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STUDIES ON A BOVINE *BABESIA* TRANSMITTED BY *HYALOMMA MARGINATUM RUFIPES* KOCH, 1844

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ABSTRACT

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A *Babesia* sp. was recently observed in *Hyalomma marginatum rufipes* and found to be transmissible to bovines. Further observations were carried out on this parasite and a study made of the morphology of stages in both erythrocytes and tick haemolymph. Apart from *Babesia divergens*, intra-erythrocytic parasites were not readily distinguishable from bovine *Babesia* spp. Merozoites in tick haemolymph morphologically resembled those of *Babesia bigemina*, but they were significantly larger. This *Babesia* sp. proved to be highly infective for adult *H. m. rufipes*, with transmission taking place transovarially and next generation nymphae and adults transmitting the infection.

Features of the infection were its very low pathogenicity, even in splenectomized animals, and the tendency of parasitized erythrocytes to accumulate in capillaries.

Serologically, this species could be differentiated from *Babesia bigemina*, *B. divergens*, *B. bovis* and *B. major*. A serological survey of 25 farms showed a wide distribution of this species in South Africa and its high rate of transmission on most properties. It was concluded that this is a true but hitherto undescribed bovine *Babesia* sp. and the name *Babesia occultans* n. sp. is proposed.

Résumé

ÉTUDES SUR UN BABESIA *BOVIN TRANSMIS PAR* HYALOMMA MARGINATUM RUFIPES, *KOCH*, 1884

Une espèce de Babesia *a récemment été observé chez* Hyalomma marginatum rufipes *et il a été trouvé être transmissible aux bovins. D'autre observations furent entreprises sur ce parasite et une étude de la morphologie des stades erythrocytaires et dans l'haemolymphe du tique fut faite. A part le* Babesia divergens, *des parasites intra-erythrocytaires ne furent pas vraiment discernables du* Babesia *bovin spp. Les merozoites dans l'haemolymphe du tique ressemblaient morphologiquement à ceux de* Babesia bigemina, *mais ils étaient significativement plus grands. Ce* Babesia *sp. s'avéra être hautement infectieux pour l'adulte* H.m. rufipes *avec la transmission prenant place transovariallement et la génération suivante de nymphes et d'adultes transmettant l'infection.*

Les caractéristiques de l'infection furent sa très faible pathogénicité, même chez les animaux splenectomisés, et la tendance des erythrocytes parasitises à s'accumuler dans les capillaires.

Sérologiquement cette espèce pourrait être différenciée de Babesia bigemina, B. divergens, B. bovis *et* B. major. *Une enquête sérologique de 25 fermes a montré une large distribution de cette espèce en Afrique du Sud et son degré élevé de transmission sur la plupart des propriétés. Il en a été déduit que ceci est une vraie espèce bovine de* Babesia, *mais non encore décrit et le nom de* Babesia occultans *n.sp. est proposé.*

INTRODUCTION

In 1976 a batch of engorged female *Hyalomma marginatum rufipes* Koch, 1844 collected from cattle in Northern Transvaal was sent to this Institute to be tested for acaricide resistance. Some of these ticks were found to have *Babesia* large merozoites (vermicules) in their haemolymph and their progeny transmitted a *Babesia* sp. to splenectomized cattle (Thomas & Mason, 1981).

In the present study this *Babesia* was investigated further to determine whether it was a true bovine parasite and, if so, whether it was a new bovine *Babesia* sp. Attempts were made to infect adult *H. m. rufipes* ticks by feeding them on infected cattle and to transmit the parasite with the resulting larval, nymphal and adult ticks back to cattle. Morphological, serological and cross-immunity studies were undertaken in an attempt to distinguish the parasite from other bovine *Babesia* spp. One attempt was also made to transmit it transovarially with *Boophilus microplus* (Canestrini, 1887).

MATERIALS AND METHODS

Babesia *spp.*

The *Babesia* sp. studied below was originally obtained from a farm in the Northern Transvaal in 1976 (Thomas & Mason, 1981). After passage it was stored as a frozen blood stabilate with 8% dimethyl sulphoxide as a cryoprotectant in the gas phase of a liquid nitrogen container. The parasitaemia of the blood when it was collected for storage was approximately 0,2%.

The strains of *Babesia bigemina* (Smith & Kilborne, 1893) and *Babesia bovis* (Babés, 1888) used, have been described by Potgieter (1977). The strains of *Babesia divergens* (M'Fadyean & Stockman, 1911) and *Babesia major* (Sergent, Donatien, Parrot, Lestoquard & Plantureaux, 1926) were obtained from Dr R. E. Purnell, formerly of the Institute for Research on Animal Diseases, Compton, England.

Tick strains

The strain of *H. m. rufipes* used in this study was obtained from the Barkly West District, Cape Province, in 1978. Until used in this study it had been fed for several generations on rabbits (larval-nymphal state) and splenectomized cattle (adults) and was known to be *Babesia*-free (Potgieter, 1980, personal communication).

The Wonderboom strain of *B. microplus* used was obtained from a farm near this Institute in 1973 and was also *Babesia*-free. It was known to be a suitable transmitter of both *B. bigemina* and *B. bovis* (Potgieter, 1977).

