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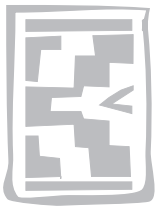

Natural infection rates and transmission of *Theileria annulata* by *Hyalomma anatolicum anatolicum* ticks in the Sudan

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ABSTRACT

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Hyalomma anatolicum anatolicum nymphs were collected from two localities in the Sudan: Eddamer in Northern Sudan and Wad-Medani in Central Sudan. They were allowed to moult to adult ticks, which were assessed for *Theileria* infection in their salivary glands using Feulgen stain. At Eddamer, 49.6 % of 123 ticks examined were infected with *Theileria* and the mean intensity of infection was 1.3 (i.e. the number of infected acini/number of infected ticks). At Wad-Medani, 8.6 % of 162 ticks were infected and the mean intensity of infection was 7.9. The prevalence of infection was higher in female than in male ticks at both localities. When adult *H. a. anatolicum* were applied onto two susceptible calves, both animals developed the severe form of theileriosis.

Keywords: *Hyalomma anatolicum*, infection rates, Sudan, *Theileria annulata*, transmission

INTRODUCTION

It has been shown that *Hyalomma anatolicum anatolicum* is the principal vector of *Theileria annulata*, the cause of tropical theileriosis in the Sudan, although other *Hyalomma* ticks are also known to be able to transmit the disease (Um El Hassan, Jongejan & Morzaria 1983). The natural infection rate in the salivary glands of *Hyalomma* ticks is an important parameter in the study of the epidemiology of tropical theileriosis in cattle (Sangwan, Chaudhri, Sangwan & Gupta 1994). Moreover, infection rates can be a useful indicator of transmission of *Theileria* and have been used as an index in combination with routine serological and parasitological surveys in cattle (Young 1981).

Different methods have been used to study infection rates of *Theileria* species in salivary glands of ticks (Walker, Susan, Meckellar, Bell & Brown 1979). In general, histological and histochemical methods for detecting the infection have the disadvantage that it is not possible to determine with certainty the parasite species infecting the tick (Melrose, Brown & Sharma 1980). This has been overcome by using PCR targeting a *T. annulata*-specific gene such as the one encoding the 30-kDa major merozoite surface antigen (Tams-1) (D'Oliveira, Van der Weide, Jacquiet & Jongejan 1997).

In the Sudan, Walker, Latif, Morzaria & Jongejan (1983) studied the natural infection rate for *Theileria* in ticks from Shambat (Khartoum State) and Nishishiba (Central Sudan). They found that 38 % and

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86 % of them were infected with *Theileria* with a mean of 37 and 19.5 parasite masses per tick in Shambat and Nishishiba, respectively. Similarly, Jongejan, Morzaria, Mustafa & Latif (1983) determined the infection rates of *Hyalomma marginatum rufipes* ticks fed on calves experimentally infected with *T. annulata* and showed that the infection rate can reach 100 %.

The purpose of this study was to assess the natural infection rates of *Hyalomma a. anatolicum* with *T. annulata* at two localities in the Sudan, and to attempt to transmit the infection experimentally to calves.

MATERIALS AND METHODS

Collection and maintenance of ticks

Fully engorged nymphs were collected at two localities, Eddamer (17°58´N, 34°96´E), Northern Sudan and Wad-Medani (14°4´N, 33°51´E), Central Sudan. Particular attention was paid to wall crevices, masses of cobwebs and dried cow-pats which were likely to provide sheltered moulting sites. The ticks were maintained in the laboratory in an incubator at 28 °C and 85 % relative humidity for moulting. After moulting, they were identified according to the descriptions provided by Hoogstraal (1956), Hoogstraal & Kaiser (1959) and Okello-Onen, Hassan & Essuman (1999). Only adult *H. a. anatolicum* were included in this study. The ticks were maintained in flat-bottomed plastic tubes, each of which was labelled with the date of collection and locality.

Feeding of ticks on rabbits and dissection

In order to allow the ticks to harden their cuticles after moulting, they were left in the incubator for a further 10 days. Thereafter, they were applied onto rabbits (crosses of New Zealand X Local) using ear bags according to the methods described by Bailey (1960). The feeding on rabbits was carried out in order to stimulate the maturation of *Theileria* sporozoites (Cunningham, Brown, Burridge & Purnell 1973). A total of 180 ticks from Eddamer and 240 from Wad-Medani were placed on six rabbits. They comprised an equal number of male and female ticks. The day of application of ticks was designated as Day 0 of feeding. On Day 3, the attached ticks were gently removed using a pair of forceps and immediately dissected.

