protected 50% of the inoculated cells. As controls, a positive and a negative horse reference serum were included routinely. The following equine herpesvirus strains were used in neutralization tests (NT): EHV-1 (MAR87), EHV-2 (LK4), EHV-4 (T252), EHV-9, and EHV-1 zebra strain T965. Equine herpesvirus 1, 2, and 4 were kindly supplied by P. Thein (München, Germany), EHV-9 was kindly provided by H. Fukushi (Department of Veterinary Microbiology, Faculty of Agriculture, Gifu University, Yanagido, Japan), and G. Allen (Department of Veterinary Science, Gluck Equine Research Center, Universitiy of Kentucky, Lexington, Kentucky, USA) kindly provided us with the EHV-1 zebra isolate T965. Titers ≥1:10 were considered positive and indicative of recent exposure to the respective virus or reactivation of latent virus. The titer level set as evidence of viral exposure was based on our extensive experience of equine herpesvirus serologic diagnostics and by using negative zebra sera as a control.

For detection of EAV-specific neutralizing antibodies, serial twofold dilutions of complement-inactivated serum were incubated with 100 PFU of EAV strain Bucyrus/100 μl for 1 hr at 37 C. The virus strain was kindly supplied by P. Thein (München, Germany). After adding 1×10^5 Vero (monkey kidney) cells/200 μl per well the incubation was continued as described for EHV-1 and −4. After 3 days of incubation cells were fixed and the 50% plaque reduction titer was calculated. A defined positive and negative horse serum were included as controls. Normally a serum is considered positive when it has a NT titer >1:4. Because of the limited amounts of sera, we started dilutions at 1:10 and all titers ≥1:10 were considered positive.

Statistical analyses were performed using SYSTAT 9.0 (SPSS Science Inc., Chicago, Illinois, USA). All statistical tests were two-tailed.

RESULTS

Sera from seven of 49 (14%) zebras were positive for antibodies against EHV-1 (Table 1). This sample contained 36 adults and 13 juveniles. The seven individuals with positive sera included six adults (five males, one female) and one subadult. In seven of 41 sera (17%), antibodies against the EHV-1 zebra strain were detected. Sera from only one of 50 (2%) zebras were positive for antibodies against EHV-4 and this individual also was positive for exposure to EHV-1, EHV-1 zebra strain, and EHV-9. Sera from 51 individuals screened for exposure to EHV-2 were negative (data not presented). Sera from 27 of 45 (60%) zebras screened for antibodies against EHV-9 had titers ≥1:10 and seven individuals (16%) had titers ranging between 1:20 and 1:40 (Table 1). A logistic regression demonstrated that the likelihood of exposure to EHV-9 was not influenced by sex (17 of 26 males; 11 of 19 females), age (six of 11 juveniles; 22 of 34 adults), or year of sampling (eight of 16 zebras in 1999; six of 10 zebras in 2000; 14 of 19 zebras in 2001; log likelihood $G=3.116$, df=3, $P=0.38$).

Our study provides little evidence of cross-reaction between EHV-9 and EHV-1 or between EHV-9 and the EHV-1 zebra strain. Only five of 27 (19%) zebras that tested positive for EHV-9 were also positive for both EHV-1 and the EHV-1 zebra strain (Table 1). Two sera neutralized both EHV-9 and the EHV-1 zebra strain and a different serum reacted with EHV-9 and EHV-1. In contrast, 17 sera reacted only with EHV-9 and none neutralized only EHV-1 and the zebra strain, respectively. Paired comparison of titers obtained from the same individual for EHV-9 and EHV-1 revealed that the probability of an animal having similar titers against these viruses

TABLE 1. Reciprocal neutralizing antibody titers against equine viruses in Burchell's zebras from the Serengeti ecosystem (Tanzania). Only zebras seropositive for one or more virus are shown.

Zebra number	EHV-1[a]	Zebra strain[a]	EHV-4[a]	EHV-9[a]	EAV[a]
4	20	nd[b]	–	nd	–[c]
7	–	–	–	–	10
8	10	–	–	10	–
9	nd	nd	–	–	40
10	10	10	–	20	–
11	–	–	–	10	–
13	–	–	–	–	10
16	–	–	–	10	–
17	–	nd	–	10	–
19	–	–	–	10	–
21	–	nd	–	10	–
22	–	10	–	20	–
23	–	10	–	10	–
24	20	10	–	20	–
26	20	20	–	20	–
27	–	–	–	10	–
28	–	–	–	–	20
29	–	–	–	10	–
30	–	–	–	–	10
31	–	–	–	10	–
33	–	–	–	10	–
34	–	–	–	–	40
35	–	–	–	20	32
36	–	–	–	10	32
39	–	–	–	10	32
40	–	–	–	20	32
41	–	–	–	10	–
42	20	20	10	40	–
44	–	–	–	10	–
45	10	10	–	10	32
46	–	–	–	10	16
47	–	–	–	10	–
50	–	–	–	10	–
51	–	–	–	10	–

[a] EHV-1 = equine herpesvirus type 1; zebra strain = EHV-1-related zebra isolate T965; EHV-2 = equine herpesvirus type 2; EHV-4 = equine herpesvirus type 4; EHV-9 = equine herpesvirus type 9; EAV = equine arteritis virus.
[b] nd = not done.
[c] – = negative; <1:10.

was highly unlikely (Sign test, $P<0.00001$, $n=43$). A similar result was obtained from a paired comparison of titers against the EHV-1 zebra strain and EHV-9 (Sign test, $P<0.00001$, $n=41$). In contrast, individuals had similar titers against EHV-1 and the EHV-1 zebra strain (Sign test, $P>0.05$, $n=41$).

