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Epidemiology of African horsesickness: Antibodies in free-living elephants (*Loxodonta africana*) and their response to experimental infection

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ABSTRACT

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The presence of low levels of group- and type-specific antibodies against African horsesickness virus in the serum of some free-living elephants was reconfirmed. Experimental infection resulted in conflicting results. No detectable viraemia nor virus could be demonstrated in the organs of the six elephant calves and none of them mounted significant levels of neutralizing antibodies against the virus. On the other hand, all calves showed a slight rise in ELISA titres. This rise, however, was modest when compared with the rise in experimentally infected zebra. The presence of low levels of group- and type-specific antibodies in the serum of some free-living elephants is judged to be the result of natural hyperimmunization due to frequent exposure to infected biting insects. Elephants should therefore, despite the presence of low levels of antibodies, be regarded as poorly susceptible and unlikely to be a source of African horsesickness virus.

Keywords: African horsesickness, antibodies, epidemiology, experimental infection, free-living elephants, *Loxodonta africana*, response

INTRODUCTION

Knowledge of the involvement of wildlife in the epidemiology of viral diseases is limited primarily to species which develop clinical signs of the disease or specific antibodies which indicate susceptibility. Such animals may act as virus reservoirs and/or amplifiers of the virus. In the case of African horsesickness (AHS) which affects mainly Equidae, horses are the most susceptible. Zebra show a mild febrile reaction to experimental infection (Erasmus, Young, Pieterse & Boshoff 1978) and may be viraemic for several weeks (Erasmus *et al.* 1978; Barnard, Bengis, Keet & Dekker 1994). Large zebra populations may act as a virus reservoir through continuous circulation of the virus in the animals (Barnard 1993). The outbreak of AHS in Spain is believed to be the result of the transportation of zebra from Namibia in 1987 (Lubroth 1988; Mellor, Boned, Hamblin & Graham 1990).

In elephants, complement-fixing (CF) antibodies (Davies & Otieno 1977; Erasmus *et al.* 1978; Mushi 1990) and inconclusively low levels of neutralizing antibodies against AHS virus have been demonstrated (Mirchamsy & Hazrati, 1973; Erasmus *et al.* 1978).

Erasmus *et al.* (1978) infected two elephants with AHSV 3, with conflicting results. Both elephants, which already possessed CF antibodies at the time of infection, showed a definite rise in CF activity. However, no detectable viraemia or development of neutralizing antibodies could be demonstrated. Lubroth (1992) concluded that available data does not clarify the role of elephants in the epidemiology of AHS and

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underlines the need for further field and experimental studies.

The purpose of the present investigation was to acquire a better understanding of the role of elephants in the epidemiology of AHS.

MATERIALS AND METHODS

Serum samples from culled elephants

Serum obtained from blood collected from elephants during the 1993 culling operation in the Kruger National Park (KNP), South Africa, was available for serological tests. Fourteen samples, with ELISA titres varying from negative to high, were selected for micro-neutralization (MN) tests. For comparison, serum collected in 1992 from 14 zebras in the KNP, aged nine to 18 months, was tested simultaneously. The shoulder heights of elephants were taken as a rough indication of their age.

Elephant calves for experimental infection

Elephant calves, approximately 4–6 years old, captured in the KNP during culling in the winter of 1994, were used for experimental infection. The calves were kept in bomas at Skukuza in the KNP. Eighteen of these calves were bled and their sera tested for the presence of type-specific antibodies against AHSV.

Samples from experimentally infected elephant calves

Blood in EDTA from experimentally infected elephant calves and blood collected without anti-coagulant was used for virus isolation and serological tests. The samples for virus isolation were collected on the day of infection and again on days 4, 7, 8, 9, 10, 21 and 35. Blood samples for serum were collected before infection and at approximately weekly, and later at two-weekly, intervals. A final collection of blood, spleens and lymph nodes was done when the calves were slaughtered 50 d after experimental infection. Sera from experimentally infected zebra obtained previously (Barnard *et al.* 1994) were tested in parallel for comparative purposes.

Experimental infection

Virulent AHSV 1 (29/62) of passage level six in suckling mouse brains was used to infect the elephant calves. One millilitre of infective cell-culture material containing 5 x 10^6 $CCID_{50}$/mℓ was inoculated intravenously into each of six calves in September, when *Culicoides* vectors are relatively scarce at Skukuza.

