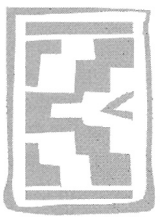

RESEARCH COMMUNICATION

The prevalence of different African horsesickness virus serotypes in the Onderstepoort area near Pretoria, during an outbreak of African horsesickness in South Africa in 1995/1996

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ABSTRACT

BREMER, C.W., GERDES, G.H., AITCHISON, H., LOUW, I., GREYLING, R.R. & WELGEMOED, J. 2000. The prevalence of different African horsesickness virus serotypes in the Onderstepoort area near Pretoria, during an outbreak of African horsesickness in South Africa in 1995/1996. *Onderstepoort Journal of Veterinary Research,* 67:65–70

During 1995/1996 parts of South Africa experienced exceptionally high rainfall. Large numbers of *Culicoides* midges were seen and an outbreak of African horsesickness (AHS) followed. In the Onderstepoort area, near Pretoria in Gauteng, a number of horses died of suspected AHS. Virus isolation and typing was done from blood and/or organ samples of 21 suspected cases as well as from five zebra which were kept in the area. Virus was isolated from 14 of the 21 suspected cases but not from the zebra. The neutralizing antibody response of the zebra to the nine different African horsesickness virus (AHSV) serotypes was determined. Results indicated the highest prevalence of serotypes 2 and 4 followed by serotypes 1, 6 and 9. Reverse transcription polymerase chain reaction (RT-PCR) was performed on total RNA extracted from blood samples of the zebra. AHSV RNA was indicated in three of five zebra by agarose gel electrophoresis analysis of amplicons and in four of five zebra after Southern blot hybridization using a ^{32}P-labelled probe. RT-PCR can be used together with serological techniques in studies of AHS to further clarify the epizootiology of the disease.

Keywords: African horsesickness outbreak, horses, serotyping, RT-PCR, virus isolation, zebra

INTRODUCTION

African horsesickness (AHS) is an infectious disease of equids which is enzootic in sub-Saharan Africa. Mortality rates as high as 95% for horses have been reported (Maurer & McCully 1963). The infectious agent is African horsesickness virus (AHSV), a dsRNA virus belonging to the *Orbivirus* genus of the Reoviridae family (Borden, Shope & Murphy 1971). The dsRNA genome consists of ten segments encoding seven structural and four non-structural proteins (Van Staden & Huismans 1991; Van Staden, Theron, Greyling, Huismans & Nel 1991; Grubman & Lewis 1992).

The disease is non-contagious and haematophagous arthropods of the genus *Culicoides* were implicated as vectors when AHS was induced in a susceptible horse after injection of an emulsion of field-caught *Culicoides* (Du Toit 1944). Du Toit (1945, cited by Wetzel, Neville & Erasmus 1970) also demonstrated transmission of AHSV via *Culicoides* from an infected to a susceptible horse.

AHS was clearly present in Africa before horses were introduced and the disease only became apparent after horses were taken into specific regions of the

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continent (Theiler 1921). For this reason a reservoir for the virus was sought amongst wild indigenous animals. AHSV antibodies have been found in zebra, elephant and in various carnivores (Davies & Lund 1974; Davies & Otieno 1977; Erasmus, Young, Pieterse & Boshoff 1978; Barnard 1993; Williams, Du Plessis & Van Wyngaardt 1993; Alexander, Kat, House, House, O'Brien, Laurenson, McNutt & Osburn 1995). Zebra were also shown to be susceptible to experimental infection with a virulent AHSV strain (Erasmus *et al.* 1978).

During the 1995/1996 summer season, a large outbreak of AHS was reported in South Africa. Large numbers of *Culicoides* midges occurred as a result of the exceptionally high rainfall during this season. As a rule < 3% of light trap collections contain > 50000 *Culicoides*. On 18 March 1996, however, over a million midges were captured in a single night catch on Kaalplaas, a farm in the greater Onderstepoort area near Pretoria, in Gauteng. In this same area, encompassing the Onderstepoort Veterinary Institute (OVI), Veterinary Faculty of the University of Pretoria and the farm Kaalplaas, a number of horses died and several AHSV isolates were made. The horses were either regularly immunized or, among the experimental animals, fully or partially susceptible to AHSV.

