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SEROLOGICAL RESPONSE IN FIELD CATTLE IMMUNISED AGAINST *BABESIA BERBERA*

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SUMMARY

Serological tests were performed on 184 *calves before and after immunisation with live* Babesia berbera *parasites. Ninety per cent of the animals were negative at time of immunisation. Titres of* 1 : 64 *and* 1 : 256 *appeared* 14 *days after immunisation. From day* 34 *till* 615 *after immunisation all animals tested were positive. Three out of* 24 *calves challenged with live parasites after immunisation reacted with fever or parasites in spite of circulating antibody titres of* 1 : 256 *or higher.*

INTRODUCTION

Protection of cattle against clinical disease caused by *Babesia berbera* is accomplished in Israel by inoculating calves with blood drawn from a donor animal carrying a low-level, chronic infection. In most cases the calves experience a mild clinical and parasitological response during the first 2 weeks after inoculation. This is followed by a latent infection that appears to protect the animals against natural challenges in tick-infested pastures (Sergent, Donatien, Parrot and Lestoquard, 1945). In order to avoid occasional severe reactions and even possible death from the vaccine, the calves are treated with a single dose of Diampron (Amicarbalide, May and Baker, Dagenham, UK) from 9 to 14 days after vaccination (Pipano, Raz and Klinger, 1970). Questions may arise as to the potency of a marginally infective live vaccine used under field conditions where delays of several hours at high temperatures may occur between inoculations of different herds. Since the initial reaction to vaccination is generally mild and of short duration, clinical indications of successful immunisation may be missed in individual animals if a vaccinated herd is inspected only once or twice during the first 2 weeks. Furthermore, the effect on the immune response of a one-shot drug treatment as practised in Israel is not yet fully elucidated. For these reasons serological evaluation of the response to immunisation could be of considerable help in determining the success of a vaccination programme.

We have previously described an indirect immunofluorescence test for antibodies to *B. berbera* (Goldman, Pipano and Rosenberg, 1972). In the present paper we report use of this test to determine the serological response over an extended period of time of field herds immunised against this parasite.

MATERIALS AND METHODS

Several hundred calves from 1 to 6 months of age distributed among three dairy herds were vaccinated. In each herd one group was vaccinated in February or March and a second group born since the first was immunised, in July. The vaccine consisted of an intramuscular inoculation of 5 ml citrated blood from a single chronically infected donor animal. On the day of immunisation and at intervals thereafter (up to 615 days) blood was drawn for serological examination. Due to the practical difficulties involved in follow-up of such a large number of field animals, not all animals were bled at all intervals. As a consequence, the present paper is based on results obtained with 184 calves for whom an initial and at least one subsequent serological test were available.

Blood smears to be examined for parasites were taken from 154 animals on the day of immunisation and again on days 9, 11 and 14 after immunisation (a.i.) at which times temperature was also measured. The smears were stained with Giemsa and examined for 10 minutes each with the 2 mm oil immersion objective. Animals responding to the primary inoculation with fever of over 40°C were treated immediately with "Diampron" (10 mg/kg). All the others were treated similarly following the last clinical examination on day 14 a.i.

A challenge dose of parasites (5 ml of blood from the same donor that provided the original inoculum) was administered to 24 animals 4 and 5 months later. The animals were checked for fever and parasites on days 9, 11, and 14 after the challenge.

All three herds were located in the northern part of the Sharon coastal plain near the foothills of Samaria. In previous years outbreaks of babesiasis had occurred in two of the herds but at the time of vaccination no clinical cases were apparent. At all three farms the calves were inspected for ticks at the time that blood smears were taken. No ticks were found on any of them.

Immunofluorence tests

The indirect fluorescent antibody method was used, essentially as described previously (Goldman *et al.*, 1972) but with the following technical modification: antigen smears, removed from storage at −20°C, were permitted to dry in a desiccator jar before being fixed in water-free acetone. Acetone from freshly opened bottles, or used acetone stored over $CaCl_2$, gave dependably good fixation. Acetone containing enough water to leave a film of moisture on the slide upon removal from the fixative yielded poorly reacting parasites in the fluorescence test.

