Hyalomma anatolicum anatolicum successfully survives in diverse habitats extending from central parts of the Sudan to North Africa, southern Europe, the Middle East, Russia, China and India. The tick develops behavioural or morphogenetic diapause in cold climates (Belozerov 1982) but can multiply throughout the year in hot climates (Latif 1985). Its feeding behaviour depends on the type of host. Thus, it undergoes a typical three-host feeding cycle on large domestic animals while on small hosts (unusual hosts) two to three-host types occur (Latif 1985). Hyalomma a. anatolicum maintains a range of economically important infections transmissible to domestic animals and humans. Transstadially it transmits Theileria annulata (tropical theileriosis in cattle), Theileria lestoquardi (malignant sheep theileriosis) (Hooshmand-Rad & Hawa 1973; Latif 1994), Theileria equi (equine babesiosis) and the Crimean Congo haemorrhagic fever virus to humans (Hoogstraal 1979).

Babesia caballi, the cause of equine babesiosis is transmitted transovarially (Donelly, Joyner & Frank 1980; Abdoon, Osman & Elwasila 1992). Furthermore, H. a. anatolicum adult
ticks collected from the field were found to harbour various developing forms of *Trypanosoma theileri*-like parasites (Table 1). O’Farrell (1913a) in the Sudan was the first to observe *T. theileri*-like parasites in the haemolymph and tissues of the tick *H. a. anatolicum* [misidentified as *Hyalomma aegyptium* (Hoogstraal & Kaiser 1960)]. Morzaria, Latif, Jongejan & Walker (1986) in the Sudan demonstrated the first biological transmission of *T. theileri* to cattle by the tick *H. a. anatolicum*. Although various developing stages of *T. theileri*-like parasites were found in the adult *H. a. anatolicum* (Table 1), none of these studies has confirmed experimentally the susceptibility of the tick to the trypanosome. Therefore, the objective of the present study was to demonstrate the susceptibility of *H. a. anatolicum* to *T. theileri* and to assess its vectorial capacity.

**MATERIALS AND METHOD**

*Trypanosoma theileri* parasite isolation

A total of 127 crossbred cattle on two dairy farms in Soba Agricultural Scheme, Khartoum, Sudan were screened for the presence of trypanosome parasites. Blood was obtained from the jugular vein of cattle using EDTA-treated vacutainers and was processed immediately for buffy coat examination using the haematochrit centrifugation technique (HCT). The buffy coat was examined under light microscope for the presence of motile trypanosomes. Thin blood smears were also prepared and stained with Giemsa stain.

A Friesian calf, obtained from a dairy farm near Khartoum on which regular chemical tick control was practised, was used. Blood smears from this animal were free of blood parasites. It was inoculated with 20 ml pooled blood obtained from four cows which were found infected with trypanosome parasites on one of the two farms. The blood was given by intravenous (10 ml) and by the subcutaneous injections (10 ml). The calf was examined for the presence of trypanosomes daily starting a week before receiving the blood inoculation and thereafter for a period of 4 weeks. Parasite examination was carried as mentioned above. The packed cell volume (PCV) was also recorded.

**RESULTS**

Using HCT, the incidence of *Trypanosoma* infection in cattle on the two farms was found to be 4.7% (six out of 127). The parasitaemia was very low (one parasite per 70 µl blood) and no parasites were seen in the stained blood smears.

The calf which received the infected blood developed *T. theileri* and *Trypanosoma vivax*-mixed infections 6 days after inoculation. However, only two *T. vivax* parasites were seen on one occasion. The peak levels of parasitaemia (42 parasites per 70 µl blood) were recorded on days 12 and 13 and was due to *T. theileri* infection. *Trypanosoma theileri* parasites in the blood smears measured 100–120 µm (Fig. 1) and the morphology was suggestive kept in the laboratory until moulting. The *Trypanosoma* infection rate in 60 adult ticks was assessed by severing one leg of each tick and smearing the clear haemolymph which exuded from the wound on a microscope slide. The smears were air-dried, fixed in methanol and stained in Giemsa for microscopic examination.

*In vitro* isolation of trypanosomes from infected ticks

A total of 12 adult ticks dropped as nymphs from the calf were washed with 70% alcohol, two to three legs of each tick were severed and the haemolymph was collected in tissue culture growth medium containing Minimum Essential Medium (MEM), 10% fetal calf serum (FCS), heat-inactivated L-15 medium and 10% heat-inactivated FCS. The cultures were incubated at 27°C and examined daily.
### TABLE 1 Reports of *Trypanosoma*-like flagellates found in the tick *Hyalomma* species

