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ISOLATION AND TRANSMISSION OF AN UNIDENTIFIED *BABESIA* SP. INFECTIVE FOR CATTLE

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ABSTRACT

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Engorged adult female ticks submitted from farms in South Africa were routinely screened for protozoan parasites by examination of haemolymph smears. An unidentified *Babesia* sp. was found in *Hyalomma marginatum rufipes* and its transmission to susceptible cattle was achieved both biologically (tick feeding) and mechanically (injection of infected blood). Attempts to transmit this species to susceptible rabbits and a horse using similar methods did not produce evidence of infection.

This *Babesia* sp. was of low pathogenicity, even in splenectomized cattle. Morphologically, intra-erythrocytic piroplasms and merozoites in tick haemolymph resembled other bovine *Babesia* spp. in many respects. Although it could be classified as a large *Babesia*, it was intermediate in size between the other species.

Résumé

ISOLEMENT ET TRANSMISSION D'UN BABESIA *SP. NON-IDENTIFIÉ ET INFECTIEUX POUR LE BOVIN*

Les tiques femelles adultes engorgées provenant de fermes en Afrique du Sud ont été examinées routinièrement pour les parasites protozoaires par examen de frottis de haemolymphe. Un Babesia *sp. non-identifié a été trouvé dans l'*Hyalomma marginatum rufipes *et sa transmission aux bovidés susceptible a été accomplie tant biologiquement (par tiques) que mécaniquement (injection de sang infecté). Des tentatives de transmission de cette espèce au lapin ainsi qu'à un cheval en utilisant des méthodes similaires ne produisirent pas d'évidence d'infection.*

Ce Babesia *sp. fut peu pathogène, même chez le bovin splenectomisé. Morphologiquement, les piroplasmes et mérozoites intra-erythrocytaires dans l'haemolymphe de la tique, ressemblent à d'autres* Babesia *sp. bovins à beaucoup d'égards. Bien qu'il put être classifié comme un grand* Babesia *il était intermédiaire en taille entre les autres espèces.*

INTRODUCTION

Two *Babesia* spp., namely, *Babesia bigemina* and *Babesia bovis*, are known to infect cattle in South Africa and both are economically important (De Vos, 1979). Only *Boophilus* spp. are currently accepted as proven vectors of these parasites (Potgieter & Els, 1976; Potgieter & Els, 1977; Potgieter, 1977).

Ticks, collected off cattle and submitted to this laboratory for testing for resistance to acaricides, were at the same time examined for the presence of protozoan parasites.

During the course of the study large merozoites of a *Babesia* sp. were found in the haemolymph of an engorged adult female, *Hyalomma marginatum rufipes* (Koch, 1844), a two-host tick species (Knight, Norval & Rechav, 1978), collected on a farm near Ellisras in the Northern Transvaal. There is serological evidence that this species can act as a vector of *Rickettsia conori* (Philip, Hoogstraal, Reiss-Gutfreund & Clifford, 1966), but, to our knowledge, it has not so far been incriminated as a vector of any *Babesia* spp. A related tick, *Hyalomma marginatum marginatum,* has, however, been reported to be a vector in domestic animals of several *Babesia* spp., including *Babesia equi, Babesia caballi* and *Babesia canis* (Morel, 1980).

A series of transmission experiments, which form the basis of this report, were undertaken to establish the identity of the *Babesia* sp. found in *H. m. rufipes.*

MATERIALS AND METHODS

Experimental animals

All the cattle used in this experiment were reared at Onderstepoort, had been maintained under tick-free conditions, were splenectomized, and were free of exposure to any *Babesia* infection. The horse had not been splenectomized, but had been maintained under tick-free conditions and, as far as could be ascertained, it had not been exposed at any time to *Babesia* infections. The rabbits were born and raised under laboratory conditions and were also tick-free.