Serological tests

The presence of group-specific antibodies against AHSV was measured by enzyme-linked immunosorbent assay (ELISA) (Williams 1987) with minor modifications and agar-gel immunodiffusion (AGID) (Blackburn & Swanepoel 1988). The presence of type-specific antibodies against serotypes 1–9 of AHSV was measured by MN (Barnard 1993). Briefly, twofold dilutions of inactivated serum in 96-well microtitre plates (Nunc, Denmark) were used to neutralize 30–100 CCID50 of virus per well.

Virus isolation

Blood samples collected on days 0–10 were processed at Skukuza and inoculated within hours of collection onto cell cultures. Duplicates of these samples as well as the other samples were kept at 4 °C until the last samples became available and they could be processed at the Onderstepoort Veterinary Institute.

For virus isolation, 0,5 mℓ of washed and packed erythrocytes and the clarified supernatant of macerated organs were inoculated onto VERO- and CER-cell monolayers in 25-mℓ plastic flasks (Nunc, Denmark). The cultures were incubated at 37 °C and examined daily for cytopathic changes indicative of viral multiplication. Two blind passages, 7–10 d apart, were made before a sample was regarded as negative.

RESULTS

Antibodies in culled elephants

Sera from 63/80 (79%) elephants reacted positively in the ELISA for AHSV. The titres of almost 65% of the positive samples were less than 10000. In comparison, 34/34 (100%) zebra samples reacted positively and their ELISA titres were significantly higher, with more than 84% having a titre of 10000 or higher (Fig. 1).

Twenty-six per cent of 14 serum samples from elephants tested for the nine types of AHSV, reacted positively with virus-neutralizing titres of 20 or higher. With a similar number of zebra-serum samples, 95% gave positive reactions (Fig. 2). The highest virus-neutralizing titre obtained with elephant serum was 160 in 4/126 tests, while more than 49% of zebra-serum samples neutralized AHSV at dilutions of 160 or higher.

All the elephant-serum samples (80) tested negatively in the AGID test, whereas all the zebra samples were positive.

Presence of antibodies related to height (age)

The relationship between antibodies against AHSV in elephants and the shoulder height (age) of elephants is shown in Fig. 3. Positive reactors occurred

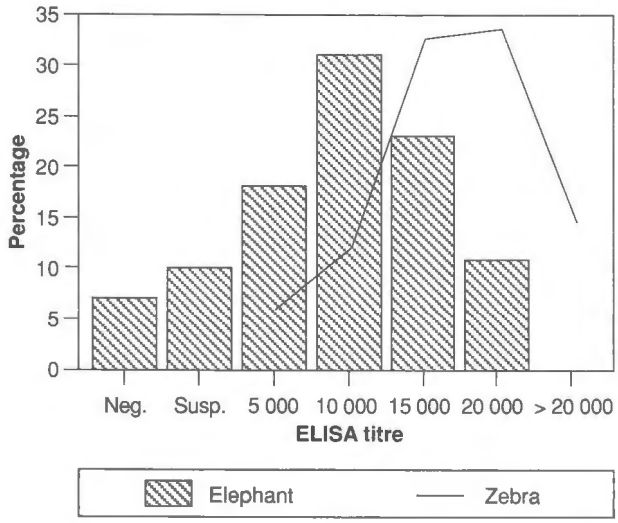

FIG. 1 ELISA antibodies against AHSV in 80 elephants and 34 9–12-months-old zebra in the Kruger National Park, South Africa

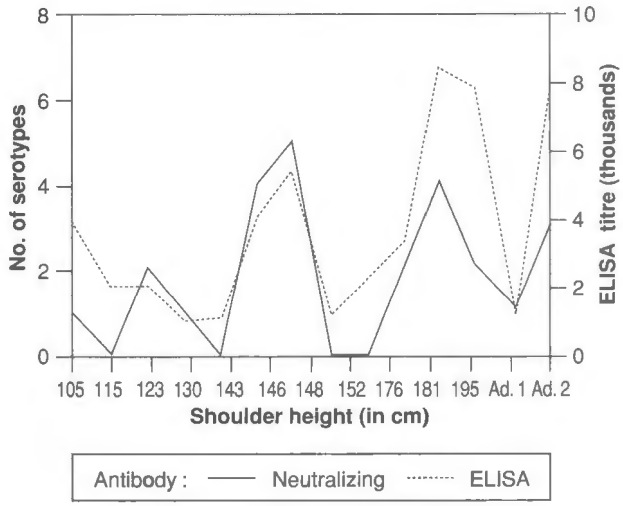

FIG. 3 Height (age) of 14 elephants in relation to group and type-specific antibodies against African horsesickness virus