Experimental animals

A total of 17 animals were used in this work and all but one, (an Afrikander *Bos indicus*), were *Bos taurus*. Nine of these animals were born and reared under tick-free conditions at these laboratories and 8 were splenectomized when 4–5 months old. A further 8 calves were obtained from a farm with minimal tick numbers and were kept tick-free by weekly dipping until required. All 8 calves were intact and serologically negative for *B. bigemina* and *B. bovis*,

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but were carriers of *Theileria mutans* (Theiler, 1906). Three of these calves were treated with 0,05 mg/kg body mass of prednisolone* from Day 0 of infection for 5 days. This procedure with betamethasone has been shown to induce subclinical relapses of *B. argentina* (*B. bovis*) (Callow & Parker, 1969).

Most of the experimental animals were infected artificially by the intravenous injection of infected blood stabilate thawed at room temperature after storage in 5 mℓ volumes in a liquid nitrogen container with 8% dimethyl sulphoxide as cryoprotectant (Table 7). Some animals received 100–500 mℓ of fresh blood injected intravenously from parasitaemic animals.

Quantification of reactions

Blood smears: Thick blood smears (Mahoney & Saal, 1961) as well as routine thin blood smears were made each day from the date of infection until parasites were not detectable in the smears for at least 2 weeks. The smears were fixed in methanol and stained with Giemsa's stain for 35 minutes. The thick blood smears were used to determine the prepatent period and were examined for a maximum of 10 minutes. The percentage parasitaemias were determined either by counting the number of parasites in 1 000 erythrocytes in thin smears or, in the case of very low parasitaemias, 100 optical fields. In the latter case fields with a uniform distribution of erythrocytes were scanned, the parasites counted, and the parasitaemia calculated from the average number of erythrocytes per field.

Febrile reaction: Rectal temperatures were taken daily between 08h00 and 11h00. In this study temperatures of 39,5 °C and lower were considered normal.

Packed cell volume (PCV) depression: The PCV depression was determined using Clay Adams microhaematocrit tubes and a Damon microfuge. These determinations were commenced when parasitaemias became patent and were continued until post-reaction values rose above 30%.

Feeding and maintenance of ticks

Immature stages of *H. m. rufipes*, which is a 2-host tick (Knight, Norval & Rechav, 1978), were fed for rearing purposes on the ears of rabbits, and adults on the ears of cattle, using the methods of Neitz, Boughton & Walters (1971). Immature *H. m. rufipes* were also fed, more successfully, on the backs of rabbits in cloth containers (back-pockets) attached to the fur with contact adhesive**.

B. microplus was fed on the body of cattle as described by Potgieter (1977).

Detection of infection in ticks

Ten days after engorgement on parasitaemic animals female ticks were screened for *Babesia* infection by making haemolymph smears. The 2nd leg of each tick was amputated on 1 side and haemolymph smears prepared as described by Burgdorfer (1970). The smears were air-dried, fixed in methanol, stained in Giemsa's stain and examined for large merozoites (LM) of the *Babesia* parasite.

Transmission with ticks

Three experiments were performed to study the ability of *H. m. rufipes* to transmit the *Babesia* sp. transovarially from adults to larvae, nymphae and adults respectively. One attempt was made to transmit the same parasite with *B. microplus* transovarially.

Transmission by H. m. rufipes *from adult to larva*

Infection of ticks: A non-splenectomized 2-year-old ox (2892) was infested on one ear with 25 male and 25 female ticks on Day 0 and again on Day 4. The other ear was infested with the same number of ticks on Days 2 and 6. On Day 2 the animal was inoculated with 5 mℓ of thawed infected blood stabilate.

Transmission: The eggs of infected ticks were sieved and thoroughly mixed. They were then mass-measured into 0,2 g aliquots (approximately 3 200 eggs) and allowed to hatch in small glass tubes (25 × 10 mm). Twenty-four days after the first eggs hatched 6 tubes of larvae were placed in 3 back pockets attached with Genkem adhesive to the neck of a splenectomized 10-month-old calf (3722). The next day a further 6 tubes were placed in bags and applied to the calf's ears. On Day 10 the calf was thoroughly washed with water-soluble pyrethrin* to kill all the ticks before larvae moulted and nymphae attached.

Transmission by H. m. rufipes *from adult to nymph*

Infection of ticks: A splenectomized 12-month-old calf (3458) was infested on the left ear with 25 male and 25 female *H. m. rufipes* that had been reared on rabbits. On Day 2, 25 male and 25 female *H. m. rufipes* were placed on the right ear, and on Day 5 the left ear received 15 male and 15 female ticks and the right ear another 10 male and 10 female ticks. Finally, 50 male ticks were placed on the tail 10 days after the initial infestation. Five mℓ of thawed stabilate was injected intravenously on Day 2. As this animal accidentally received 10 mg/kg body mass of tetracycline** on Day 9, a further 5 mℓ of infected blood stabilate was inoculated on Day 10. Prednisolone administered intramuscularly at 0,1 mg/kg given daily for 10 days, commencing on Day 10.

Transmission: The eggs of infected ticks were mixed and mass-measured in 0,2 g aliquots. After hatching, the larvae were placed in back pockets on rabbits (one tube each). Eighteen days after infestation 200 pharate or freshly moulted nymphae were removed and placed in 2 back pockets on a splenectomized 7-month-old calf (4018) 24 h later.

Transmission by H. m. rufipes *from adult to adult*

Infection of ticks: Infected ticks were obtained from the same batch as was used in the previous experiment.

