Onderstepoort J. vet. Res., 55, 33–35 (1988)

ISOLATION OF PURE *BABESIA EQUI* AND *BABESIA CABALLI* ORGANISMS IN SPLENECTOMIZED HORSES FROM ENDEMIC AREAS IN SOUTH AFRICA

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ABSTRACT

DE WAAL, D. T., VAN HEERDEN, J., VAN DEN BERG, S. S., STEGMANN, G. F. & POTGIETER, F. T., 1988. Isolation of pure *Babesia equi* and *Babesia caballi* organisms in splenectomized horses from endemic areas in South Africa. *Onderstepoort Journal of Veterinary Research*, 55, 33–35 (1988).

Both *Babesia equi* and *Babesia caballi* are endemic in large parts of South Africa. Attempts were made to obtain pure local isolates of both *B. equi* and *B. caballi* for the purpose of developing serological tests to study the epidemiology of equine babesiosis in this country.

The indirect fluorescent antibody test was used to screen horses for *B. equi* and *B. caballi* in an endemic area. Seven horses and 3 donkeys between 3 and 36 months of age that tested negative were subsequently splenectomized.

The splenectomy operation was performed through the abdominal approach. A 100 % survival rate was achieved through this method, probably because it reduced the risk involved in the operation.

Blood collected from naturally infected horses and passaged in fully susceptible spenectomized horses and a donkey, under laboratory conditions, produced 2 isolates of *Babesia caballi* and 1 of *B. equi*. Microscopical and serological examinations confirmed that these were pure isolates.

INTRODUCTION

Babesia equi and *Babesia caballi* both occur in the Republic of South Africa. Only *Rhipicephalus evertsi evertsi*, one of the more common ticks found on horses, has as yet been identified as a stage-to-stage vector of both *B. equi* (Theiler, 1906) and *B. caballi* (De Waal & Potgieter, 1987). Mixed infections make it very difficult to obtain pure isolates of these parasites for the purpose of antigen production and reference sera.

To obtain heavily infected blood it is necessary to use splenectomized horses. The role of the spleen as an immunological defence mechanism against *Babesia* infections is well documented (Todorovic, Ferris & Ristic, 1967; Roberts, Kerr & Trucey-Patte, 1972; Zwart & Brocklesby, 1979). The function of the spleen in the control of *Babesia* infections is based primarily on its phagocytic function, its anitbody response against antigens in the circulation and its ability to deal with antigenic variation (Taliaferro, 1956). Splenectomy before infection with *Babesia* normally results in a severe clinical reaction and death. Removal of the spleen during the chronic phase in the case of bovine babesiosis may result in recrudescing parasitaemias that may lead to varying degrees of disease and even death (Uilenberg, 1969).

This paper reports on the isolation of 2 local isolates of *B. caballi* and 1 of *B. equi*, using splenectomized horses. The technique for splenectomy as well as the pre- and post-operative care are briefly described.

While on the Government property, these animals grazed natural veld. The tick control practised consisted of fortnightly treatments with cypermethrin[1] in the summer.

MATERIALS AND METHODS

Experimental animals

Horses, born on a Government property comprising portions of the farms De Onderstepoort and Haakdoornboom (28° 8′ E, 25° 38′ S), adjacent to the Veterinary Research Institute (VRI), Onderstepoort, and kept in insect-proof stables of this Institute from the age of 6 months, and donkeys born and raised at this Institute, were screened for the presence of antibodies against *B. equi* and *B. caballi*, as described below.

Seven horses and 3 donkeys (between 3½ and 36 months of age) were selected, treated with an acaricide and transferred to tick-free stables.

Serology

Both the indirect fluorescent antibody (IFA) test (Madden & Holbrook, 1968) and the complement fixation (CF) test (Herr, Huchzermeyer, Te Brugge, Williamson, Roos & Schiele, 1985) were used to screen the serum samples for the presence of *B. equi* and *B. caballi* antibodies. No *B. caballi* antigen was available for the CF test.

The *B. caballi* isolate used to produce the IFA antigen and the *B. equi* reference sera were obtained from the Reagents Section of the Scientific Services Laboratory, Ames, Iowa, United States of America (USA).

Both batches of the *B. equi* antigen for the IFA and CF tests were obtained from a local isolate which was tested with reference sera obtained from the USA. No cross-reaction occurred with the IFA antigen and *B. caballi* positive sera.

Splenectomy procedure

Prior to splenectomy, the experimental horses and donkeys were starved for 24 h.

