



High infection rates of the tick *Hyalomma anatolicum anatolicum* with *Trypanosoma theileri*

A.A. LATIF¹, M.A. BAKHEIT², AMNA E. MOHAMED² and E. ZWEYGARTH³

ABSTRACT

LATIF, A.A., BAKHEIT, M.A., MOHAMED, AMNA E. & ZWEYGARTH, E. 2004. High infection rates of the tick *Hyalomma anatolicum anatolicum* with *Trypanosoma theileri*. *Onderstepoort Journal of Veterinary Research*, 71:251–256

A crossbred calf (3 months old) obtained from a farm where regular control of ticks was practised and found to be free of blood parasites was inoculated with 20 ml pooled blood collected from four field cattle which had very low *Trypanosoma theileri* parasitaemias (one parasite per 70 µl blood as determined by the haematocrit centrifugation technique). *Trypanosoma theileri* was present in the blood 6 days after injection and a peak parasitaemia of 42 parasites per 70 µl blood was recorded by day 12. *Hyalomma anatolicum anatolicum* nymphs were applied on the ears of the calf on day 8 and they dropped engorged by days 13 and 14. The resulting adult ticks were examined for the presence of *T. theileri* by severing a leg and making a smear of the clear haemolymph which exuded from the wound. The smear was fixed in methanol and stained with Giemsa stain. The infection rate with *T. theileri* in the ticks was 43.3% (26 out of 60). The intensity of infection was very high and various developmental stages of the flagellates were observed (epimastigotes, sphaeromastigotes, trypomastigotes and other intermediate stages). The haemolymph from 12 ticks was also collected in tissue culture medium and the trypanosomes survived for 25 weeks before eventually dying. The results demonstrated unequivocally the high vectorial capacity of the tick *H. a. anatolicum* for *T. theileri*.

Keywords: *Hyalomma anatolicum anatolicum*, infection rate, *Trypanosoma theileri*

INTRODUCTION

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 (Belozеров 1982) but can multiply through-

out 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 (Donnelly, Joyner & Frank 1980; Abdoon, Osman & Elwasila 1992). Furthermore, *H. a. anatolicum* adult

¹ Faculty of Science, Midrand Graduate Institute, P.O. Box 2986, Halfway House, 1685 South Africa

² Faculty of Veterinary Science, University of Khartoum, P.O. Box 13314, Khartoum North, Sudan

³ Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa

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 haematocrit 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.

Tick infectivity with *Trypanosoma theileri*

Laboratory-reared *H. a. anatolicum* nymphs were allowed to feed on the calf when the *Trypanosoma* parasites were detected in the buffy coat and blood smears. The engorged nymphs were collected and

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.

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



FIG. 1 Blood stream form of *Trypanosoma theileri* from the infected calf

TABLE 1 Reports of *Trypanosoma*-like flagellates found in the tick *Hyalomma* species