Transmission: As in the previous experiment, the larvae were fed on rabbits but the ticks in this case were allowed to engorge as nymphae, detach and moult in the acaridarium to adults. Four weeks after the first appearance of adults, 50 females and 50 males were placed on each ear of a splenectomized 2-year-old ox (3060).

Attempted infection of and transmission with Boophilus microplus

The larval progeny of 8 female *B. micoplus* were applied to the body of a 12-month-old splenectomized calf (3455) and 15 days later 5 mℓ of blood stabilate was injected intravenously. Intramuscular injections of 0,05 mg/kg of prednisolone were given daily from Days 15–19 post-infestation. Females that engorged

* Deltacortril, Pfizer
** Genkem Ank 508, General Chemical Corporation

* C&B concentrate, Avima
** Terramycin 100, Pfizer

were screened for LM 10 days after engorgement and their progeny were tested for infection by feeding them on an 8-month-old splenectomized calf (4022).

Infectivity of the Babesia *for and its effect on* H. m. rufipes

Once infected ticks had been identified by the screening of the haemolymph, the proportion of infected ticks collected on a particular day was correlated with the blood parasitaemia of the previous day. It was assumed that these ticks acquired the infection during the last day of engorgement as is the case with the transmission of *B. bigemina* by *B. microplus* (Callow, 1968).

After ovipositing, the mass of these ticks and their eggs were determined. The eggs were then thoroughly mixed and divided into 0,2 g batches.

The eggs in 3 of these batches were counted in methanol and the remainder were allowed to hatch. After hatching, the larvae in another 3 batches were counted in methanol. This procedure was also carried out for some uninfected ticks so that it was possible to assess the effects of the *Babesia* infection on egg production and egg hatch. Statistical analysis was carried out, using Student's t-test on egg production and the Mann-Witney U test on egg hatch.

Parasite morphology and dimensions

Studies on the morphology and dimensions of the parasites were made from thin blood smears and from tick haemolymph smears. The dimensions of the intra-erythrocytic stages of the *Babesia* were determined at peak parasitaemias from an intact and a splenectomized prednisolone-treated animal with the aid of an ocular micrometer. The parasites selected for measurements were pairs that had evidently just completed division. One member of each of 25 pairs was measured in each animal. For comparative purposes similar measurements were taken of paired organisms in blood smears of animals infected with *B. bigemina, B. bovis, B. major* and *B. divergens.*

One hundred LM in the haemolymph of engorged female *H. m. rufipes* were also measured. For comparative purposes the same number of measurements were made of LM of *B. bigemina* from the haemolymph of *B. decoloratus* females infected as part of another project.

The data on parasite dimensions were analysed by Student's t-test.

Serology

The antigenic characteristics of the *Babesia* sp. were investigated by the indirect immunofluorescent antibody test. This test is considered to be sufficiently specific for taxonomic purposes and, as reviewed by Zwart & Brocklesby (1979), it has been used successfully in several studies on bovine *Babesia* spp. The technique used was essentially a modification of that used by Joyner, Donnelly, Payne & Brocklesby (1972).

Antigen preparation: Thick blood smears were made in 12 wells on each teflonized glass slide prepared beforehand as described by Morzaria, Brocklesby & Harradine (1977). Two batches of antigen were prepared by infecting 5–6-year-old, splenectomized animals and treating them with prednisolone at the rate of 0,05 mg/kg body mass for 5 days, commencing on the day of infection. Blood showing a parasitaemia 1–2% was used for antigen production. The smears were sealed in plastic bags and stored at −20 °C until required and fixed in cold acetone before use.

For control purposes antigen preparations were also made of blood infected with *B. bigemina, B. bovis, B. divergens* and *B. major*. Blood showing parasitaemias of 1–5% were used for antigen preparation.

Sera: Sera of 9 animals recovered from *Babesia* sp. infections were tested against antigens of the 5 *Babesia* spp. Each serum was tested in a series of doubling dilutions starting at 1:40. The titre was taken as the reciprocal of the highest dilution giving specific fluorescence.

Sera, positive for *B. bigemina, B. bovis, B. major* and *B. divergens,* were titrated and tested as controls. Serum of an uninfected animal was used as the negative control.

Conjugate: Commercial rabbit anti-bovine globulin, conjugated with fluorescein isothiocyanate*, was used as the optimal working dilution as determined by titration (1:80).

Microscopy: Fluorescence was observed with a Leitz Orthoplan microscope using the same incident light illumination system described by Joyner, Payne, Takahashi, Brocklesby & Irvin (1979). A 50 × water immersion objective was used throughout (total magnification 500×).

Sensitivity control: The sensitivity of the test was controlled by using antisera to *B. bigemina* and *B. bovis* with known titres to the homologous antigens.

Serological survey: To obtain some information on the distribution and prevalence of this *Babesia* sp. in cattle in South Africa, sera were obtained from 25 localities for serological screening (Fig. 1). Some of the farms were known to be outside the normal distribution range of *H. m. rufipes* described by Howell, Walker & Nevill (1978).

Sera were tested only of animals 1–2 years of age, born on the respective properties. These sera were screened at a set dilution of 1:40 on antigen smears of this *Babesia* sp., *B. bovis,* and *B. bigemina.*

Other taxonomic parameters

Differential counts were made on 5 different occasions of parasites in thin blood smears made from capillary blood and from the general circulation of 1 splenectomized animal. Blood was obtained from the tip of the tail and from the vein on the ventral aspect of the base of the tail respectively, as described by Hoyte (1971). In each case the number of parasites seen in 100 optical fields was counted and the respective means calculated. Statistical analysis was carried out with Student's t-test.

