

Prevalence of antibodies against some equine viruses in zebra (*Zebra burchelli*) in the Kruger National Park, 1991–1992

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ABSTRACT

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The presence of antibodies against equine encephalosis virus (EEV) and equid herpesvirus 1 and 4 in zebra in the Kruger National Park (KNP) was demonstrated. The ability of zebra to maintain immunity against EEV is illustrated by the appearance of neutralizing antibodies in most zebra foals within months of losing their maternal immunity. This occurs in every month of the year, even in winter. The high proportion of serologically positive foals in winter is ascribed to the presence of large numbers of susceptible foals and sufficient numbers of *Culicoides* vectors even at that time of the year.

The high prevalence of antibodies against both herpesviruses is similar to the situation in horses and suggests that herpesvirus infection is endemic among zebra in the KNP.

No evidence of infection with either A/equine/H3N8 or equine arteritisvirus could be found.

INTRODUCTION

The rapid expansion of the game industry in South Africa has resulted in large-scale movement of game and the reintroduction, into certain areas, of species that had disappeared. A sound knowledge of the involvement of game in the epidemiology of those diseases that might pose a threat to the permanence of our indigenous game and the health of farm animals, is essential. It will ensure the continued health of translocated game and prevent losses as a result of viral diseases.

African horsesickness virus (AHSV), equine encephalosis virus (EEV), equine influenza virus A/equine/

H3N8 (Equi 2), equid herpesvirus 1 (EHV 1), equid herpesvirus 4 (EHV 4) and equine arteritis virus (EAV) are some of the more important viral infections of horses present in South Africa. Equine encephalosis, first isolated in 1967 (Erasmus, Adelaar, Smit, Lecatsas & Toms 1970), appears to be confined to Africa. This insect-transmitted orbivirus may cause abortions or peracute deaths in horses as a result of cardiac failure. Although serological investigations have revealed a high prevalence of exposure to the virus, clinical disease occurs infrequently (Erasmus, Boshoff & Pieterse 1978).

Equi 2 entered South Africa in 1986. After rapid dissemination among the highly susceptible horse population, active immunization resulted in the disappearance of clinical disease.

Viral arteritis was believed to be absent from the country until it was diagnosed in 1987 in a Lippizaner herd and in a small number of Thoroughbred horses (Erasmus 1988). Recently, serological evidence of a widespread infection with this virus among South African donkeys was obtained (Paweska & Barnard 1993).

Apart from the closely related EHV 1 and 4 (Studdert, Simpson & Roizman 1981; Allen & Turtinen 1982; Turtinen, Allen, Darlington, & Brayns 1981), an alpha herpesvirus (AHV) has also been isolated from donkeys (Browning, Ficorelli, & Studdert 1988). Equid herpesvirus 1 is associated with respiratory and neurological disease (Whitwell & Blunden 1992), while EHV 4 is primarily responsible for respiratory infection. It is only occasionally isolated from aborted fetuses (Studdert & Blackney 1979; Allen, Yeargan, Turtinen, Bryans & McCollum 1983).

Knowledge of the involvement of free-living zebra in the epidemiology of viral diseases of *Equidae* is very limited, with the exception of their involvement in AHS (Davies & Lund 1974; Davies & Otieno 1977; Erasmus, Young, Pieterse & Boshoff 1978; Barnard 1993).

Experimental infection of zebra with Equi 2 resulted in the development of only a slight nasal discharge (B.J. Erasmus 1992, Onderstepoort Veterinary Institute, unpublished data 1992). Clinical signs among free-living zebra have not yet been reported. Although no evidence of herpesvirus infections of free-living zebra has been presented, abortion and perinatal foal mortality (Wolf, Meehan, Basgall, Allen & Sundberg 1986), and myelitis associated with a herpesvirus infection (Montali, Allen, Bryans, Phillips & Bush 1985) were reported from two zoological parks in the United States of America.

The purpose of this investigation was to obtain information on the prevalence of antibodies in free-living zebra against the more significant viral diseases of horses present in South Africa.

MATERIALS AND METHODS

Specimens

Blood was collected from free-living zebra in the KNP. They were immobilized with etorphine hydrochloride, M99 (R & C Pharmaceuticals [Pty] Ltd), and xylazine hydrochloride, Rompun (Bayer), at 6–12-week intervals, from August 1991 to May 1992. The blood, collected in vacutainer tubes, was allowed to clot, and the serum was decanted and stored at -20°C , till tested.

To determine the approximate date of infection, it was assumed that zebra foals lose their passive immunity against EEV at 5–6 months of age, as is the case with AHS (Barnard 1993). The age of the foals, which varied from 3–12 months, was determined according to the eruption and wear of the teeth (Smuts 1974a). The approximate time of infection was calculated in months from the time when the foals had presumably lost their passive immunity to 14 d before the date of sampling.

Viruses

The viruses used in the serological tests, together with their origins, are given in Table 1.