Five zebra were kept in the vicinity of these horses. Four of the five had been purchased from the Kruger National Park (KNP) in February 1987 by the OVI and were initially stabled at the Institute. These zebra were experimentally infected with equine influenza virus and also with equine encephalosis virus before the end of that year. In 1989 the animals were relocated to Kaalplaas and a foal was born to one of the zebra mares during December 1995.

We were interested in determining which AHSV serotypes were circulating in horses and zebra in the area. Furthermore, we wanted to investigate whether reverse transcription polymerase chain reaction (RT-PCR) (Bremer & Viljoen 1998) was sufficiently sensitive to detect AHSV in naturally infected asymptomatic zebra. Other RT-PCRs for the detection of AHSV have also been described (Zientara, Sailleau, Moulay, Plateau & Cruciere 1993; Mizukoshi, Sakamoto, Iwata, Ueda, Kamada, & Fukusho 1994; Sakamoto, Punyahotra, Mizukoshi, Ueda, Imagawa, Sugiura, Kamada & Fukusho 1994; Stone-Marschat, Carville, Skowronek & Laegreid 1994; Zientara, Sailleau, Moulay & Cruciere 1994). Differentiation of serotypes by RT-PCR followed by restriction length polymorphism studies of amplicons have been described (Zientara *et al.* 1993; Zientara, Sailleau, Moulay & Cruciere 1995; Zientara, Sailleau, Plateau, Moulay, Mertens & Cruciere 1998). We did not attempt to use these procedures for serotyping because sequence variations in different field isolates of the same serotype could cause problems when making use of restriction enzymes.

Isolation of AHSV was attempted from blood and/or organ samples of 21 of the horses in the Onderstepoort area according to procedures described previously (Bremer & Viljoen 1998). Serotyping was done using a modification of the plaque reduction neutralization test (Huismans & Erasmus 1981). The results are shown in Table 1. From these 21 cases, AHSV serotype 1 was isolated from two, serotype 2 from six, serotype 4 from four, serotype 6 from one and serotype 9 from one of the cases. From seven of them no virus was isolated. As a result of the large midge population during this season the virus inoculum per horse was probably much higher than normal and the high level challenge virus could have overcome vaccine induced antibodies resulting in more deaths. Isolation from samples of horses from other parts of the country revealed that serotypes 2 and 4 were dominant followed by serotypes 1, 6, 7 and 9, respectively (unpublished departmental data).

In order to obtain blood samples from the five zebra, the animals were immobilized on 5 June 1996. A TelinjectBlowgun Vario IV with Telinject darts was used and 2–3 mg etorphine hydrochloride (M99 C Vet) and 40 mg azaperone (Cyron Laboratories, Johannesburg) was administered to each animal. Blood samples were obtained from the jugular vein in 10 mℓ Vac-U-Test glass tubes with or without heparin. A long acting penicillin was administered as an antibacterial prophylactic. In order to isolate virus, blood samples (0,02 mℓ) were injected intracerebrally into 1–3 day-old mice. No nervous signs were observed and no deaths occurred. As no virus was recovered, the virus serotypes circulating in the zebra could not be determined as in the case of the horses. Unsuccessful virus isolation was probably due to the high antibody titres (> 160) (Table 2) and the length of time that had lapsed since the last recorded equine AHSV isolate.

We then attempted RT-PCR to determine whether AHSV viral RNA could be detected in the zebra. Total RNA was extracted from blood and was subjected to RT-PCR as described by Bremer & Viljoen (1998). Agarose gel electrophoresis of the RT-PCR amplicons (Fig. 1A) revealed the presence of 230 bp amplicons in samples obtained from two of the zebra mares (lanes 2 and 3) and the foal (lane 4). To further increase the sensitivity and to indicate AHSV specificity, Southern blot hybridization using a ^{32}P labelled DNA copy of the NS2 gene of AHSV3 was performed (Fig. 1B). Samples of all three mares (lanes 1, 2 and 3) and the foal were positive (lane 4). No AHSV RNA was detected in the male (lane 5). Some residual plasmid DNA from which the NS2 gene was excised, was also labelled and hybridized to the molecular size marker DNA (lane 7).

