

RESEARCH COMMUNICATION

THE TRANSTADIAL TRANSMISSION OF *BABESIA CABALLI* BY *RHIPICEPHALUS EVERTSI EVERTSI*

D. T. DE WAAL and F. T. POTGIETER, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

DE WAAL, D. T. & POTGIETER, F. T., 1987. The transstadial transmission of *Babesia caballi* by *Rhipicephalus evertsi evertsi*. *Onderstepoort Journal of Veterinary Research*, 54, 655-656 (1987).

Rhipicephalus evertsi evertsi larvae were fed on the ears of rabbits. Seven days after larval infestation, unfed, newly moulted nymphae were manually removed to infest a splenectomized donkey showing a patent *Babesia caballi* infection. Engorged nymphae were collected from the donkey and the ensuing adult ticks were placed on a susceptible horse. The horse contracted a *B. caballi* infection showing a prepatent period of 19 days after tick infestation. A very low parasitaemia, (highest score 2), which was patent for only 10 days, was recorded. The lowest packed cell volume recorded was 16 %.

INTRODUCTION

Babesia equi was first described as "anthrax fever" in South Africa in 1883 by Wiltshire (1883, cited by Henning, 1949), and Theiler (1901; 1902) was first to demonstrate that equine piroplasmosis and horse sickness were different disease entities.

Although equine babesiosis is widespread in the Republic of South Africa (RSA), very little is known about its epidemiology. Both *B. equi* and *B. caballi* occur in the RSA, but *B. equi* is thought to be more widespread (Henning, 1949; Littlejohn, 1963) and is more commonly diagnosed in clinical cases.

No record of the first description of *B. caballi* in South Africa could be found, apart from a casual reference by De Kock (1920) to the presence of *B. caballi* in the blood of some of his experimental horses. Even today very little is known in this country about the distribution and importance of *B. caballi*, mainly because most field reports do not include a specific diagnosis when clinical cases are recorded.

Horses, donkeys and mules are all susceptible to both forms of the disease (Theiler, 1905; 1906a), and, *B. equi* has been reported from zebra (*Equus burchelli*) (Neitz, 1933).

Twelve species of ixodid ticks in the genera *Dermacentor*, *Rhipicephalus* and *Hyalomma* have been identified as transstadial vectors of *B. caballi* and *B. equi*, while 8 of these species were also able to transmit *B. caballi* infections trans-ovarially (Neitz, 1956; Stiller & Frerichs, 1979; Stiller, Frerichs, Leatch & Kutler, 1980).

The transstadial transmission of *B. equi* from the immature to the adult stage by the two-host tick, *R. e. evertsi*, was reported by Theiler (1906b). Circumstantial evidence indicated that *R. e. evertsi* may also transmit *B. caballi*, but it has never been shown in the laboratory.

This paper reports on the transstadial transmission of *B. caballi* by *R. e. evertsi*, one of the most common and most widespread ticks found on horses in this country.

MATERIALS AND METHODS

Experimental animals

The animals used in this study tested negative for the presence of antibodies against *B. caballi* and *B. equi* with the indirect fluorescent antibody (IFA) test. They included a 20-month-old splenectomized donkey and a 12-month-old intact horse, both born and reared under strict tick-free conditions at this laboratory.

Babesia caballi

The K-isolate of *B. caballi* was made at Onderstepoort

from a naturally infected horse (De Waal, Van Heerden, Van den Berg, Stegman & Potgieter, 1987).

R. e. evertsi feeding and maintenance

The Mkuze strain of *R. e. evertsi* has been maintained in the laboratory for several generations by feeding all stages of this two-host tick on the ears of rabbits, using the methods of Neitz, Boughton & Walters (1971). For the purpose of this investigation, the nymphal and adult stages were fed on the shoulder region of the horse and donkey under cloth patches attached to the hair with contact adhesive.¹

The non-feeding stages of the ticks were maintained in an acaridarium at 25 °C and 85 % relative humidity.

Quantification of Babesia caballi reactions

Clinical signs: Clinical signs of disease, rectal temperatures and haematocrit determinations were taken daily between 08h00 and 10h00.

