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SEROLOGICAL RESPONSE OF CATTLE TO INFECTION WITH *BABESIA BIGEMINA* AND *BABESIA BOVIS* IN SOUTHERN AFRICA

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ABSTRACT

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The indirect fluorescent antibody test was used to measure the antibody response of cattle for 8 weeks after infection with either *Babesia bigemina* or *Babesia bovis*, or a combination of both species. Serological cross-reactions were observed between the 2 species, but these were most marked when *B. bigemina* antigen was used. In animals infected with both *Babesia* spp., the *B. bigemina* reaction appeared to suppress the *B. bovis* reaction.

INTRODUCTION

The indirect fluorescent antibody (IFA) test has been shown to be useful in the identification and diagnosis of *Babesia* spp. (Zwart, Van den Ende, Kouwenhoven & Buys, 1968; Brocklesby, Zwart & Perié, 1971; Goldman, Pipano & Rosenberg, 1972; Joyner, Donelly, Payne & Brocklesby, 1972; Leeflang & Perié, 1972; Goldman & Rosenberg, 1974; Todorovic & Long, 1976; Fujinaga, Minami & Ishihara, 1980).

The IFA test has also been used successfully in this Institute to differentiate between *Babesia bigemina* and *Babesia bovis* during field surveys. It was noted during these surveys that animals in areas where *B. bovis* does not occur occasionally showed low-level, positive reactions to this species. This phenomenon was assumed to be due to the serological cross-reactions between *B. bigemina* and *B. bovis* described by Todorovic & Long (1976), Fujinaga *et al.* (1980) and Gray & De Vos (1981).

In this study we attempted to investigate further the phenomenon of serological cross-reactions between *B. bigemina* and *B. bovis* under controlled conditions in the laboratory, using the IFA test.

MATERIALS AND METHODS

Animals

A total of 14 cross-bred *Bos indicus* cattle, 18–24 months old, were obtained from a nearby government experimental farm. They were serologically negative for *B. bigemina* and *B. bovis* before use and were kept tick-free during the course of this investigation.

Parasite strains

The strains of *B. bigemina* and *B. bovis* used in this study were those issued at the time by this Institute in the bivalent babesiosis vaccine.

B. bigemina: The P strain (De Vos, Combrink & Bessenger, 1982 b) was isolated from *Boophilus decoloratus* ticks collected in the Pongola district, Natal. It was unmodified, and was stored in liquid nitrogen as an infected blood stabilate with DMSO as cryoprotectant. The technique used to freeze the blood was described by De Vos *et al.* (1982 b). Each 2 mℓ ampoule contained approximately 4×10^7 parasites when frozen.

B. bovis: The S strain (De Vos, 1978) was isolated from *B. microplus* ticks collected in the Pretoria district, Transvaal. This strain was modified by a series of 10 rapid passages in splenectomized calves (De Vos, Bessenger & Fourie, 1982 a) and stored as blood stabilate of Passage 11 in liquid nitrogen. Each 1 mℓ ampoule contained approximately 1×10^7 parasites when frozen.

Method of infection

The ampoules of stabilate were transported on dry ice and kept frozen until just before use. They were then thawed rapidly in water (*c.* 40 °C) and inoculated subcutaneously into each animal, as outlined below.

Experimental procedure

The animals were divided into 3 groups of 4 animals each and a 4th control group of 2 animals.

Group 1: Each animal was inoculated subcutaneously with 2 mℓ of thawed *B. bigemina* + 2 mℓ of *B. bovis* stabilate.

Group 2: Four animals were each inoculated with 2 mℓ of thawed *B. bigemina* stabilate.

Group 3: Four animals were each inoculated with 2 mℓ of thawed *B. bovis* stabilate.

Group 4: Two uninfected control animals.

Thin and thick (Mahoney & Saal, 1961) blood smears were made daily from all the animals after infection, stained with Giemsa's stain and examined for blood parasites. The animals were also bled weekly for serological tests for 8 weeks after infection.

Serology

The IFA technique, essentially a modification of the technique described by Joyner *et al.* (1972), was used as described by Gray & De Vos (1981).

All the sera were stored at −20 °C until tested. Titrations were made, starting at 1:40 and using doubling dilutions, and the titre was taken as a reciprocal of the highest dilution giving specific fluorescence. All the sera were tested against antigens of both *B. bigemina* and *B. bovis*.

Commercial rabbit anti-bovine globulin*, conjugated with fluorescein isothiocyanate at a dilution of 1:80, was used throughout. The fluorescence was observed with a Leitz Orthoplan microscope, using a 50 water immersion objective and incident light excitation.

RESULTS

Infectivity of stabilates

In Group 1, where *B. bigemina* and *B. bovis* were inoculated simultaneously, *B. bigemina* had a mean prepatent period of 3,5 ± 0,58 days, while the *B. bovis* parasites were first seen after 17,5 ± 2,08 days.

The animals inoculated with *B. bigemina* only (Group 2) had a mean prepatent period of 3,0 ± 0,93 days, while those inoculated with *B. bovis* only (Group 3) had a mean prepatent period of 7,25 ± 1,71 days.