General anaesthesia was performed by intravenous premedication with xylazine[2], at a dosage rate of 1 mg/kg, 5–10 min before induction. Induction was effected by a single bolus of thiopentone[3] at a dosage rate of 3 mg/kg. This was followed by intubation and maintenance on a closed circuit system with 2 % halothane[4].

Splenectomy was performed through a transabdominal approach with the horse in lateral recumbency. The method followed was essentially that described by Quinlan, De Kock & Marais (1935), but with the following differences: Preparation of the horses and donkeys did not include feeding a concentrated, non-bulky diet 8 days prior to the operation, nor were experimental animals subjected to purging before the operation. Only the *M. abdominis obliquus internus* was incised, the remaining muscles being entered through a grid incision. Catgut[6] was used for most of the ligating except for bigger blood vessels which were tied down with poliglactin 910[5]. Nylon[7] was used for suturing the skin. No bandage was applied post-operatively and surgical stitches were removed 10 days after the operation.

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Recieved 2 October 1987—Editor

[1] Curatik cattle dip, Agricura

[2] Rompun, Bayer

[3] Intraval sodium, Maybaker

[4] Fluothane, ICI

[5] Vicryl, Ethicon

[6] Chromic Catgut, Ethicon

[7] Ethilon, Ethicon

All the splenectomized animals were monitored on a daily basis for clinical signs of disease. Thick (Mahoney & Saal, 1961) and thin bloodsmears were prepared and quantified according to the methods described by De Waal & Potgieter (1987) for a period of 30 days after the operation.

Babesia isolates

Two batches of *B. caballi* infected blood were obtained, one from a clinically affected horse on a farm in the Lynnwood area, Pretoria. Ten mℓ of this blood was cryopreserved in liquid nitrogen, as described below. The other was obtained from Horse 67 born on the Government property, adjacent to the VRI, that had antibodies to *B. caballi* only. There was microscopically no detectable parasitaemia when 800 mℓ of blood, collected in 14 % citrate phosphate dextrose (CPD) anticoagulant solution, was transfused into a splenectomized donkey 162.

The *B. equi* isolate originated from a clinically affected Percheron that contracted a natural infection at the VRI.

Establishing the purity of the different field isolates

Two horses, No. 339 and 414, and a donkey, No. 162, all splenectomized, were infected with the different *Babesia* field isolates and the ensuing reactions are summarized in Table 2. Rectal temperatures, parasitaemias, as well as haematocrits were determined daily, as described by De Waal & Potgieter (1987). Serum samples were collected before and on Day 21 post-infection.

Cryopreservation

During the peak reaction 250 mℓ of blood was collected from each animal and cryopreserved, according to the method of De Vos, Combrink & Bessenger (1982).

RESULTS

Serology

Sixty-nine serum samples were collected from animals at the Research property and the VRI. Seventeen of these samples (25 %) tested negative for *B. equi* on both the IFA and CF tests. Sixty out of 69 (87 %) serum samples yielded negative IFA results for *B. caballi*, whereas only 1 sample (1,4 %) tested positive for *B. caballi* and negative for *B. equi*.

TABLE 1 Serological results of animals selected for splenectomy

Animal No.[b]	Age at splenectomy (months)	Reciprocal *B. equi* titre	
		IFA	CF
43	4	—	—
43[a]		—	—
65	3,5	80	—
65[a]		—	—
45	5,5	80	—
45[a]		—	—
46	3,5	80	—
46[a]		—	—
(d)162	3	—	—
(d)182	3	—	—
(d)379	19	—	—
339	36	—	—
179	12	—	—
414	27	—	—

[a] repeat test after 14 days

[b] were also tested against *B. caballi* with the IFA test and all yielded negative results

d = donkey

TABLE 2 Results of the isolation and purity of *B. equi* and *B. caballi* in splenectomized animals

Animal No. (age)	Infection (i.v.)	*Babesia* isolate	Prepatent period (days)	Parasitaemia score at day of cryopreservation	Febrile reaction			Lowest haematocrit (%)	Reciprocal IFA titre 21 days p.i.[b]		Outcome (day)
					First (day)	Maximum (°C)	Duration (days)		*B. equi*	*B. caballi*	
339 (36 mths)	5 mℓ cryopreserved blood	W-isolate *B. caballi*	14	10	16	40,7	1	35	•	•	Died (18)
162 (20 mths)	800 mℓ fresh blood from 67	K-isolate *B. caballi*	7	5	10	41,2	2	24	—	320	Recovered
414 (36 mths)	5 mℓ cryopreserved blood	OP-isolate *B. equi*	5	15	8	41,1	4	20	•	•	Died (11)
43 (6 mths)	Recrudescent parasitaemia after splenectomy	*B. equi*	13	10	16	39,5	1	30	—	—	Recovered

• Not done

[b] Post Infection

A total of 10 sero-negative animals (7 horses and 3 donkeys) (Table 1) were selected for splenectomy. Their ages ranged between 3½ and 36 months. Three of these animals, between 3½ and 5½ months of age had low IFA titres (1/80). On a retest later all were found to be negative, which could indicate the presence of maternal antibodies in these animals.