Parasitaemia: Thick blood smears (Mahoney & Saal, 1961) as well as thin blood smears were made daily for at least 7 days from the date of infection until parasites were no longer detectable in the thick smears. Smears were prepared from the tip of the tail and air-dried. The thin smears were fixed in methanol and both thick and thin smears stained with 10 % Giemsa for 35 min. Parasites in thick blood smears were not quantified, but their presence was used to determine prepatent periods and the duration of patent parasitaemias. Since the parasitaemias recorded were very low (< 0,005 %), the following scoring system was used (Timms, Dalglish, Barry, Dimmock & Rodwell, 1983).

- Parasites detected in thick blood smear only = 1
- 1-5 parasites in 100 microscope fields, with a uniform distribution of approximately 350 erythrocytes = 2
- 6-80 parasites in 100 microscope fields = 5
- 1-6 parasites in a field = 10
- more than 6 parasites in a field = 15

A smear was considered negative after a 5 min examination.

Infection and transmission of B. caballi

The donkey was infected intravenously on Day 0 with 500 ml of *B. caballi* blood having a parasitaemia score of 2.

The larval progeny of 1 *R. e. evertsi* female tick was fed on the ears of a rabbit. On Day 7 post-larval infestation, prior to moulting, approximately 100 engorged larvae were removed and transferred directly to the donkey (Day 6 post-infection with *B. caballi*), which at that stage had a parasitaemia score of 10.

Twenty-five engorged nymphae were collected between Days 9–15 from the donkey and allowed to moult in the acaridarium. Twenty days post-moulting, the 25 adult *R. e. evertsi* ticks (13 females and 12 males) were placed on the shoulder region of the horse, as described above, and allowed to engorge.

RESULTS AND DISCUSSION

The donkey developed a patent *B. caballi* parasitaemia 24 h after infection. Twenty-five engorged nymphal ticks were collected from the animal, which fed during a declining parasitaemia score ranging between 10–1, from Day 9–15 post nymphal infestation.

In this investigation, the life cycle of *R. e. evertsi*, a two-host tick, was interrupted during the pre-moulting stage: (a) because it is difficult to synchronize the infection with the duration of the tick feeding, (b) because of the very short period of patent parasitaemias that we had encountered before, and (c) it is difficult to keep the cloth containers intact for longer than 7–8 days. The feeding period of the nymphae (9–15 days) was not adversely affected when they were removed as engorged larvae. Rechav, Knight & Norval (1977) reported a larval feeding period of 5–8 days, followed by a pre-moulting stage of 4–5 days on the host, while the nymphae engorged for 5–8 days before dropping off.

By interrupting the normal larva-nymph-adult, two-host life cycle, good synchronization was achieved with the duration of a patent parasitaemia in the infected animal.

Of the 25 adult ticks used to infest the horse to test transmission, 9 engorged females were collected between Days 10–12 after infestation.

The first parasites were observed in a thick blood smear of the horse on Day 19. This was followed the next day by a temperature rise (40 °C) which persisted for 2 days. The parasitaemia remained low, the highest score being 2, and remained patent for 10 days only. Although a 2nd temperature peak (41,2 °C) was seen on Day 27, there was no noticeable increase in the parasitaemia. A marked drop in the haematocrit occurred on Day 27, when it dropped from 27 % (Day 26) to 17 % (Day 27). The lowest haematocrit was 16 % on Day 28, and it slowly subsequently increased to pre-infection levels. The horse recovered without treatment.

Babesiosis induced by *B. caballi* is regarded as a mild disease (Valladares, 1914; Littlejohn, 1963). However, Yakimoff & Jodorsky (cited by Littlejohn, 1963) reported a mortality rate of 85 % in untreated cases. In our experience with *B. caballi* infections in 8 splenectomized horses (D. T. de Waal, unpublished data, 1983–1985), a near 100 % mortality was recorded. A most interesting finding was that, although parasitaemias recorded in all 10 cases studied hitherto never even reached a score of 10, a marked drop in hematocrit was noted in all these cases. The horse in this study recovered without treatment but, judging by its poor physical condition, emaciation, listlessness and general weakness, it is doubtful if it would have survived under field conditions.

These findings shed new light on the epidemiology of equine babesiosis in the RSA, since *R. e. evertsi*

transmits both *B. equi* (Theiler, 1906b) and *B. caballi* from the nymphal to the adult stage. This tick occurs in large parts of this country (Howell, Walker & Nevill, 1978) and this would imply that *B. caballi* is probably just as widely distributed as *B. equi*. This, however, needs to be confirmed,

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