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Experimental Transmission of *Babesia bigemina* by *Boophilus decoloratus* in Cattle

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Abstract: Experimental transmission of *B.bigemina* between cattle and ticks *Boophilus decoloratus* were carried out in the laboratory. Body bags were applied on the bodies of two calves infected with *B. bigemina*.These were the four month old splenectomized male Friesian calf number 10B and the two month old intact male Ayrshire calf number 28B. *B. decoloratus* larvae were then introduced into these body bags to make contact with the calves, Engorged female ticks were then collected several days later as the dropped from the calves. Haemolymph smears of the engorged females *B. decoloratus* derived from calves 28B and 10B revealed 93 (35%) and 112 (34%) infection rates with kinetes of *B. bigemina* respectively. Twelve percent mortality rate was reported for ticks derived from calf 28B while 5% was reported for ticks derived from calf 10B before the commencement of egg laying. The blood of the two calves were found to show declining packed cell volume (PCV) values and red blood cell counts. *B. decoloratus* was found to transmit *B. bigemina* in cattle with a high level of success.

Key words: Experimental Transmission · Boophilus decoloratus · Babesia · Bigemina · Bovine Babesiosis

INTRODUCTION

Arthropod vectors of *Babesia* are all hard ticks of the family Ixodidae. In general, each species of *Babesia* is associated with a single vector species which may differ from region to region [1]. Hoyte [2] observed that in Australia and South America, the vector of *B. bovis* is *Boophilus microplus*. Reports of the development of *Babesia* species in tick vectors are numerous, such as those of *B. bigemina* in *B. microplus* [3,4]. *B. argentina* in *B. microplus* [5] and *B. canis* in *Haemaphysalis teachi* [6].

Simitch *et al*. [7] in their study in Europe showed that *B. ovis* infect cattle in those areas where *Rhipicephalus bursa* were present, whereas *B. divergens* was the main cattle parasite where *Ixodes ricinus* were present. Morzaria [8] described some stages in the development of *Babesia major* in *H. punctata. B. microti* is transmitted by ixodid ticks [9].

B. bovis and *B. bigemina* are apicomplexan hemoparasites of cattle that are transmitted by one-host *Boophilus* ticks [10, 11]. Several tick species including the one-host tick *B. decoloratus* have been reported to transmit *B. bigemina* in South Africa. Throughout East Africa, *B. decoloratus* and *R. appendiculatus* have been implicated as vectors of *B. bigemina* but there is no experimental evidence for this [12].

Purnell *et al*. [13] attempted to transmit *B. bigemina* with *R. appendiculatus* without success. Morzaria *et al*. [12] in their experiment established *B. decoloratus* as a natural vector of *B. bigemina* in Kenya. They found that female *B. decoloratus* ticks became infected during feeding on an animal with a patent *B. bigemina* parasitaemia.

The objective of the present study was to assess the transmission of *B. bigemina* between cattle and experimentally reared *B. decoloratus*. This studies will enhance the planning of appropriate control strategies for bovine babesiosis due to *B. bigemina*.

MATERIALS AND METHODS

Rearing of Babesia-Free Batch of *Boophilus decoloratus:* Several engorged uninfected *B. decoloratus* were brought from the South Africa Bureau of Standard Field Station (SABS), South Africa and reared in

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the *Babesia* Research Laboratory, Imperial College, London. The engorged ticks were placed in Petri dishes and maintained in an incubator from the date of arrival at 26-28°C. Humidity was kept at 85% using saturated potassium chloride (KCl). Dates of egg laying and larval emergence were monitored.

After complete larval emergence, the larvae were transferred from the incubator at 26-28°C to another incubator at 15-17°C under the same humidified condition. The essence of this sharp reduction in the temperature of incubation was to reduce the activity of the larvae under this non-feeding stage of its life history. The larvae were then sent at appropriate time to the Centre for Tropical and Veterinary Medicine (CTVM), Edinburgh to establish infection with *B. bigemina*.

Infection of Calves with *B. Bigemina:* Two calves maintained at the Centre for Tropical and Veterinary Medicine, Edinburgh were used for the experiment. The inoculations of *B. bigemina*, application of ticks, collection of ticks and monitoring of the calves were done by researchers at CTVM.

