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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

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ABSTRACT

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In the Kruger National Park 75 % of zebra foals are born in October-March and they lose their passive immunity against African horsesickness virus (AHSV) when they are 5–6 months old. One month later infection with different serotypes of AHSV amounts to 31 % and thereafter infections increase rapidly to almost 100 % before the foals are 12 months old. The capability of zebra to maintain AHSV is clearly illustrated by the continuing infections during every month of the year with a peak period in winter. This peak is ascribed to the presence of large numbers of susceptible foals in the presence of active *Culicoides* species.

INTRODUCTION

African horsesickness (AHS) was first recorded in South Africa in 1719 among horses introduced from Europe and the Far East into the Cape Province (Alexander & Mason 1941). Contrary to general belief AHS does not occur regularly in most parts of the country and the occurrence of the disease is typically terminated abruptly by the first frost in early winter. However, in the subtropical north-eastern Transvaal lowveld, where frost usually does not occur, the disease can occur during every month of the year. The seasonal occurrence of the disease in other parts of South Africa such as the Transvaal Highveld is attributed to increases in the *Culicoides* population in summer to a level suitable for virus transmission and the absence of or low *Culicoides* numbers in winter and spring. Outbreaks in successive years do not continue from where they stopped and are often due to different serotypes of the virus.

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This pattern suggests the existence of a reservoir of virus in the warmer north-eastern region of the country and/or adjacent countries.

The 9 distinct serotypes of AHSV, which all occur in South Africa, are according to isolations made from horses not equally abundant (G.H. Gerdes, Onderstepoort Veterinary Institute, unpublished data 1992). During the past 11 years the rarer serotypes 3 & 5 were isolated less then 10 times each while the most abundant serotypes 1, 4 & 7 were isolated on 43, 37 and 25 occasions respectively. AHSV-9, associated with the Middle East, was not isolated from horses during this period. This is probably due to a relatively low virulence of this serotype (Howell & Erasmus 1963).

The susceptibility of zebra (*Equus burchelli*) to experimental infection (Erasmus, Young, Pieterse & Boshoff 1978) and the presence of antibodies against AHSV in free-living zebra (Davies & Lund 1974; Davies & Otieno 1977) suggests that zebra

may possibly, under certain conditions, be incriminated as a reservoir for AHSV. This is supported by the 1987 outbreak of AHS in Spain which apparently followed the importation of infected zebra to a Safari Park near Madrid (Lubroth 1987). The purpose of this investigation was to examine the zebra population in the Kruger National Park (KNP) over a period of 1 year for the presence of serotype specific antibodies as proof of infection by AHSV and to demonstrate whether such a population could maintain the virus.

MATERIAL AND METHODS

Zebra

Free-living zebra in the KNP in an area of approximately 60 x 100 km south of the Olifants River (Fig. 1) as well as a few zebra to the east of Lower Sabie were used for the collection of serum. Samples were collected at intervals of approximately 6–12 weeks from August 1991–May 1992. For sampling the zebra were immobilized with etorphine hydrochloride (M99) and xylazine hydrochloride (Rompun).

To determine the age when zebra lose their passive immunity 4 foals, 1 of 3 months and 3 of 4 months, were confined in open stables immediately next to the KNP at the Hans Hoheisen Research Unit at Orpen. The zebra were not protected from infection by free-living *Culicoides* and were not too far removed from free-roaming zebra in the KNP and adjoining game reserves.

The foals were sampled 3 times; once at capture, and again at 1 & 3 months.

Sera from 6 Cape mountain zebra (*Equus zebra*) from Cradock, in the Eastern Cape, an area believed to be free from African horsesickness, was included as negative control.

Age determination

In planning the investigation it was deemed necessary to focus on presumably susceptible zebra. Consequently specimens were collected from zebra less than 12 months of age. To obtain sufficient numbers of zebra of this age animals for immobilization were selected on body size. The approximate age was determined by the eruption and wear of the teeth (Smuts 1974a).

Specimens

Blood was collected in vacutainer tubes without anticoagulant. The blood was allowed to clot and the serum was decanted and stored at 4 °C till testing.