A brain smear was also prepared from brain biopsy material of a subpatently infected animal, obtained as described by Johnston & Callow (1963). The smear was fixed in methanol, stained with Giemsa's stain and examined for infected erythrocytes in the capillaries.

Pathogenicity of Babesia *sp.*

No detailed studies on the pathogenicity of the *Babesia* sp. were carried out, but routine observations were made each time an animal was infected with blood stabilate, fresh blood or by tick transmission. The parameters observed were: prepatent period, maximum parasitaemia, duration of parasitaemia, day of first temperature, maximum temperature, duration of temperature and lowest PCV.

* Miles Laboratories

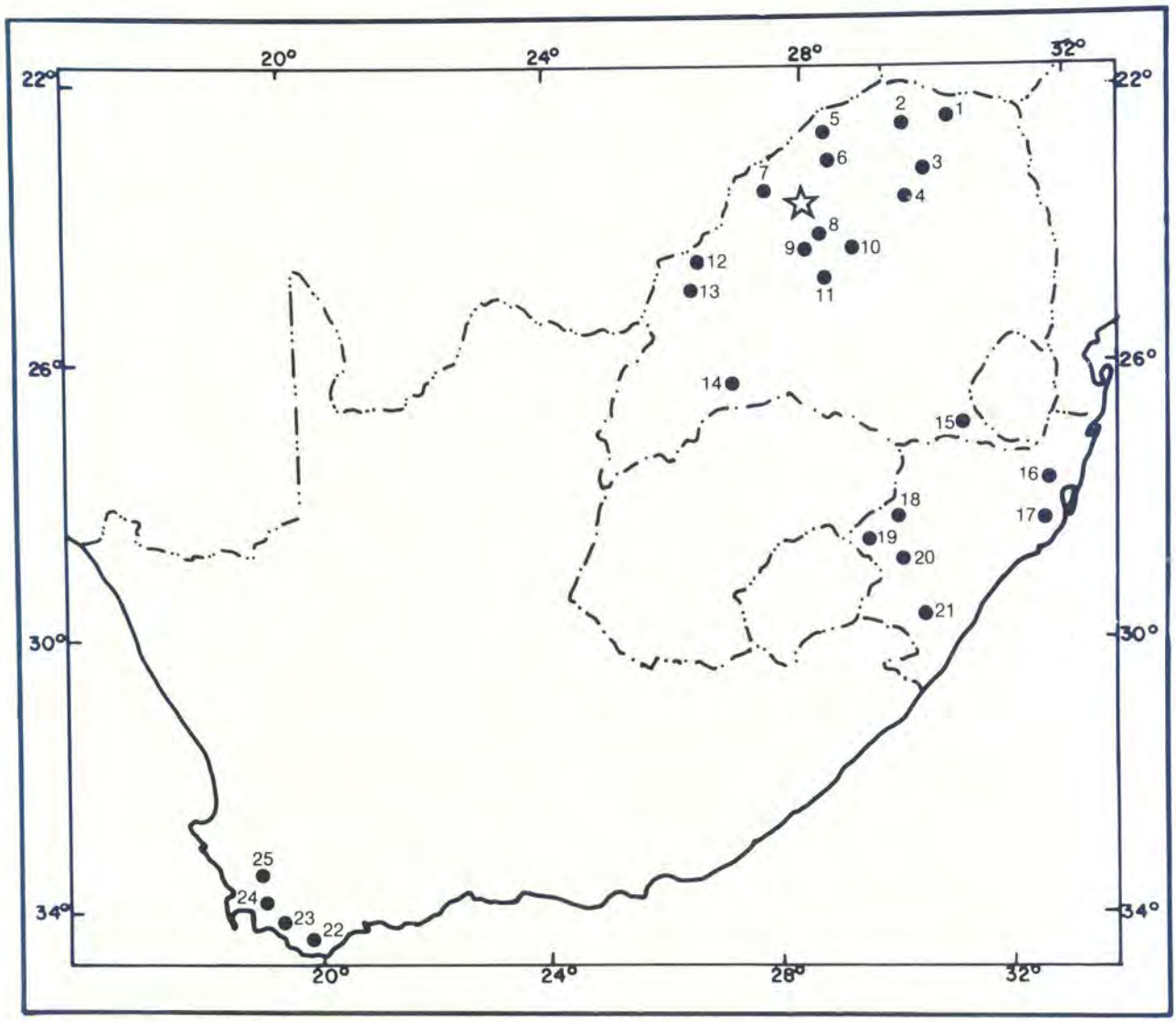

FIG. 1 Distribution of 25 farms where cattle were screened serologically for bovine *Babesia* spp. Star indicates farm where *Babesia occultans* n. sp. was isolated in 1976

Results and Discussion

Infection of H. m. rufipes

Female *H. m. rufipes* that engorged on an infected intact ox (2892) and on an infected splenectomized calf (3458) became infected with the *Babesia*, as the presence of LM in the haemolymph 10 days after engorgement showed. Of the ticks that engorged on patent parasitaemias, 69,2% from the intact ox and 20,6% from the splenectomized calf became infected.

Transovarian transmission by H. m. rufipes

Transmission by larvae: Very few of the ±10 000 larvae that were applied to the neck and ears of Calf 3722 attached and fed. Those that did were killed by treatment with an acaricide on Day 10. During the ensuing 21 days this animal showed no signs of reaction and remained serologically negative for the *Babesia* sp.

