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THE IMMUNIZATION OF CALVES AGAINST HEARTWATER: SUBSEQUENT IMMUNITY BOTH IN THE ABSENCE AND PRESENCE OF NATURAL TICK CHALLENGE

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ABSTRACT

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Cattle, vaccinated as calves with *Cowdria ruminantium*-infected tick stabilate, were challenged 6, 12 and 24 months later. In the absence of tick challenge, vaccination of calves induced a partial immunity against subsequent challenge at 12 and 24 months. In animals exposed to ticks, the resistance was no better than that of control, unvaccinated cattle. When they were challenged at 6 months of age there was no difference between vaccinated and unvaccinated calves, either in the absence or presence of tick challenge, and all the animals manifested a high degree of natural resistance. This study therefore suggests that the value of vaccinating Afrikander-cross calves in heartwater endemic areas should be further investigated.

The indirect fluorescent antibody (IFA) test proved to be a valuable means of monitoring the serological response of vaccinated animals and detecting the sero-conversion of animals exposed to tick infection. On one hand, there was good correlation between the febrile reaction and the results of the IFA test on the sera of vaccinated and control cattle challenged with the heartwater agent, in that all sero-positive animals were resistant to challenge. On the other hand, though, a considerable percentage of the animals that were serologically negative were also resistant to challenge.

INTRODUCTION

The phenomenon that calves under the age of one month are more resistant to heartwater than adult cattle makes it possible to immunize them at an early age, and this procedure has been widely practised since it was first recommended in 1945 (Neitz & Alexander). These workers reported that out of 1 374 Aberdeen Angus calves immunized at pasture in a heartwater endemic region, 8,8 % contracted heartwater in the subsequent 3 years against 24,6 % of 195 unvaccinated controls. The efficacy of this procedure, however, is not beyond all doubt and losses due to natural tick-transmitted heartwater of cattle immunized as calves are reported from time to time.

To further evaluate the method, it was therefore decided to compare the immunity of animals, vaccinated as calves and subsequently reared on a farm practically free from the arthropod vector, with the immunity of animals also vaccinated as calves but subsequently maintained on a farm in a heartwater endemic area. At the same time the experiment served as a first field trial to evaluate a new method in the preparation of a vaccine against heartwater by using *Amblyomma hebraeum* nymphae infected with *Cowdria ruminantium* (Bezuidenhout, 1981). Furthermore, the recent development of the indirect fluorescent antibody (IFA) test (Du Plessis, 1981; 1982a) facilitated the serological monitoring of the experiment.

MATERIALS AND METHODS

Animals

The Afrikander-Simmentaler- or Afrikander-Hereford-cross calves of both sexes at the age of 1–4 weeks used were kept at pasture on 2 government experimental farms. Both farms are situated in heartwater endemic areas, but on one farm (Kaalplaas) tick infestation is minimal as a result of an intensive dipping programme over several years prior to the experiment, so that the heartwater vector, *A. hebraeum*, is practically absent. According to official records, no clinical cases of or mortalities due to heartwater have been recorded on this farm for the past 5 years.

On the other farm (Mara), tick control over the past 8 years consisted of the fortnightly dipping of all cattle 2 years old and older during spring, summer and autumn, and 3-weekly dipping during winter. Calves from weaning until 2 years of age were dipped strictly every week. The animals in this experiment were also subjected to this dipping programme. Over the past 12 years, some 187 cattle died from naturally-acquired heartwater at Mara. The diagnosis was confirmed with positive brain smears in all except 11 of these cases.

Immunization of calves

The *C. ruminantium*-infected tick filtrate used to immunize the calves was prepared and tested according to the method discribed by Bezuidenhout (1981).