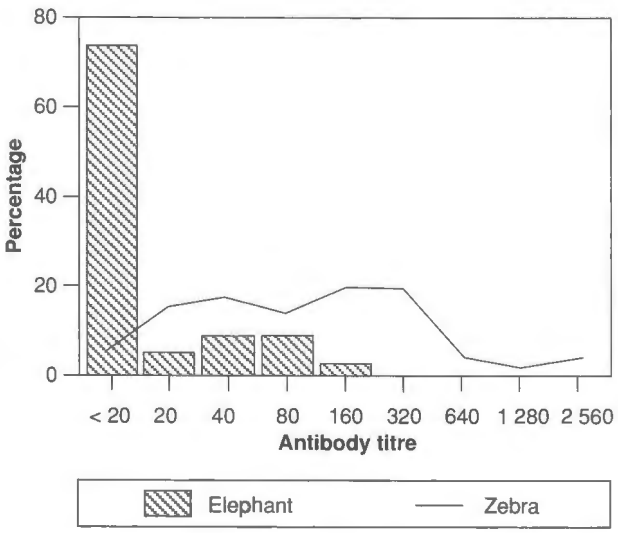

FIG. 2 Neutralizing antibodies against AHSV in 14 elephants and 14 9–18-months-old zebra in the Kruger National Park

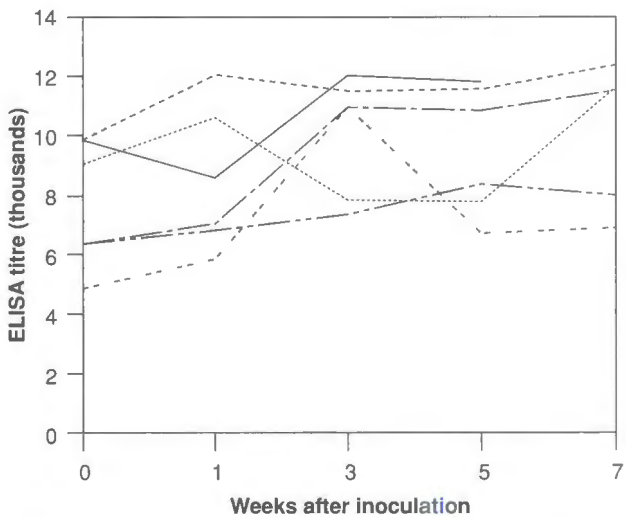

FIG. 4 ELISA titres against African horsesickness virus in six experimentally infected elephant calves

TABLE 1 Antibodies against AHSV in elephant calves at the time of infection

Number of calf	ELISA results	Antibody titres against AHSV								
		1	2	3	4	5	6	7	8	9
1	Positive	–[1]	–	10	10	40	–	40	40	40
2	Positive	–	10	20	–	40	–	160	20	160
3	Positive	10	–	20	10	40	–	10	20	80
4	Positive	–	–	10	10	20	20	40	–	80
5	Positive	–	20	–	10	10	20	20	–	–
6	Positive	–	–	40	10	–	20	10	–	40

[1] = Negative

among elephants of different ages. Some elephants of at least 10 years old reacted almost negatively in both ELISA and MN tests, while other much younger calves reacted positively in both tests. However, there seems to be a close association between ELISA titres and the number of AHSV serotypes in which neutralizing antibodies are present. Elephants with high ELISA titres often showed neutralizing antibodies against multiple serotypes, while elephants with low ELISA titres reacted with a limited number of serotypes.

Experimentally infected elephant calves

All the available calves possessed ELISA antibodies against AHSV 4 weeks before experimental infection. However, five of them tested completely and one partially negative for neutralizing antibodies against AHSV 1 (Table 1) and they were consequently used for experimental infection with AHSV 1.

Three weeks after infection, neutralizing antibodies in four calves showed a slight increase and stayed at that level for the following three weeks (Table 2).

The ELISA titres of all the calves increased slightly after infection and remained more or less stable during the next 5 weeks (Fig. 4).