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

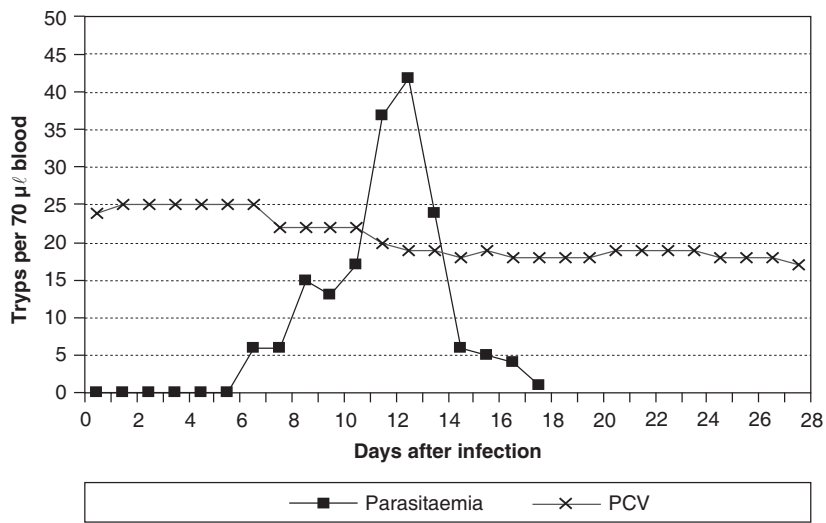


FIG. 2 *Trypanosoma theileri* parasitaemia and PCV in the infected calf

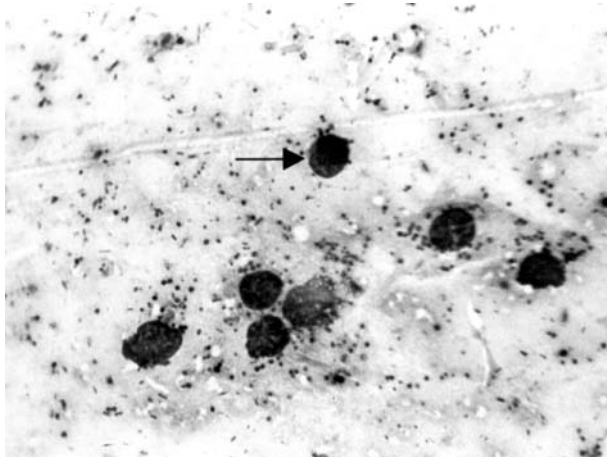


FIG. 3 Haemolymph smear from an uninfected *H. a. anatolicum* adult tick. The arrow indicates a haemocyte

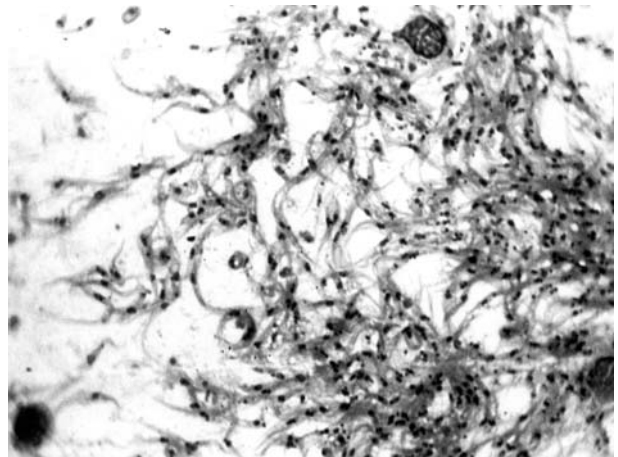
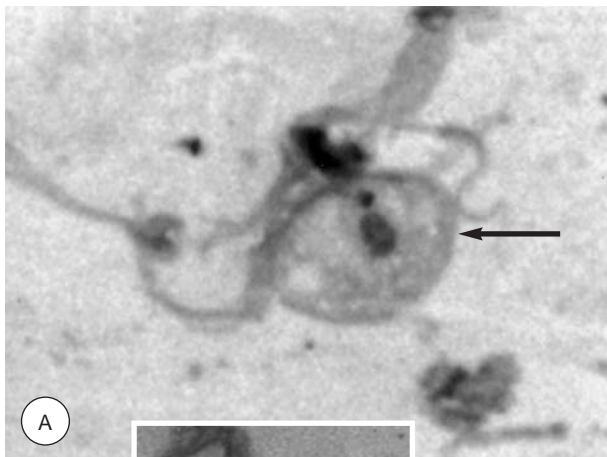
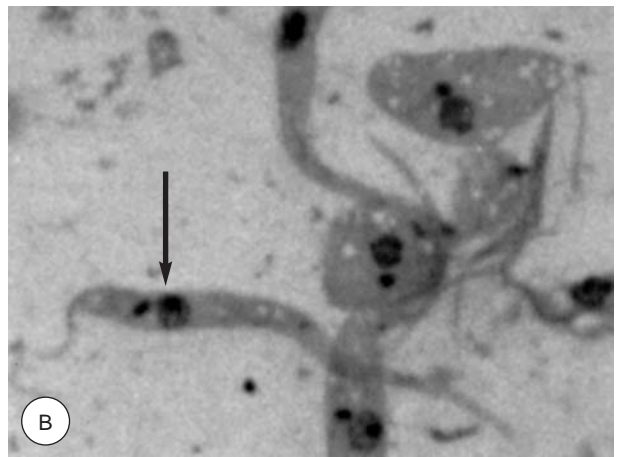


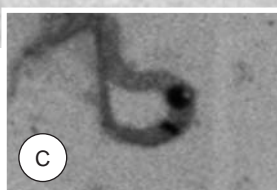
FIG. 4 Haemolymph smear showing a high infestation with different developmental stages of *T. theileri*