Serological tests

Agar gel immunodiffusion (AGID) (Blackburn & Swanepoel 1988), complement fixation (CF) (McIntosh 1965) and an enzyme-linked immunosorbent

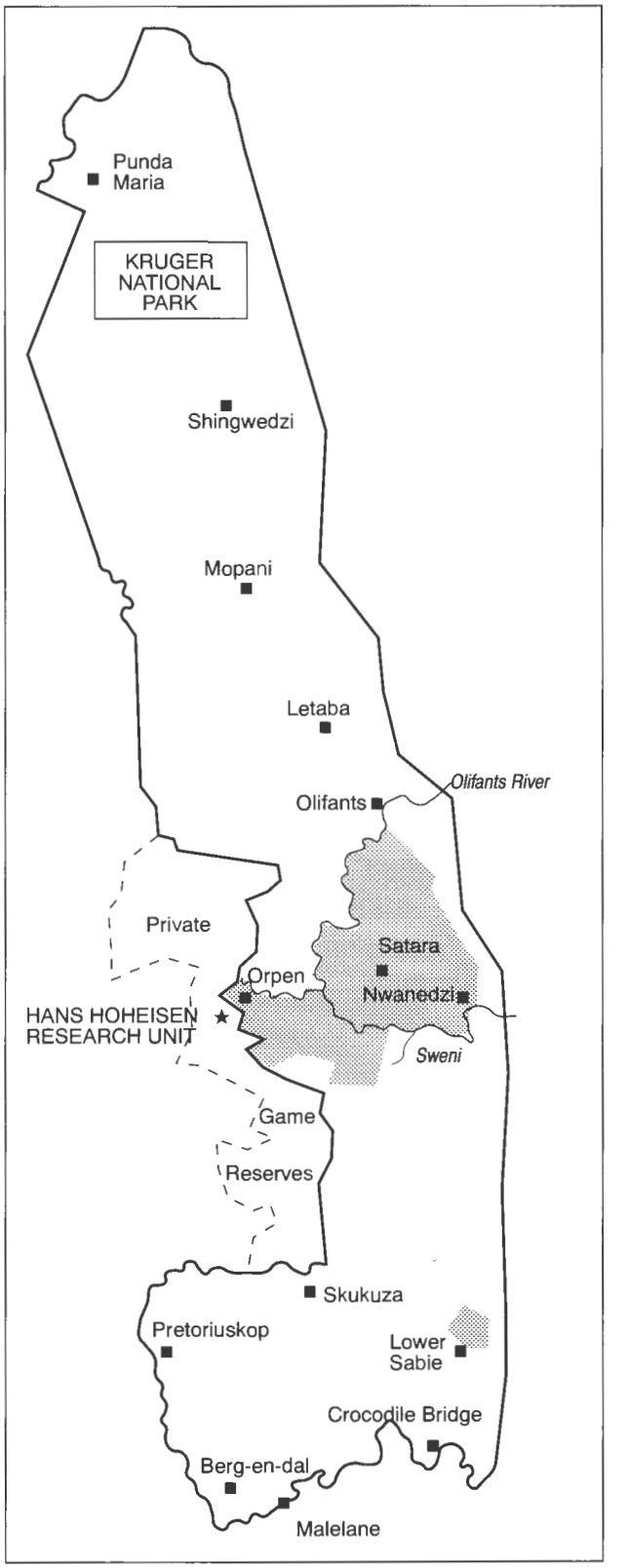

FIG. 1 Free-living zebra in an area of approximately 60 x 100 km ( ) in the Kruger National Park were used for the collection of serum and 4 zebra were confined at the Hans Hoheisen Research Unit at Orpen

assay (ELISA) (Williams 1987) with minor modifications were utilized as group specific tests. Microneutralization (MN) was used to determine serotype specific antibody titres. In the test 30–100 CID50 was used to neutralize two-fold serum dilutions in 96-well microtiter plates.

The serum-virus mixtures were kept for approximately 30 min at room temperature prior to the addition of a Vero cell suspension containing sufficient cells to form a monolayer within 24 h.

The test plates were sealed with cellotape and incubated at 37 °C in an atmosphere of 5 % CO_2 in air until cytopathic changes in controls, seeded with half the amount of virus used in the test, were clearly visible. The test plates were then washed with tap water, fixed with alcohol, stained with a 2 % solution of neofuchsin and read. The end point titre was taken as the dilution of serum where cytopathic changes were similar to those controls containing negative serum and seeded with 50 % less virus.

To exclude the possibility of false-positive neutralization as a result of cross-reactions with other serotypes, antibody titres of 20 and less were disregarded in those cases in which high titres were encountered with the cross-reacting serotype.

Passive immunity

It was assumed that foals would have a passive immunity to most or all of the 9 serotypes of AHSV and that infection could ensue at any time after they had lost this immunity. To determine this age, serum of foals 3–9 months of age, was tested for the presence of group- and type-specific antibodies.