Tick haemolymph smears

Engorged female ticks collected from various farms were identified and kept in an acaridarium at 26 °C and 80% RH. Haemolymph smears were prepared from each tick 10–14 days after collection by amputating the distal portion of one or more legs. The smears were prepared as described by Burgdorfer (1970), fixed in methanol and then stained with 10% Giemsa's stain for 30 min. Conventional light microscopy was used for examining the smears for protozoan parasites.

Measurement of merozoites and piroplasms

Parasites were measured by taking photographs at a fixed magnification of piroplasms, merozoites and an object micrometer*, enlarging these photographs 5 times and then making comparative measurements, using the photograph of the micrometer as a scale. Only paired forms of the intra-erythrocytic piroplasms were measured as described by Uilenberg, Rombach, Perié & Zwart (1980). Fifty parasites were measured in each case.

Transmission experiments

Tick feeding and subinoculation programmes were undertaken in an attempt positively to identify the protozoan parasite seen in the haemolymph of *H. m. rufipes.* Female ticks with positive haemolymph smears were allowed to oviposit and the ensuing stages were reared according to the methods of Neitz, Boughton & Walters (1971).

Experiment 1 was designed to determine the ability of the immature progeny of the original infected *H. m. rufipes* females to transmit the *Babesia* sp. to various hosts. Immatures were fed on rabbits, a horse (96) and 2 splenectomized oxen (419 and 8544).

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Experiment 2 was performed to determine the ability of the ensuing adults to transmit the *Babesia* sp. to an ox. Approximately 100 adults originating from immatures which had fed on a rabbit in Experiment 1 were fed on a splenectomized ox (6996).

Experiment 3 was conducted to determine if the *Babesia* sp. could be transmitted artificially by sub-inoculating blood from one bovine to another. To this end 500 mℓ of blood was collected in ACD (citric acid, sodium citrate, dextrose) anticoagulant from an infected ox (419) and injected intravenously into a splenectomized cow (8625).

Experiment 4. In a further observation on host specificity, 50 mℓ of blood collected in ACD from Cow 8625 was injected intravenously into the horse used in Experiment 1.

Examination of smears

Thick and thin blood smears were taken daily from all the animals. The thick smears were prepared and stained as described by Mahoney & Saal (1961). Thin smears were fixed and stained as described above. All smears were examined for intra-erythrocytic parasites. When parasites were present, the parasitaemia was quantitated as outlined by De Vos (1978).

Results and Discussion

Babesia infections in field ticks

Merozoites of *Babesia* spp. were found in the following tick species:

(a) *Boophilus decoloratus:* Twenty-three of the batches were infected with merozoites morphologically similar to those described for *B. bigemina* by Riek (1964) and by Potgieter & Els (1977). The exact number of ticks that were infected in each batch was not determined and the incidence of observed infections could not therefore be calculated.

(b) *Boophilus microplus:* One of the batches of ticks examined was infected with merozoites. The merozoites corresponded morphologically with those described for *B. bovis* by Riek (1966) and Potgieter (1977).

(c) *H. m. rufipes:* One of the batches of ticks examined was infected with merozoites of an unidentified *Babesia* sp.

Transmission of Babesia *sp. isolated from* H. m. rufipes

Experiment 1: After immature ticks were allowed to feed, engorged nymphae were collected from the rabbits and oxen. None were collected from the horse. The horse and rabbits remained clinically normal and no parasites were found in their thick and thin blood smears.

Ox 419, however, became infected with *Babesia* sp. and piroplasms were present in detectable numbers in thin blood smears from Day 16 post-infestation. The maximum parasitaemia observed (0,2%) was accompanied by a febrile reaction lasting 3 days (maximum temperature 41,8 °C). Parasites were not seen in thin blood smears after Day 20. The second ox (8544) also became infected and the first piroplasms were seen in thin blood smears on Day 42 after infestation. There was no febrile reaction and the animal remained clinically normal. The parasitaemia was very low (< 0,005%), but remained for several weeks at a detectable level in thick blood smears.