The Two Calves Were:

- A four month old splenectomised male Friesian calf number 10B previously inoculated with *B. bigemina* piroplasms from the *in vitro* culture of the *Babesia* laboratory.
- A two month old intact male Ayrshire calf number 28B infected with 1ml of *B. bigemina* Muguga blood stabilate. The 1ml blood stabilate was prepared from calf 634 (at 2.2% parasitaemia in 5% Dimethyl Sulfoxide) and 1ml of RBC in autologous serum at 50% PCV. The blood stabilates were injected into calf 28B 17 days following larval application.

Monitoring of Calves and Application of Ticks: Daily rectal temperatures of both calves were monitored. Blood smear, PCV and RBC counts of calf 28B were examined three times weekly before inoculation of parasites. After inoculation of parasites, they were examined daily. Blood smear, PCV and RBC counts of calf 10B were examined three times weekly.

Body bags were applied to the bodies of the calves by methods previously described by Thompson[14] and Hodgson *et al*. [15] with modifications. 14,000 *B. decoloratus* larvae were applied using the same procedure on calf 28B but in this case the *B. bigemina* stabilates were injected after 17 days of its application. Engorged female ticks were collected as they dropped from the calf and dispatched in sample bottles plugged with wet cotton wool to the *Babesia* laboratory.

Screening of the Engorged Females for the Kinetes of *B. Bigemina*: Giemsa stained haemolymph smears of the engorged ticks were examined for the presence of kinetes. This was done by draining haemolymph from the third or fourth pair of legs onto a glass slide within the first 10 days of dropping from the host. They were fixed in absolute ethanol for one minute, stained with 10% Giemsa and examined under the oil immersion objective [16].

The degrees of infectivity of the ticks were ranked as 1+ for those with one kinete per microscopic field, 2+ for those with two to three kinetes per field and 3+ for those with four kinetes and above per microscopic field.

RESULTS

Experimental Transmission of *Babesia bigemina* in the Laboratory

Rearing of *Babesia* Free *Boophilus decolaratus:* The *Babesia* free engorged *Boophilus decoloratus* ticks brought from SABS commenced egg laying a day after arrival and continued for 12 days. The round, brownish eggs were laid in masses. Each tick was estimated to lay 1000-3000 eggs. Larvae emergence commenced 2 weeks after egg laying and lasted for about 12 days. The larvae were minute, very mobile organisms bearing 3 pairs of legs as against the 4 pairs found in the adult.

Infection of the Calves: Calf 28B which was infected 17th day following application of larvae showed signs of *B. bigemina* in the blood smear from the 19th day till the end of the experiment. From the first to the 19th day of the experiment, the PCV and the RBC counts ranged between 31-35% and 8.55-9.97 x 10^6/µl respectively. The PCV and RBC counts dropped after the 19th day to the end of the experiment ranging from 23-27% and 6.91-7.71 x 10^6/µl respectively (Table 1). The rectal temperature of calf 28B ranged between 38.2-40.2°C throughout the duration of the experiment (Table 1).

Before the commencement of the experiment, calf 10B (the 4 month old splenectomized male Friesian calf) was earlier challenged with *B. bigemina* piroplasm from *in vitro* tissue culture maintained in the laboratory. The rectal temperature, RBC count and packed cell volume (PCV) of the calf monitored throughout the course of the experiment ranged between 38.4-39.8°C, 6.33-7.85 x 10^6/µl and 24-28% respectively (Table 2).

Table 1: Experimental infection of calf 28B with *B. bigemina* (African) blood stabilate and subsequent application of *Boophilus decoloratus* larvae

Day	Examination of blood smear of calf for the piroplasm	PCV of calf (%)	RBC count of calf ($X10^6/\mu1$)	Temp of calf (°C)	Ticks
0	-				Approx. 1500 larvae applied
1	-				
2	-				
3	-	34	9.27	39.4	
4	-				
5	-				
6	-				
7	-	35	11.0	38.8	
8	-				
9	-				
10	-	35	9.83	38.2	
11	-				
12	-	35	9.55	38.9	
13	-				
14	-	33	9.46	39.0	
15	-				
16	-				
17	-	34	9.48	39.1	Inoculation of *B. bigeminia*
18	-				
19	+	32	9.97	39.1	
20	+				14 dropped
21	+	27	7.71	40.2	100 dropped
22	+	26		39.7	78 dropped
23	+	25		39.4	75 dropped
24	+	23	6.91	37.7	
-	=	negative for *B. bigemina*			
+	=	positive for *B. bigemina*			