Exposure of zebra

Infection of zebras with various serotypes of AHSV and the approximate time of infection was determined by assessing the appearance of type-specific antibodies in zebras 1, 2–3, 4 & 6 months after they had lost their passive immunity.

RESULTS

Age groups

A total of 123 zebra were sampled in August, September, October, December, January, April and May 1991–1992. They were sorted according to age into 7 groups of 3, 4, 5, 6, 7–8, 9 & > 9 months old foals.

Microneutralization tests

It was anticipated that cross-reactions between different serotypes might hamper the interpretation of results but this was rarely the case. Cases in which cross-reactivity could have played a role were relatively few. In zebra more than 6 months of age antibody titres in excess of 320 were frequently encountered. In cases where a titre of ≤ 20 against 1 serotype and a titre of ≥ 640 against the cross-reacting serotype was found, the low titre was assumed to be the result of a cross-reaction and consequently omitted. This may have resulted in false negative results. However, as the aim was to determine without any doubt the presence of specific antibodies, it was considered to be more appropriate to incorporate false negative rather than false positive results. Where both titres were of a low or high level neither was regarded to be the result of cross-reaction.

Passive immunity

In the confined foals the level of passively acquired neutralizing antibodies (Fig. 2) declined till they were 5–6 months old. At this age their antibody titres against most of the serotypes were ≤ 20. The antibody titres of foal H1 against 5 of the 9 serotypes were ≥ 640 at 3 months and 1 month later the titres against 7 serotypes declined to ≤ 320. When this foal was 6 months old it reacted negatively to all but 2 serotypes. At 4 months the number of serotypes with titres of ≤ 320 in foals H2, H3 & H4 were 8, 9 and 9 respectively and by 5 months the antibody titres for most serotypes had declined to ≤ 20.

An increase in antibody titres was demonstrated in serum collected when the foals were 7 months old. The 3 older foals showed increased titres for serotypes 5, 8 & 9.

In free-living foals the percentage of positive reactions obtained with AGID, ELISA, and MN declined with age (Fig. 3). Three of 3 3-month-old foals reacted positively to the group specific AGID test and the ELISA and the number of positive reactions reached a low in foals of approximately 5 months old. Negative results were obtained with CF at 3 months and in 14 of 15 5-month-old foals. With the ELISA 20 % reacted positively and 35 % gave a suspicious reaction at the latter age.

In 6-month-old foals the number of positive reactions obtained in all the tests showed an increase and at 7–8 months more than 55 % of the foals reacted positively to all the tests. In 9-month-old zebras the incidence of positive reactions to all tests was in excess of 80 %. The incidence obtained with CF and ELISA at this age was almost 100 % while it was slightly lower with neutralization.

These results show that zebra foals lose their passive immunity when they are approximately 5 months old and can then become infected. Therefore the period of exposure was calculated in months over 5 months and it was assumed that 6-month-old foals had been exposed for approximately