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TABLE 1 Titres of animals infected simultaneously with *B. bigemina* and *B. bovis* stabilate

Animal No.		2305		2394		2459		2633	
Antigen		*B. bigemina*	*B. bovis*	*B. bigemina*	*B. bovis*	*B. bigemina*	*B. bovis*	*B. bigemina*	*B. bovis*
Weeks after infection	0	<40	<40	<40	<40	<40	<40	<40	<40
	1	<40	ND	<40	<40	<40	<40	<40	40
	2	80	<40	160	80	160	80	160	80
	3	160	80	640	80	320	640	320	160
	4	160	160	640	160	320	320	320	320
	5	320	320	640	320	320	640	640	640
	6	320	640	640	160	160	640	640	640
	7	640	640	1 280	320	1 280	320	320	320
	8	640	640	640	640	640	320	320	640

TABLE 2 Titres of animals infected with *B. bigemina* only

Animal No.		1539		2307		2498		2870	
Antigen		*B. bigemina*	*B. bovis*	*B. bigemina*	*B. bovis*	*B. bigemina*	*B. bovis*	*B. bigemina*	*B. bovis*
Weeks after infection	0	<40	<40	<40	<40	<40	<40	<40	<40
	1	40	<40	<40	<40	<40	<40	<40	<40
	2	160	<40	40	<40	160	80	320	40
	3	640	40	320	80	640	160	640	80
	4	320	40	320	<40	640	ND	640	ND
	5	320	80	160	<40	640	80	160	40
	6	320	40	320	40	320	40	320	40
	7	160	40	320	40	320	40	320	<40
	8	320	<40	160	40	640	40	80	<40

TABLE 3 Titres of animals infected with *B. bovis* only

Animal No.		2221		2338		2511		2580	
Antigen		*B. bovis*	*B. bigemina*	*B. bovis*	*B. bigemina*	*B. bovis*	*B. bigemina*	*B. bovis*	*B. bigemina*
Weeks after infection	0	<40	<40	<40	<40	<40	<40	<40	<40
	1	<40	<40	<40	<40	<40	<40	<40	<40
	2	160	<40	<40	<40	80	<40	160	80
	3	1280	80	320	80	640	160	320	160
	4	1280	160	1280	80	1280	160	640	<40
	5	2560	80	640	80	320	80	320	<40
	6	640	160	640	80	1280	160	640	80
	7	1280	80	640	<40	1280	160	640	160
	8	1280	80	640	<40	640	160	1280	80

Blood smears of the 2 uninfected controls remained negative for the duration of the experiment.

Serology

After simultaneous inoculation of *B. bigemina* and *B. bovis*, fluorescent antibodies to both species were detected from 2–3 weeks after infection (Table 1). Antibodies to *B. bigemina* reached maximum titres of 640–1280 6–7 weeks after infection, while antibodies to *B. bovis* reached a titre of 640 between Weeks 5 and 8 in the different animals.

Animals inoculated with *B. bigemina* only reached peak titres of 320–640 with the homologous antigen by Week 3 (Table 2). These titres persisted for 1–3 weeks before declining to levels of 160–320 by Week 8. The reactions of *B. bigemina* antisera with *B. bovis* antigen reached peak titres of 80–160 at about the same time as maximum reactivity was observed with the homologous antigen. These titres also declined to <40–40 by Week 8.

The titres of the animals infected by *B. bovis* only, reached peak titres of 640–2560 with the homologous antigen between Weeks 4 and 5, but declined to 640–1280 at the end of the investigation 8 weeks after infection (Table 3). The titres of *B. bovis* antisera with the heterologous *B. bigemina* antigen reached peaks of 80–160 between Weeks 3 and 5 and were still at these levels in 3 out of the 4 animals at Week 8.

The uninfected controls throughout failed to show specific fluorescence for either of the *Babesia* spp. at a dilution of 1:40.

Discussion

Both the *B. bigemina* and *B. bovis* stabilates used in this investigation proved to be highly infective. The prepatent periods of *B. bigemina* infections were comparable in animals infected with this species either on its own or combined with *B. bovis*. However, the prepatent periods of *B. bovis* infections were very much shorter in the animals infected with this species alone. The presence of *B. bigemina* complicated the search for *B. bovis* in the thick blood smears of the animals infected with both species, but this did not explain the markedly prolonged prepatent periods in these animals. We therefore conclude that the concomitant but more rapid *B. bigemina* infection had a suppressive effect on the development of *B. bovis*.

The specificity of the IFA technique used in this study increased during the 8 weeks following infection. False positive *B. bovis* reactions appeared to be of importance for only a short period after *B. bigemina* infections, but 2 out of 4 animals still had specific antibodies at a titre of 40 8 weeks after infection. False positive *B. bigemina* reactions, however, were more persistent, as 3 out of the 4 animals infected with *B. bovis* still showed specific fluorescence 8 weeks after infection at titres of 80-160.

Goldman *et al.* (1972) observed no cross-reactions between *B. bigemina* and *B. bovis (= berbera)* in serological tests performed with the IFA test. They concluded that the 2 *Babesia* species were antigenically distinct. The results reported by Leeflang & Perié (1972), however, indicated a "limited relation" between the 2 species. Todorovic & Long (1976) also reported possible cross-reactions in certain naturally infected cases. In Australia, the IFA test is regarded as a reliable test for the study of *B. bovis* (Johnston, Pearson & Leatch, 1973), but it is considered to be lacking in sensitivity and specificity for *B. bigemina* (Callow, 1979).

The results reported above explain the occasional finding during a recent serological survey of low level *B. bovis* reactions in areas where it was not known to occur (De Vos, unpublished observations, 1981). *B. bigemina* is more widespread in South Africa than *B. bovis*, and its infection rate is generally much higher than that of *B. bovis* on infected farms. We therefore do not consider false positive *B. bigemina* reactions to be of major significance in surveys of bovine babesiosis in South Africa. As a result of this investigation it was decided to accept only titres of 80 or greater as positive in serological surveys of bovine babesiosis in this country.

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