Serological tests

A microneutralization test (MN) for EHV 1 and EHV 4 was carried out according to the method of Thomson, Mumford, Campbell, Griffiths & Clapham (1976). The method of Morailon & Morailon (1978)—with minor modifications (Paweska & Barnard 1993)—was employed for EAV. In the MN tests for EEV, 30–100 CID_{50} of virus was used to neutralize two-fold dilutions of inactivated serum in 96-well microtitre plates (Nunc Denmark). The serum-virus mixtures were maintained at 37°C for 1 h; then a cell suspension containing sufficient CER cells to form a monolayer within 24 h was added to the test wells.

TABLE 1 Viruses used in the serological tests, and their origins

Virus	Serotype	Isolate	Origin
EEV	Cascara Gamil Kaalplaas Bryanston Kyalami Langebaan Potchefstroom	— M9/71 7088 M17/76 7084 M44/76 EP8/91	B.J. Erasmus, OVI ^a B.J. Erasmus, OVI B.J. Erasmus, OVI B.J. Erasmus, OVI B.J. Erasmus, OVI B.J. Erasmus, OVI G.J. Gerdes, OVI B.J. Erasmus, OVI
A/equine/H3N8		Huguenot	B.J. Erasmus, OVI
EHV 1		Kentucky D	J.T. Brayns, Lexington, USA
EHV 4		Kentucky F	J.T. Brayns, Lexington, USA
EHV		Bucyrus	J.T. Brayns, Lexington, USA

^a Onderstepoort Veterinary Institute

TABLE 2 Virus-neutralizing antibody-prevalence rates against EHV 1 and EHV 4 in zebra foals in the Kruger National park

Antibody titre	Virus-neutralizing antibody prevalence rates in zebra			
	Equid herpesvirus 1		Equid herpesvirus 4	
	No.	%	No.	%
Negative	8	13	5	8
4 ^a	5	8	3	5
8	9	15	4	7
16	14	23	9	15
32	15	25	5	8
64	6	10	11	16
128	4	7	7	11
256	—	—	7	11
512	—	—	10	16
Total positive	53	87	56	92

^a Reciprocal of the endpoint dilution

The plates were sealed with translucent adhesive tape and incubated at 37 °C in an atmosphere of 5% CO₂ in air, until cytopathic changes permitted reading of the results. The cells were stained with a 2% solution of neofuchsin. The titres were expressed as the highest serum dilution completely inhibiting cytopathic effects. In EEV, serum with a MN titre of ≤ 20 was considered negative.

A micro-haemagglutination-inhibition (HAI) test (Swanepoel, Blackburn, Efstratiou & Condy 1977) was used to screen sera for the presence of antibody against Equi 2.

RESULTS

Equine encephalosis

Neutralizing antibodies against all seven serotypes were present in zebra of all ages (Fig. 1). The average prevalence of antibody increased from 18% in 6-month-old foals to 60% in 12-month-old foals. The highest prevalence against a single serotype (88%) was against Bryanston in 12-month-old foals. Cascara and Kyalami were the least prevalent in 6-, 7- and 9-month-old foals.

The prevalence of serotype-specific antibodies against EEV in 6- and 7-8-month-old foals exposed at different times during 1991–1992, declined from 60% in foals exposed in June/July, to 10% in foals exposed in January/February. It increased again to 40% in foals exposed during April/May.

Herpesviruses

Titres of virus-neutralizing antibodies against both herpesviruses are shown in Table 2. Of the 61 serum

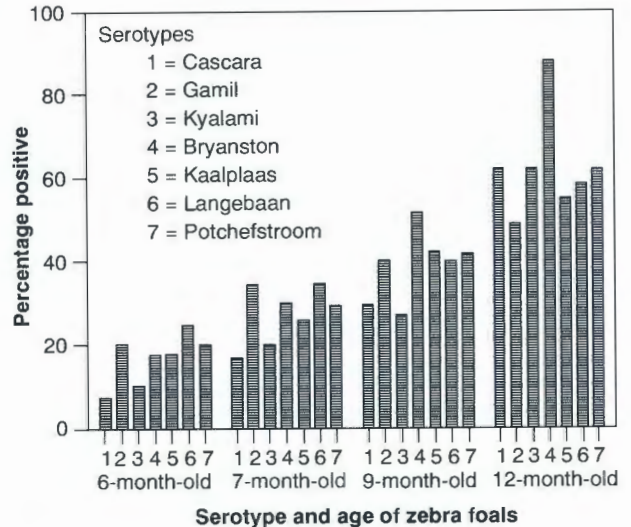


FIG. 1 Prevalence of antibodies against EEV in zebra foals in the KNP, 1991–1992

specimens tested, 53 (86.9%) reacted positively against EHV 1 and 56 (91.8%) neutralized EHV 4. Usually the virus-neutralizing titres against EHV 4 were two- to eight-fold higher than the titres against EHV 1.