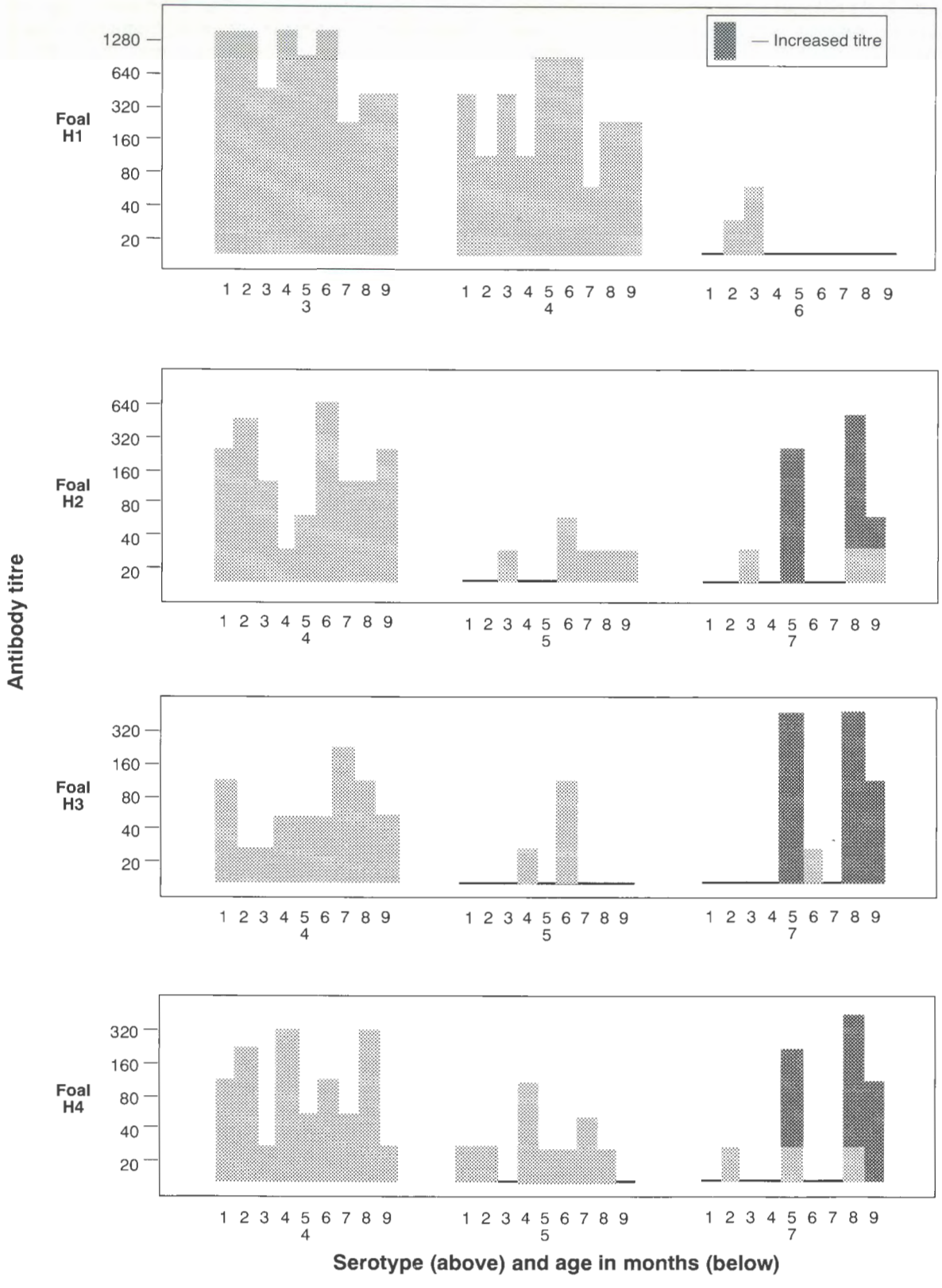

FIG. 2 Neutralizing antibodies in foals confined at the Hans Hoheisen Research Unit at Orpen

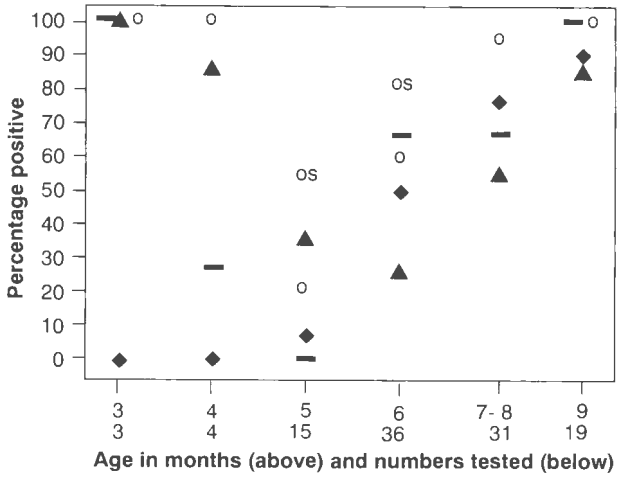

FIG. 3 Antibodies against AHSV in free-living zebra 3–9 months of age, determined with agar gel immunodiffusion (—), complement fixation (♦), enzyme-linked immunosorbent assay (o), (os- positive + suspicious ELISA reactions) and microneutralization (▲)

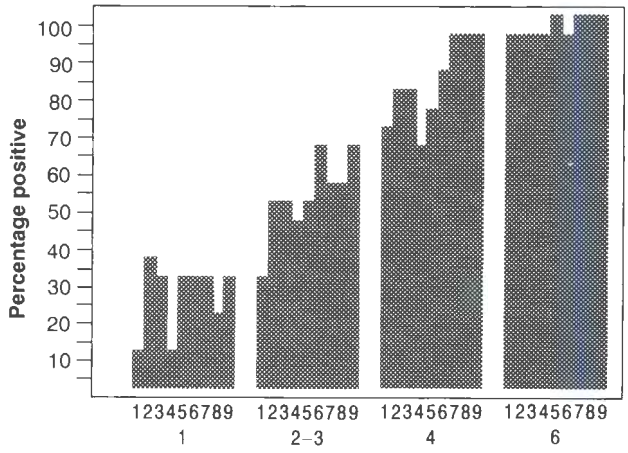

FIG. 4 Neutralizing antibodies against 9 serotypes of AHSV in zebra exposed for 1, 2–3, 4 and 6 months in the Kruger National Park 1991–1992

1 month, 7-month-old foals for 2 months and so forth and the time of exposure was taken as the month or months directly preceding the month in which sera were collected.