Splenectomy

All the experimental animals survived the operation, which lasted on average approximately 1 h. All the animals developed a post-operative swelling at the site of the surgical incision.

The blood smears of all the splenectomized animals, except that of the Horse 43, remained negative throughout. The blood smears of Horse 43 was negative for *B. equi* on post-operative Day 13. The parasitaemia rose to a height of 1,2 % on Day 22. No clinical signs of disease were ever observed, nor was any babesiacidal treatment applied. A very low IFA response, which lasted for only approximately 6 months, was recorded in this animal.

Purity of isolates

The results of the ensuing reactions of Horse 339 and Donkey 162, infected with the *B. caballi* isolates, designated the W and K isolate respectively, and of Horse 414, infected with *B. equi*, designated the OP isolate, are summarized in Table 2.

Discussion

A study of the epidemiology of equine babesiosis in this country is envisaged. For this reliable serological tests are needed and pure local isolates of both *Babesia* spp. involved should be available. Splenectomized horses fully susceptible to *B. equi* and *B. caballi* are essential for the isolation of field-collected parasites and the production of antigen from known pure infections.

Very little is known about the epidemiology of these 2 *Babesia* parasites, and to complicate matters they share at least one common tick vector, namely, *R. e. evertsi* (Theiler, 1906; De Waal & Potgieter, 1987). With the antigens and reference sera obtained from the USA we were able to screen 64 horses and 5 donkeys and identify sero-negative animals.

Subsequent splenectomy of these sero-negative animals proved the test to be reasonably reliable, as only 1 horse (No. 43) out of 10 had a mild relapse and a poor IFA response. This is contrary to the observation made by Quinlan *et al.* (1935) that splenectomy of a carrier horse of *B. equi* usually resulted in an acute elevation in the number of parasitized cells which is invariably fatal. It is interesting to note that Horse 43 died when challenged with the OP isolate of *B. equi* 26 months later.

Uilenberg (1969) reported that splenectomy of carrier cattle infected with *Babesia bigemina* or *Babesia argentina* does not always result in a severe form of the disease, and he concluded that not all carriers of *B. bigemina* or *B. argentina* can be detected by splenectomy only.

Three of the foals (3½–5½ months old), when selected for splenectomy, had low IFA titres for *B. equi*. It is possible that these antibodies were due to the passive transfer of maternal antibodies rather than to infection of these foals. Donnelly & Philips (1982) reported maternal antibodies to *B. equi* and *B. caballi* in foals up to the age of about 4 months. When these foals were retested later, they were all found to be sero-negative, and on subsequent splenectomy only Horse 43 had a recrudescent parasiteamia.

The near 100 % success rate in the identification of a number of *Babesia* susceptible animals can be ascribed to the selection of young animals which had been exposed to regular tick control and thus the posibility of their contracting babesiosis was fairly limited. Further more, a reliable serological test was used to identify these sero-negative animals. Although false negative results have been reported with the IFA test, it was found to be species specific and sensitive enough to detect non-infected animals in 9 out of the 10 cases in this study.

The use of relatively young animals facilitated the delivery of the spleen through an abdominal incision. The spleen in younger animals is considerably smaller than that of older and mature animals, and can thus be handled better by the operators. It is also noticeable that the operation performed on the 36-months-old animals lasted nearly twice as long as that on the younger animals. Using the abdominal instead of the transthoracic approach probably reduces the risk involved in the operation.

Splenectomized horses yielded a relatively greater number of infected cells that facilitate the study of the morphology of the parasites. Where possible, the purity of the isolates was also confirmed serologically.

However, these findings should ideally be confirmed by xenodiagnosis with a vector tick that transmits only 1 of the parasite species involved. This is not possible at the moment because the only known and laboratory-proven tick vector identified so far locally is *R. e. evertsi*, which transmits both parasites.

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