On each farm 30 calves were inoculated intravenously with 5 mℓ of infective inoculum and 30 were left as controls. Rectal temperatures were recorded early each morning. Thirteen days after the inoculation of the infective inoculum, 20 mℓ of blood was collected in heparin from each of the 30 Kaalplaas calves and 10 mℓ was injected intravenously into each of 2 heartwater susceptible sheep. This procedure was repeated 2 days later and the same sheep were injected with the blood from the same calf. The temperatures of the sheep were recorded daily and the sheep that survived were challenged with heartwater-infected sheep blood (Ball 3 strain) one month later.

Six, 12 and 24 months after immunization, 10 vaccinated and 10 control animals on each farm were challenged by inoculating them intravenously with 10 mℓ of sheep blood infected with a field strain of *C. ruminantium* isolated from a goat from the Messina district in the Northern Transvaal. The strain was passaged in sheep several times and the infected blood was then collected, tested for infectivity and absence of contaminating micro-organisms and stored as previously described (Du Plessis, 1982 b). The early morning rectal temperatures of all animals challenged were recorded.

Serum samples, collected from all the experimental animals on the day the vaccine was administered as well as each time that the challenge inoculations were given, were stored at −18 °C.

Indirect fluorescent antibody test

The IFA test was carried out as described in a previous publication (Du Plessis, 1982 a), except that antigen slides were stored at −18 °C and that all sera were tested at a dilution of 1:20.

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RESULTS

Febrile reactions

The febrile reactions of the calves following both immunization and challenge were arbitrarily divided into 4 categories as previosly described (Du Plessis & Bezuidenhout, 1979). Animals that showed Categories I and II reactions were considered susceptible, and those in Categories III and IV as resistant.

Reactions following immunization

Only 12 out of 30 Kaalplaas calves vaccinated with the tick stabilate showed mild febrile reactions (Category III), while the other 18 showed no febrile reaction at all (Category IV). Likewise, only 14 out of 30 Mara calves showed Category III reactions and the rest Category IV reactions.

Sheep, inoculated with blood from 12 out of 30 Kaalplaas calves drawn 13 and 15 days after they had been inoculated with the tick stabilate, either died from heartwater of developed severe febrile reactions and clinical signs of the disease. The sheep that reacted and survived were found to be solidly immune against challenge. There was no correlation between these 12 calves and the 12 animals that had shown a mild febrile reaction to the immunizing inoculation.

Reactions to challenge

The reactions to challenge of Kaalplaas and Mara cattle immunized as calves and the non-immunized controls are summarized in Table 1. It can be seen that at 6 months after immunization there was no significant difference between immunized and control calves, either in the Kaalplaas or Mara animals. At 2 years after immunization there was only a slight difference between the immunized and control Kaalplaas groups, but no difference in the case of the Mara animals. It is important to note that on both occasions a high percentage of control animals were resistant, particularly in the case of the Mara cattle.

At the 12 month challenge period, however, an appreciably greater number of Kaalplaas animals that had been immunized as calves were resistant to challenge than were the control animals. There was no difference between the Mara groups.

IFA test results

From the results given in Table 1 it can be seen that the serum of 23 out of 60 Mara calves, collected on the day of immunization, gave a positive reaction. Many of the positive reactions were possibly due to passively transferred colostral antibodies. All the sera of the Kaalplaas calves were negative. Three months after immunization, 27 Kaalplaas and 21 Mara calves that had been vaccinated were serologically positive. The serological response at this stage did not correlate with the febrile reactions exhibited by the calves at immunization, since several calves that showed a mild febrile reaction were serologically negative, whereas others that had failed to react altogether were serologically positive.

Furthermore, there was no correlation between the presence of the heartwater agent in the blood of Kaalplaas calves and their serological response. The sera of only 10 out of 12 calves, the blood of which had elicited heartwater in sheep, gave a positive reaction with the IFA test 3 months after immunization, while that of 17 out 18 other calves, the blood of which had not been infective, were also serologically positive. There was therefore no correlation between the presence of the heartwater agent in the blood of Kaalplaas calves and their serological response.

