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THE INABILITY OF A SOUTH AFRICAN *BABESIA OVIS* VACCINE STRAIN TO INFECT *BOOPHILUS MICROPLUS*

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ABSTRACT

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A strain of *Babesia bovis* that had been attenuated by rapid syringe passage through a series of 23 splenectomized calves was unable to infect its vector *Boophilus microplus*. An attempt to transmit the attenuated Australian *Babesia bigemina* G strain with a South African strain of *B. microplus* was likewise unsuccessful. The epidemiological implication of these observations in terms of babesiosis control is discussed.

INTRODUCTION

Bovine babesiosis is of economic importance in areas of the Republic of South Africa in which the 2 vector ticks *Boophilus decoloratus* and *Boophilus microplus* occur. The disease is controlled by the use of a blood vaccine containing both *Babesia bigemina* and *Babesia bovis* parasites.

Progress has been made as far as the virulence of the current vaccine strains is concerned. The virulence of the *B. bovis* S strain was reduced through rapid needle passage in splenectomized calves (De Vos, Bessenger & Fourie, 1982). Three slow needle passages of a local *B. bigemina* strain in intact calves failed to show a reduction in virulence and consequently the highly passaged attenuated Australian *B. bigemina* G strain (Dalgliesh, Callow, Mellors & McGregor, 1981) was imported. Its efficacy as a possible vaccine strain was tested in the laboratory before it was incorporated into the Onderstepoort babesiosis vaccine (De Vos, Combrink & Bessenger, 1982). Blood-passaged *Babesia* strains show reduction or loss of their infectivity to ticks (O'Sullivan & Callow, 1966; Dalgliesh & Stewart, 1977; Stewart, 1978; Dalgliesh, Stewart & Rodwell, 1981).

The purpose of this study was to investigate the transmissibility of the South African S strain of *B. bovis* (De Vos, 1978) and the imported Australian *B. bigemina* G strain, both currently used in the vaccine, with a local strain of *B. microplus*.

MATERIALS AND METHODS

Animals

The cattle used in this study were all cross-bred (Africander × Hereford) and susceptible to *Babesia* spp. They were reared and maintained under tick-free conditions.

Thick blood smears (Mahoney & Saal, 1961) and thin blood smears were made daily from 1 week prior to tick infestation to 1 month after tick drop. The smears were fixed in methanol, stained with 10 % Giemsa stain for 30 min and examined for *Babesia* parasites with a light microscope.

Ticks

A non-infected *B. microplus* strain that had been maintained thus for several years was used. The non-parasitic stages were kept in an acaridarium at 26 °C and 85 % relative humidity.

Engorged females were collected from the floor of the pens at 10h00 daily. They were washed, counted, placed individually in glass vials stoppered with cotton wool and transferred to the acaridarium. Nine to thirteen days after collection haemolymph smears were prepared as described by Burgdorfer (1970), fixed in methanol and then stained with 10 % Giemsa stain for 30 min. Only those ticks that had been collected when the host had a patent parasitaemia were examined for tick stages of the parasite (vermicules).

Strains of Babesia bovis

(a) *Field Strain*

The F strain (De Vos, 1978) was isolated by infesting cattle with *B. bovis*-infected ticks collected in the Eshowe District, Natal. Their larval progeny were fed on a bovine for 72 h and then removed. A tick stabilate was prepared according to the methods of Potgieter & Van Vuuren (1974). This material has been stored in the gas phase of a liquid nitrogen refrigerator since 1973.

(b) *Vaccine Strain*

The S strain (De Vos, 1978) was passaged rapidly 10 times in splenectomized calves (De Vos, Bessenger & Fourie, 1982) and then passaged rapidly a further 13 times in splenectomized calves (M. P. Combrink, personal communication, 1985). Blood containing organisms of this 23rd passage was collected and a stabilate prepared according to the method of De Vos, Combrink & Bessenger (1982).

Strains of Babesia bigemina

(a) *Field Strain*

The P strain was isolated by infesting cattle with *B. bigemina*-infected *Boophilus decoloratus* collected in the Pongola District, Natal. The larval progeny of infected female ticks were fed on a calf. From Day 14–Day 19 post infestation, a total of ± 300 nymphae and adults were removed. Stabilates were prepared from these ticks according to the method of Potgieter & Van Vuuren (1974). This material has been stored in the gas phase of a liquid nitrogen refrigerator for 10 years.

(b) *Vaccine Strain*

The Australian G strain (Dalgliesh, Callow, Mellors & McGregor, 1981) was imported in 1981 for the purpose of vaccine production. On receipt, the blood was inoculated into an intact calf. Splenectomy of this calf 12 weeks later was followed by a very mild parasitaemic relapse. As the number of parasites/mℓ was too low for stabilate production the parasite was further passaged in a splenectomized calf and a stabilate prepared when the calf reacted (De Vos, Combrink & Bessenger, 1982).

