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

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ABSTRACT


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 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 Haakdoomboom, 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extent, 4 attempts to transmit this infection transovarially with H. m. rufipes failed (T. E. Mason & F. T. Potgieter unpublished observations, 1984).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 eversi eversi 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 a farm near 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 cypermethrin¹, 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 kit²), B. bovis, B. bigemina, Theliera spp. (Joyner, Donnelly, Payne & Brocklesby, 1975) 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

¹ Curative cattle dip, Agricura
² Leukassay* E, 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 stabili­ate for further study.

**Experimental animal**

All the laboratory animals used in this study were born and reared under strict, tick-free conditions in the stable of the Division of Protozoology at the VRI, Onderstepoort. These animals were 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 Depart­ment 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 stimulate collected from Animal 9430.

Since it is known that corticosteroids suppress immunity in blood protozoal infections (Smitd & Squires, 1951; Singer 1954; Jackson, 1955; Sherman & Rubé, 1967; Callow & Parker, 1969), predni­s-lone, 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 nymphs were collected and allowed to moult in the acaridarium. Six months after moult­ing, 100 male and 100 female ticks were placed in a back­pocket 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-infec­tion (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 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 efluvaine, after blood was collected to prepare a blood stimulate. This was stored in liquid nitrogen. The parasitaemia at the time of treatment was 0.56 %.

**Injection 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 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* kinetotes 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 *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
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 $1/160$ 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 harboured 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 recrudescence 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-reactions 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 $Babesia belicerae$ which is transmitted by $Hyalomma anatolicum$ in Russia.

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