Transmission by nymphae: Most of the 200 nymphae, fed as larvae on rabbits, attached to Calf 4018, but only 38 engorged nymphae were recovered. A temperature of 40,3 °C was recorded on Day 12 after infestation and the first *Babesia* parasites were observed in a thick blood smear on Day 13.

Transmission by adults: Out of a total of 100 female and 100 male *H. m. rufipes* fed on Ox 3060, 72 engorged female ticks were recovered. This animal also became infected and parasites were detected for the first time in a thick blood smear on Day 10. The animal's temperature remained within normal limits.

Infection of and transmission by B. microplus

A total of 57 *B. microplus* engorged females that had engorged on *Babesia* sp. parasitaemias ranging from 0,2–3,2% were collected from Calf 3455. No LM were detected in the haemolymph of these ticks 10 days after engorgement. The larval progeny of 12 of the females that had engorged on a parasitaemia of 3,2% yielded a total of 44 engorged female *B. microplus* when fed on a susceptible animal. One month after infestation the animal was still serologically negative for this *Babesia* sp. Blood smears also remained negative throughout the period.

Thomas & Mason (1981) reported the successful transmission of this *Babesia* sp. with immature *H. m. rufipes* but did not attempt to determine which of the larval and/or nymphal stages of this two-host tick were involved. In the present study transmission by larvae was not demonstrated, but then larvae failed

TABLE 1 Proportion of engorged female *H. m. rufipes* and *B. microplus* with large merozoites (LM) of *B. occultans* n. sp. in haemolymph in relation to blood parasitaemia on last day of engorgement

Tick species	Bovine No.	Days after initial infestation	Number of ticks collected	Number of ticks with LM	Range of parasitaemias on last day of engorgement (%)
H. m. rufipes	Calf 3458	9	1	1	0–0,005
		10*	8	8	0,005–0,17
		11	6	0	0,17–0,002
		12	10	0	0,002–0
		13	9	0	0–0,005
		14	11	0	0,005–0,001
		15	5	0	0,001
		16	5	1	0,001–0,014
		24	3	2	0,014–0,005
	Ox 2892	10	11	10	0,03–0,017
		11	11	7	0,017–0,001
		13	17	10	0,001–0,008
B. microplus	Calf 3455	23	14	0	0,2–0,4
		24	15	0	0,4–1,2
		25	15	0	1,2–3,2
		26	12	0	3,2–3,0

* Treated accidentally with tetracycline

to attach in significant numbers. Since nymphae did transmit the infection, the observation of Thomas & Mason (1981) was confirmed. It should be noted, however, that the immature stage of *H. m. rufipes* is not commonly found on cattle (Knight *et al.*, 1978) and transmission of this *Babesia* sp. from cattle to cattle must of necessity be by the transovarian route from adult to adult tick.

The infectivity of the Babesia *for and its effect on* H. m. rufipes

This *Babesia* proved to be highly infective for *H. m. rufipes,* since ticks became infected when engorging on animals showing very low parasitaemias (Table 1). For example, adult ticks that fed on the intact ox (2892) engorged on parasitaemias that ranged from 0,03–0,001% but LM were found in 58,8–90,2% of the engorged females collected on the different days. The proportion of ticks that became infected after feeding on the splenectomized calf (3458) was comparatively small. However, it can be seen from Table 1 that, although it was still present in blood smears, there seemed to be a loss of infectivity of the parasite for ticks after the calf was accidentally treated with tetracycline on Day 10. After the calf had been treated with prednisolone and super-infected with the *Babesia*, a few more ticks became infected. It should be noted that the demonstration of LM in the haemolymph of engorged females is not an accurate method of detecting *Babesia* infection in ticks (Mahoney & Mirre, 1971), so it is probable that the actual infection rate of *H. m. rufipes* with this *Babesia* was even higher than that determined here.

Infected ticks were quite severely affected by the parasite and their egg production and egg viability were considerably reduced even when engorgement took place on very low parasitaemias (Table 2). Very few infected ticks died before ovipositing, but several died after laying only a few eggs. All these individual ticks had medium to large numbers of LM in the haemolymph 10 days after engorgement. In some cases eggs produced by infected ticks were laid in a partly liquefied state and formed a cement over the genital opening and capitulum, thus bringing oviposition to an end. The percentage hatch of eggs was also considerably reduced. It is known that both *B. bovis* and *B. bigemina* have similar detrimental effects on *B. microplus* (Riek, 1964, 1966).

TABLE 2 Effect of infection with *Babesia occultans* n. sp. on egg production and egg viability of *H. m. rufipes*

Ticks infected on	Egg production* ±standard error		Median percentage hatch of eggs in pooled batches	
Calf 3458	0,4084 ±0,0750	b		
Ox 2892	0,4554 ±0,0390	b	42,2	c
		a		c
Uninfected ticks	0,7029 ±0,0132	a, b	76,1	c

a Student's t-test $P<0,001$

b Student's t-test $P<0,01$

c Mann-Witney U test $P<0,05$

* Egg production $= \frac{\text{Egg mass}}{\text{Spent female mass} + \text{egg mass}}$

The infectivity of this parasite for adult *H. m. rufipes*, despite the very low parasitaemias observed in infected cattle, seems to indicate the existence of a well-developed bovine-tick-parasite relationship.