It is evident from Table 1 that only 5 out of the 9 Kaalplaas calves that were serologically positive at 3 months post-immunization were still positive at 6 months when they were challenged. In the group challenged 12 months after vaccination, only 2 out of 10 were positive. No serological positives were detected amongst the immunized group challenged at 24 months.

The sera of 3 out of the 5 Mara calves found positive at 3 months gave a positive reaction in the IFA test at 6 months after immunization. In the group challenged 12 months after vaccination, the 2 animals that had been sero-positive 3 months after immunization were still positive, but the sera of 3 other animals were also positive, most probably as a result of tick infection. Two years after immunization, the serum of only one out of 9 animals vaccinated as calves was positive. It is therefore clear that cattle, serologically positive according to the IFA test, become negative, even with the possibility of tick re-infection.

TABLE 1 Reactions to challenge and results of IFA test on sera of Kaalplaas and Mara cattle immunized as calves and of unvaccinated controls

Tick challenge	No. of animals		Age in months at challenge	Reaction category				Number of sera positive to IFA test at 1–24 months of age				
	Immunized	Controls		I	II	III	IV					
				Susceptible		Resistant		1[1]	3	6	12	24
Absent (Kaalplaas)	9[2]	—	6	0	1	5	3	0	9	5	—	—
	—	10	6	0	3	4	3	0	—	1	—	—
	10	—	12	2	1	3	4	0	10	—	2	—
	—	10	12	5	4	1	0	0	—	—	0	—
	10	—	24	0	0	5	5	0	8	—	—	0
		9[2]	24	2	1	1	5	0	—	—	—	0
	Total 29	29	—	9	10	19	20	0(60)	27(30)[3]	6(19)	2(20)	0(19)
Present (Mara)	10	—	6	0	2	3	5	1	5	3	—	—
	—	10	6	0	1	4	5	5	4	7	—	—
	10	—	12	2	3	3	2	5	2	2	5	—
	—	10	12	3	2	3	2	5	3	3	3	—
	9[2]		24	0	0	2	7	5	2	1	2	1
		9[4]	24	0	0	1	8	2	5	5	8	4
	Total 29	29	—	5	8	16	29	23(60)	21(59)	21(59)	18(39)	5(18)

[1] Serum collected on day of immunization

[2] One calf in group died of a cause other than heartwater before being challenged

[3] 27(30) = A total of 27 out of 30 serum samples tested were positive

[4] One control animal died of naturally acquired heartwater 13 months after the commencement of the experiment

The results of the IFA test on the sera of the Mara control animals differed significantly from those of the Kaalplaas animals. The serum of only one of the latter was found to be positive, whereas those of 28 control Mara cattle were positive as a result of tick infection at some stage over the course of the 2 years (Table 2). It is important to note, though, that they were only transitorily positive and often became negative during the ensuing months.

TABLE 2 IFA test results of Mara control cattle and their reactions to challenge

Animal No.	Age in months at challenge	IFA test at 1-24 months of age					Reaction category
		1	3	6	12	24	
1	6	−	+	−			IV
2		+	+	−			III
3		+	−	−			IV
4		+	+	+			IV
5		−	+	+			III
6		+	−	+			IV
7		+	−	+			IV
8		−	−	+			III
9		−	−	+			II
10		−	−	+			III
11	12	−	−	−	−		II
12		+	−	−	+		IV
13		−	+	−	−		III
14		−	−	+	−		I
15		−	−	−	−		I
16		−	+	+	−		II
17		+	−	+	+		IV
18		+	+	−	−		I
19		+	−	−	−		III
20		+	−	−	+		III
21	24	+	−	+	+	−	IV
22		+	+	+	+	−	IV
23		−	+	+	+		
24		−	+	−	+	−	IV
25		−	−	+	−	+	IV
26		−	+	−	−	−	IV
27		−	−	−	+	−	IV
28		−	+	−	+	+	III
29		−	−	+	+	+	IV
30		−	−	−	+	+	IV

The correlation between the febrile reaction and the IFA test on the sera of 58 vaccinated and 58 control cattle challenged at Kaalplaas and Mara is indicated in Table 3. It can be seen that, in the case of 60 animals (52 %), there appeared to be a good correlation between the reaction to challenge and the IFA test. The other 56 (48 %), however, were serologically negative and yet failed to react to challenge.

