

RESEARCH COMMUNICATION

Epidemiology of African horsesickness: Duration of viraemia in zebra (*Equus burchelli*)

B.J.H. BARNARD¹, R. BENGIS², D. KEET² and E.H. DEKKER²

ABSTRACT

BARNARD, B.J.H., BENGIS, R., KEET, D. & DEKKER, E.H. 1994. Epidemiology of African horsesickness: Duration of viraemia in zebra (*Equus burchelli*). *Onderstepoort Journal of Veterinary Research*, 61:391–393

The viraemic period of African horsesickness is significantly longer in experimentally infected zebra than in horses. The virus could be isolated 40 d post-infection from blood and 48 d post-infection from spleen. The introduction of zebra into African horsesickness-free countries should therefore be considered carefully, and preferably be restricted to serologically negative zebra.

INTRODUCTION

In 1987, an outbreak of African horsesickness (AHS) was confirmed in Spain. The origin of the virus is believed to have been ten zebra imported from Namibia, five of which were taken to a safari park where the first signs of disease were detected approximately 26 d after their arrival (Lubroth 1988; Mellor, Boned, Hamblin & Graham 1990). This interval, together with the time the zebra were in transit by sea, indicates that the viraemic period of AHS in zebra is significantly longer than the approximately 7 d in horses infected experimentally with virulent virus. It may also be more extended than the 27 d previously recorded for zebra (Erasmus, Young, Pieterse & Boshoff 1978).

The purpose of this investigation was to confirm this supposition.

MATERIALS AND METHODS

Zebra

Seven approximately 6-month-old, free-living zebra foals in the Kruger National Park (KNP) were immobilized with etorphine hydrochloride (M99) and xylazine hydrochloride (Rompun) and confined to insect-proof stables at night when *Culicoides* vectors are active. During the day, from approximately 3 h after sunrise to 3 h before sunset, the foals were allowed to exercise in bomas outside.

Serological tests

The presence of antibodies against all nine serotypes of AHSV in foals at capture, and the serological response to infection, were determined by micro-neutralization (Barnard 1993). Briefly, two-fold dilutions

¹ Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110 South Africa

² Directorate of Animal Health, Skukuza, Kruger National Park, 1350 South Africa

of inactivated serum in 96-well microtitre plates (Nunc Denmark) were used to neutralize 30–100 plaque-forming units of virus per well.

Experimental infection

Three serotypes of AHSV of a low passage level were used for infection (Table 1). One ml of infective cell-culture material containing $10^{4.5}$ – $10^{5.5}$ CCID₅₀/ml of AHSV was inoculated intravenously into susceptible foals.

Samples

The zebra were immobilized on the days indicated (Table 3) for collection of samples. Blood in heparin was used for virus isolation, while serum obtained from blood samples without anticoagulant was utilized for neutralization tests.

Spleen and cervical prescapular lymph nodes were collected when the zebra were killed (Table 3).

Virus isolation

For virus isolation, 0.5 ml of packed erythrocytes and clarified supernatant of macerated organs were inoculated within 3 d of collection onto CER cell (developed by Tsunemasa Motanashi at the Nippon Institute for Biological Science, Tokyo) monolayers in 25 ml plastic flasks (Nunc Denmark). The cultures were incubated at 37 °C and three blind passages, 7–10 d apart, were made before a sample was regarded as negative. Virus in cultures showing cytopathic changes was identified in a micro-neutralization test employing type-specific reference serum obtained from Dr B.J. Erasmus, World Reference Centre for African horsesickness, Onderstepoort.

RESULTS

Zebra foals and susceptibility

The foals adapted reasonably well to confinement, but foals 6 and 7 developed septicaemia and died on day 22. Unfortunately no organ samples were collected from these foals. None of the foals was completely susceptible to AHSV prior to infection, and most of them possessed antibodies against multiple serotypes 2 weeks before exposure (Table 2). However, all of them tested negatively for at least one serotype which was then used for experimental infection.

Serological response

Specific neutralizing antibodies developed in all foals.

Virus recovery

Virus was recovered from the blood of all foals (Table 3). Two of three foals, foals 3 and 4, injected with

TABLE 1 African horsesickness virus serotypes used for experimental infection of zebra foals kept in insect-free stables in the Kruger National Park

AHS virus		No. of passages	
Serotype	Isolate	Suckling mice	Cell culture
2	82/61	3	4
3	13/63	3	4
7	31/62	5	2

TABLE 2 Neutralizing antibodies against African horsesickness virus in captured zebra foals

Foal no.	Antibodies against AHSV serotypes								
	1	2	3	4	5	6	7	8	9
1	40	–	20	20	80	160	80	20	40
2	80	320	–	–	80	–	–	80	–
3	160	–	1280	320	320	640	1280	160	40
4	40	160	–	80	20	40	–	–	40
5	160	640	–	80	640	40	–	1280	40
6	40	–	–	640	160	320	80	320	10
7	40	80	–	640	160	320	80	320	10

both serotypes 3 and 7, yielded both viruses. In both foals serotype 3 appeared during the first half of viraemia, while type 7 was isolated in the last half of the period. The level of viraemia seemed to be low, as cytopathic changes could, in several isolation attempts, be detected only in the second passage. Plaques, when present in primary cultures, were usually limited to less than ten plaques per flask, indicating a virus titre of less than 10 plaque-forming units/ml of blood.