Parasite morphology and dimensions

The appearance of the parasite in blood smears was that of a typical *Babesia*, with pairs of piriform merozoites occurring in the centre of erythrocytes.

Single parasites were common, particularly when parasitaemias were low. The size of recently divided intra-erythrocytic merozoites was found to be:

(a) Intermediate between those of the 2 other African bovine babesias, the large *B. bigemina* and the small *B. bovis;*

(b) similar in size to *B. major* merozoites; and

(c) much larger than *B. divergens* merozoites (Table 3).

Although statistically significant differences in length or width or both were found between the *Babesia* sp. and the 4 bovine babesias (Table 3), only *B. divergens* could visually be distinguished with certainty from the *Babesia* sp. It should be noted that merozoites of the *Babesia* sp. from splenectomized and corticosteroid-treated animals were sligthly shorter (not significantly) and wider (significantly) than those from relatively insusceptible intact animals in which the parasites were frequently pyknotic. These differences were readily detected by visual examination.

The LM of this *Babesia* sp. in the haemolymph of *H. m. rufipes* appeared very similar to the LM of *B. bigemina* described in detail by Potgieter & Els (1977). In this study LM of the *Babesia* sp. were found to be slightly larger than those of *B. bigemina* (Table 4), which were about the same size as those measured by Morzaria & Brocklesby (1977). These authors also measured the LM of *B. major* and their results show that these LM are the largest of the 3 species.

Serology

The results obtained with the IFA test are summarized in Table 5. As can be seen from this table there was close agreement between results obtained with sera of the new *Babesia* sp. tested against 2 batches of antigen prepared from 2 different infected animals. No positive reactions were observed when the same sera were tested against *B. bigemina, B. bovis, B. major* and *B. divergens* antigens. Conversely, however, sera of the *Babesia* sp., *B. bigemina* and *B. major* gave positive reactions with *B. bovis* antigen, but the titres were lower than those obtained with *B. bovis* sera. The same applied when sera of the *Babesia* sp., *B. bigemina* and *B. bovis*, were tested with *B. major* antigen.

This *Babesia* sp. is therefore serologically distinct from other recognized bovine *Babesia* spp. to the extent that positive reactions obtained with the *Babesia* sp. antigen can be considered to be specific. Theoretically, this species may interfere with results when antigens of *B. bovis* (or *B. major*) are used. This, however, was not confirmed in the serological survey.

TABLE 3 Dimensions of intra-erythrocytic merozoites of bovine babesias from blood

Species of *Babesia*	Type of bovine	Mean length ± standard error (µm)	Mean width ± standard error (µm)
B. occultans n. sp (1)	Intact ox	2,88±0,155	1,22±0,047
B. occultans n. sp (2)	Splenectomized cow + prednisolone	2,57±0,1016	1,68±0,076
B. bigemina	Splenectomized calf	3,29±0,070	1,49±0,044
B. bovis	Splenectomized calf	2,29±0,061	1,10±0,036
B. major	Splenectomized cow	3,25±0,049	1,51±0,057
B. divergens	Splenectomized cow	1,22±0,037	0,93±0,013

Student's t-test

	Length	Width		Length	Width
B. occultans n. sp (1) / *B. occultans* n. sp (2)	P>0,05	P<0,001	*B. occultans* n. sp / *B. major*	P<0,001	P>0,05
B. occultans n. sp (2) / *B. bigemina*	P<0,001	P<0,05	*B. occultans* n. sp / *B. divergens*	P<0,001	P<0,001
B. occultans n. sp (2) / *B. bovis*	P<0,05	P<0,001			

TABLE 4 Dimensions of large merozoites (vermicules) of *B. occultans* n. sp. and *B. bigemina* from tick haemolymph

Species of *Babesia*	Species of tick	Mean length ±standard error (µm)	Mean width ±standard error (µm)
B. occultans n. sp	*Hyalomma m. rufipes*	13,66±0,095	2,57±0,042
B. bigemina	*Boophilus decoloratus*	12,07±0,122	2,27±0,035
*B. major**	*Haemaphysalis punctata*	15,53	3,00

Student's t-test

	Length	Width
B. occultans n. sp / *B. bigemina*	P<0,001	P<0,001

* Measured by Morzaria & Brocklesby (1977)

TABLE 5 Reciprocal titres of babesial antisera using antigens of *B. occultans* n. sp. *B. bigemina, B. bovis, B. major* and *B. divergens* in the indirect fluorescent antibody test

Serum		Antigens					
		B. occultans n. sp. (Antigen 1)	*B. occultans* n.sp. (Antigen 2)	*B. bigemina*	*B. bovis*	*B. major*	*B. divergens*
B. occultans n.sp.....	1	640	320	<40	<40	<40	<40
	2	80	80	<40	<40	<40	<40
	3	160	160	<40	<40	<40	<40
	4	640	640	<40	<40	<40	<40
	5	640	ND	<40	<40	<40	<40
	6	640	320	<40	<40	<40	<40
	7	160	320	<40	<40	<40	<40
	8	160	160	<40	<40	<40	<40
	9	160	320	<40	<40	<40	<40
B. bigemina.....	1	<40	<40	320	<40	<40	<40
	2	<40	<40	320	<40	<40	<40
	3	<40	<40	320	<40	<40	<40
B. bovis.....	1	40	80	80	320	<40	<40
	2	80	160	80	>640	40	<40
	3	160	160	80	640	40	<40
B. major.....	1	ND	80	80	40	160	40
	2	80	80	80	40	160	40
B. divergens.....	1	<40	ND	<40	<40	40	160
	2	<40	ND	<40	<40	<40	80

ND—Not done

The results of the serological survey of 25 farms are summarized in Table 6. Positive sera were recorded on 23 of the farms, including 3 where all the samples tested were negative for *B. bigemina* and 18 where the presence of *B. bovis* could not be confirmed. On 19 of the farms 50% or more of the samples tested were positive for this species. Thus, despite the low parasitaemias seen in experimentally infected cattle, the rate of transmission in the field appears to be at least as good as that of *B. bigemina*.