Table 2: Experimental infection of calf 10B with *in vitro* cultured *B. bigemina* (African) and simultaneous application of *Boophilus decoloratus* larvae

Day	Examination of blood smear of calf for the piroplasm	PCV of calf (%)	RBC count of calf ($X10^6/\mu1$)	Temp of calf (°C)	Ticks
0	+	24	6.33	39.7	Approx. 14000 tick larvae applied
3	+	24	6.72	39.8	
6	+	27	6.94	39.2	
9	+	28	7.56	39.2	
12	+	26	7.44	39.0	
15	+	27	6.84	39.4	
18	+	26	7.36	39.2	
21	+	28	7.85	38.4	3 dropped
22	+				23 dropped
23	+				126 dropped
24	+	28	7.59	38.9	175 dropped
+	=	positive for *B. bigemina*			

Table 3: Summary of the degree of infectivity of the ticks applied on the two strains of calves

Strains of calf in which *B. decoloratus* larvae were applied / No of engorged ticks recovered	Number (%) of ticks infected and the degree of infectivity			Total number of ticks infected (% overall prevalence)	Number (%) of dead ticks
	3+	2+	1+		
10B / 329	23 (7)	28 (9)	61 (19)	112 (34)	15 (5)
28B / 267	33 (12)	28 (11)	32 (12)	93 (35)	32 (12)

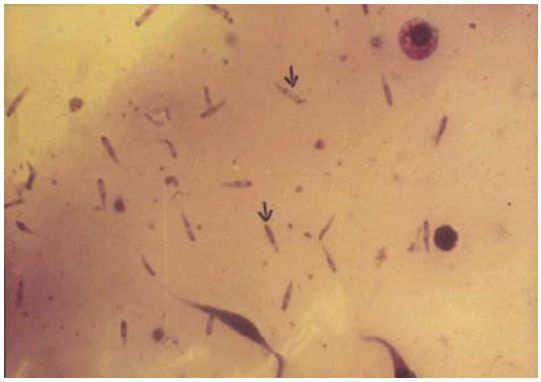

Plate 1: Kinete stage of *B. bigemina* at 3+ degree of infectivity derived from haemolymph smear of engorged *B. decoloratus*. (Magnification x 1000)

Infection of the Ticks: Haemolymph smears of the 329 engorged female ticks derived from calf 10B revealed 112 (34%) infection rate with the kinete stage of *B. bigemina*. The degree of infectivity ranked as 1+, 2+ and 3+ were 19%, 9% and 7% respectively (Table 3). Plate 1 illustrates kinetes at 3+ degree of infectivity derived from the haemolymph of *B. decoloratus*.

Haemolymph smears of the 267 engorged female ticks derived from calf 28B revealed 93 (35%) infection rate with the kinete stages of *B. bigemina*. The degree of infectivity ranked as 1+, 2+ and 3+ were 12%, 11% and 12% respectively (Table 3). The number of dead ticks recovered from calf 10B and 28B after dropping were 15 (5%) and 31 (12%) respectively.

DISCUSSION

Experimental evidence indicate that the haemolymph smears of engorged *Boophilus decoloratus* derived from calves 10B and 28B revealed 34% and 35% infection rates with *B. bigemina* respectively. Mahoney and Mirre [16] found 36% infection rate of ticks infected with *B. bigemina* through a similar haemolymph examination. In earlier studies, the prevalence of the bovine *Babesia*within the progeny of infected female ticks was found to be very low [16-18]. Although all the ticks applied on the calves were subjected to the same sets of condition on the different calves, not all were infected, probably due to physiological or genetic variations in the strains of *B. decoloratus*. Another likely reason for the low percentage of infected ticks was attributed to the fact that some of the female ticks completed engorgement when the parasite density in the bovine host blood was below a particular critical level [10]. Thus, the proportion of ticks infected with *B. bigemina* reflects the parasite rate of their bovine host. In contrast, in strains of *B. bovis* that was highly adapted to ticks, low cattle parasitaemia (0.1%) produced infection in 100% of the ticks [10,19].

