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AN INVESTIGATION INTO THE CLINICAL PATHOLOGICAL CHANGES AND SEROLOGICAL RESPONSE IN HORSES EXPERIMENTALLY INFECTED WITH *BABESIA EQUI* AND *BABESIA CABALLI*

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ABSTRACT

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Serologically negative horses, as determined with the indirect fluorescent antibody test (IFA), were infected with *Babesia equi* and 60 days later with *Babesia caballi*. The only clinical signs of disease observed in these animals were a febrile reaction and slight icterus. Haematological changes included a drop in haematocrit and haemoglobin concentration, as well as lowered platelet counts. The serum concentrations of albumin, iron and phosphorus were lowered. Mildly elevated serum bilirubin and fibrinogen concentrations were observed. Antibody titres were determined with the IFA and complement fixation (CF) tests. Antibodies to *B. equi* were first detected between Days 10–19 and 12–38 with the IFA and CF test, respectively, while the corresponding IFA periods for *B. caballi* were 6–8 days after infection. The parasitaemia of both *B. equi* and *B. caballi* infections never reached the 1 % level.

INTRODUCTION

Equine babesiosis (piroplasmosis) is a tick-borne disease caused by *Babesia equi* and/or *Babesia caballi*. According to Henning (1949) and Littlejohn (1963), *B. equi* is the most widely distributed of the 2 parasites in the Republic of South Africa. The most common clinical signs of equine babesiosis are fever, pale mucous membranes and icterus (Henning, 1949). The packed cell volume usually decreases rapidly. Haemoglobinuria, a prominent clinical sign of *B. equi* infection, is seldom seen in *B. caballi* infections (Holbrook, Frerichs & Allen, 1973). Anaemia, thrombocytopaenia and a rise in plasma fibrinogen levels have been reported in experimental acute *Babesia caballi* infections (Holbrook *et al.*, 1973). Parasitaemia in clinical cases is usually demonstrated by staining of blood smears with dyes such as Giemsa's stain and Diff-Quik[1]. The carrier state is, however, not readily demonstrable by examination of stained blood smears. A sound, reliable serological test would be more reliable in the diagnosis of the carrier state. Both the complement fixation (CF) and the indirect fluorescent antibody (IFA) tests have been used in the diagnosis of infections with *B. equi* and/or *B. caballi* (Hirato, Ninomiya, Uwano & Kutii, 1946, cited by Donelly, Joyner, Graham-Jones & Ellis 1980; Madden & Holbrook, 1968). The CF test, however, tends to indicate a lower prevalence of antibody titres than the IFA test (Donelly *et al.*, 1980), but according to Ristic (1981) it appears to be a useful serological tool for epidemiological studies.

The aim of this study was to follow the clinical, haematological, blood chemical and serological response in horses experimentally infected with *B. equi* and *B. caballi*.

MATERIALS AND METHODS

Experimental animals

Horses were obtained from stock 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. These animals grazed freely on natural veld, were treated fortnightly with an acaricidal dip[2] during summer, but not at all during the winter months.

At the time of weaning at 6–8 months of age foals were transferred to insect-proof stables at the Veterinary Research Institute, Onderstepoort. Three intact horses No. 180, 198 and 235, respectively 17, 17 and 16 months of age, were selected from this group, treated with cypermethrin[2] and transferred to tick-free housing conditions for the duration of the investigation. These animals tested serologically negative with the IFA test for *B. equi* and *B. caballi*. Routine blood smear examinations before infection were negative for blood parasites.

Strains of Babesia *species*

The *B. equi* strain used was first isolated from a local intact carrier donkey and needle passaged through another susceptible splenectomized donkey before cryopreservation in liquid nitrogen, according to the method of De Vos, Combrink & Bessenger (1982).

The *B. caballi* strain was obtained from the Reagents Section of the Scientific Service Laboratory, Ames, Iowa, United States of America.

Experimental infection

All 3 horses were infected by the intravenous injection of 5–10 mℓ of infected blood stabilates, which were thawed at 37 °C immediately prior to injection. Horses were first infected with *B. equi* and 60 days later with *B. caballi*.

