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ADULT *AMBLYOMMA HEBRAEUM* BURDENS AND HEARTWATER ENDEMIC STABILITY IN CATTLE

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ABSTRACT

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Several conclusions of importance to studies on the epidemiology of heartwater were drawn from an investigation in which the numbers of adult *Amblyomma hebraeum* ticks, to which a closed herd of Hereford cattle were exposed over a period of 6½ years, were manipulated. With a tick *Cowdria ruminantium* infection rate of 3–5 %, an endemically stable situation was created by dipping the herd only when an average of 10 adult male and female *A. hebraeum* ticks were counted on 10 animals. When the average was increased to 15 during the calving period, 97 % of calves acquired a tick-mediated immunity at the age of 6 months.

Because only aduly ticks confined to the hindquarters are counted, this procedure is recommended as a feasible and practical guideline to stock owners wishing to determine a dipping programme that would ensure endemic stability.

The indirect fluorescent antibody test gave a true reflection of the infection rate through ticks in calves 3–6 months old, but not in older animals that had been re-infected more than once. This is because on one hand antibody may persist for 2 years after withdrawal from tick exposure and on the other the artificial re-infection of cattle with a tick acquired immunity is not always followed by a rise in antibody titres and may even result in seronegativity.

Four cows infected and re-infected through ticks, remained immune to challenge for 2 years after withdrawal from tick exposure.

Within the confines of one farm 3 isolates of *C. ruminantium* that differed in pathogenicity and immunogenicity were recovered from ticks. One of these isolates was almost non-pathogenic to cattle.

INTRODUCTION

In a previous study done on 23 farms where heartwater occurs endemically, it was found that the immunity of cattle, as reflected by a positive reaction in the indirect fluorescent antibody (IFA) test, was determined largely by the tick loads to which they were subjected (Du Plessis & Malan, 1987a). Shortcomings in this investigation were firstly that only one tick count was done on the majority of these farms. Secondly, there was no correlation between the incidence of heartwater reported by the farmer and the immune status of the animals. The disease was no less prevalent in some of the herds where 80–100 % of the animals were serologically positive than on several farms where the seropositivity was 60 % or less.

Furthermore, although it was evident that the seropositivity of the animals on the farms where strategic tick control was practised was higher than on the farms where total tick control was practised, it was not possible to obtain an indication of the approximate number of ticks required to maintain a situation of endemic stability under a strategic tick control programme.

The recent discovery of serological cross-reactions between *Cowdria ruminantium* and *Ehrlichia* spp. (Du Plessis, Camus, Oberem & Malan, 1987; Holland, Logan, Mebus & Ristic, 1987) questions the ability of the IFA test to determine the heartwater immune status of a herd. Only if the reaction to this test could be correlated with the resistance to artificial challenge of cattle with a tick acquired immunity monitored serologically over a reasonable period, would it be possible to determine the degree of interference by *Ehrlichia* with epidemiological studies on heartwater. A substantial number of serologically positive animals that react to challenge would certainly suggest interference.

It was therefore decided that observations over the course of several years on a closed herd of cattle, under good management and extensive grazing conditions in a heartwater endemic area, in which exposure to the tick vector can be manipulated at will, would hopefully elucidate at least some of these problems.

MATERIALS AND METHODS

Experimental animals

A herd of Hereford cattle kept as a closed herd for the past 50 years on the government experimental farm, Mara, in the arid Northern Transvaal bushveld, where heartwater occurs endemically, was chosen. During the course of the investigation the animals, comprising approximately 50 cows and heifers and 2 bulls, were run in a multiple camp system according to the principle of controlled selective grazing on an area totalling 975 ha. With an expected calving rate of 90 %, approximately 36 calves were born between the 15th of October and the 15th of December each year. Cows that were culled because of old age or poor performance were replaced by the best performing heifers.

Tick counts

Tick counts were performed weekly. Adult

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Amblyomma hebraeum males and females in the peri-anal and vulvar region, the perineum and on the udder were counted on 10 cows and heifers of each of 3 groups selected randomly each week.

Tick control

At the commencement of the investigation the cows and heifers were randomly divided into 3 groups so that the age distribution was similar in the 3 groups. Group 1 was dipped in a synthetic pyrethroid flumethrin[1], if an average of 2 or more adult *A. hebraeum* ticks were counted on them and Groups 2 and 3 if 8 or more and 32 or more, respectively, were counted. Calves were dipped with their mothers from the age of 8 weeks.