TABLE 3 Correlation between the IFA test on the sera of 116 Kaalplaas and Mara cattle and their reaction to challenge

IFA test	Reaction category		Degree of correlation	Number of animals
	I & II	III & IV		
+ ive	+		Poor	0
+ ive		+	Good	22
− ive	+		Good	38
− ive		+	Poor	56

DISCUSSION

It is difficult to conclude from the results obtained in this study whether the vaccination of young calves contributes to their resistance against tick-transmitted heartwater in their later lives because, apart from the vaccine-induced resistance, several other factors have to be taken into consideration.

One of these factors is the influence of natural tick challenge on the eventual immunity of the animals after they had been immunized as calves. The only group of immunized animals in this study that showed a significantly greater resistance than their respective control group were those at Kaalplaas, challenged 12 months after immunization. Here the possibility of tick challenge can practically be excluded, since only one serum sample of a control animal was found positive over the course of 2 years. There was no clear evidence of an increased resistance in the Kaalplaas cattle challenged 2 years after immunization. It is impossible to say whether immunization played a role in the group challenged 6 months after vaccination, because of the marked non-specific resistance shown by the controls.

At no stage over the course of the 2 years was there any difference between the vaccinated and control animals challenged at Mara. There the evidence of natural tick infection of the control cattle was obtained with the IFA test and the contraction of the natural disease by one of them.

Almost 50 % of the control Mara cattle were serologically positive when they were challenged. This does not necessarily reflect the maximum number of animals that had been exposed to natural infection, since it has previously been found that antibodies detectable with the IFA test do not persist for much longer than 6 months (Du Plessis, 1982 a), an observation which was confirmed in the case of the immunized Kaalplaas animals used in this study. It can therefore be assumed that the greater resistance to challenge of both vaccinated and control Mara cattle over that of the Kaalplaas cattle can be attributed to the natural tick challenge and not to the vaccination of the calves.

Even if allowance is made for the fact that a greater number of Mara control cattle that were found to be serologically positive had, in fact, been exposed to tick infection, it would appear that the tick challenge was only moderate. If the rate of sero-positivity of the 14 out of 29 (48 %) controls that were serologically positive when they were challenged is compared with that recorded in other heartwater endemic areas and reported in a previous publication (Du Plessis, 1982 a), it would appear that the heartwater immune status of cattle at Mara tends to be unstable. This observation is not incompatible with the average annual heartwater mortality rate of 16 animals over the past 12 years.

Another factor which complicates an evaluation of the vaccination of calves against heartwater is the natural resistance to challenge exhibited by a significant number of 6- and 24-month-old control Kaalplaas animals. This observation confirms an earlier report (Du Plessis & Bezuidenhout, 1979) that resistance factors other than specific immunity play a role in heartwater epidemiology. The high degree of natural resistance of calves up to the age of 6 months has also been observed in a subsequent study (Bezuidenhout, Spickett & Du Plessis, unpublished observations, 1984), the results of which are relevant to the conclusions drawn in the present study on the immunity of cattle vaccinated as calves *(vide infra)*.