Clinical response

Infected experimental animals were subjected to daily physical examinations and the following parameters were used to evaluate the clinical response to infection: respiratory rate, heart rate, colour of mucous membranes, capillary refilling time, cardiac rhythm, lung sounds, presence or absence of borborygmi, sweating and abdominal pain. A daily flow-chart was maintained. Rectal temperatures were recorded daily between 08h00 and 10h00. A temperature of between 37,5 °C–38,5 °C was considered normal.

Parasitaemias

Thick (Mahoney & Saal, 1961) and thin bloodsmears were prepared daily and the parasitaemia was determined as described by De Waal & Potgieter (1987).

Clinical pathology

Blood was collected at regular intervals in Vacutainer tubes[3] for the determination of the concentrations of sodium (Na), potassium (K), urea, creatinine, total proteins (TP), albumin (Alb), phosphorus, bilirubin, iron

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[1] Harleco

[2] Curatik Cattle Dip, Agricura

[3] Vac-u-test, Radem Laboratory Equipment, RSA.

TABLE 1 Results of clinical pathological investigations in Horse 180 infected with *B. equi* and *B. caballi*

Days P.I.	Hb g/ℓ	RCC $\times 10^{12}$/ℓ	Ht	Fibrinogen mg/dℓ	FDP μg/mℓ	Platelet count $\times 10^9$/ℓ	Blood smear results		Temp °C	TP g/ℓ	Alb g/ℓ	Phosphorus mmol/ℓ	Bilirubin μmol/ℓ	Fe μmol/ℓ	ALP U/ℓ	LDH U/ℓ	GGT U/ℓ
							B. caballi score	*B. equi* score									
−2	10,9	8,20	0,29	130	N	248000	0	0	38,0	55	29	1,74	6	33,0	508	307	2
0	*	*	*	120	N	*	0	0	38,4	57	27	1,83	7	30,3	510	279	4
3	10,8	8,20	0,30	170	N	183000	0	0	38,4	61	29	1,68	13	36,7	495	296	8
4	12,0	9,00	0,32	141	N	195000	0	0	37,7	*	*	*	*	*	*	*	*
7	11,7	9,20	0,32	53	N	210000	0	0	38,0	*	*	*	*	*	*	*	*
10	11,8	9,16	0,33	118	N	90000	0	0	37,8	56	29	1,93	7	38,5	525	338	7
12	11,6	8,90	0,31	125	N	235000	0	0	37,8	*	*	*	*	*	*	*	*
14	11,4	8,76	0,31	105	N	277000	0	0	38,3	55	27	1,63	7	33,8	553	292	4
17	11,2	7,69	0,29	*	N	232000	0	0	37,5	54	28	1,61	10	20,8	638	289	7
19	11,7	8,46	0,29	120	N	272000	0	0	37,0	61	29	1,64	11	46,4	712	323	a
61	11,0	7,73	0,29	92	N	176000	0	0	37,8	50	30	*	*	*	628	315	3
66	11,1	8,80	0,30	100	N	187000	0	0	38,5	*	*	*	*	*	*	*	*
68	9,5	6,89	0,21	*	N	149000	1	0	38,8	59	29	0,80	53	6,70	471	269	a
70	10,6	7,44	0,29	165	N	89000	1	0	38,5	48	26	*	*	*	440	378	1
73	8,7	6,06	0,23	127	N	64000	2	0	40,4	47	25	*	*	*	432	567	a
75	6,8	4,68	0,18	150	N	84000	5	0	41,4	40	24	1,36	74	16,8	317	779	4
77	6,2	3,78	0,17	155	N	90000	2	0	39,5	53	24	1,71	28	54,9	325	652	a
80	8,4	5,71	0,22	175	N	140000	0	0	38,0	58	24	1,80	15	36,4	412	486	12
82	9,0	5,99	0,23	145	N	227000	*	0	37,9	58	27	1,88	10	31,2	430	409	10
89	9,1	6,34	0,25	120	N	*	1	0	*	57	25	*	12	35,9	534	345	9
91	9,5	6,86	0,26	*	*	172000	0	0	*	59	25	1,67	11	26,3	535	303	4

* Not done
N = Negative
a = Below detectable levels

TABLE 2 Results of clinical pathological investigations in Horse 198 infected with *B. equi* and *B. caballi*