Sera were tested in four-fold dilutions starting at 1 : 16, the minimum positive dilution based upon comparison with known negative controls. Readings were made on a Wild fluorescence microscope equipped with a dark-field condenser. The light source was a 100 W quartz iodine filament bulb operated at 10 V. The primary filter was an interference type with peak transmission at 480 μm and the secondary was a matched yellow glass, both obtained from Optisk Laboratorium, Lyngby, Denmark.

RESULTS

Table I shows the immunofluorescence titres found in each of the herds separately in sera drawn on the day of immunisation. In all three herds most of the sera was negative, the range being from 87 to 94 per cent of the calves immunised. No *B. berbera* parasites were found on blood smears taken at this time.

TABLE I

Titres found on day of immunisation in the three herds studied

Herd	Neg.	1 : 16	1 : 64	1 : 256 and higher	Total
R	73 (87%)	2	2	7	84
G	43 (94%)	0	1	2	46
M	49 (91%)	4	0	1	54

Since the pattern of response after immunisation was similar in all three herds, results at the various time intervals have been pooled. Table II summarises titres found in all three herds from day 0 to day 615 a.i. On day 0, 165 of the 184 calves

tested were negative, with the rest showing titres of up to 1 : 256. By day 14 only one out of nine calves tested was negative, the other eight showing titres of 1 : 64 or 1 : 256. From day 34 onward no more negative results were encountered out of 295 samples tested and only one serum was positive at 1 : 16. All the rest titred at 1 : 64, 1 : 256 or higher.

The percentage of sera positive at 1 : 256 or higher was greatest in tests conducted 61 and 69 days a.i. (87 per cent) and remained high through the period 147–181 days later (81 per cent). In subsequent tests the percentage of titres in this range dropped, being 40 per cent of 15 animals tested at day 615 a.i.

TABLE II

Titres found in all herds combined from day 0 *to day* 615 *after immunisation*

Titre	0	14	34	61, 69	147, 181	206, 208, 216	353	503	615
Neg.	165	1	0	0	0	0	0	0	0
1 : 16	6	0	0	0	0	1	0	0	0
1 : 64	3	3	19	11	5	27	27	6	9
1 : 256 or higher	10	5	18	74	21	40	23	8	6
	(54%)	(56%)	(49%)	(87%)	(81%)	(59%)	(46%)	(57%)	(40%)
Total animals tested	184	9	37	85	26	68	50	14	15

Table III shows the clinical and parasitological response following the initial immunisation and the challenge inoculation 4 and 5 months later. Of 154 animals checked after the primary inoculation, 96 (62·3 per cent) showed fever (temperature higher than 39·5°C) and 49 (31·8 per cent) showed parasites. Two of the calves with fever had titres of 1 : 256 on the day of immunisation and one calf with fever and parasites, had a titre of 1 : 16. The rest of the reacting calves were negative serologically when immunised. After the challenge inoculation, two out of 24 calves (9·1 per cent) showed fever and one (4·2 per cent) revealed parasites. All three of the reacting calves had titres of 1 : 256 or greater when they received the challenge dose.

TABLE III

Clinical and parasitological response of calves following primary and challenge inoculations of living B. berbera *parasites*

When tested	No. tested	Calves showing	
		Fever	Parasites
Following primary immunisation	154	96 (62·3%)	49 (31·8%)
Following challenge	24	2 (9·1%)	1 (4·2%)

DISCUSSION

It is clear that the immunofluorescence test is useful for assessing the results of vaccination against *B. berbera* in field herds. During the period 14 to 165 days after immunisation essentially all of the animals tested yielded positive serologic results, although 90 per cent of the animals were negative at the time of vaccination. In most cases titres were 1 : 64, 1 : 256 or greater in a test procedure where 1 : 16 is the

lowest positive result. Thus, for mass screening purposes testing sera at a single dilution of 1 : 16 or 1 : 32 could provide information about clearly positive or negative animals relatively quickly and reliably.