TABLE 1 The number of cases of different AHSV serotypes isolated from necropsy samples in the Onderstepoort area during the 1995/1996 outbreak of AHS. Where known age and vaccination status are included

Date	Number	Age	Sample	Date of vaccinations	Serotypes in vaccine	Serotype isolated
18/12/95	379	6 years	Spleen	28/11/89 09/12/89 17/10/91 13/11/91 18/11/92 11/02/94 02/03/94 24/10/95	1, 3, (4), (5)[a] 2, 6, 7,8 Unknown 2, 6, 7, 8 1, 3, (4), (5)[a] 1, 3, (4), (5)[a] 2, 6, 7, 8 1, 3, (4), (5)[a]	Negative
08/02/96	420	4 months	Spleen/lung	Not vaccinated	–	1
14/02/96	325	5 years	Spleen/lung	17/02/93 11/02/94 02/03/94 23/10/95 16/11/95	2, 7, 6, 8 1, 3, (4), (5)[a] 2, 6, 7, 8 1, 3, (4), (5)[a] 2, 6, 7 ,8	Negative
15/02/96	296	6–8 months	Spleen/lung	13/09/95	1, 3, 4	2
16/02/96	456	3–4 months	Spleen/lung	Not vaccinated	–	6
16/02/96	498	10 years	Spleen	31/03/88 19/04/88 05/05/88 17/11/88 28/11/89 09/12/89 09/10/90 18/11/92 11/02/94 02/03/94 23/10/95 16/11/95	1, 3, (4), (5)[a] 4 2, 6, 7, 8 2, 6, 7, 8 1, 3, (4), (5)[a] 2, 6, 7, 8 Unknown 1, 3, (4), (5)[a] 1, 3, (4), (5)[a] 2, 6, 7, 8 1, 3, (4), (5)[a] 2, 6, 7 ,8	Negative
19/02/96	113	5 years	Spleen/lung	21/12/93 24/10/95 16/11/95	1, 3, 4 1, 3, (4), (5) 2, 6, 7, 8	Negative
21/02/96	295	1 year	Spleen	13/09/95 30.11.95	1, 3, 4 2, 6, 7 ,8	4
22/02/96	183	4½ years	Spleen/lung	27/10/92 26/11/92 11/02/95 24/10/95 16/11/95	1, 3, (4), (5)[a] 2, 6, 7, 8 1, 3, (4), (5)[a] 1, 3, (4), (5)[a] 2, 6, 7, 8	2
22/02/96	329	± 2 ½ years	Spleen	10/08/95	1, 3, 4	2
22/02/96	432	3 months	Spleen	Not vaccinated	–	2
23/02/96	204/96	5 years	Spleen	No record	No record	1
26/02/96	212/96	18 years	Spleen	Vaccinated annually Except 1994— no history	–	9
28/02/96	185	3–4 years	Spleen/lung	26/9/94 27/12/94	1, 3, 4 1, 3, (4), (5)[a]	Negative
06/03/96	80	5 years	Spleen/blood	13/02/91 10/02/91 05/12/91 03/01/92 18/11/92 12/02/94 23/10/95 16/11/95	6 1, 3, 4 1, 3, (4), (5)[a] 2, 6, 7, 8 1, 3, (4), (5)[a] 1, 3, (4), (5)[a] 1, 3, (4), (5)[a] 2, 6, 7, 8	2

TABLE 1 *(continued)*

Date	Number	Age	Sample	Date of vaccinations	Serotypes in vaccine	Serotype isolated
19/03/96	416	5 months	Spleen	Not vaccinated	–	4
19/03/96	460	5 months	Spleen	Not vaccinated	–	4
23/03/96	79	4–5 years	Spleen/lung	18/07/96 27/12/95	4 5	2
26/03/96	172	4–5 years	Spleen/blood	02/12/94	Recombinant serotype 4[b]	Negative
12/04/96	368	No record	Spleen	Not vaccinated	–	4
15/04/96	88	No record	Spleen	No record	–	Negative

[a] The commercial polyvalent AHSV vaccine 1 (part of the dual vaccine obtainable from Onderstepoort Biological Products) usually consists of serotypes 1, 3, 4 and 5. In the recent past serotypes 4 and/or 5 were not included in certain batches. In most instances batch numbers of vaccines used to immunize horses were not known

[b] No further information on challenge and further vaccinations was available

TABLE 2 Neutralizing antibody response induced to different AHSV serotypes in the five zebra (zebra 1, 2, 3 = mares; zebra 4 = foal; zebra 5 = male)

Zebra no.	AHSV serotypes								
	1	2	3	4	5	6	7	8	9
1	> 160	160	> 160	> 160	80	> 160	> 160	> 160	> 160
2	> 160	160	> 160	> 160	80	> 160	> 160	80	80
3	> 160	160	> 160	> 160	160	> 160	> 160	40	> 160
4	80	160	< 20	20	< 20	< 20	< 20	< 20	20
5	20	< 20	20	> 160	40	> 160	> 160	40	> 160