Days P.I.	Hb g/ℓ	RCC $\times 10^{12}$/ℓ	Ht	Fibrinogen mg/dℓ	FDP µg/mℓ	Platelet count $\times 10^9$/ℓ	Blood smear results		Temp °C	TP g/ℓ	Alb g/ℓ	Phosphorus mmol/ℓ	Bilirubin µmol/ℓ	Fe µmol/ℓ	ALP U/ℓ	LDH U/ℓ	GGT U/ℓ
							B. caballi score	*B. equi* score									
−2	11,8	9,20	0,32	135	N	150000	0	0	38,4	55	30	1,69	10	22,5	364	292	8
0	*	*	*	110	N	*	0	0	39,1	55	27	1,62	11	26,0	346	285	6
3	13,0	9,60	0,36	150	N	144000	0	0	37,7	60	31	2,08	20	36,9	361	291	7
4	12,2	9,44	0,33	128	N	155000	0	0	37,4	*	*	*	*	*	*	*	*
7	12,4	9,38	0,34	97	N	160000	0	0	37,0	*	*	*	*	*	*	*	*
10	11,1	8,26	0,31	170	10	94000	0	2	38,7	58	29	1,36	34	16,5	388	302	7
12	9,6	7,02	0,27	170	N	55000	0	10	38,0	49	24	*	*	*	*	442	*
14	7,7	5,82	0,21	150	N	105000	0	5	39,0	56	27	1,52	59	34,7	332	295	5
17	10,8	7,30	0,27	*	N	106000	0	1	37,0	54	27	1,88	15	41,8	399	315	11
19	11,7	7,97	0,29	133	N	150000	0	0	37,0	62	28	1,51	11	24,7	431	317	9
61	14,2	9,58	0,37	117	N	180000	0	0	38,0	50	28	*	*	*	473	321	4
66	14,5	9,93	0,39	112	N	125000	0	0	37,5	55	30	1,82	12	20,9	501	262	3
68	13,1	9,03	0,37	*	N	101000	1	0	37,3	59	28	1,16	22	9,8	448	275	2
70	12,2	8,53	0,33	190	N	91000	2	0	38,4	59	30	1,74	22	40,1	447	344	3
73	12,5	8,53	0,33	175	N	54000	2	0	39,5	60	28	0,76	77	8,6	411	739	6
75	9,8	6,79	0,27	180	N	86000	2	0	37,8	57	27	0,92	65	7,0	258	738	5
77	8,6	6,18	0,23	150	N	104000	2	0	38,2	51	25	1,29	48	34,0	265	736	7
80	10,0	9,96	0,27	168	N	77000	1	0	38,1	54	23	*	*	*	307	526	11
82	10,6	7,13	0,28	115	N	93000	*	0	38,0	58	26	1,83	13	27,1	314	418	6
89	10,8	7,50	0,30	121	N	*	1	0	*	61	27	*	13	26,5	408	353	7
91	10,8	7,60	0,30	*	*	150000	1	0	*	60	25	1,69	14	20,1	390	418	9

* Not done
N = Negative

TABLE 3 Results of clinical pathological investigations in Horse 235 infected with *B. equi* and *B. caballi*