Two years after the commencement of the experiment the tick control programme was changed drastically in order to artificially create a state of endemic instability. For a period of 2 years Groups 2 and 3 were dipped weekly. To gain an indication of the tick onslaught, Group 1 was dipped if an average of 0,5 or more ticks were counted. In order to bring the tick control of Group 1 on a par with that of the other 2 groups, the animals in Group 1 were also dipped weekly for a period of 2½ months from August to November 1988.

To once again move out of an endemically unstable into a stable situation, it was decided not to dip the whole herd for a period of 6 months unless an average of 4 or more adult female ticks were counted on 10 specific animals. Because of low tick numbers, this resulted that in practice the animals were not dipped once during the 6-month period. The decision to base the dipping frequency on the average number of female ticks was made when almost only male ticks were observed after the weekly dipping. In order to enhance the increase in the numbers of ticks, only females were taken into consideration.

Because of an increase in the number of udder abscesses and signs of tick worry in the newborn calves, the dipping frequency until the completion of the investigation in May 1991 was finally fixed at an average of 10 ticks (both males and females) calculated in a manner similar to that used at the commencement of the experiment. In order to expose the last calf crop to larger numbers of ticks, an average of 15 ticks was used as guideline from October 1990 until the youngest calf was 3 months old in March 1991.

Serology

In May and November over the course of 6½ years a blood sample was collected from each animal and the serum submitted to the IFA and conglutinin tests. Three 4-fold dilutions, starting from a 1:20 dilution of each serum were tested in the IFA test as previously described (Du Plessis & Malan, 1987b). Sera of the newborn calves, collected when the youngest calf was 3 months old, were also tested. The first serum samples were collected in November 1984.

Optimal utilization of the IFA test. In order to determine the age-group, the serology of which would give a true reflection of the immune status of the herd and the rate at which animals become infected through the tick, two steps were taken.

Firstly, 10 7-month-old oxen and 4 cows, 5–10 years old, were withdrawn from tick exposure and housed free from ticks for 1–2 years. Serum samples, collected at appropriate intervals, were tested until antibody could no longer be detected or in the case of the older animals for a period of 2 years. At this stage the 4 cows were challenged with the Mara 87/7 isolate and another serum sample tested one month later.

Secondly, 33 8-month-old calves and 11 cows, 4–10 years old, were likewise withdrawn from tick exposure and challenged with the Mara 87/7 isolate. The 11 older animals included the 4 cows referred to above. Fourfold serial dilutions of serum samples collected one month after challenge were subjected to the IFA test.

Infection rate in calves. To determine the rate at which calves become infected through the tick during the first 6 months of their lives, serum samples from 35 calves, born during the period October–December 1989, were collected fortnightly from 4 weeks up to the age of 12 weeks and thereafter at monthly intervals. Fourfold serial dilutions were tested.

Immunity tests

The calves were weaned at 6½ months of age. The bull calves were castrated and transferred to the Veterinary Research Institute, Onderstepoort. The immunity of the first two consignments of 17 and 19 calves was tested by injecting them intravenously with 5 mℓ of sheep blood infected with the Ball 3 stock of *C. ruminantium*. Early morning rectal temperatures were recorded for 4 weeks. The next 4 groups of bull calves were challenged with the Mara 87/7 isolate. An additional 30 cows and heifers, 1½–15 years of age, were challenged at Mara with the Mara 87/7 isolate.

C. ruminantium *infection rate of ticks*

On most occasions of serum collection, adult *A. hebraeum* ticks, both males and females, were collected and 20–40 of them assayed individually for infection with the heartwater agent according to the method described earlier (Du Plessis, 1985b). Because sufficient numbers of ticks were not available on the experimental animals, ticks were collected from cattle in a camp adjacent to those of the experimental herd in 1986 and 1987.

C. ruminantium *in game animals at Mara*

In order to determine whether game on Mara possibly serve as a source of infection to ticks, heparinized blood was collected from 20 adult and juvenile impala (*Aepyceros melampus*) and 5 scrub hares (*Lepus saxatilis*) and immediately frozen in liquid nitrogen. At the laboratory the specimens were thawed and 0,3 mℓ of blood of each sample injected i.v. into each of 3 mice. Four weeks later the mice were bled and the sera subjected to the IFA test at a dilution of 1:10. Fourfold serial dilutions of

[1] Bayticol, Bayer

the plasma of the 20 impala and 5 scrub hares were likewise tested in the IFA test, using anti-goat and anti-rabbit fluorescein-conjugated immunoglobin[2] as conjagates, respectively.