Equine arteritis and Equi 2

No serological evidence of infection of zebra with EAV and Equi 2 could be found in the 102 specimens tested.

DISCUSSION

The prevalence of antibodies against EEV and EHV in zebra in the KNP and, consequently, their susceptibility to infection with these viruses, are clearly demonstrated by the serological results. No indication of infection by Equi 2 and EAV could be found.

There is no evidence that EEV occurs outside Africa and zebra are probably a factor in the persistence of the infection. Virus-neutralizing antibodies against EEV appear in a high proportion of foals shortly after they have presumably lost their maternal immunity at 5–6 months of age. These antibodies also appear in zebra exposed in every season of the year (Fig. 2). This indicates a continuous circulation of these viruses between hosts and vectors and is attributed to the unbroken presence of the vector, *Culicoides* spp., throughout the year in the subtropical climate (R. Meiswinkel 1992, Onderstepoort Veterinary Institute, unpublished data 1992). The declining antibody prevalence rate in summer (Fig. 2), and a high rate in winter, is similar to that observed with AHSV (Barnard 1993). It is attributed to the presence of large numbers of susceptible zebra in winter with few

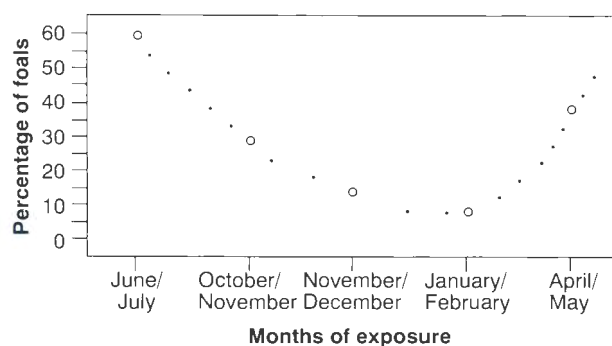


FIG. 2 Prevalence of type specific antibodies against EEV in 6–8-month-old zebra in the KNP, 1991–1992

susceptible foals in summer. Although zebra foals may be born any time of the year, over 75% are born from October to March (Smuts 1974b). This means that most foals become susceptible in winter. Despite a decrease in *Culicoides* activity, sufficient numbers remain to ensure infection of zebra. Consequently, EEV circulates between host and vectors throughout the year.

The prevalence of antibodies against EHV 1 and EHV 4 in zebra in the KNP is very similar to that in horses (Sabine, Feilen, Herbert, Jones, Lomas, Love & Wild 1983; Erasmus 1966). This indicates a similar pathogenesis. An important property of herpesviruses is their ability to establish latency in their host. Such latency becomes a source of virus (Rock 1992) that can cause frequent reinfections resulting in a high degree of cross-reactivity (Thomson *et al.* 1976; Edington, Bridges, Broad & Griffiths 1988). This property of herpesvirus could explain the high prevalence of virus-neutralizing antibody against EHV 1 in the zebra.

A herpesvirus serologically related to EHV 1 has been isolated from a zebra foetus at the Lincoln Park Zoo in Chicago. However, DNA-restriction-endonuclease analysis indicated that it was distinct from strains of EHV 1 isolated from horses (Wolf *et al.* 1986). In the zebra tested, the prevalence of antibodies against EHV 4 was only slightly higher than that against EHV 1. However, the titres of antibody against EHV 4 were often two- to eight-fold higher than titres against EHV 1 (Table 2). This may suggest that the virus present in the KNP is serologically more closely related to EHV 4 than to EHV 1.

In addition to Equid herpesviruses, an alpha herpesvirus isolated from donkeys (Browning *et al.* 1988) must also be considered as a virus potentially pathogenic for zebra. To elucidate the matter, efforts should be made to isolate the herpesvirus from zebra in the KNP.

The absence of evidence of EAV and Equi 2 infection among zebra in the KNP may merely be an indication that they have not yet been exposed to these viruses. However, the development of only a slight nasal discharge in zebra after experimental infection with EIV illustrates the susceptibility of zebra to EIV (B.J. Erasmus, Onderstepoort Veterinary Institute, unpublished data 1992). It is possible that natural infection may result in a more severe disease. Furthermore, the population dynamics of zebra, characterized by foaling in every season of the year, is ideal for the development of an endemic disease situation.

The recent detection of antibodies to EAV in donkeys must be borne in mind when donkeys are allowed onto game farms. It is known that zebra may breed with donkeys and the possibility exists that EAV may thus be transmitted to zebra.

In the light of these observations, exposure of zebra to donkeys and horses should be avoided. It is unlikely that such a situation would occur in the KNP, but zebra on private game farms, where horses are sometimes kept for recreational purposes, are undoubtedly at risk. The introduction of donkeys and horses onto game farms should be restricted to animals proven to be free of EAV and EIV.

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