Days P.I.	Hb g/ℓ	RCC $\times 10^{12}$/ℓ	Ht	Fibrinogen mg/dℓ	FDP μg/mℓ	Platelet count $\times 10^{9}$/ℓ	Blood smear results		Temp °C	TP g/ℓ	Alb g/ℓ	Phosphorus mmol/ℓ	Bilirubin μmol/ℓ	Fe μmol/ℓ	ALP U/ℓ	LDH U/ℓ	GGT U/ℓ
							B. caballi score	*B. equi* score									
−2	14,1	10,54	0,39	150	N	161000	0	0	37,8	60	32	*	15	28,2	502	305	8
0	12,6	10,24	0,33	135	N	216000	0	0	38,8	57	29	1,56	13	26,5	486	409	4
3	14,4	10,78	0,39	145	N	206000	0	0	38,5	61	32	1,84	20	30,5	511	368	10
4	12,6	10,36	0,34	126	N	230000	0	0	37,6	*	*	*	*	*	*	*	*
7	12,9	10,94	0,35	60	N	260000	0	0	37,6	*	*	*	*	*	*	*	*
10	12,9	10,52	0,34	184	N	251000	0	0	38,4	57	39	1,96	14	29,5	487	366	10
12	12,2	9,80	0,34	150	N	230000	0	1	*	53	28	*	*	*	509	390	5
14	12,8	9,98	0,35	160	N	305000	0	*	38,4	58	28	1,67	12	27,4	481	306	4
17	12,1	8,64	0,31	*	N	274000	0	2	38,0	55	28	1,43	19	23,4	*	*	*
19	11,6	8,41	0,28	130	N	278000	0	2	37,0	61	30	1,74	18	32,0	521	292	5
61	13,2	9,89	0,36	170	N	269000	0	0	38,0	56	32	*	*	*	517	312	1
66	13,5	9,73	0,36	140	N	227000	0	0	38,4	58	31	1,34	11	23,5	529	339	4
68	12,5	9,50	0,34	*	N	148000	1	0	38,8	59	29	1,03	39	7,2	503	289	4
70	12,2	8,88	0,31	130	N	189000	2	0	39,0	60	30	1,83	27	29,2	473	391	4
73	11,2	8,23	0,30	175	N	35000	5	0	40,0	55	26	1,33	48	13,2	387	706	1
75	9,7	7,34	0,27	200	N	136000	2	0	41,0	59	28	1,02	33	18,9	312	927	3
77	9,8	6,43	0,27	147	N	66000	2	0	38,8	57	26	1,24	*	24,1	351	764	2
80	10,2	7,45	0,26	185	N	136000	1	0	38,1	52	22	*	*	*	413	542	10
82	*	*	*	145	N	*	1	0	37,8	66	30	1,63	12	31,2	487	487	15
89	11,3	10,22	0,32	145	N	*	1	0	*	60	27	*	12	24,2	542	326	6
91	11,9	10,14	0,33	*	*	206000	1	0	*	61	27	2,75	10	24,3	565	300	3

* Not done
N = Negative.

