

STUDIES ON THE INFECTIVITY OF *BOOPHILUS DECOLORATUS* MALES AND LARVAE INFECTED WITH *BABESIA BIGEMINA*

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ABSTRACT

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Babesia bigemina was transmitted by male *Boophilus decoloratus* and also by intravenous inoculation of a homogenate prepared from infected incubated larval ticks.

INTRODUCTION

It has been known for some time that *Babesia bigemina* is transmitted transovarially by *Boophilus microplus* and *B. decoloratus* and that the nymphae and adults of the following generation transmit the infection to cattle (Riek, 1964; Callow, 1968; Morzaria, Young & Hudson, 1977; Potgieter & Els, 1977). The parasite, it would seem, is not sufficiently developed for transmission to occur during the larval stage of these 2 tick species and, in the past, transmission by the male was not really considered important.

Recent work in Australia has shown that artificial transmission can be achieved with parasites obtained from eggs and larvae of *B. microplus* (Dalglish & Stewart, 1978), and also that male *B. microplus* are capable of transmitting *B. bigemina* naturally (Dalglish, Stewart & Callow, 1978).

The purpose of the present study was to determine whether these findings also apply to *B. decoloratus*, the main vector of *B. bigemina* in South Africa.

MATERIALS AND METHODS

B. decoloratus and *B. bigemina* strains

The *B. decoloratus* and *B. bigemina* strains used in this study have been described by Gray & Potgieter (1981).

Experimental animals

The cattle used for the study involving male *B. decoloratus* were born and reared under tick-free conditions and had been splenectomized when 4-10 months old. The cattle used for the other study, the transmission of parasites derived from larvae, were intact calves obtained from an experimental farm. They were treated with an acaricide* once a week until brought into tick-free stables for experimentation. All were found to be serologically negative for *B. bigemina* and *B. bovis* by the indirect fluorescent antibody (IFA) test, as described by Gray & De Vos (1981), prior to their being used in this experiment.

Rectal temperatures were determined daily and temperatures below 39,5 °C were considered to be normal. Thick and thin blood smears were made daily from blood obtained from the tip of the tail and stained with Giemsa's stain. Thick blood smears (Mahoney & Saal, 1961) were examined to determine prepatent periods and thin blood smears to determine percentage parasitaemias.

Feeding and maintenance of ticks

In these studies ticks were confined by double-layered cloth containers (back pockets) about 90 mm in diameter attached to clipped areas on the animal's neck, back or rump with a contact adhesive.** The tubes containing the ticks were placed in the inner layer of the back pocket, the top of which was then fastened with a rubber

castration ring. The cotton wool bungs of the tubes were removed with a needle through the wall of the back pocket. The non-parasitic stages of the ticks were maintained in an acaridarium at 25 °C and 85% R.H.

Transmission of *B. bigemina* by male ticks

Infection of ticks. Haemolymph smears of engorged females from a clean strain of *B. decoloratus* which had fed on an ox showing a patent *B. bigemina* parasitaemia were examined for the presence of vermicles (Gray & Potgieter, 1981). The larval progeny of 6 of these infected females were used to infect an 8-month-old susceptible splenectomized calf, following the method described by Gray & Potgieter (1981). When the engorged females started to drop from this calf 23 days later, 100 male *B. decoloratus* were removed manually. The ticks were carefully sorted to exclude any female ticks and then kept overnight in tubes in the acaridarium. At this stage the animal had a patent *B. bigemina* parasitaemia of <0,005%.

Intrastadial transfer of male ticks

The day after the collection of the ticks a 7-month-old splenectomized calf was infested with *B. decoloratus* males. Approximately 50 males were placed in a back pocket on the rump of the animal and the remaining 50 were placed, unconfined, on the back of the neck.

Transmission of *B. bigemina* with incubated larvae

Infectivity of larval homogenate. The eggs from 25 female *B. decoloratus* infected with *B. bigemina* were sieved, mixed, and divided into aliquots of 0,2 g. Three weeks after the first larvae hatched, 4 tubes of unfed larvae were incubated for 7 days at 37 °C. Two tubes of those containing the incubated larvae were then triturated, following the method of Potgieter & Van Vuuren (1974), and 2 ml of freshly prepared larval homogenate was injected intravenously into a calf.