Dissection was carried out under a stereoscopic microscope. Both salivary glands of each tick were gently freed from the posterior tracheas, placed on a clean microscope slide with a drop of normal saline. They were then fixed in absolute methanol for 10 min and air dried. The slides were labelled to indicate the locality of origin and sex of the tick.

Feulgen stain

The Feulgen stain used to stain the salivary gland smears was prepared as has been described previously (Anonymous 1983). The smears were first hydrolyzed for 1 h in 5N HCL and subsequently stained in the Feulgen stain for 1 h. Thereafter, they were gently washed with distilled water, heat dried at 60 °C overnight, and then mounted with Depex under a coverslip.

Examination of the slides

The slides were examined under the x40 objective lens of a standard microscope. The parasitized acinar cells were distinguished by their large hyperplastic nuclei, which stained intensely red. Acini in which there were infected cells containing densely stained sporozoites and sporoblasts that pushed the nuclei to one side were counted.

The infection rate was expressed as follows:

$$\text{Infection rate (\%)} = \frac{\text{Number of infected ticks}}{\text{Total number of ticks dissected}} \times 100$$

In addition, two other infection parameters were determined according to the method of Sangwan *et al*. (1994). These concerned the mean intensity of infection (number of infected acini / number of infected ticks) and the mean abundance of infection (number of infected acini / number of ticks examined).

Transmission of *T. annulata* by *H. a. anatolicum*

For this purpose, ticks collected as fully engorged nymphs from Eddamer and Wad-Medani and from the same batch that had been subjected to the infection rate assessments as set out above were used. After moulting to adults, transmission of *T. annulata* was carried out by applying 14 females and 17 males from Eddamer or Wad-Medani to both ears of two calves number 382 and number 387, respectively. Sera from the calves were tested before application of the tick for *T. annulata* antibodies using the indirect fluorescent antibody test (IFA)(Salih 2003). After the ticks had been applied both calves were monitored on a daily basis using clinical and parasitological parameters.

Data analysis

The data on the infection rates were subjected to chi-square in order to test the variation between males and females, and the localities.

RESULTS

Theileria infection rates in ticks

The number of *H. a. anatolicum* examined for *Theileria* parasites infections and the number of *Theileria* parasite masses found in their salivary glands at the two localities are shown in Table 1. A total of 123 and 162 *H. a. anatolicum* collected from Eddamer and Wad-Medani, respectively, were dissected to assess the infection rate in their salivary glands. Forty-two females (61.8 %) and 19 males (34.5 %) from Eddamer were infected, whereas from Wad-Medani 13 females (15.8 %) and 1 male (1.25 %) were positive. The prevalence of infection was higher in female than male ticks at both localities (Table 1). The mean number of infected acini was significantly higher in females than in males ($P < 0.05$). In addition, the intensity and abundance of infection were also significantly higher in females than in males ($P < 0.01$).

Transmission of *T. annulata* by *H. a. anatolicum*

Prior to tick application both calves were shown to be negative for *T. annulata* antibodies using the IFA test. *Theileria annulata* was successfully transmitted to both calves (Table 2). The period that elapsed for the onset of fever after transmission by ticks from Wad-Medani and Eddamer was 8 and 16 days, respectively. Calf 387 died of tropical theileriosis, whereas Calf 382 recovered from the infection without treatment. Macroschizonts and piroplasms appeared earlier in Calf 387 than in Calf 382 (Table 2).

DISCUSSION

Infection rates of *Theileria* in ticks are considered a useful indicator for the level of *Theileria* challenge in combination with data on *Theileria* infection rates in cattle (Young 1981). The infection rate in *H. a. anatolicum* (49.6 %) collected in Eddamer indicated that cattle are subjected to a high challenge with *T. annulata* sporozoites and that ticks feeding as nymphs have a high chance of becoming infected with the parasite. In comparison, at Wad-Medani a

TABLE 1 Infection rate, intensity and abundance of infection of *Theileria* in salivary glands of male and female *H. a. anatolicum* ticks collected as nymphs from Eddamer and Wad-Medani

Localities	Ticks	No. examined	No. positive (%)	Mean no. of infected acini	Mean abundance of infection	Mean intensity of infection
Eddamer	Females	68	42 (61.8)	81.0	1.2	1.9
	Males	55	19 (34.5)	12.0	0.2	0.6
	Both sexes	123	61 (49.6)	46.5	0.7	1.3
Wad-Medani	Females	82	13 (15.8)	54.0	2.0	13.7
	Males	80	1 (1.25)	2.0	0.7	2.0
	Both sexes	162	14 (8.6)	28.0	1.4	7.9