Sera from 12 of 51 (24%) zebras were positive for EAV and all except one individual with antibodies were adults (11 of 25 adults; one of 14 juvenile). Seropositive individuals were found in each year of the study (two, three, and seven individuals in 1999, 2000, and 2001, respectively), and both males and females were exposed to EAV (eight of 31 males; four of 20 females). An individual exposed to EAV was also seropositive for EHV-1 (Table 1). In general, evidence of multiple exposure to viruses was found in 10 of 51 animals and six of these cases involved exposure to both EHV-9 and EAV.

DISCUSSION

Although much has been published on various aspects of EHV and EAV infection in domestic horses, little is known about the epidemiology and clinical effects of these viruses in wild equine species. This is especially true for Burchell's zebra, an abundant species with an extensive distribution in Africa. In this study, we demonstrated for the first time EHV-9-specific neutralizing antibodies in sera from Burchell's zebras. Exposure to EHV-9 in the large migratory population in the Serengeti occurred in all 3 yr of study, in males and females, and in juveniles and adults. The high level of exposure to EHV-9 among Serengeti zebras indicates that Burchell's zebras in East Africa may represent a natural host for this virus. We also found that the Serengeti zebra population had a low level of exposure to EHV-1, the EHV-1 zebra strain, and EHV-4, and a moderate level of exposure to EAV. Several individuals had evidence of exposure to more than one virus. We detected no evidence of exposure to EHV-2 in this population.

Barnard and Paweska (1993) applied a microneutralization test to 61 sera from Burchell's zebras in the Kruger National Park and their results suggest a far higher level of exposure to EHV-1 (87%) and EHV-4 (92%) than we found in the same species in the Serengeti National Park (EHV-1, 11%; EHV-4, 2%). Because of

high levels of exposure, Barnard and Paweska (1993) suggested that both EHV-1 and EHV-4 were endemic among zebras in Kruger National Park. Although results from serologic surveys may not be directly comparable, the study of Borchers and Frölich (1997) that applied the same serologic methods as those used in this study indicates that the level of exposure to EHV-1 among mountain zebras (14% of 21) in Namibia is similar to that among Serengeti Burchell's zebras but that Namibian zebras have a comparatively higher level of exposure to EHV-4 (29% of 21) than Serengeti zebras. Evidence for exposure to EHV-2 was absent in Tanzania, and restricted to one zebra in Namibia. At the time of these two earlier serologic surveys of zebra populations, the antigenically related EHV-9 was not known. Clinical signs of EHV-1 infection in domestic horses are thought to be more severe than in wild equine species, although rare cases of the neurologic form of EHV-1 infection have been found in captive zebras (Montali et al., 1985; Kahrmann et al., 1993).

Cross-neutralization of EHV-9 has been reported using rabbit hyperimmune anti-EHV-1 and −4 sera (Fukushi et al., 1997). In contrast, our study does not suggest extensive serologic cross-reactions of EHV-9 with EHV-1, the EHV-1 zebra strain, or EHV-4. Paired comparison of titers from the same individual against EHV-1 and EHV-9 and against EHV-9 and the EHV-1 zebra strain for more than 40 zebras indicated that the Serengeti zebras were significantly more likely to show exposure to EHV-9 than EHV-1 or the related zebra variant. Although 27 zebras were positive for EHV-9, only five of these were also positive for EHV-1 and the EHV-1 zebra strain, and only one of these was positive for EHV-4. It is possible that antibody titers attributed to EHV-9 in these five cases might represent cross-reaction elicited by infection with EHV-1 or the zebra-specific related virus. We suggest this because our horse reference serum from a nonvaccinated horse with a confirmed EHV-1-induced abortion (Chowdhury et al., 1986) neutralized EHV-1, EHV-1 zebra strain, EHV-4, and EHV-9 (data not presented), thus providing an unequivocal example for serological cross-reaction. To check our interpretation that Serengeti zebras are predominantly exposed to EHV-9, and also probably to EHV-1 or the zebra-related variant of EHV-1, we tested a large sample of sera from other equine species: nine donkey and nine horse sera from Tanzania, 12 mountain zebra sera from Namibia, and 55 horse sera from Germany (data not presented). Unlike sera from Serengeti zebras, none of these 85 sera was exclusively positive for EHV-9 and all sera that were positive against EHV-9 were positive also for EHV-1 and in rare cases also for the zebra strain. This provides further evidence that Serengeti zebras that were only found with positive titers against EHV-9 were most likely exposed to a virus more similar to EHV-9 than EHV-1. Nevertheless, to clarify whether EHV-9 or a related virus has induced the EHV-9 titers revealed by this study, the isolation and genetic characterization of the virus from Serengeti zebras is required.

Equine arteritis virus usually occurs as a mild or subclinical infection in domestic horses and donkeys and infection typically induces a protective and long-lasting immunity. Our results demonstrate a moderate level of exposure to EAV in the Serengeti zebra population during the 3 yr of this study. In contrast, Barnard and Paweska (1993) found no evidence for exposure to EAV among a collection of 102 sera from Burchell's zebras in Kruger National Park.

In conclusion, the high prevalence of EHV-9- and EAV-specific neutralizing antibodies in the Burchell's zebra population of the Serengeti National Park demonstrates that exposure to these viruses is widespread within this large migratory zebra population. However, the consequences and epidemiology of these viruses within this zebra population are unclear. Because EHV-9 is virulent to captive Thom-

son's gazelles (Fukushi et al., 1997), transmission of this virus in the Serengeti ecosystem from zebras to Thomson's gazelles via contaminated pastures is possible, suggesting that EHV-9 may be a mortality factor in the population dynamics of this gazelle species. However, currently we have no data indicating an exposure to EHV-9 in gazelles.

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