Virus isolations from spleens were restricted to zebras in which viraemia had been demonstrated, and the virus was recovered on days 20, 31, 35, 40 and 48 after infection, when the last foal was killed.

No virus could be isolated from lymph nodes.

Duration of viraemia

Virus isolation from blood yielded positive results (Table 3) on day 4, when the first samples were collected. On days 10–24, virus was isolated from 21 of 31 blood samples, and foal 5 yielded virus in seven of seven attempts during the first 24 d after infection. By contrast, virus appeared irregularly in the blood of foal 3, from which it could be isolated only on days 10, 24 and 40.

DISCUSSION

Zebra foals in the KNP lose their passive immunity to AHSV when they are 5–6 months old and then become serologically positive to all nine serotypes

TABLE 3 Duration of viraemia and isolation of African horsesickness virus from spleens of experimentally infected zebra foals

Foal no.	Serotype injected	Isolated African horsesickness virus														
		No. of day on which blood was collected												Spleen		
		4	6	10	13	17	20	22	24	31	35	40	48	Virus	Day	
1	2	+ ¹	+	T2 ²	+	+	+								T2	20
7	3	+	+	- ³	T3	+	-	# ⁴								
6	2	-	-	-	T2	+	-	#								
5	3, 7	+	+	+	+	+	+		+	-					T7	31
4	3, 7	-	-	-	T3	+	+		T7	-	-				T7	35
2	3, 7	-	-	-	-	+	-		T7	-	-				T3	40
3	2	-	-	T2	-	-	-		T2	-	-	T2	-		T2	48

¹ AHSV isolated, but serotype not determined

² Serotype of AHSV recovered

³ No virus isolated

⁴ # Died on day 22. No samples collected

within a few months (Barnard 1993). In the present investigation, all the captured foals were serologically positive to most serotypes. This restricted the selection of virus serotypes for experimental infection to one or two for a specific zebra, and necessitated the use of three serotypes for the infection of seven foals. Three foals, 2, 4 and 5, were infected with two serotypes to mimic the natural situation in the large zebra population of the KNP, where simultaneous infections with more than one serotype are likely to occur. Unfortunately the death of foals 6 and 7 limited the number of foals which could be examined over a period longer than 22 d post-infection. However, the results obtained provided additional information on the viraemic period of AHSV in zebra.

The effectiveness of virus isolation may be influenced by several factors, including a combination of low virus titres, the time interval from collection of blood to virus isolation and the presence of cross-reacting antibodies against multiple serotypes of AHSV in the serum of the experimental foals. Although blood was processed within 3 d of collection, only low titres of virus were recovered. However, it was possible to regularly isolate virus from several zebra with antibodies against multiple serotypes. The titre of AHSV recovered was approximately 10^1 CCID₅₀/ml or less. This level of virus seems to be the rule in zebra with antibodies against multiple serotypes and confirms a previous observation (Erasmus *et al.* 1978). This experiment unfortunately did not include completely susceptible zebra and comparable results for such zebra could therefore not be obtained.

In horses infected with virulent AHSV, clearance of virus from blood corresponds to an increase in the level of type-specific antibodies and usually occurs in less than 7 d. Therefore the 11–30 d (Table 3) duration of viraemia in zebras infected with virus of a low passage level, and the isolation of virus from blood 40 d post-inoculation, is distinctly longer than in horses.

The unexpected isolation of virus 40 d after infection, from the blood of one of two foals kept for more than 35 d, and the detection of virus in spleens of three foals on days 35, 40 and 48 after infection, is an indication that it may be a relatively common occurrence. Consequently, the importation of zebra into countries free of horsesickness should be considered carefully, and preferably be restricted to serologically negative zebra. Furthermore, the isolation of virus from foals that have already seroconverted at least 1–3 weeks previously, is of interest, and the mechanism by which AHSV in zebra blood evades neutralization, needs clarification.

ACKNOWLEDGEMENTS

The authors are grateful to the National Parks Board for providing the zebra. We also wish to thank Dr G.H. Gerdes for constructive criticism in compiling this paper and Mr M. Smuts for technical assistance.

REFERENCES

- BARNARD, B.J.H. 1993. Circulation of African horsesickness virus in zebra (*Equus burchelli*) in the Kruger National Park, South Africa, as measured by the prevalence of type specific antibodies. *Onderstepoort Journal of Veterinary Research*, 60:111–117.
- ERASMUS, B.J., YOUNG, E., PIETERSE, L.M. & BOSHOFF, S.T. 1978. The susceptibility of zebra and elephants to African horsesickness, in *Infectious Diseases. IV. Proceedings Fourth International Conference on Equine Infectious Diseases*, edited by J.T. Bryans & H. Gerber: 409–418.
- LUBROTH, J. 1988. African horse sickness and the epizootic in Spain 1987. *Equine Practice*, 10:26–33.
- MELLOR, P.S., BONED, J., HAMBLIN, C. & GRAHAM, S. 1990. Isolations of African horse sickness virus from vector insects made during the 1988 epizootic in Spain. *Epidemiology and Infection*, 105:41–42.