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# THE ISOLATION AND TRANSMISSION OF AN UNIDENTIFIED *BABESIA* SP. TO CATTLE BY *HYALOMMA TRUNCATUM* KOCH 1844

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ABSTRACT

DE WAAL, D. T., POTGIETER, F. T., COMBRINK, M. P. & MASON, T. E., 1990. The isolation and transmission of an unidentified *Babesia* sp. to cattle by *Hyalomma truncatum* Koch 1844. *Onderstepoort Journal of Veterinary Research*, 57, 229–232 (1990).

An unidentified *Babesia* sp. which causes a mild disease in cattle was isolated in a splenectomized ox that received pooled blood from field cattle.

That this organism is pleomorphic and resembles *Babesia occultans* makes it difficult to differentiate between these organisms microscopically. Initially, it was suspected that this *Babesia* could be *B. occultans*. Several attempts to transmit this parasite transovarially with *Hyalomma marginatum rufipes*, the vector of *B. occultans*, failed. Continued efforts to identify possible vectors, using *Boophilus microplus, Rhipicephalus evertsi evertsi* and *Rhipicephalus appendiculatus*, all failed. The only tick thus far identified that could have transmitted the infection transovarially in the adult stage was the two-host tick *Hyalomma truncatum*.

## INTRODUCTION

Two economically important *Babesia* spp. of cattle occur in South Africa, viz. *Babesia bigemina* and *Babesia bovis*. *B. bigemina* is transmitted by *Boophilus decoloratus* and *Boophilus microplus*, while *B. bovis* is transmitted by *B. microplus* only (Potgieter, 1977). *B. bovis* has a limited distribution, because *B. microplus* is found mainly in the high rainfall areas, while *B. bigemina* on the other hand is more prevalent with the much wider distribution of *B. decoloratus* (Howell, Walker & Nevill, 1978).

A 3rd *Babesia* sp. of cattle, *Babesia occultans*, also occurs in South Africa (Gray & De Vos, 1981). It causes a mild disease in cattle and is transmitted transovarially by *Hyalomma marginatum rufipes* (Thomas & Mason, 1981; Gray & De Vos, 1981). It cross-reacts serologically in the indirect fluorescent antibody test with *B. bovis* and may therefore interfere with results of serological surveys of the 2 economically important species (Gray & De Vos, 1981).

During 1983, a *Babesia* parasite was isolated from cross-bred Bonsmara calves born and reared under extensive farming conditions on the farm Kaalplaas (28° 08′ E, 25° 38′ S) comprising portions of the farms De Onderstepoort and Haakdoornboom, adjacent to the Veterinary Research Institute (VRI), Onderstepoort. Though the morphology of this parasite resembled that of *B. occultans* to a large extent, 4 attempts to transmit this infection transovarially with *H. m. rufipes* failed (T. E. Mason & F. T. Potgieter unpublished observations, 1984).

Single attempts to transmit this *Babesia* isolate transovarially with each of the following tick species, *B. microplus, Rhipicephalus evertsi evertsi* and *Rhipicephalus appendiculatus*, all failed (F. T. Potgieter & D. T. de Waal, unpublished observations, 1984–1987).

A tick survey of horses on Kaalplaas indicated that both *Hyalomma truncatum* and *H. m. rufipes* appeared to be the most common tick species (D. T. de Waal unpublished observations, 1987). This led us to evaluate *H. truncatum* as a vector of the unidentified *Babesia* isolate.

This paper reports on the successful transovarial transmission of this *Babesia* with *H. truncatum* from adult to adult.

## MATERIALS AND METHODS

### Babesia *isolate*

During 1983, cattle, fully susceptible to *Babesia* and *Anaplasma* infections, were urgently required for live blood vaccine production. A group of approximately 6-month-old, cross-bred Bonsmara calves were obtained from Kaalplaas, where they had been bred under extensive ranching conditions. A strict, fortnightly tick control programme is followed, except during the winter months, May to July, when the animals are not dipped.