Experiment 2: Approximately 100 adult *H. m. rufipes* were fed on Ox 6996, and 38 engorged females were collected. Sixteen days after infestation, the ox became febrile (41,4 °C) with a low-grade parasitaemia (maximum 0,2%). Pyrexia continued for 6 days, whilst the packed cell volume (PCV) dropped from 40 to 24%.

Experiment 3: Cow 8625 became febrile (40,8 °C) 6 days after injection of 500 mℓ of blood from an infected animal. The fever persisted for 4 days with a maximum parasitaemia of 1%. On Day 12 after infection the PCV had dropped to 16%. Parasites were seen intermittently in thick blood smears for a further 46 days, after which observations were stopped.

Experiment 4: As in the case of Experiment 1, no parasites were seen in the blood smears of Horse 96.

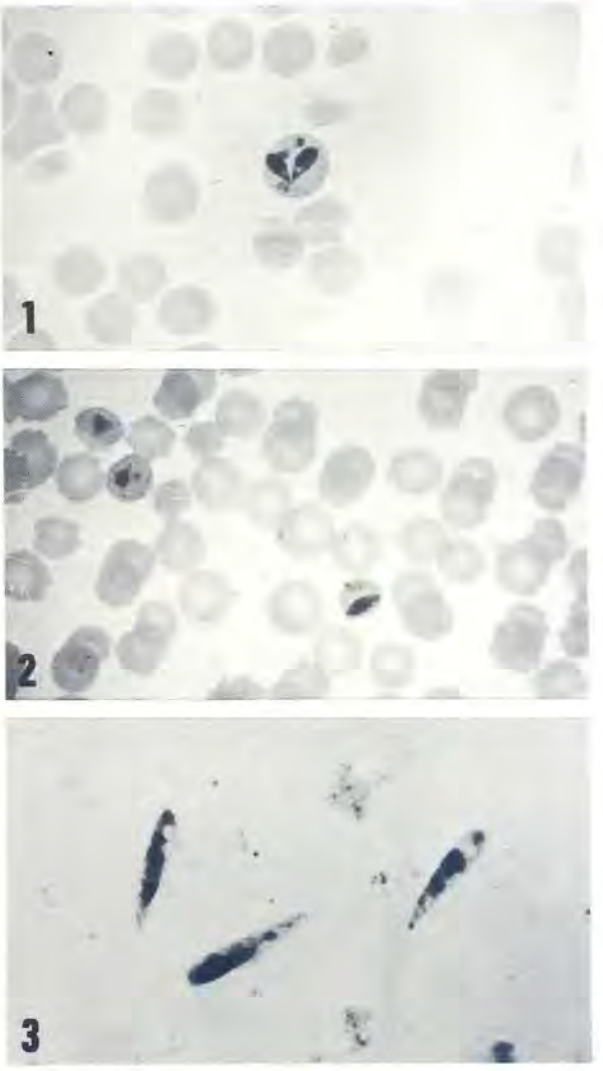

FIG. 1 Paired piroplasms of *Babesia* sp. in bovine erythrocyte: ×1250
FIG. 2 Single intra-erythrocytic parasites: ×1250
FIG. 3 Merozoites of *Babesia* sp. in smear preparation of haemolymph of *H. m. rufipes*: ×1250

Morphology of Babesia *transmitted by* H. m. rufipes

Paired piroplasms in the erythrocytes ranged from pyriform (Fig. 1) to oval in shape. A total of 100 were 2,4–3,7×1,3–2,1 μm (mean 2,70×1,59 μm) in size, and this parasite therefore falls into the category of the "large" babesias (Mahoney, 1977).

Single parasites were predominantly round to oval in shape, although half-moon and pleomorphic forms were also common (Fig. 2).

The dimensions of merozoites in smear preparations of tick haemolymph ranged from 11,3–14,1 μm in length and 2,4–3,0 μm in width (mean 12,7× 2,62 μm). Most of these merozoites had a typical club shape with the shorter ones tapering less towards one end than the longer forms (Fig. 3). The nuclei were located near the middle of the parasite in 98% of cases, and near the rounded end in the remaining 2%.

