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THE RETENTION OF *BABESIA BIGEMINA* INFECTION BY *BOOPHILUS DECOLORATUS* EXPOSED TO IMIDOCARB DIPROPIONATE DURING ENGORGEMENT

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ABSTRACT

GRAY, J. S. & POTGIETER, F. T., 1981. The retention of *Babesia bigemina* infections by *Boophilus decoloratus* exposed to imidocarb dipropionate during engorgement. *Onderstepoort Journal of Veterinary Research*, 48, 225–227 (1981).

Babesia bigemina was retained in the vector *Boophilus decoloratus* for a complete generation despite the use of the babesicide, imidocarp dipropionate, to prevent reinfection. This drug did not sterilize ticks of the *B. bigemina* infection as has been suggested for *B. bovis*.

Résumé

LA RÉTENTION DE L'INFECTION À BABESIA BIGEMINA *PAR* BOOPHILUS DECOLORATUS *SOUMIS AU DIPROPIONATE D'IMIDOCARB PENDANT L'ENGORGEMENT*

Babesia bigemina *a été retenu dans le vecteur* Boophilus decoloratus *pendant une génération complète malgré l'utilisation du babesicide, dipropionate d'imidocarb, pour prévenir la ré-infection. Cette drogue ne stérilisa pas les tiques de l'infection à* B. bigemina *comme cela avait été suggéré pour* B. bovis.

INTRODUCTION

Transovarial transmission of *Babesia bigemina* in *Boophilus decoloratus* was first demonstrated by Theiler (1905) and confirmed in later experiments (Theiler, 1907). In further work Theiler (1909) showed that the infection was apparently retained by the ticks even when they were fed on horses, which are not susceptible to *B. bigemina*. Similar studies were carried out by Callow (1965), who showed that *Boophilus microplus* retained *B. bigemina* when fed on sheep and horses. Callow (1965) did not rule out the possibility of reinfection and in fact an intra-erythrocytic stage of *B. bigemina* was seen in a splenectomized sheep. The suggestion has also been made that reinfection of engorging females from their own salivary secretions may take place (Callow, 1979). However, Potgieter & Els (1977) suggested that transovarial transmission without reinfection may occur, since large merozoites (vermicules) of *B. bigemina* were found in the haemolymph of ovipositing female *B. decoloratus* that had engorged on animals after the *B. bigemina* infections they had initiated had apparently been sterilized by therapy. In the present study this work has been extended by feeding *B. bigemina*-infected ticks on an animal that had been treated with imidocarb dipropionate.* The possibility that this drug might sterilize ticks of their *Babesia* infections, as suggested by Kuttler, Graham & Trevino (1975) and Brocklesby (1979), was also investigated.

MATERIALS AND METHODS

B. decoloratus *strain*

The tick strain used in this study was originally obtained from Pongola, northern Natal, and was found to be infected with *B. bigemina*. Uninfected ticks were obtained from this strain (Potgieter, 1977), and have since been maintained as such in the laboratory.

The non-parasitic stages of the tick were kept in an acaridarium at 25 °C and 85% RH.

B. bigemina *strain*

The *B. bigemina* strain used was originally carried by *B. decoloratus* from Pongola as described above. The particular parasites used in this study had only undergone one syringe passage since the last time they had been transmitted by ticks.

Experimental animals

The cattle used in this study were *Bos taurus* breeds born and reared under tick-free conditions. They were all splenectomized when 4–10 months old. Rectal temperatures and thick and thin blood smears, subsequently stained with Giemsa, were taken daily.

Infestation of cattle with ticks

The standard procedure used in this laboratory was followed. Small cotton wool stoppered tubes containing *B. decoloratus* larvae were attached with Unna's paste to the animal's back in the shoulder region (Potgieter, 1977) and then the neck, back and sides were covered with a piece of cloth (50 × 50 cm). This was also attached with Unna's paste. The tube plugs were removed after the paste had dried.

Detection of B. bigemina *infection in ticks*

Engorged female ticks from each infestation were screened for infection with *B. bigemina* 10 days after engorgement by amputating the right or left second leg and smearing the drop of haemolymph that exuded onto a glass slide. These smears were air-dried, fixed in methanol, stained with Giemsa and examined for vermicules or large merozoites (LM) of *B. bigemina*.

Experimental procedure

Infection of ticks. A splenectomized 15-month-old ox was infested with the larval progeny of 9 uninfected female *B. decoloratus*. On Day 18 after infestation 5 mℓ of frozen *B. bigemina*-infected blood, which had been stored over liquid nitrogen with 10% dimethyl sulphoxide as cryoprotectant, was thawed at room temperature and injected intravenously into the animal. Engorged female *B. decoloratus* dropped from Day 19–26 and those that survived to oviposition were screened for infection with *B. bigemina*. Those that were found to be infected were put to one side and allowed to oviposit. The remainder were destroyed.