The failure of very young calves to react either clinically or serologically to the inoculation of a virulent infective inoculum was also reflected in the observation that the blood of only 12 out of 30 Kaalplaas calves was infective to susceptible sheep 13 to 15 days after the calves had been vaccinated without their having shown a febrile reaction. This manifestation of the young calf resistance is in all probability also reflected in the fact that the sera of only 62 % of the Kaalplaas and Mara calves were positive to the IFA test 3 months after immunization. The sera of 10 out of 12 Kaalplaas calves that had

been proved to have the infective agent in circulation were IFA positive, and these animals were all resistant to subsequent challenge. But it is clear that other calves, the blood of which had not been infective, were also sero-positive and resistant to subsequent challenge and they had therefore also become infected. There is, therefore, no clear correlation between the replication of the heartwater agent in calves with a high degree of natural resistance and their serological response to and their eventual immunity against challenge with the agent. The relationship between the non-specific resistance of young calves and their immunity to heartwater is, therefore, poorly understood, and the ever increasing reports of artificially-immunized calves that die from natural heartwater within months of their vaccination, necessitates further studies on this relationship.

An earlier study suggested that conglutinin may be responsible for the non-specific resistance of older cattle to heartwater (Du Plessis & Bezuidenhout, 1979). Levels of this serum protein were determined in the present study, but the findings were omitted because, although some supported the earlier observation, others were contradictory. However, they pointed to the necessity for further studies either to confirm of disprove the conglutinin hypothesis, because the present study illustrates the difficulty in assessing the acquired immunity of cattle, whether artificial or natural, if non-specific factors are also involved.

Irrespective of whether non-specific resistance factors are taken into consideration, the present study suggests that in the absence of natural tick challenge, vaccination of calves induces a partial immunity against subsequent artificial challenge. In animals exposed to even a moderate tick challenge, however, the resistance of animals vaccinated as calves was no better than that of control unvaccinated cattle. Since there is a need for heartwater control, mainly in areas where the disease occurs endemically and in view of the evidence obtained in this study, the question arises whether young calf vaccination ensures an adequate and durable immunity in adult cattle kept under enzootically unstable conditions. It may well be, though, that calves vaccinated under one month of age have some degree of immunity during the ensuing months which, in conjunction with natural resistance and tick challenge, will ensure protection against heartwater.

This is the first recorded study on the immunity of cattle vaccinated as calves with infected tick stabilate. In a subsequent trial (Bezuidenhout, Spickett & Du Plessis, unpublished observations, 1984), carried out at Kaalplaas and involving 3 groups of calves of the same age and breed as those used in the present study, the immunity of one group immunized with tick stabilate was compared with that of a second group immunized with *C. ruminantium*-infected sheep's blood according to standard procedures. A third control group was left unvaccinated. When these animals were challenged a year after vaccination, it was found that the resistance of both vaccinated groups was significantly better than that of the unvaccinated control group. There was, however, no significant difference between the immunity of the 2 vaccinated groups. These findings are in agreement with those made in the present study in which tick stabilate only was used and the results obtained in it can therefore be extrapolated to calves immunized with sheep's blood.

Subsequent to a previous study (Du Plessis, 1982 a), this investigation provided another opportunity to evaluate the applicability of the IFA test in the study of the epidemiology of heartwater. It was reported then, and it was again found, that sera which initially gave a positive IFA reaction became negative within 3 to 6 months after artificial infection. This observation was made on the sera of vaccinated Kaalplaas cattle as well as on the sera of tick-infected Mara animals. This phenomenon as well as the observation in this study that serologically negative cattle were resistant to challenge can be explained by the suggested concept of a cell-mediated immunity in heartwater (Du Plessis, 1982 b), in which serum antibodies play a minor role, if any.

Although animals that react negatively in the IFA test may be resistant to challenge, their serum may nevertheless be free from antibodies detectable with the IFA test. The fact that immune cattle become sero-negative cannot be ascribed to a lack of sensitivity of the test, but rather indicates that the failure of IFA-detectable anitbodies to persist is inherent to the nature of immunity in heartwater of cattle. The IFA test proved to be a useful tool in this study, because it served to monitor the serological response of vaccinated animals.

Observations made in this study support the specificity and sensitivity of the test, since all the animals that were serologically positive when they were challenged displayed a high degree of immunity.

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