TABLE 2 Transmission of *Theileria annulata* to experimental calves by *H. a. anatolicum* adults collected as nymphs from the field

Animal no.	Locality	Challenge by adult ticks	Days to fever	Duration of fever (days)	Prepatent periods			
					Rectal temp. (°C)	Schizont (days)	Piroplasm (days)	Days to death
382	Eddamer	14 females 17 males	16	3	40.8	18	19	Recovered
387	Wad-Medani	14 females 17 males	8	11	40.4	11	16	23

much lower infection rate (8.6 %) was observed, which may indicate that cattle are subjected to a lower challenge with *T. annulata* in this area and that ticks feeding as nymphs have a lower chance of becoming infected. The fact that lower numbers of ticks were infected at Wad-Medani, but with higher numbers of parasites per tick may reflect an endemically unstable situation compared with Eddamer where most ticks were infected but with lower numbers of parasites per tick.

In a previous study in the Sudan, *H. a. anatolicum* was shown to have high natural infection rates with *Theileria*, ranging between 38 % and 86 % (Walker *et al*. 1983). Infection rates of *H. a. anatolicum* with *T. annulata* were subsequently estimated, using the same method as used in this study, to be 80 % in the ticks collected from the field (Kuku area, Khartoum) (El-Imam 1999) and 96 % in ticks fed experimentally on infected calves (Bakheit 1998). The high infection rate in ticks collected from Eddamer may be influenced by the animal husbandry system used. This consist of small enclosures in which susceptible crossbred (Zebu x Friesian) cattle are maintained, and which creates an ideal microhabitat for *H. a. anatolicum* ticks. Hence, opportunities for animals to become infected and develop immunity to *T. annulata* at a younger age are greater than at Wad-Medani where semi-intensive cattle raising is practiced. It is interesting, however, to note that earlier investigations by Walker *et al*. (1983) indicated very high (86 %) infection rates in Nishishiba (a village nearby Wad-Medani). At the time of that investigation, however, the drought which prevailed at the time of the current investigations had not yet started and there was no influx of animals carrying ticks from *H. a. anatolicum* infested areas for grazing in the El Gezira Agricultural Scheme, which is situated in the Wad-Medani area. This might have resulted in the interbreeding of different tick populations and the development of more heterogenous and resistant offspring, in contrast to a more a stable population in the Eddamer area. This hypothesis may be supported by the fact that *H. a. anatolicum* has now established in the Blue Nile Province (south of Wad-Medani), an area where, in the early 1980s, this tick was not present (Salih, Hassan, El Hussein & Jongejan 2004).

The finding that the intensity of infection was higher in female than in male ticks is in agreement with the fact that the total number of type III acini, which are arranged distally to the main salivary gland ducts, is higher in females than in males (Ochanda, Young, Welles, Medley & Perry 1996). Male ticks detach irregularly to re-attach next to feeding females in order to mate, which may contribute to a lower number of sporozoites in the male ticks. Female ticks may thus play a more important role in transmission than male ticks (Ochanda *et al*. 1996). Moreover, the development of *Theileria* parasites in vector ticks depends on several extrinsic and intrinsic factors which remain to be elucidated as stated by Sangwan *et al.* (1989, cited by Sayin, Karaer, Dincer, Cakmak, Inca, Yukari, Eren, Vatansever, Nalbantoglu & Melrose 2003).

It is known that *H. a. anatolicum* can also transmit other *Theileria* spp. such as *Theileria lestoquardi* (Hooshmand-Rad & Hawa 1973) and *Theileria equi* (Schein, Rehbein, Voigt & Zweygarth 1981). The results of the transmission experiment show that *H. a. anatolicum* collected from the field are infected with *T. annulata* and caused tropical theileriosis in susceptible calves, confirming earlier observations made by Um El Hassan *et al.* (1983). Since histological staining methods are insufficient to differentiate between *Theileria* species, in our ongoing studies larger number of ticks from a wider survey area are being examined by the PCR-based methods of D'Oliveira *et al.* (1997).

In conclusion, this study confirms the importance of *H. a. anatolicum* ticks as vectors of *T. annulata* and the need to protect susceptible animals against tropical theileriosis possibly through vaccination with attenuated cell culture vaccines such as the one currently under development in the Sudan.

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