DISCUSSION

The involvement and importance of wild animals in the epidemiology of insect-transmitted viral diseases, can be adequately assessed where large numbers are available and under circumstances of optimal exposure to the virus. In the KNP, the abundance and variety of wild animals in a well-preserved ecosystem favours endemic occurrence of viruses. We therefore believe that conditions to study the susceptibility of elephants to AHS virus and their involvement in the epidemiology of AHS, are fulfilled in the KNP, with 7500 elephants, a continual cycling of AHS virus in approximately 33000 zebra and active vectors, *Culicoides* species, present in every month of the year (Barnard 1993). Furthermore, the sharing of grazing and communal drinking places where these animals tend to congregate, provides ideal opportunities for the exchange of viruses.

Culicoides imicola, the only South African midge positively known to transmit AHSV, is rare in most areas in the KNP. There are, however, several other *Culicoides* species which can potentially fulfil this role (Meiswinkel & Braack 1994). These authors have captured at least 21 *Culicoides* species in light traps, including five *Avaritia* species which have an intimate relationship with elephants. These five species breed in the dung of elephants, feed on the elephants and most likely also on other species of game, including zebra. The other 16 species, which include *C. imicola*, belong to the sector of the genus *Culicoides*, whose immature stages develop in groundwater habitats.

The antibody levels in free-living elephant in the KNP are insignificant when compared to those of zebra. In the nine- to 18-month-old free-living zebra bled in 1992, group- and type-specific antibodies against AHS virus could be demonstrated in 100 and 95%, respectively. As most of the elephants sampled in 1993 were at least 3, and some more than 10 years old, they must undoubtedly have been exposed to large numbers of AHSV-infected midges on numerous occasions. If they are genuinely susceptible to AHSV, a similar or even higher percentage with antibodies against AHS virus, could be expected if one considers their age. This, however, was not the case. Sixty-three percent reacted positively in the group-specific ELISA and only 26% reacted positively in 126 MN tests. In addition, antibody levels in their serum were significantly lower than those in zebra serum. This low number of serologically positive elephants, together with the insignificant antibody levels, is insufficient evidence, however, of the susceptibility of elephants to AHSV.

A previous attempt to determine the susceptibility of two elephants to AHS virus provided inconclusive results (Erasmus *et al.* 1978). In the present investigation, similar results were obtained with six elephant calves. No viraemia could be detected and their immune response was limited to an insignificant rise in antibody levels. This poor response is in direct contrast to the superior response obtained with zebra in a similar study (Barnard *et al.* 1994) and is a clear indication that elephants are poorly susceptible to AHSV.

No reason was provided for the previously reported low levels of neutralizing antibodies against AHSV in elephants (Mirchamsy & Hazrati 1973; Davies & Otieno 1977; Mushi 1990). Several reasons should be considered. Collection of samples too soon after infection may fail to detect specific antibodies, while serum collected too late may display only low levels of group-specific antibodies. This may be valid when only a few samples are examined. In the present investigation where samples from 80 continuously exposed elephants were tested, this seems doubtful.

Another explanation that should be borne in mind is the possible existence of a natural inability of elephants to produce high levels of antibodies against viruses. This, however, is regarded as extremely unlikely in the case of viruses pathogenic for the species. Encephalomyocarditis virus which recently caused several deaths among elephants in the KNP, stimulated the development of high levels of neutralizing antibodies in the same elephants tested for the presence of antibodies to AHS virus (B.J.H. Barnard 1984, Onderstepoort Veterinary Institute, unpublished data). On the other hand, elephants infected experimentally

TABLE 2 Neutralizing antibody titres against AHSV 1 in elephant calves after experimental infection in the Kruger National Park, South Africa

No. of calf	Weeks after infection					
	−4	0	1	3	5	7
1	−[1]	–	–	10	10	#
2	–	–	–	10	10	10
3	10	–	–	–	10	10
4	–	–	–	10	10	10
5	–	–	–	–	–	–
6	–	–	–	10	10	10

[1] = Negative
\# = Dead

with foot-and-mouth-disease virus to which they are not susceptible under natural conditions, developed a local reaction as well as viraemia. Their immune response, however, was poor and the decline in antibody concentration was rapid compared with that of other species (Howell, Young & Hedger 1972).

The inability to demonstrate precipitating antibodies in 80 serum samples may be ascribed to undetectable low levels or total absence in their serum of precipitating antibodies against AHS virus.

A likely explanation for the low levels of antibodies is a form of natural hyperimmunization caused by numerous episodes of exposure to *Culicoides* infected with AHSV. This hypothesis is supported by the higher levels of ELISA titres seen in elephants with neutralizing antibodies against multiple serotypes.

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