Infection of ticks

Four cattle were each infested with ± 24 000 larvae over a period of 6 days and then infected intravenously with the different *Babesia* stabilates, as indicated in Table 1.

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Tick transmission

Larvae bred from females which had engorged at the time the 4 animals (Table 1) were showing patent parasitaemias were used to infest 4 other susceptible oxen (Table 2). Each of these animals was also infested with approximately 24 000 larvae.

RESULTS

It is clear from the results summarized in Tables 1 & 2 that normal infection and transmission occurred with *B. microplus* and the 2 field strains (F & P strains). However, the local *B. bovis* S strain, which had been rapidly passaged in splenectomized calves, as well as the Australian *B. bigemina* G strain, which had been slowly passaged in intact cattle, failed to complete their life-cycles in the same strain and batch of *B. microplus* that transmitted the unmodified field strains under the same conditions.

TABLE 1 Infection of *B. microplus* with vaccine and field strains of *B. bovis* and *B. bigemina*

Animal No. *Babesia* spp. and strain	Infection with *Babesia* spp. stabilate: Days post larval infestation	Parasitaemia during collection of engorged females (% parasitized erythrocytes)	No. of infected ticks as determined by haemolymph examination
9387/5 *B. bovis* (S strain)	5 mℓ blood stabilate: Day 18	0,1–1,9	0/248
9513/7 *B. bigemina* (G strain)	5 mℓ blood stabilate: Day 18	0,1–56,8	0/116
9509/8 *B. bovis* (F strain)	1 mℓ tick stabilate: Day 22	0,1–0,3	64/107
9481/9 *B. bigemina* (P strain)	1 mℓ tick stabilate: Day 15	0,1–48,0	18/36

TABLE 2 Transmission of *B. bovis* and *B. bigemina* by *B. microplus*

Animal No.	*Babesia* spp.	Total No. of engorged females collected	Parasitaemia (% parasitized erythrocytes)
9479/9	*B. bovis* (S strain)	120	None
9589/3	*B. bigemina* (G strain)	1 071	None
9537/9*	*B. bovis* (F strain)	None	Day 18: 0,1– Day 21: 12,0
9581/0	*B. bigemina* (P strain)	388	Day 18: 0,1– Day 32: 2,5

* Animal died on Day 23

DISCUSSION

Hoyte (1965) and Callow (1968) found that the last day or 2 that the tick spends on the host constitute the most important period for the acquisition of the infection. Hoyte (1965) also suggested that there is a direct correlation between the rate of infection of the ticks and the host's parasitaemia. In the present study patent parasitaemia and engorged female tick drop were synchronized to ensure optimum conditions for tick infection.

Australian workers found that they were unable to transmit needle-passaged strains of *B. bovis* in intact and splenectomized cattle with *B. microplus* (O'Sullivan & Callow, 1966; Stewart, 1978; Dalgliesh & Stewart, 1977). Stewart (1978) studied the differences in the life-cycles of a repeated needle passaged *B. bovis* and an unmodified strain and came to the conclusion that continuous blood passaging may result in the selection of parasites incapable of penetrating the gut epithelial cells of the tick.

Similar results were obtained with the *B. bigemina* strain by Dalgliesh, Stewart & Rodwell (1981) when they demonstrated a markedly reduced proportion of ticks infected with this slow passaged strain. Although the observations in the present study are limited, our results support the Australian findings.

Dalgliesh & Stewart (1977) went further to demonstrate that 2 needle-passaged vaccine strains of *B. bovis* did not regain their infectivity to ticks during longstanding infections in cattle, as suggested by Mahoney (1974).

It is concluded that *Babesia* strains modified through needle passage may show either reduced or complete loss of tick infectivity. However, the mechanisms involved in the latter phenomenon are not clearly understood at this stage.

The possible epizootiological implications of issuing a vaccine that is not tick transmissible should be considered. In enzootic areas where the vector ticks are established such a vaccine offers no advantage. On the contrary, continuous use of such a vaccine could lead to lowered infection rates in tick populations and this would result in a reduction of the tick challenge (superinfection of vaccinates) which is thought to be essential for maintaining immunity in herds (O'Sullivan & Callow, 1966). The latter is especially true in the case of *B. bigemina* infections (Löhr, 1972). In this regard it would be prudent to study the actual duration of immunity after a single vaccination in the absence of tick challenge for both species under local conditions.

The use of a tick transmissible vaccine in enzootic areas would probably be far more effective for the creation of enzootic stability by maintaining or increasing the infection rates in tick populations.

When isolated babesiosis outbreaks occur sporadically in epizootic areas and vaccination is indicated, a non tick-transmissible vaccine would be ideal as it would not promote the infection of ticks. It would therefore reduce the possibility of spreading the disease.

If evidence of loss of immunity in vaccinated herds is forthcoming the influence of the loss of infectivity to ticks of the *Babesia* vaccine strains should be considered as a possible contributing factor.

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