A



B



C

FIG. 5 Amastigote (a), epimastigote (b), trypomastigote (c) stages in haemolymph of an infected tick

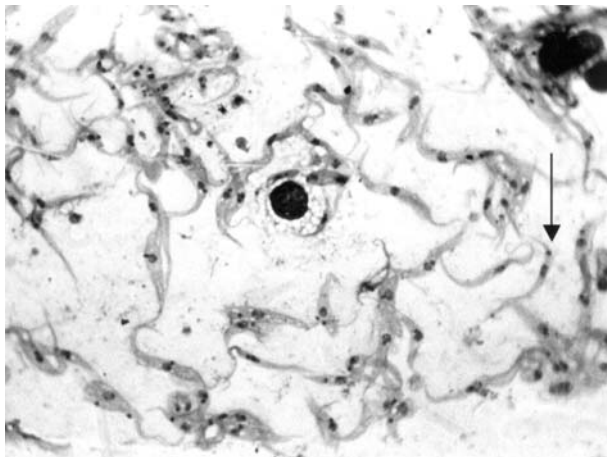


FIG. 6 Epimastigote (arrow), the most common form of *T. theileri* in the haemolymph

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

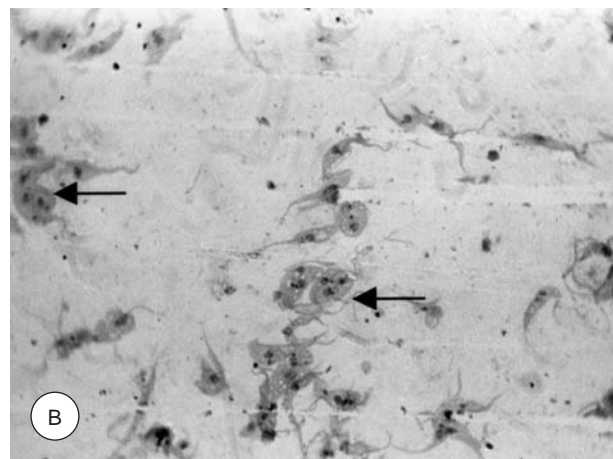
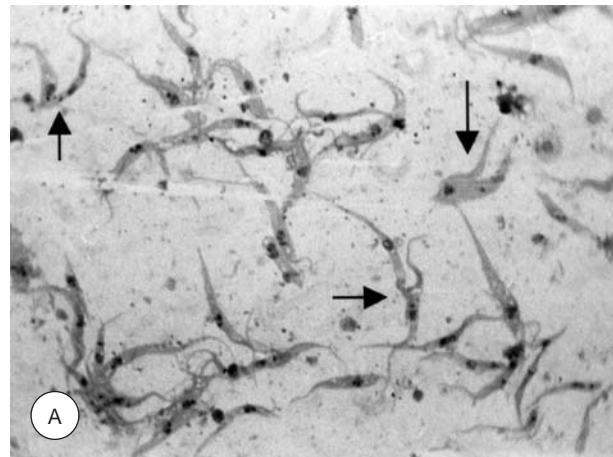


FIG. 7 Different dividing stages of trypanosomes in the haemolymph (arrows)

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 *et al.* (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 probably 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.