TABLE 6 Percentage cattle positive for 3 *Babesia* spp.

Farm No.	Number of sera	*B. occultans* n. sp (%)	*B. bigemina* (%)	*B. bovis* (%)
1.....	25	84	88	0
2.....	25	100	60	0
3.....	30	87	53	0
4.....	30	90	87	0
5.....	25	90	96	0
6.....	25	84	100	0
7.....	25	52	28	0
8.....	25	8	88	0
9.....	28	50	89	61
10.....	25	0	52	0
11.....	29	59	86	31
12.....	30	57	7	0
13.....	30	50	7	0
14.....	30	50	57	0
15.....	30	57	30	0
16.....	25	84	88	68
17.....	10	40	60	20
18.....	20	20	0	0
19.....	22	91	100	0
20.....	18	61	17	0
21.....	21	100	0	0
22.....	14	21	0	0
23.....	20	0	0	0
24.....	20	100	95	85
25.....	20	80	70	0

Six of the farms studied fall well outside the recognized area of distribution of *H. m. rufipes*. Of these 5 had serological evidence of the *Babesia* sp. (20–100% positive), which seems to indicate the existence of another vector, such as *Hyalomma truncatum* Koch, 1844. Its existence was confirmed on all 5 of the positive farms.

Pathogenicity

Insignificant reactions to the *Babesia* sp. occurred in 8 intact, untreated calves. Elevated body temperatures were seen in 4 of them, but in 4 seroconversion was the only evidence of infection. Thick bloodsmears remained negative throughout the observation period.

Even in 2 splenectomized calves and 1 intact corticosteroid-treated calf this parasite failed to cause clinical reactions apart from elevated body temperatures. The parasitaemias in these animals failed to reach 0,2% (Table 7). A 5-year-old splenectomized cow, however, developed a parasitaemia of almost 2%, but it had no clinical disease apart from a fever lasting 3 days. The only 3 animals developing clinical disease necessitating therapeutic intervention had all been splenectomized and treated with corticosteroids.

It is evident, therefore, that this parasite is of very low virulence and unlikely to be the cause of disease in the field.

As summarized in Table 8, this parasite has a tendency to accumulate in capillaries. This tendency is confirmed by the presence of readily detectable parasites in capillaries of brain biopsy material obtained from a subpatent carrier of this species. Accumulation in capillaries has taxonomic significance and has been used by Hoyte (1976) to differentiate *B. bovis* and its synonyms from other recognized bovine *Babesia* spp. *B. bovis* has a marked tendency to accumulate in capillaries.

TABLE 7 Reactions of splenectomized and intact cattle to infections with *B. occultans* n. sp.

Bovine No.	Type animal	Physiological manipulation	How infected	Prepatent period (days)	Maximum parasitaemia (%)	Duration of parasitaemia (days)	Day of first temperature	Maximum temperature (°C)	Duration of temperature (days)	Lowest PCV (%)	Treatment
60	7 year old cow	splenectomized prednisolone*	blood fresh.....	4	7,5	10	5	41,5	4	20	euflavine (Day 7)
2355	calf.....	splenectomized prednisolone*	blood stabilate..	5	3,2	8	7	41,1	6	18	euflavine (Day 11)
1205	6 year old ox..	splenectomized prednisolone*	blood stabilate..	5	1,0	3	8	41,7	1	28	imidocarb (Day 8)
1191	5 year old cow	splenectomized.....	blood fresh.....	3	1,9	6	3	41,1	3	30	none
3458	calf.....	splenectomized prednisolone†	blood stabilate..	6	0,17	22	6	41,0	4	30	tetracycline (Day 9)
4367	calf.....	intact prednisolone*.....	blood stabilate..	7	0,17	4	7	40,1	2	27	none
2892	2 year old ox..	intact.....	blood stabilate..	6	0,04	8	6	41,3	2	30	none
3060	2 year old ox..	splenectomized.....	adult ticks.....	10	0,01	4	—	38,8	0	30	none
4018	calf.....	splenectomized.....	nymphal ticks..	13	0,03	10	12	41,3	5	30	none

* Deltacortril (Pfizer) 0,05 mg/kg body mass for 5 days from day of infection
† Deltacortril (Pfizer) 0,1 mg/kg body mass for 10 days from Day 10 post-infection

TABLE 8 Mean number of erythrocytes infected with *B. occultans* n. sp. in thin blood smears from capillary and venous blood

	Mean number of infected cells/100 optical fields ±standard error
Capillary blood.....	29,4±5,636
Venous blood.....	2,0±0,707

Student's t-test $P<0,002$

Conclusion

This *Babesia* sp. shares many features with other bovine *Babesia* spp. but differences, particularly with regard to serology and virulence, signalize it as a species hitherto undescribed in South Africa. Its wide distribution and high prevalence on infected farms seems to indicate that a well-established host-parasite relationship exists between bovine and parasite. It is conceivable, however, that this *Babiesia* sp. originated from, for example, and African antelope, as has been suggested for *Theileria taurotragi* of cattle (Grootenhuis, Young, Dolan & Stagg, 1979).