In order to determine to which serotypes zebra had been exposed, serum samples were analyzed by the plaque reduction neutralization test (Huismans & Erasmus 1981). Readings < 20 were considered negative and all readings above this as positive. Serum from the three mares (zebra 1, 2 and 3, respectively) had antibodies to all nine serotypes. (Table 2). These mares had most likely been exposed to all AHSV serotypes in the KNP or in the Onderstepoort area in this and previous seasons. Barnard (1993) described infection of zebra by all nine serotypes in the KNP. The male (zebra 5) had relatively low levels of antibodies directed against serotypes 1, 2 and 3. As there was a high prevalence of serotype 2, according to the serotypes isolated from horses in the area, it was unexpected that the level of antibodies directed against serotype 2 was so low. The reason for the antibody profile of the male differing from the females is unclear. The foal was susceptible and having its first exposure seroconverted to serotype 2 and also to 1. These serotypes were also isolated from horses that died in the Onderstepoort area at the time (Table 1). The antibody profile in the foal, which was approximately 6 months old at the time of bleeding, differed from that of the mares, probably indicating that it had already lost its maternal antibodies against most serotypes. The antibodies present at the time of sampling were most likely induced by exposure to AHSV serotypes transmitted by *Culicoides* midges infected from an unknown source or from infected zebra or horses in the vicinity. Unfortunately it was not possible to obtain blood samples of the foal at a later stage in order to follow the antibody profile. Barnard (1993) observed a loss of maternal antibodies in zebra foals at 5–6 months of age.

From virus serotyping results of samples obtained from the Onderstepoort area and from the antibody profile of the zebra foal, the serotypes occurring in this area were seen to be mainly serotypes 2 and 4 with a lower prevalence of serotypes 1, 6 and 9. The zebra probably were not AHSV reservoirs in this situation as there was only a single fully susceptible zebra foal present. A large susceptible population pool in a frost-free area with continuous vector activity is required for the zebra to act as reservoirs (such as in the KNP) (Barnard 1993). It is not clear how AHSV is introduced into the area each season (Meiswinkel 1998).

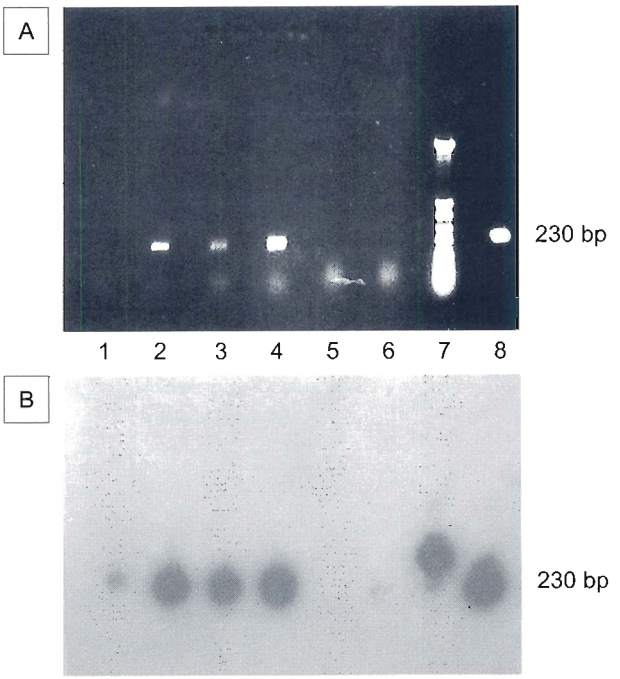

FIG. 1 Agarose gel electrophoresis (A) and Southern blot hybridization (B) of RT-PCR amplicons obtained from RNA extracted from the blood of five zebra and an AHSV infected horse

Lanes 1: zebra 1
Lanes 2: zebra 2
Lanes 3: zebra 3
Lanes 4: zebra 4
Lanes 5: zebra 5
Lanes 6: negative control (water)
Lanes 7: *Hinf* 1 digested pAT153
Lanes 8: positive control (AHSV infected horse)

The detection of AHSV RNA in zebra by RT-PCR indicates that this procedure has the potential to be used together with serological techniques to identify reservoirs of AHSV.

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