Before the calves were transferred to the laboratory, they were treated with cypermethrin[1], and then kept in quarantine under strict, tick-free conditions. Once in quarantine, sera from these calves were tested serologically for the presence of *Brucella* (Herr, Huchzermeyer, Te Brugge, Williamson, Roos & Schiele, 1985), bovine leukosis virus (BLV) (Bovine Leukaemia Antibody Test kit)[2], *B. bovis, B. bigemina, Theileria* spp. (Joyner, Donnelly, Payne & Brocklesby, 1972) and *Anaplasma* (Potgieter & Van Rensburg, 1983). Thick and thin blood smears were also examined.

Only calves that tested serologically negative for *Brucella*, BLV and harboured no blood parasites, as determined by blood smear examination and serology, were selected and splenectomized. Routine blood smear examinations, conducted for a period of 60 days after splenectomy, revealed no haemoparasitic relapses in these animals.

Before the calves were used for vaccine production, they were finally tested biologically. The calves were first treated with corticosteroids, according to the method of Callow & Parker (1969). One-hundred mℓ of blood was then collected from each of 32 calves, pooled into 3 batches from 10, 10 and 12 calves respectively, and subinoculated intravenously into 3 susceptible splenectomized cattle. Animal 9401, which received pooled blood from the group of 12 calves, contracted a *Babesia* infection. To identify the source of the infection a further subinoculation test was done, using pooled blood from smaller groups of the suspect group of 12 calves.

*Babesia* parasites were again identified on blood

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[1] Curatic cattle dip, Agricura

[2] Leukassay *B, Janssen

smears from only 1 animal (9430) that received pooled blood from 5 calves. On Day 9, blood was collected by venepuncture into 20 % acid citrate dextrose (ACD) and cryopreserved in liquid nitrogen, with 10 % DMSO as cryoprotectant, as a stabilate for further study.

*Experimental cattle*

All the laboratory animals used in this study were born and reared under strict, tick-free conditions in the stables of the Division of Protozoology at the VRI, Onderstepoort. These animals are splenectomized at approximately 6–8 months of age and used as donors for redwater vaccine. At the time of this experiment they were between 12 and 18 months of age.

*Tick strain, feeding and maintenance*

The Warrenton strain of *H. truncatum* (a two-host tick) used in this study was obtained from the Division of Entomology, VRI. It had been maintained in the laboratory since 1980 by feeding the larval-nymphal stages on rabbits and the adults on sheep (Heyne, Elliott & Bezuidenhout, 1987).

The non-feeding stages were maintained in an acaridarium at 25 °C and 80 % relative humidity.

*Experimental procedure*

To infect ticks with the *Babesia* isolate, Animal 9781 was infested on the left ear with 50 adult, *Babesia*-free (De Waal, unpublished observation, 1987) *H. truncatum* ticks on Day 0. At the same time it was infected intravenously with 5 mℓ of the *Babesia*-infected blood stabilate collected from Animal 9430.

Since it is known that corticosteroids suppress immunity in blood protozoal infections (Smidt & Squires, 1951; Singer 1954; Jackson, 1955; Sherman & Rubé, 1967; Callow & Parker, 1969), prednisilone[3], administered intramuscularly at a dose of 200 mg from Day 2 at 48 h intervals until death, was used in an attempt to increase the parasitaemia during tick engorgement.

Body temperature, packed cell volume (PCV) and blood smears were monitored and prepared as described by De Waal & Potgieter (1987).

The engorged female *H. truncatum* ticks, collected from Animal 9781, were placed in the acaridarium and allowed to lay eggs. Between Day 7 and Day 10 post-engorgement, the female ticks were screened for *Babesia* infection by microscopic examination of haemolymph smears, as described by Burgdorfer (1970).