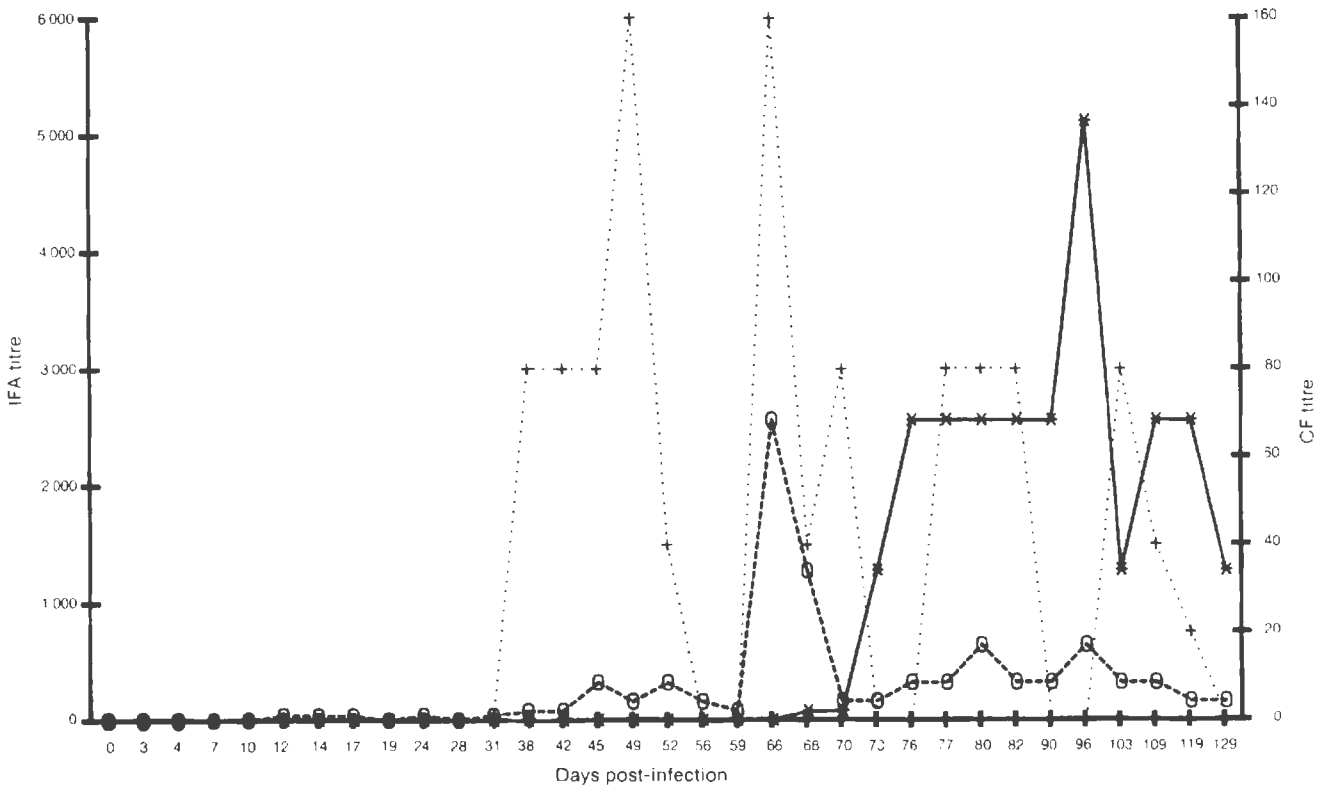

FIG. 1 IFA and CF titres after experimental infection of Horse 180 with *Babesia equi* and *Babesia caballi*
O---O---O *Babesia equi* IFA titre
* *—* *Babesia caballi* IFA titre
+ ... + ... + *Babesia equi* CF titre

(Fe), alkaline phosphatase (ALP), aspartate transaminase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), alanine transaminase (ALT) and gammaglutamyltransferase (GGT), by routine methods (Van Heerden, Dauth, Jarvis, Keffen, Denny, Dreyer & Kriek, 1985).

Blood for fibrinogen determination was collected in sodium citrate (3,8 %) Vacutainer tubes[4]. The test procedure was performed on plasma, with the Coagachek Fibrinogen Test Kit[4] on the Clotek II instrument[4].

Blood specimens for fibrinogen degradation products (FDP) determination were collected in vacutainer tubes for FDP assay. The test procedure was done with the Thrombo-Wellcotest FDP Kit[5].

Blood specimens for haematological investigations were collected in Vacutainer tubes[3]. The red cell count (RCC) was performed on the Coulter Counter Model DN[6], calibrated specifically for performing equine red blood cell counts. A 1:100 000 dilution was used. The white cell count (WCC) was performed on a 1:500 dilution. Haemoglobin-concentration (Hb) was performed on the Coulter Haemoglobinometer[6]. The haematocrit (Ht) was performed with the Heraeus-Christ Hämofuge Cat No 775[7]. Platelet counts were obtained from a specially calibrated Sysmex Platelet Coulter, Model PL-110[8].

The results obtained from haematological and blood chemical investigations during the experiments were compared to baseline results obtained from the same animals prior to experimentation (Tables 1, 2 & 3).

[4] Hyland Diagnostics, Pennsylvania.
[5] Welcome Diagnostics, Dartford, England.
[6] Coulter Electronics, Hialeah, USA.
[7] Heraeus-Christ, West Germany.
[8] Toa Medical Electronics, Japan.

Serological tests

Sera for these tests were collected into 10 mℓ Vacutainer tubes[3] at regular intervals during the reaction period. The blood was left for 24 h at room temperature before centrifugation at 200 g for 5 min and the sera poured into 5 mℓ glass vials and stored at -20 °C until tested.

Indirect fluorescent antibody tests

The technique used was essentially that described by Madden & Holbrook (1968). A commercial fluorescein conjugate[9] was used at a 1/80 dilution. Twofold serum dilutions were prepared and the titre was taken as the reciprocal of the highest serum dilution giving specific fluorescence.

Complement fixation test

The technique used was described by Herr, Huchzermeyer, Te Brugge, Williamson, Roos & Schiele (1985).

RESULTS

The results are summarized in Tables 1–3.

Infection of horses with B. equi

The only clinical sign of disease recorded was a slight febrile reaction in 1 of the experimental animals. The first *B. equi* parasites were detected in the blood of Horses 198 and 235, respectively, 9 and 12 days after infection. The highest parasitaemia score recorded was 10 in Horse 198 on Day 12 post-infection. No parasites were observed in the blood of Horse 180 during this observation period. Horses 198 and 235 showed a drop in Ht and Hb concentration and RCC, and a drop in platelet numbers in the case of Horse 198, which corresponded with the demonstration of a parasitaemia by the

[9] Fite Anti-horse IgG (H&L) Bio-Yeda.