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Infestation of imidocarb-treated animal. A 19-month-old splenectomized ox was infested with the larval progeny of 6 female *B. decoloratus* that had been shown to have LM of *B. bigemina* in their haemolymph. This animal had been used for *B. bigemina* and *Babesia bovis* vaccine production and had received 5 mg/kg imidocarb dipropionate intramuscularly 2 weeks before infestation for the sterilization of any remaining *Babesia* infection. Another 3 mg/kg of this drug was administered the day before infestation with ticks to ensure that the animal remained insusceptible to infection with *B. bigemina* throughout the period of infestation. Imidocarb dipropionate protects against clinical infections of *B. bigemina* for up to 12 weeks (Callow & McGregor, 1970), and Taylor & McHardy (1979) showed that 3 mg/kg prevented establishment of *B. bigemina* for at least 21 days after treatment.

The engorged females that resulted from this infestation were collected and allowed to oviposit in the acaridarium.

To ensure that no transmission of *B. bigemina* to this animal had taken place 500 mℓ of fresh blood was subinoculated intravenously into a susceptible ox on Day 34 after infestation with infected ticks.

Transmission of B. bigemina *with progeny of infected ticks*

A 7-month-old susceptible calf was infested with larvae from 6 infected female ticks. These larvae were from the same batch of larvae that were used to infest the imidocarb-treated animal and were used to prove that these ticks were capable of transmission.

Transmission of B. bigemina *with progeny of imidocarb-exposed ticks*

A 7-month-old susceptible calf was infested with the larval progeny of 10 female *B. decoloratus* that had engorged on the imidocarb-treated animal to determine whether they had retained the infection with *B. bigemina* in the absence of reinfection.

Results

Infection of ticks

Infestation of imidocarb-treated animal with B. bigemina *infected larvae.* No *B. bigemina* were seen in thick or thin blood smears of this animal at any stage. Examination of blood smears was discontinued 60 days after infestation with *B. decoloratus* larvae. No temperature reaction was observed during this period.

Furthermore, the animal that received 500 mℓ of blood from the imidocarb-treated ox on Day 34 after infestation, showed no temperature reaction, and no parasites were detected in blood smears that were examined for 24 days.

A total of 223 engorged female *B. decoloratus* were collected 24–33 days after infestation and of these 28,2% were found to have LM of *B. bigemina* in their haemolymph.

Transmission of B. bigemina *with progeny of infected ticks*

The larvae fed on the non-treated animal transmitted *B. bigemina* successfully. The parasites were first seen in thick blood smears on Day 23 after infestation.

The infection was terminated on Day 24, when the parasitaemia had reached 0,1%, with 3,5 mg/kg diminazene*. One hundred and fifty engorged female *B. decoloratus* were collected 24–28 days after infestation and 32,9% of these were found to be infected with LM of *B. bigemina.*

Tick-transmission by progeny of imidocarb-exposed ticks

B. bigemina was successfully transmitted to a susceptible calf by larval progeny of the ticks that dropped from the imidocarb-treated animal. The prepatent period was 26 days after infestation.

The infection was terminated with 3,5 mg/kg diminazene on Day 29, when the parasitaemia was 1,2%.

Discussion

This study has shown that *B. bigemina* is retained by the vector, *B. decoloratus,* for at least one generation without reinfection by intra-erythrocytic stages of the parasite. There remains the possibility that the ticks may have reinfected themselves from their own salivary secretions as suggested by Callow (1979). This possibility cannot be ruled out at this stage, but the probability that the babesicide, imidocarb dipropionate, was ingested with the blood by the ticks may well have resulted in the death of any parasites in the lumen of the tick.

The suggestion that, as in the case of *B. ovis* in *Rhipicephalus bursa*, the presence of LM in the haemolymph indicates that the infection has been acquired by that particular tick generation (Buscher, 1975) does not seem to apply to *B. bigemina* in *B. decoloratus.* The study of Potgieter & Els (1977) showed that LM of *B. bigemina* were formed in all 3 stages of the tick through successive cycles of schizogony, and it is apparent from this work that the infection could be transmitted transovarially without reinfection from the bovine. The present study seems to confirm this suggestion. It remains to be seen whether the level of infection persists in the absence of reinfection in successive tick generations or whether it declines to zero.

The proportion of engorged female *B. decoloratus* that dropped from the imidocarb-treated animal and proved to be positive for LM of *B. bigemina* in the haemolymph was only slightly less than the proportion of LM-positive females from the animal in which intra-erythrocytic stages of *B. bigemina* was demonstrated and which presented an opportunity for reinfection.

This study has also demonstrated that imidocarb, given at the recommended prophylactic dose of 3 mg/kg, does not seem to sterilize *B. decoloratus* of an existing *B. bigemina* infection as has been suggested by Kuttler *et al.* (1975) for *B. bovis* and possibly *B. bigemina.* It must be borne in mind, however, that it appears as if *B. bovis* does not persist in an infective form beyond the larval stage of *B. microplus* (Potgieter, 1977; Mahoney & Mirre, 1979). This implies that engorging adult females must acquire the infection in each generation from an infected bovine to complete their life cycle in order to infect the larvae of the next generation. This basic difference in the life cycles of *B. bovis* and *B. bigemina* must be considered an important aspect in epidemiological studies and disease control.

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We concluded that it is not possible to eliminate a *B. bigemina* infection in a population of *B. decoloratus* in the short term by treating cattle prophylactically with imidocarb dipropionate. It is not known at this stage whether long-term prophylactic use of this drug in a cattle herd would result in a decline in the *B. bigemina* infection rate of the *B. decoloratus* populaton.

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