On the day of immunisation 10 per cent of the calves already showed serologic titres. Since no clinical babesiosis was detected in the herds at that time and since the animals had not yet been put out to pasture, it is likely that most of these reactions were due to maternally transmitted antibody. Other work in our laboratory and elsewhere (Ross and Lohr, 1970) has shown that antibody titres to *Babesia* derived from colostrum may persist for several months under conditions where transmission by ticks is not taking place.

Table II shows that circulating antibody to *B. berbera* appeared rapidly following vaccination with a small dose of living parasites and chemotherapy 9 to 11 days later. Antibody titres reached a peak during the period 2 to 6 months after immunisation and regressed thereafter but moderately high titres were still present after 20 months. Whether the persistence of circulating antibody for almost 2 years after a single vaccinating inoculation was due only to the vaccination, or whether it represented the reinforcement effects of a low level of natural transmission during the period, cannot be answered by this study. Johnston and Tammemegi (1969), working with *Babesia argentina*, found no difference in the immunofluorescence serology of immunised calves kept in tick-free or tick-infected paddocks over a period of 13 months. From a practical standpoint, it is significant that a single immunisation of calves raised in an area of low endemicity for *B. berbera* resulted in a long-life serologic titre.

Although the usefulness of the serologic test in revealing exposure to *B. berbera* is clear, the value of a serologic titre in indicating the immune status of an animal is not absolute. Out of 96 calves showing fever after the primary vaccination two had immunofluorescence titres of 1 : 256 on the day of immunisation. This is a lower percentage than the overall 10 per cent serologically positive reactors found in the entire group vaccinated but it shows that even a relatively high titre is not an indication of absolute resistance to infection. Similarly, at the time of the challenge inoculation two out of 24 calves showed fever and one showed parasites, although all three reacting animals had serological titres of 1 : 256 or greater. Johnston and Tammemegi (1969) found that challenge with a heterologous strain of *B. argentina* could result in a parasitaemia in the presence of circulating antibodies. Our results extend this observation to include challenge even with a homologous strain.

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RÉPONSE SÉROLOGIQUE DE BOVINS IMMUNISÉS CONTRE *BABESIA BERBERA*

Résumé—Des épreuves sérologiques ont été effectuées sur cent quatre vingt quatre veaux avant et après immunisation avec des *Babesia berbera* vivantes.

Quatre vingt dix pour cent des animaux ont réagi négativement au moment de l'immunisation. Des taux d'anticorps de 1 : 64 et 1 : 256 sont apparus quatorze jours après l'immunisation. Du 34e au 615e jour après l'immunisation, tous les animaux testés ont réagi positivement.

Trois veaux sur vingt quatre soumis à l'épreuve avec des parasites vivants après l'immunisation ont réagi par de la fièvre ou l'apparition de parasites, malgré des taux d'anticorps circulant de 1 : 256 ou plus.

RESPUESTA SEROLOGICA EN GANADO DE CAMPO IMMUNIZADO CONTRA *BABESIA BERBERA*

Sumario—Pruebas serologicas fueron llevadas a cabo en 184 terneros antes y despues de la immunización con parásitos vivos de *Babesia berbera*. Noventa y cuatro por ciento de los animals fueron negativos al momento de la immunizacion. Titulos de 1 : 64 y 1 : 256 aparecieron alrededor de 14 dias despues de la immunización. Desde los 34 hasta los 615 dias despues de la immunización todos los animales muestreados fueron positivos. Tres de 24 terneros desafiados con parasitos vivos despues de la immunización reaccionaron con fiebre ó parásitos a pesar de tener títulos de anticuerpos circulantes de 1 : 256 o mas.