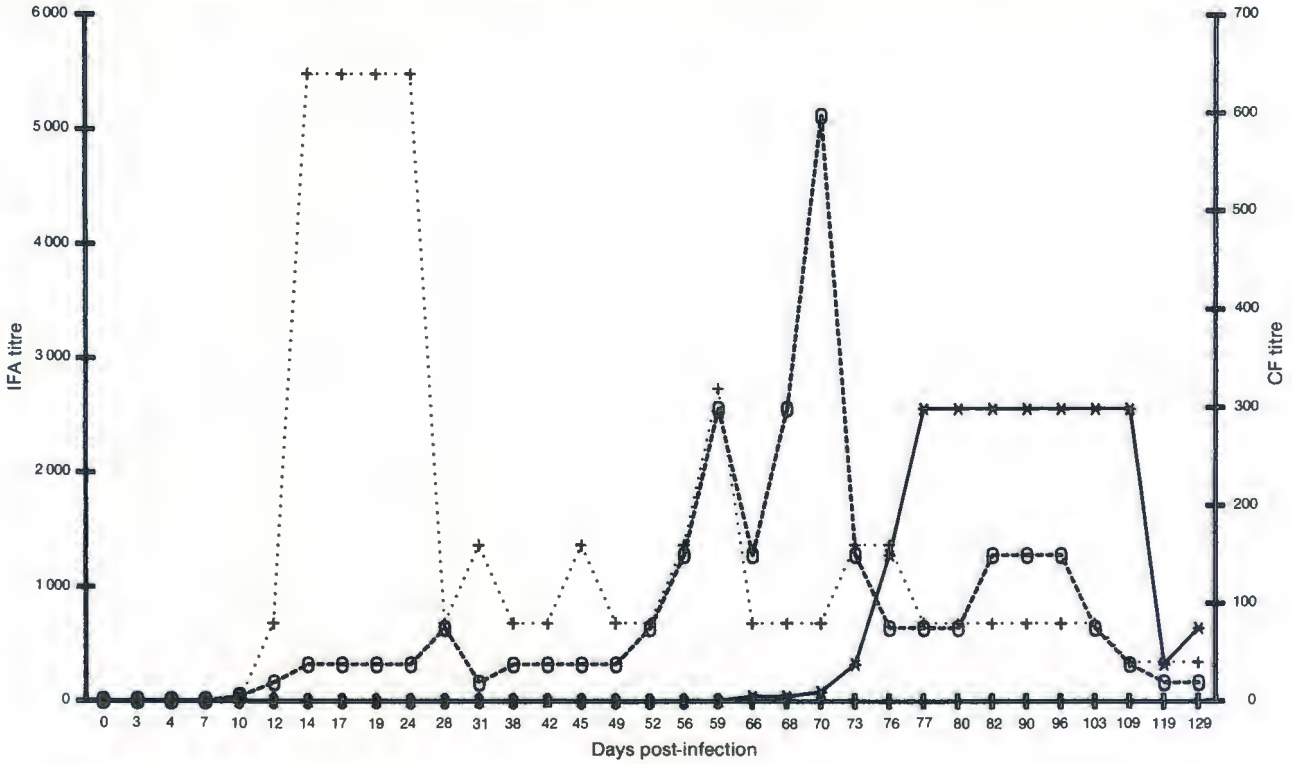

FIG. 2 IFA and CF titres after experimental infection of Horse 198 with *Babesia equi* and *Babesia caballi*
O---O---O *Babesia equi* IFA titre
——* *Babesia caballi* IFA titre
+...+...+ *Babesia equi* CF titre