Forty days after the eggs hatched the larval progeny of 3 female ticks was fed on a rabbit. The engorged nymphae were collected and allowed to moult in the acaridarium. Six months after moulting, 100 male and 100 female ticks were placed in a back pocket on Animal 9899. Blood smears, body temperature and PCV were monitored, as described previously. Engorged female ticks, collected from this animal, were again screened between Day 7 and Day 10 post-engorgement for *Babesia* infections.

## RESULTS

*Isolation*

Before splenectomy, all 32 calves were serologically negative for *B. bovis* and *B. bigemina,* and no haemoparasitic relapses could be identified during routine blood smear examinations of these animals for a period of 60 days after splenectomy.

Subinoculation of pooled blood, however, from 12 of these splenectomized calves to Animal 9401 resulted in a febrile reaction on Day 7 post-inoculation (p.i.) and *Babesia* parasites were identified in thick blood smears on Day 5 p.i. As it was thought that this parasite was probably *B. occultans,* the animal was treated on Day 8 p.i. with diminazene[4] to sterilize the infection (D. T. de Waal & M. P. Combrink, unpublished observations, 1986). Further subinoculation of pooled blood from 5 of these 12 calves to Animal 9430 again resulted in a febrile reaction on Day 7, *Babesia* parasites being visible in thick blood smears on Day 5. The animal was treated on Day 9 p.i. with euflavine[5], after blood was collected to prepare a blood stabilate. This was stored in liquid nitrogen. The parasitaemia at the time of treatment was 0,56 %.

*Infection of adult ticks*

The first *Babesia* parasites were detected on thick blood smears of Animal 9781 on Day 7 p.i. The parasitaemia rose quickly to reach a peak of 20 % on Day 12, when the animal died. On Day 10 p.i., the temperature rose to 41,5 °C and remained above normal till death. The PCV dropped on Day 11 p.i. to 17 % and was 8 % on Day 12, when the animal died.

The *H. truncatum* ticks engorged during a rising parasitaemia (from 1,8 % to 20 %). A total of 19 replete females were collected. No kinetes could be detected in the haemolymph smears of these ticks made between Day 7 and Day 10 post-engorgement.

*Transmission with adult ticks*

Out of the total of 200 *H. truncatum* adult ticks fed on Animal 9899, 47 engorged females were recovered between Day 9 and Day 14 post-tick infestation. A febrile reaction occurred on Day 9 after tick infestation and lasted for 8 days. The first parasites were seen in this animal in a thick blood smear on Day 10. The parasitaemia increased steadily over the next 7 days to reach a peak score of 2 (between 1–5 parasites in 100 microscope fields, with a uniform distribution of 350 erythrocytes) on Day 14, and thereafter it declined and the animal recovered without treatment. The highest temperature recorded was 41,6 °C on Day 14 p.i. The PCV fluctuated within normal limits (between 25–35 %).

*Babesia* kinetes were observed in 8 of the 47 female ticks collected from Animal 9899.

*Morphology of* Babesia

The appearance of this parasite in blood smears was that of a typical *Babesia,* with pairs of piriform merozoites and single parasites, varying in shape and size, occurring in erythrocytes (manuscript in preparation). Because of the low parasitaemias normally encountered in these infections it is difficult to distinguish, on morphological grounds alone, this parasite from the other large *Babesia (B. occultans* and *B. bigemina).* Even in the high parasitaemia blood smears from Animal 9781 the pleomorphic nature of this parasite was evident.

## DISCUSSION

The farm Kaalplaas, where these animals originated, is situated in an endemic area for babesiosis and anaplasmosis. The cattle on Kaalplaas are maintained under a minimal disease situation. No epidemics of tick-borne diseases so as have occurred on

[3] Delta-cortril, Pfizer

[4] Berenil, Hoechst

[5] Euflavine, Centaur

this farm for many years and the animals are not vaccinated against tick-borne diseases so as to serve as a potential source of susceptible cattle for experimental purposes and quality control of certain other vaccines.