Neutralizing antibodies in zebra of different ages

Antibodies against all the serotypes were found. A marked increase in the positive reactions to all the serotypes was observed in zebra of the different age groups and the animals were consequently exposed for increasing periods of time (Fig. 4). After an exposure period of 1 month the occurrence of type specific antibodies varied from 13 % for AHSV-1 and AHSV-4 and 37 % for AHSV-2. In zebras exposed for 2–3 months the incidence of type specific antibodies against AHSV-1 (33 %) was also the lowest. After an exposure period of 4 months the incidence of AHSV-7, 8 & 9 was almost 100 % with serotypes 1 & 4, the lowest at 75 % & 70 % respectively. In zebras exposed for 6 months the incidence for all the serotypes was 97 % or more.

Serum collected from 6 Cape mountain zebras from Cradock reacted negatively to all the serological tests.

Seasonal occurrence of type-specific antibodies

The percentage of zebra with antibodies against the different serotypes of AHSV reached the lowest point in zebra exposed during summer (Fig. 5). The incidence in zebra exposed for 1 month dropped from 40 % in August and September 1991 to 10 % in December and 0 % in February 1992; that for zebra exposed for 2–3 months declined from 65 % in June/July to 25 % in December/January. In March–April 1992 the incidence increased to the same level as in June of the previous year.

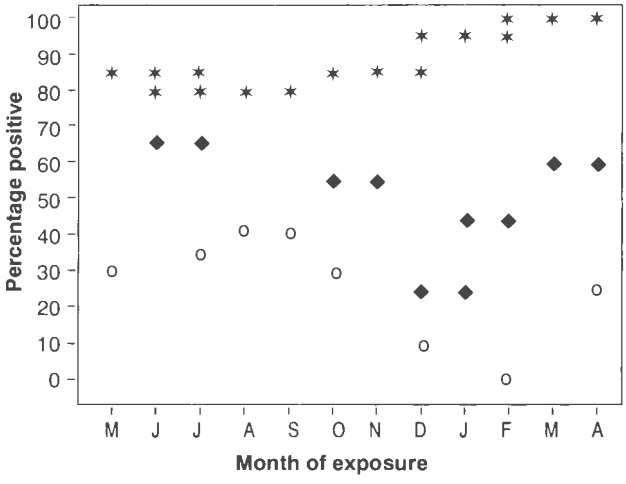

FIG. 5 Type-specific antibodies against AHSV in zebras exposed during different times for periods of approximately 1 (o), 2–3 (♦) and 4 (⋆) months in the Kruger National Park in 1991–1992

The incidence of type-specific antibodies in zebra exposed for 4 months was high and varied from 80–95 %.

DISCUSSION

The continued circulation of an insect-transmitted virus depends on the constant availability of susceptible hosts and competent vectors in sufficient numbers.

Although the number of zebra in the KNP inclines to fluctuate, the population of approximately 33 000 in 1991 is regarded as one of the largest in Africa (Viljoen 1991). Zebra keep to small family groups consisting of a stallion and 1 or more mares and their foals (Smuts 1974b). Stallions rejected from family

groups often form bachelor groups. Herds of zebra are made up of many non-territorial groups which move freely over large areas, sharing such areas with other groups and species of game. This movement was increased during the period under investigation. Food was scarce as a result of a severe drought, and isolated showers of rain which resulted in temporary grazing in localized areas prompted the zebra to move about continuously in search of food. However, despite the drought, the zebra which are predominantly grazers and partial to feeding on short grass, were in good condition.

Age determination was problematic. With the relatively large numbers of zebras of different ages sampled, it became obvious that body size does not correlate well with the eruption and wear of teeth. Furthermore, marked differences in the time of eruption of teeth on the left and right sides were observed in at least 15 % of zebra. This was most striking in zebras 3–6 months of age. It is therefore possible that the estimated age of some zebra may differ slightly from the actual age. However, the stepwise increase in antibody titres, obtained with the MN test (Fig. 4) in zebra exposed for increasing periods of time is a good indication of the difference in age.