Twelve percent mortality rate was reported for ticks derived from calf 28B while only five percent was reported for ticks derived from calf 10B before the commencement of egg laying. Thus a higher death rate was found in ticks derived from calf 28B than ticks from calf 10B. This was attributed to the fact that the larvae of ticks applied on calf 10B were exposed to *B. bigemina* from the onset of the experiment; that is calf 10B was infected prior to application of larvae. Hence the engorged ticks were more adapted to the *B. bigemina* in calf 10B, thus facilitating a higher survival rate. On the contrary, calf 28B picked up the *B. bigemina* on the 19th day of the experiment when the ticks were almost dropping from the calf. Hence they could not cope with the sudden infection of the calf with *B. bigemina*, therefore their higher mortality rate. Friedhoff and Smith [10] found that one strain of *B. decolaratus* had mortality rates of more than 80% of the engorged female ticks after alimentary infection with *B. bigemina*. Gray [20] reported that the ingestion of large numbers of *babesias* may have a negative effect upon female tick survival and reproduction. However, others found little effect of *B. bigemina* and *B. bovis* on *B. microplus* [21, 22].

After the inoculation of the calves with the *B. bigemina*, there was a drastic fall in the PCV of the two calves. This was sequelled from the report collected from the centre for Tropical and Veterinary Medicine, Edinburgh. Calf 28B which was *Babesia* free for the first 19 days of the application of *B. decoloratus* larvae, had a PCV of 31-35%, After acquiring the infection the PCV dropped to 23-27%. Also, since calf 10B was previously infected before the commencement of the experiment, the PCV dropped to 24-28% throughout the duration. Similarly, calf 28B had RBC count of 8.55-11.0 x 10^6/µl from day 1 to day 19 of the experiment. The RBC count dropped after day 19 to 6.91-7.71 x 10^6/µl. Also RBC count of calf 10B ranged from 6.33-7.85 x 10^6/µl throughout the duration of the experiment. Hence there appears to be a relationship between bovine babesiosis, PCV and RBC count. It was observed that the destruction of the red blood cells by the parasites has an adverse effect on the RBC count and PCV because they both showed marked decline. Animals vaccinated with crude soluble parasite antigens essentially showed patent *Babesia* infections of shorter duration and a less marked fall in PCV values [23].

Wright [24] carried out a comparative study of the haematology of experimentally induced *B. bovis* and *B. bigemina* infections in cows. Six animals were each infected with *B. bigemina* by the inoculation of 500ml of blood from a carrier animal. There was rapid fall in the total erythrocyte count from 7.6 x 10^6/mm^3 on day 4 to a minimum of 2.1 x 10^6/mm^3 on day 11. The value then persisted at about this level until all the animals had died. Lohr *et al*. [25] also made a comparative study of the haematological reactions to experimental *B. bigemina* infection in intact cattle in Kenya. Their results were in agreement with those of Wright [24] and also with that of the present study.

Calf 10B in which the uninfected *B. decoloratus* larvae were applied was earlier splenectomized before being inoculated with *B. bigemina* from the *in vitro* culture. The essence of the splenectomy was to ensure that the calf losses resistance immediately and then acquire the *B. bigemina* infection. Zwart and Brocklesby [26] found that the spleen is important in non-specific and immunological defence of the host against *Babesia* species; reflected in the histopathological changes found in the spleen during *Babesia* infection. Hence, it was necessary to ensure that the calf adequately acquired the parasite by reducing their immune resistance. The present investigation shows that bovine babesiosis has a negative impact on the physiological functions of infected animals. This is evident in the reduction of the PCV values and the RBC counts of infected calves. Hence, the control of this infection should be given adequate attention in the livestock industry. Moreover, *B. decoloratus* has able proven to be the vector of *B. bigemina*. Eventhough, vectors of *B. bigemina* vary from region to region, in the control of bovine babesiosis, emphasis should be placed on the elimination of *B. decoloratus* and all other tick species in any field studies. The present studies will however stimulate field studies in various regions where the vector species have not yet been established.

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