The calves that were selected were screened on blood smear examination and serological tests for haemoparasites, before being splenectomized. Serological screening was performed at a $^{1}/_{80}$ dilution against *B. bovis* and *B. bigemina* antigens only. However, after splenectomy, which should have resulted in a recrudescence of any latent *Babesia* infection (Riek, 1963), all the animals remained negative on regular blood smear examination for a period of 60 days. Only after biological testing of these animals did it come to light that 1 or more of them haboured a *Babesia* infection. Retrospectively, it was later confirmed, with the IFA test using *B. occultans* antigen, that 2 animals had positive titres to *B. occultans*. Serological cross-reactions do occur between the *Babesia* isolate and *B. occultans* (De Waal, Combrink & Potgieter, 1989). Low pathogenicity, low parasitaemia levels and the fact that blood smears were examined only 3 times per week, could have caused recrudescent parasitaemias to escape detection in these 2 animals after splenectomy.

Excluding the 2 animals that were treated early in the infection during the biological test, this parasite caused very mild reactions and low parasitaemias in splenectomized animals.

The failure of repeated attempts to transmit this *Babesia* isolate with *H. m. rufipes*, the vector of *B. occultans*, may have been due to the fact that the parasitaemias were too low to infect the ticks. It was therefore decided to further suppress the animals' immunity with corticosteroids during the tick feeding in subsequent attempts. This proved to be successful as a maximum parasitaemia of 20 % was produced when *H. truncatum* was tested as a possible vector.

In spite of the high parasitaemia, no *Babesia* kinetes could be identified in the adult female *H. truncatum* ticks, but their ensuing adult progeny were able to transmit the infection. It has, however, been reported elsewhere that the demonstration of *Babesia* kinetes in the haemolymph of engorged female ticks cannot be regarded as an accurate method of demonstrating *Babesia* infection in ticks (Mahoney & Mirre, 1971).

The tick vector, *H. truncatum*, is widely distributed in this country. It occurs throughout the northern and western parts of the Republic of South Africa (RSA). In the northern part of Kwazulu and the adjoining lower lying areas of Swaziland it occurs in areas of drier climatic conditions. It is absent in the higher lying and more humid areas of the Cape Midlands, Eastern Cape, Orange Free State, Transvaal, Natal, Swaziland and the entire Lesotho (Theiler, 1956).

The host range of the adult ticks include cattle, horses, sheep and goats, as well as some of the larger game species. The larval-nymphal stages are found on various bird species and to a lesser extent on hares and rodents (Howell *et al.*, 1978).

Other diseases transmitted by this tick species include; sweating sickness in cattle, sheep and pigs (Neitz, 1959), babesiosis *(Babesia caballi* infection) in horses (De Waal, 1990) and Crimean-Congo haemorrhagic fever in humans (Swanepoel, Shepherd, Leman, Shepherd McGillivray, Erasmus, Scarle & Gill, 1987).

Gray & De Vos (1981) reported the presence of *B. occultans* in a serological survey on 5 out of 6 farms which fall well outside the recognized area of distribution of the only known vector, *H. m. rufipes*. They concluded that another possible vector could exist, such as *H. truncatum*. Considering the serological cross-reaction between this *Babesia* isolate and *B. occultans*, it is possible that the antibodies they demonstrated were against the former and not against *B. occultans*. The presence of *H. truncatum* was confirmed on all 5 of the positive farms mentioned above.

This parasite shares many morphological features with other bovine *Babesia* spp., but it does differ with regard to serology, virulence and vector specificity. This indicates that it might be a hitherto unrecognized bovine *Babesia* sp. Further attention will now be given to the serological cross-reactions between the different *Babesia* spp., immunological cross protection between species, as well as the relationship of *B. occultans* and this *Babesia* sp. to *Babesia beliceri* which is transmitted by *Hyalomma anatolicum* in Russia.

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