<table>
<thead>
<tr>
<th>Author/country</th>
<th>Flagellate species</th>
<th>Tick species</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>O’Farrel (1913b), Sudan</td>
<td><em>Crithidia hyalommae</em></td>
<td><em>Hyalomma aegyptium</em></td>
<td>Transovarian transmission suggested</td>
</tr>
<tr>
<td>Carpano (1932), Russia</td>
<td><em>Trypanosoma theileri</em></td>
<td><em>H. a. anatolicum</em></td>
<td>Suggested ticks to be vectors</td>
</tr>
<tr>
<td>Muratov &amp; Cheissin (1959), Russia</td>
<td><em>C. hyalommae</em> (O’Farrel,1913)</td>
<td><em>H. detritum, H. a. anatolicum</em></td>
<td>Found the flagellates in the ticks</td>
</tr>
<tr>
<td>Arifdzhanov &amp; Nikitina (1961), Russia</td>
<td><em>C. hyalommae</em> (O’Farrel,1913)</td>
<td><em>H. a. anatolicum</em></td>
<td>Transovarian transmission suggested</td>
</tr>
<tr>
<td>Kirmse &amp; Taylor-Lewis (1976), not stated</td>
<td><em>T. lewisi, T. evansi, T. vivax</em></td>
<td><em>H. a. excavatum</em></td>
<td>Very limited survival period</td>
</tr>
<tr>
<td>Shastri &amp; Deshpande (1981), India</td>
<td><em>T. theileri</em></td>
<td><em>H. a. anatolicum</em></td>
<td>Successful transmission by tick homogenates</td>
</tr>
<tr>
<td>Shastri &amp; Krishnamurthy (1981), India</td>
<td><em>Blastocrithidia hyalommae</em></td>
<td><em>H. a. anatolicum</em></td>
<td>Epimastigotes found in eight nymphs and adults</td>
</tr>
<tr>
<td>Morzaria et al. (1986), Sudan</td>
<td><em>T. theileri</em>-like flagellate</td>
<td><em>H. a. anatolicum</em></td>
<td>Successful transstidal transmission in calf</td>
</tr>
</tbody>
</table>
Infection rates of *Hyalomma anatolicum anatolicum* with *Trypanosoma theileri*

FIG. 2 *Trypanosoma theileri* parasitaemia and PCV in the infected calf

FIG. 3 Haemolymph smear from an uninfected *H. a. anatolicum* adult tick. The arrow indicates a haemocyte

FIG. 4 Haemolymph smear showing a high infestation with different developmental stages of *T. theileri*

FIG. 5 Amastigote (a), epimastigote (b), trypomastigote (c) stages in haemolymph of an infected tick
of the species (Soulsby 1982). The parasites were not seen after day 17. The calf was clinically sick and the PCV dropped from 25 to 17% (Fig. 2).

Most of the *H. a. anatolicum* nymphs fed and the time when they dropped engorged from the calf coincided with the *Trypanosoma*-parasitaemia high peaks. The infection rate with *Trypanosoma* parasites in the adult tick haemolymph was high; 26 out of 60 (43%) ticks showed different developmental stages of *T. theileri*-like parasites. The trypanosome parasitaemia in the haemolymph was very high and no degenerative forms were recognized (Fig. 3 and 4). Several different morphological forms were seen which were suggestive of the trypomastigote, epimastigote, promastigote and amastigote stages of the parasite (Fig. 5). The promastigote/epimastigote forms were predominant (Fig. 6). Two types of cell multiplication were observed, i.e. binary fission in the epimastigotes and multiple fission in the amastigotes (Fig. 7).

The haemolymph collected in the growth medium showed a number of active *Trypanosoma* parasites. The organisms were maintained for about 100 days and had a slow rate of growth but all eventually died.

**DISCUSSION**

Our results present the first demonstration that the feeding of *H. a. anatolicum* nymphs from an uninfected colony on a calf with a high parasitaemia of *T. theileri* produced highly infected adult ticks (43% infection rate). Various developmental stages were seen and their survival was confirmed by tissue culture isolation. The study also supports the reports that various developmental stages and forms of *T. theileri* can be found in the tick *H. a. anatolicum* collected in the field (Table 1). Thus, *H. a. anatolicum* can be regarded as a vector of *T. theileri*. O’Farrell (1913a, b) in the Sudan was the first to discover flagellates, which he named *Crithidia hyalommae*, in the tick *Hyalomma aegyptium* collected from cattle. Wenyon (1926) suggested the name *T. theileri* for these flagellates. Most probably O’Farrell misidentified this tick species since *H. aegyptium* adults parasitize tortoises and the species does not occur in the Sudan (Hoogstraal 1956; Hoogstraal & Kaiser 1960). The most common cattle tick in Khartoum area is *H. a. anatolicum* (Latif 1984) but the identity of O’Farrell’s tick can only be speculated on from its host-cattle association. Seventy-three years after the reports of O’Farrell (1913a, b), Morzaria et al. (1986) were able to transmit *T. theileri*-like parasites to cattle using field collected *H. a. anatolicum* ticks. The present results demonstrate unequivocally the high vectorial capacity of the tick *H. a. anatolicum* for *T. theileri*. Interestingly all of these stud-
ies were carried out using the ticks and the parasites derived from the same area (Khartoum). The success of the tick infectivity attempt in this study can be related to the high level of *T. theileri* parasitaemia in the experimental calf. Although the prevalence of *T. theileri* in cattle is high (Farrar & Klei 1990), cattle in the field have been shown to harbour low levels of *T. theileri* parasitaemia (Elamin 1997) resulting in low infectivity of the ticks. Morzaria et al. (1986) were unable to demonstrate by xenodiagnosis the infectivity of *T. theileri* to ticks since the parasites could not be detected in the blood stream of the infected animal. The parasites isolated from the unfed ticks haemolymph showed slow growth in the tissue culture medium, although they survived for over 25 weeks, as compared to a vigorous growth shown in the earlier study of Morzaria (1986) and to the growth of the parasites derived from an animal’s blood. However, these workers preferred the ticks on rabbits for 4 days before parasite cultivation and it is probable that the parasites needed a blood factor to stimulate their further development in the growth media. The development of the trypanosomes in the various tissues of tick needs to be studied and the possibility of transovarian mode of transmission (O’Farrel 1913b) to be investigated.

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