Infectivity of larvae. The remaining 2 tubes of incubated larvae, placed respectively in 2 pockets attached to the neck of another calf, were used to test whether they would be able to transmit the infection naturally.

The animal was washed with water-soluble pyrethrin* on Day 7 after infestation to kill the larvae before they moulted to nymphae.

RESULTS

Transmission by male *B. decoloratus*

One day after infestation, 47 male *B. decoloratus* were found to have attached to the host in the back pocket. It is assumed that a similar number of the unconfined ticks had also attached. On Day 6 after infestation the animal showed a temperature of 39,5 °C and the following day, when the temperature was 40,0 °C, *B. bigemina* parasites were detected in a thick blood smear. The parasitaemia had reached a level of 1,2% on Day 9, when the animal was treated with 3,5 mg/kg dimazine**.

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* Supamix, Coopers, RSA

** Genkem Ank 508, G.C.C., RSA

* AVI C & B concentrate special, AVIMA

** Berenil, Hoechst

Transmission by incubated larvae

Infectivity of larval homogenate. *B. bigemina* parasites were detected on Day 7 in a thick blood smear of the calf that received the larval homogenate. The calf was treated with 5 mg/kg diminazene on Day 8 when the parasitaemia was 0,4%.

Infectivity of incubated larvae. Although most of the larvae were alive at the time of infestation, none were observed to have attached on Day 7. The animal did not become infected with *B. bigemina*.

DISCUSSION

Dalgliesh *et al.* (1978) demonstrated that *B. microplus* males that acquired *B. bigemina* transovarially were capable of transmitting the infection to cattle. Similar results were obtained in this study with *B. decoloratus* males which lends support to the suggestion made by Dalgliesh *et al.* (1978) that male ticks may play a role in the spread of *B. bigemina* infection.

B. microplus males are known to live up to 70 days (Seddon, 1951 cited by Dalgliesh *et al.*, 1978) and *B. decoloratus* males can survive for at least 37 days on the host (Londt, 1976).

Recently, Mason & Norval (1981) were able to demonstrate the natural transfer of larvae and adult males of *B. microplus* from infested to uninfested cattle under field conditions. Such evidence is lacking in the case of *B. decoloratus*, but, since the male ticks of both species probably behave alike, *B. decoloratus* males may thus play a role in the transmission of *B. bigemina*.

B. bigemina is naturally transmitted by the nymphal and adult stages of *B. decoloratus* (Potgieter, 1977). Stabilates prepared from pre-fed infected *B. decoloratus* nymphae have been shown to be infective to cattle when inoculated intravenously (Morzaria *et al.*, 1977; Potgieter, 1977). Morzaria *et al.* (1977) were unable to infect cattle using stabilates derived from pre-fed infected *B. decoloratus* larvae.

It was always thought that *Babesia* parasites in ticks required the stimulus of feeding to become infective for cattle. However, Dalgliesh & Stewart (1976; 1978) found that infective *B. bovis* and *B. bigemina* parasites can be extracted from *B. microplus* eggs and unfed larvae incubated at 37 °C.

A life cycle study of *B. bigemina* in *B. decoloratus* indicated that the infective forms do not appear to mature until the ticks have reached the nymphal stage (Potgieter, 1977). However, the results obtained in this study with *B. decoloratus* confirm those reported by Dalgliesh & Stewart (1978) with *B. microplus* that high temperatures may be the only stimulus required for the development of infective parasites in the tick.

In the control experiment the incubated larvae failed to engorge on the calf and it is thought that the exposure to the high temperature for 7 days was too long. Apparently, all the larvae died soon after infestation and it is not clear whether any attached at all. It is interesting to note that Dalgliesh & Stewart (1978) were similarly unable to prepare an infective extract from *B. microplus* larvae that were also kept at 37 °C for 7 days and that positive results were only obtained with extracts of larvae incubated for 4 or 5 days.

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