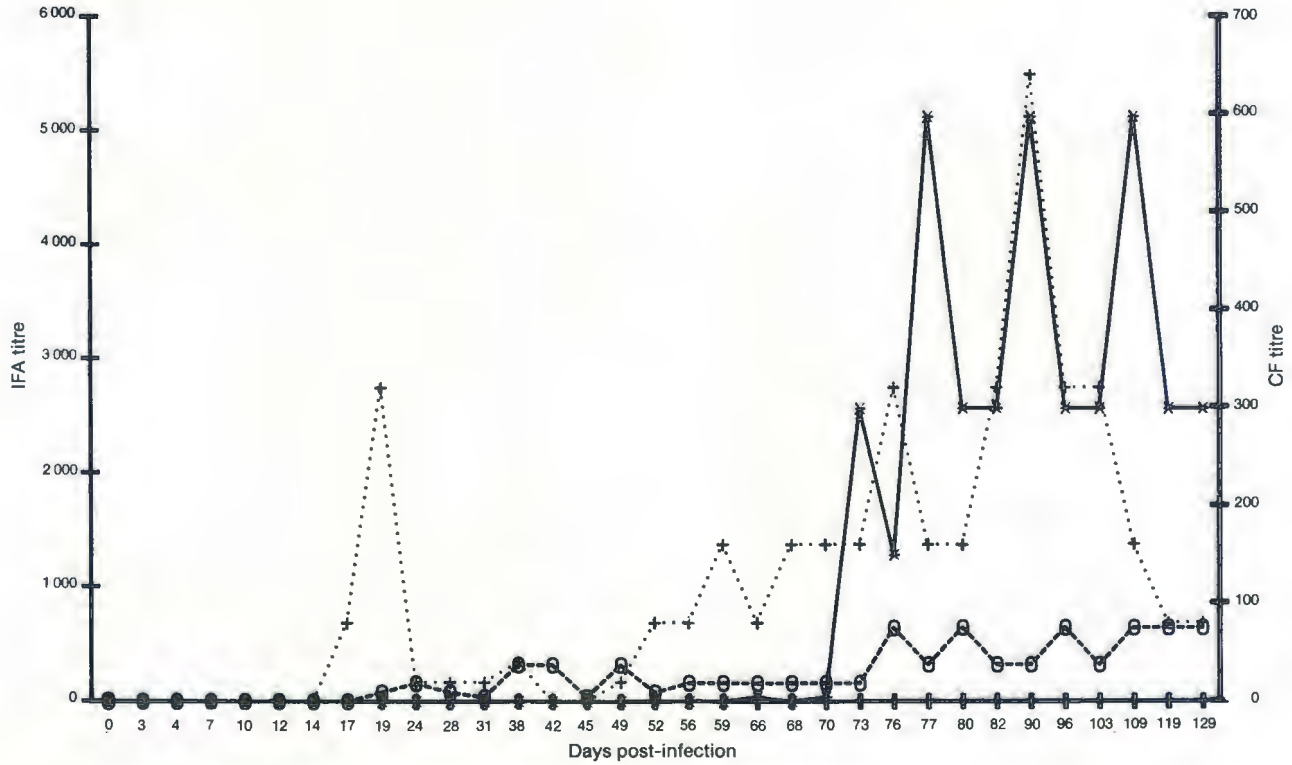

FIG. 3 IFA and CF titres after experimental infection of Horse 235 with *Babesia equi* and *Babesia caballi*
O---O---O *Babesia equi* IFA titre
——* *Babesia caballi* IFA titre
+...+...+ *Babesia equi* CF titre

examination of stained blood smears. Mildly elevated fibrinogen concentrations were associated with these changes (Tables 2 & 3).

A mild drop in albumin, iron and phosphorus concentrations, as well as an elevated serum bilirubin concentration, was observed in Horse 198 in association with the haematological changes described above (Table 2). No significant changes were recorded in the following parameters measured; white blood cell count, sodium, potassium, urea, cretinine, ALP, AST, CK and ALT.

The serological responses are summarized in Fig. 1–3.

Infection of horses with B. caballi.

An increase in rectal temperature was recorded in all 3 experimental horses within 7–13 days of infection with *B. caballi*. The only other clinical sign observed was slight icterus in all 3 horses during post-infective Days 14–17.

B. caballi parasites were first observed in thick blood smears of the horses between Days 7–8 post-infection. The highest recorded parasitaemia score was 5 on Day 13 in the blood smear of Horse 198. No *B. equi* parasites were seen on blood smears of the horses during the *B. caballi* reaction.