REFERENCES

- ABDOON, A.M.O., OSMAN, O.M. & ELWASILA, M. 1992. The epidemiology of equine piroplasmiasis in Khartoum District, Sudan. 1-Tick prevalence and their parasites. *Bulletin of Animal Health and Production in Africa*, 40:1–14.
- ARIFDZHANOV, K.A. & NIKITINA, R.E. 1961. Detection of *Crithidia hyalomma* (O'Farrel 1913) in *Hyalomma a. anatolicum* (Koch, 1844) (in Russian). *Zoologi Cheskie*, 40:20–24.
- BELOZEROV, V.N. 1982. Diapause and biological rhythms in ticks, in *Physiology of ticks*, edited by F.D. Obenchain & R. Galun. Oxford: Pergamon Press.
- CARPANO, M. 1932. Localisation du *Trypanosoma theileri* dans les organes internes des bovins, son cycle évolutif. *Annales de Parasitologie Humaine et Comparée*, 10:305–322.
- DONNELLY, J., JOYNER, L.P. & FRANK, C. 1980. Quantitative epidemiological studies on the prevalence of babesiosis in horses in Kuwait. *Tropical Animal Health and Production*, 12: 253–258.
- ELAMIN, A.E.M. 1997. Prevalence of bovine trypanosomiasis in Khartoum State. Master of Tropical Animal Health dissertation, University of Khartoum.
- FARRAR, R.G. & KLEI, T.R. 1990. Prevalence of *Trypanosoma theileri* in Louisiana cattle. *Journal of Parasitology*, 76:734–736.
- HOOGSTRAAL, H. 1956. African Ixodoidea. I. Ticks of the Sudan (with special reference to Equatoria Province and with preliminary reviews of the genera *Boophilus*, *Margaropus* and *Hyalomma*) Department of Navy, Washington, DC, USA.
- HOOGSTRAAL, H. 1979. The epidemiology of tick-borne Crimean-Congo haemorrhagic fever in Asia, Europe and Africa. *Journal of Medical Entomology*, 15:307–417.
- HOOGSTRAAL, H. & KAISER, M. 1960. Some host relationships of the tortoise tick, *Hyalomma (Hyalommastax) aegyptium* (L.) (Ixodoidea, Ixodidae) in Turkey. *Annals of the Entomological Society of America*, 53:457–458.
- HOOSHMAND-RAD, P. & HAWA, N.J. 1973. Transmission of *Theileria hirci* in sheep by *Hyalomma anatolicum anatolicum*. *Tropical Animal Health and Production*, 5:103–109.
- KIRMSE, P. & TAYLOR-LEWIS, G. 1976. Ticks as possible vectors of trypanosomes, in *Tick-borne diseases and their vectors*, edited by J.K.H. Wilde. University of Edinburgh Publication.
- LATIF, A.A. 1984. Resistance to natural tick infestations in different breeds of cattle in Sudan. *Insect Science and its Application*, 5:95–97.
- LATIF, A.A. 1985. Tick (Acari: Ixodoidea: Ixodidae) immatures feeding on cattle, camels and sheep in the Sudan, with notes on behaviour of *Hyalomma anatolicum anatolicum* larvae fed on different hosts. *Insect Science and its Application*, 6:59–61.
- LATIF, A.A. 1994. Theileriosis of sheep in the Sudan: a case report. *Proceedings of a workshop on Tropical Theileriosis held at the veterinary Association Residence, Khartoum, 4–5 May 1994*, edited by A.M. Ata-Elmanan & S.M. Kheir.
- MORZARIA, S.P., LATIF, A.A., JONGEJAN, J. & WALKER, A.R. 1986. Transmission of *Trypanosoma* sp. to cattle by the tick *Hyalomma anatolicum anatolicum*. *Veterinary Parasitology*, 19:13–21.
- MURATOV, E.A. & CHEISSIN, E.M. 1959. The discovery of *Crithidia hyalommae* O'Farrel in the ticks *Hyalomma detritum* and *Hyalomma anatolicum* in Tadjikskoi SSR (in Russian). *Doklady Akademii Tadjikskoi SSR*, 2:33–37.
- O' FARREL, W.R. 1913a. Preliminary note on a new flagellate, *Crithidia hyalommae* sp. nov., found in the tick *Hyalomma aegyptium*, Linnaeus, 1758. *Journal of Tropical Medicine and Hygiene*, 16:245–246.
- O' FARREL, W.R. 1913b. Hereditary infection with special reference to its occurrence in *Hyalomma aegyptium* infected with *Crithidia hyalommae*. *Annals of Tropical Medicine and Parasitology*, 7:545–562.
- SHASTRI, U.V. & DESHPANDE, P.D. 1981. *Hyalomma anatolicum anatolicum* (Koch, 1844) as possible vectors for transmission of *Trypanosoma theileri*, Lavern, 1902 in cattle. *Veterinary Parasitology*, 9:151–155.
- SHASTRI, U.V. & KRISHNAMURTHY, R. 1981. Occurrence of epimastogotes in the tick *Hyalomma anatolicum anatolicum*, in India. *Indian Journal of Parasitology*, 5:57.
- SOULSBY, E.J.L. 1982. *Helminth, arthropods and protozoa of domesticated animals*, 7th ed. London: Bailliere Tindall.
- WENYON, C.M. 1926. *Protozoology: A manual for medical men, veterinarians and zoologists*. London: Bailliere, Tindall & Cox.