Horse foals of mares which have been immunized or which have recovered from natural infection receive a variable degree of passive immunity through the colostrum. This immunity is restricted to the serotype of horsesickness virus against which the mare is immune and foals can become susceptible well before the age of 6–9 months (Blackburn & Swanepoel 1988).

In zebra the duration of passively acquired immunity appears to be similar to that in the horse. In the 4 zebra foals confined at the Hans Hoheisen Research Unit (Fig. 2) the neutralizing antibody titres against all the serotypes declined with age and reached the lowest level when the foals were 5–6 months old, but at 7 months the neutralizing titres against 3 serotypes were once again elevated indicating infection at least 2 weeks previously. The youngest zebra which reacted positively to CF, indicating a recent infection, was 1 of 15 foals estimated to be 5 months old. However, as explained above this foal could have been older. In the 6-month-old group the percentage of positive reactions was significantly higher. This indicates infection and susceptibility at this age to several AHSV serotypes.

Microneutralization revealed an infection rate, for all the serotypes, of almost 50 % in foals exposed for 2–3 months and close to a 100 % infection in foals exposed for 6 months (Fig. 4). This high incidence in a drought year is remarkable. As a result of the drought suitable breeding sites for *C. imicola*, the only known vector in South Africa of AHSV, were restricted and light-trap catches in areas from which samples of zebra were collected contained virtually no members of this species. However, reasonable numbers of dung-inhabiting species of *Culicoides* of the subgenus *Avaritia* were regularly present (R. Meiswinkel, Onderstepoort Veterinary Institute, unpublished data 1992). The high infection rate of zebra in the absence of *C. imicola* is an indication that this species is not the sole vector of AHSV.

Specific antibodies against 7 serotypes were demonstrated in approximately 30 % of tests on sera of foals exposed for only 1 month. After an exposure period of 2–3 months almost 50 % of tests were positive and after exposure periods of 4 and 6 months the incidence increased to 85 % and 97 % respectively. Serotypes 1 and 4 appeared to be less abundant. This was demonstrated by the absence of antibodies against these 2 types in foals exposed for 1 month and a low incidence of antibodies against the same 2 types in foals exposed for 2–3 and 4 months. This finding is in contrast to the high incidence of the same serotypes isolated from cases of horsesickness during 1981–1991 (G.H. Gerdes, Onderstepoort Veterinary Institute, unpublished data 1992). Type 1 was isolated from 43 and type 4 from 37 horses while 2 of the most abundant types in zebra 3 & 5, were isolated on only 2 and 5 occasions respectively. This ostensible difference may possibly be ascribed to an unnatural situation in horses as a result of vaccination. Furthermore, the data collected from zebra represents 1 season and it is quite likely that the situation may vary from year to year.

Infection by AHSV in all 4 seasons is evident by the appearance of type-specific antibodies in the serum (Fig. 5) of zebra exposed for 1 and 2–3 months during different months of the year. This is to be expected in a subtropical climate in which insect activity continues during the cooler winter months, but the low incidence of infection in foals exposed for 1 and 2–3 months in December–February and the high incidence in winter were not anticipated as cases of horsesickness among horses further south in the central part of Transvaal are usually encountered from December–May with the highest incidence in March and April.

The declining infection rate in summer and an increase in the occurrence of infection in winter correlate closely with the availability of susceptible zebra. Foals may be born at any time of the year. In the Kruger Park however, over 75 % of all foals are born from October–March with peak foaling in December and January while the average relative percentage of foals born in May–September is less than 2 % per month (Smuts 1974). This means that the vast majority of foals become susceptible in April–September with a peak in winter, June–July,

and with the continuous presence of *Culicoides* in the KNP circumstances remain suitable for virus multiplication and transmission.

It therefore appears very likely that AHSV is maintained by the classical method of continuous circulation of virus within a zebra population provided that the population is large enough and that a vector is continuously present in sufficient numbers to transmit virus. The presence of a large number of susceptible zebra foals in winter despite *Culicoides* activity tapering off, ensures viral circulation throughout the year.

The indication that species other than *C. imicola* may be involved in the transmission of AHSV in the KNP necessitates investigations to determine activity, behaviour and vector competence of relevant *Culicoides* species not only in the KNP but also in areas into which zebra are being reintroduced. In the latter areas the potential risk of establishing a source of AHSV in a farming area needs to be evaluated.

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