Since the *Babesia* occurs at very low parasitaemias and does not seem to cause clinical reactions in normal animals, the specific name *Babesia occultans* n. sp. is proposed.

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References

BURGDORFER, W., 1970. Haemolymph test. A technique for detection of rickettsiae in ticks. *American Journal of Tropical Medicine and Hygiene*, 19, 1010–1014.

CALLOW, L. L., 1968. The infection of *Boophilus microplus* with *Babesia bigemina*. *Parasitology*, 58, 663–670.

CALLOW, L. L. & PARKER, R. J., 1969. Cortisone-induced relapses in *Babesia argentina* infections of cattle. *Australian Veterinary Journal*, 45, 103–104.

GROOTENHUIS, J. G., YOUNG, A. S., DOLAN, T. T. & STAGG, D. A., 1979. Characteristics of *Theileria* species (eland) infections in eland and cattle. *Research in Veterinary Science*, 27, 59–68.

HOWELL, C. J., WALKER, J. B. & NEVILL, E. M., 1978. Ticks, mites and insects of domestic animals in South Africa. Part 1: Description and biology. Science Bulletin No. 393, Department of Agricultural Technical Services, Rep. of S.A.

HOYTE, H. M. D., 1971. Differential diagnosis of *Babesia argentina* and *Babesia bigemina* infections in cattle using thin blood smears and brain smears. *Australian Veterinary Journal*, 47, 248–250.

HOYTE, H. M. D., 1976. The tick-fever parasites of cattle. *Proceedings of the Royal Society of Queensland*, 87, V–XIII.

JOHNSTON, L. A. Y. & CALLOW, L. L., 1963. Intracerebral inoculation and brain biopsy in cattle. *Australian Veterinary Journal*, 39, 22–24.

JOYNER, L. P., DONNELLY, J., PAYNE, R. & BROCKLESBY, D. W., 1972. The indirect fluorescent antibody test for the differentiation of infections with *Babesia divergens* or *Babesia major*. *Research in Veterinary Science*, 13, 515–518.

JOYNER, L. P., PAYNE, R. C., TAKAHASHI, K., BROCKLESBY, D. W. & IRVIN, A. D., 1979. Serological comparison of British *Theileria mutans* and Japanese *T. sergenti*. *Research in Veterinary Science*, 26, 387–388.

KNIGHT, M. M., NORVAL, R. A. I., & RECHAV, Y., 1978. The life cycle of the tick *Hyalomma marginatum rufipes* Koch (Acarina: Ixodidae) under laboratory conditions. *Journal of Parasitology*, 64, 143–146.

MAHONEY, D. F. & MIRRE, G. B., 1971. Bovine babesiosis: Estimation of infection rates in the tick vector, *Boophilus microplus* (Canestrini). *Annals of Tropical Medicine and Parasitology*, 65, 309–317.

MAHONEY, D. F. & SAAL, J. R., 1961. Bovine babesiosis: Thick blood films for the detection of parasitaemia. *Australian Veterinary Journal*, 37, 44–47.

MORZARIA, S. P. & BROCKLESBY, D. W., 1977. A differential diagnostic criterion for *Babesia major* and *Babesia bigemina* vermicules from tick haemolymph. *Zeitschrift für Parasitenkunde*, 52, 241–243.

MORZARIA, S. P., BROCKLESBY, D. W. & HARRADINE, D. L., 1977. Evaluation of the indirect fluorescent antibody test for *Babesia major* and *Theileria mutans* in Britain. *Veterinary Record*, 100, 484–487.

NEITZ, W. O., BOUGHTON, F. & WALTERS, H. S., 1971. Laboratory investigations on the life cycle of the Karoo paralysis tick (*Ixodes rubicundus* Neumann, 1904). *Onderstepoort Journal of Veterinary Research*, 38, 215–223.

POTGIETER, F. T., 1977. The life cycle of *Babesia bovis* and *Babesia bigemina* in ticks and in cattle in South Africa. Ph. D. Thesis, Rand Afrikaans University.

POTGIETER, F. T. & ELS, H. J., 1977. Light and electron microscopic observations on the development of *Babesia bigemina* in larvae, nymphae and non-replete females of *Boophilus decoloratus*. *Onderstepoort Journal of Veterinary Research*, 44, 213–232.

RIEK, R. F., 1964. The life cycle of *Babesia bigemina* (Smith & Kilborne, 1893) in the tick vector *Boophilus microplus* (Canestrini). *Australian Journal of Agricultural Research*, 15, 802–821.

RIEK, R. F., 1966. The life cycle of *Babesia argentina* (Lignières, 1903) (Sporozoa: Piroplasmidea) in the tick vector *Boophilus microplus* (Canestrini). *Australian Journal of Agricultural Research*, 17, 247–254.

THOMAS, S. E. & MASON, T., 1981. Isolation and transmission of an unidentified *Babesia sp.* infective for cattle. *Onderstepoort Journal of Veterinary Research* 48, 155–158.

ZWART, D. & BROCKLESBY, D. W., 1979. Babesiosis: Non-specific resistance, immunological factors and pathogenesis. *Advances in Parasitology*, 17, 49–113.