The paired forms of this parasite were smaller than those of *B. bigemina* and *Babesia major* as described by Zwart, Van den Ende, Kouwenhoven & Buys (1968), Brocklesby & Barnett (1970) and Potgieter (1977) (Table 1), but larger than those of *B. bovis* (Neitz, 1941; Riek, 1966; Potgieter, 1977). Merozoites of this *Babesia* sp. in tick haemolymph were larger than those of *B. bigemina* (Riek, 1964; Potgieter & Els, 1977; Morzaria & Brocklesby, 1977), but smaller than those of *B. major* (Morzaria & Brocklesby, 1977) and *B. bovis* (Riek, 1966; Potgieter, 1977) (Table 2). Thus this species does not correspond in size with any of these 3 bovine *Babesia* spp.

TABLE 1 Relative sizes of intra-erythrocytic paired forms of bovine *Babesia* spp.

Babesia sp.	Authors	Mean length (μm)	Mean width (μm)
Babesia sp...		2,70	1,59
B. bigemina..	Zwart *et al.* (1968)....	3,68	1,90
	Potgieter (1977).....	3,7	1,8
B. major....	Zwart *et al.* (1968)....	3,31	1,90
	Brocklesby & Barnett (1970).....	3,53	
B. bovis.....	Riek (1966).....	1,8	1,2
	Neitz (1941).....	1,5	
	Potgieter (1977).....	2,0	1,2

TABLE 2 Relative sizes of merozoites of bovine *Babesia* spp. in tick haemolymph

Babesia sp.	Authors	Mean length (μm)	Mean width (μm)
Babesia sp...		12,7	2,62
B. bigemina.	Riek (1964).....	11,1	2,6
	Potgieter & Els (1977)..	10,7	3,2
	Morzaria & Brocklesby (1977).....	11,79	2,55
B. major....	Morzaria & Brocklesby (1977).....	15,53	3,00
B. bovis.....	Riek (1966).....	15,8	3,0
	Potgieter (1977).....	15,9	3,2

Babesia divergens, the 4th bovine *Babesia* sp. recognized by Hoyte (1976), is characteristically very small compared with the other species and the majority of the parasites are situated in the peripheral part of the erythrocyte. Being readily distinguishable from the *Babesia* sp. dealt with in this paper, it was therefore not considered.

Pathogenicity of Babesia *sp. isolated from* H. m. rufipes

The reactions of splenectomized cattle following infection were mild, the PCV being reduced to less than 20% in only 1 of the 4 infected animals, a 6-year-old cow. This reaction is different from that known of *Babesia* spp. in general. The literature reviewed by Riek (1968) and Zwart & Brocklesby (1979) has provided evidence that splenectomy of animals before infection often results in severe clinical reactions.

Tick vector

According to Brocklesby & Barnett (1970) and Hoyte (1976), the distribution of *B. major* is associated with temperate climate vector ticks such as *Ixodes ricinus* and *Haemaphysalis puncata*, and is therefore quite different from the distribution of *B. bovis* and *B. bigemina*. The latter are associated with tropical and sub-tropical tick vectors, and it therefore seems unlikely that *B. major* could be transmitted by a tick species such as *H. m. rufipes*.

Specificity of Babesia *sp. isolated from* H. m. rufipes

Transmission to susceptible cattle demonstrably occurred after both larval and adult stages of *H. m. rufipes* had fed. The larvae apparently did not attach and feed on a horse, nor were parasites seen in blood smears of the horse following injection of infected blood. We therefore assume that this *Babesia* sp. is probably not a parasite of the horse.

This parasite was originally thought to be *B. bigemina* (De Vos, 1979), but when differences in pathogenicity and morphology are taken into account, it seems conclusive that it is a hitherto unidentified bovine *Babesia* sp. Further research is being undertaken to clarify the identity and taxonomic position of this parasite (Gray & De Vos, 1981).

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