All the experimental animals showed a drop in the Hb concentration, Ht and RCC within 8–15 days after infection with *B. caballi*. A marked drop in platelet count was associated with these findings as well as with parasitaemia with *B. caballi*, as was evidenced by positive blood smears. Mild increases in the fibrinogen concentration was associated with these changes. An increased FDP concentration was never observed (Tables 1, 2 & 3).

Haematological changes were further associated with a drop in the concentrations of albumin, phosphorus and iron, as well as increases in the concentration of bilirubin (Tables 1, 2 & 3).

The serological results are summarized in Fig. 1–3.

Discussion

Despite the fact that experimental infection in this study did not result in overt signs of equine piroplasmosis, typical haematological features of the disease were nevertheless induced. The absence of clinical signs in the animals with haematocrit values as low as 17 % and with a percentage platelet count reduction of up to 70 % of normal can perhaps be ascribed to: (a) the relative apathogenicity of the strains of *B. equi* and *B. caballi* used, and (b) the minimal amount of physical stress imposed upon infected animals. Throughout the experiment, the animals had no exercise; they were not subjected to adverse housing conditions and methods of restraint were not different from those experienced by them prior to the experiment. The lack of clinical signs in the presence of a relatively low Ht supports the concept of the release of enzymes as an important factor in the pathogenesis of babesiosis, as was described for bovine babesiosis (Wright, 1981).

In the Republic of South Africa, where equine piroplasmosis is thought to be endemic in large areas of the country and perhaps a relatively small number of clinical cases are encountered, it is likely that certain strains of parasite would cause severe clinical reactions. Also, only animals subjected to stress, such as physical performance and concomitant infection with, for example, African horse sickness virus, would be likely to develop overt signs of disease (J. van Heerden, unpublished observations, 1976–86).

The finding of thrombocytopaenia in equines showing a parasitaemia with *B. equi* or *B. caballi* is in agreement with findings of Allen, Frerichs & Holbrook (1975b). Thrombocytopaenia has also been described in other protozoan infections in man and animals and, although various theories as to its aetiology have been proposed, the exact pathophysiological mechanisms remain unknown (Van Heerden, Reyers & Stewart, 1983). The cause for the slightly elevated fibrinogen concentrations observed in this investigation is unclear. One theory might be that of a lowgrade chronic process of disseminated intravascular coagulopathy as proposed for dogs with *Babesia canis* (Greene, 1975). Further investigation is needed, however, to elucidate the pathogenesis of a thrombocytopaenia in equine piroplasmosis.

The drop in haematocrit is in agreement with findings of Allen *et al.* (1975a) and appears to be a sensitive indicator of early parasite-induced pathological change. It remains a cheap and useful tool for the practitioner to monitor an equine patient suffering from piroplasmosis.

The appearance of IFA antibodies to *B. caballi* and *B. equi* more or less coincided with the first appearance of parasites in blood smears which is in agreement with the results of Weiland (1986). Frerichs, Holbrook & Johnson (1969) found CF antibodies only 6–8 days after parasites were observed in stained blood smears. A second IFA and CF titre peak in some of the horses at the time of the *B. caballi* reaction could be ascribed to cross-reactions during the acute phase, as observed by Holbrook *et al.* (1973) and Frerichs *et al.* (1969). The *B. caballi* IFA titres at this stage, however, were very low. The duration of antibody titres in the carrier animals was not determined. The temperature reactions in the case of *B. equi* infections were very mild, while the *B. caballi* infections resulted in more severe febrile reactions of an irregularly intermittent nature which one would expect to find in *B. equi* (Littlejohn, 1963).

Serological tests are useful tools to detect *B. equi* or *B. caballi* carrier animals. False positive and/or negative results do occur (Frerichs *et al.*, 1969; Weiland, 1986). However, these tests have proved to be highly specific under similar conditions (De Waal, Van Heerden, Van den Berg, Stegman & Potgieter, 1987). It is not clear why only mild *B. equi* reactions with a low parasitaemia occurred. In one animal (No. 180), no *B. equi* parasites were detected though seroconversion did take place.

The *B. caballi* reactions are consistent with other experiments involving antigen production for CF and IFA tests, as well as transmission studies, both utilising blood stabilates (De Waal, unpublished observations 1984–86). Further investigations repeating this study on tick-transmitted babesiosis in susceptible horses may help to clarify some of these issues.

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