Conglutinin levels

To determine the role played by conglutinin in the natural resistance to heartwater of the experimental herd, the sera collected during May each year from all the cows and heifers older than 2½ years were assayed for conglutinin activity according to the method previously described (Du Plessis & Bezuidenhout, 1979; Du Plessis, 1985a). Sera collected in November were not tested because of fluctuating levels of conglutinin before and after parturition (Ingram & Mitchell, 1970). A single batch of sera collected from animals 4–6, 8–10, 16–18 and 18–30 months of age was likewise subjected to the conglutinin test. The sera of all the animals challenged at Mara or at the laboratory were also tested for conglutinin levels.

Characterization of Mara C. ruminantium *isolates*

The 3 isolates of *C. ruminantium* obtained from ticks used in the tick infection rate assays, were characterized by determining on one hand their infectivity to mice, sheep and cattle and on the other the degree of cross-immunity between them mutually and against other stocks of *C. ruminantium*.

Stabilates of the 3 isolates were prepared in each case by inoculating a heartwater susceptible sheep i.v. with an homogenate of the spleens of the mice that had shown clinical signs of a ruffled haircoat and dyspnoea after having been inoculated with tick homogenates. The remainder of the tick homogenate was added to the spleen homogenate as inoculum. At the height of the febrile reaction, 10 mℓ of heparinized blood was sub-inoculated into a 2nd sheep. At the height of its febrile reaction, 0,5 ℓ of its blood was added to 0,5 ℓ of citrated buffered lactose peptone (BLP), dispensed in aliquots of 10 mℓ and deep-frozen in liquid nitrogen.

Mice. Although outbred, conventional mice were used for the inoculation of the original tick homogenates, inbred, specified pathogen free (SPF) BALB/C mice were used to compare the pathogenicity of the 3 isolates. Inbred, SPF mice provided a much more homogeneous environment to the isolates than outbred, conventional mice and thereby ensured a more dependable comparison. A volume of 0,2 mℓ of the sheep blood stabilates and two 10-fold serial dilutions thereof in BLP were inoculated i.v. into 3 mice per dilution. The specificity of the recorded mortalities of the mice that died was confirmed as having been due to *C. ruminantium* infection as previously described (Du Plessis & Malan, 1988)

Sheep. Three groups of 4 6-month-old heartwater susceptible Merino ewes were infected with the 3 isolates. Five mℓ of stabilate was injected i.v. into each sheep and the early morning rectal temperatures recorded. All the sheep, except those infected with the Mara 88/9 isolate, were treated with oxytetracycline[3] at a dosage rate of 5–7 mg/kg body mass on the 2nd or 3rd day of the febrile reaction and again 2 or 3 days later if the temperature on these days equalled or exceeded that recorded on the day the first treatment was given.

Five weeks after infection, 2 sheep of each group, infected with one of the 3 isolates, were challenged with one of the other 2 isolates and another 2 sheep with the 3rd isolate. Temperatures were again recorded but no treatment given. Both at infection and challenge a reaction index was calculated for each animal as previously described (Du Plessis, Van Gas, Olivier & Bezuidenhout, 1989). An additional 10 points were added to the index if the sheep showed clinical signs or was treated.

Cattle. Three groups of 4 Friesland cows 2–6 years old and raised in a region where *A. hebraeum* does not occur, were infected with the 3 isolates in a similar manner. Two animals per group were subsequently challenged with the Ball 3, and 2 with the Germishuys stock (Du Plessis *et al.*, 1989). Temperatures were recorded and reaction indices calculated. Serum samples collected on the day of infection and at challenge were subjected to both the IFA and the conglutinin tests.

Passage of the Mara 88/9 isolate through sheep and A. hebraeum

When it was observed that the sheep inoculated with the tick and mouse spleen homogenate, from which the Mara 88/9 isolate was obtained, developed only a moderate febrile reaction and recovered without treatment, the isolate was passaged 3 times in sheep and thereafter several times alternatively in sheep and *A. hebraeum* to determine whether its pathogenicity to sheep could in this manner be enhanced. Five mℓ of heparinized blood collected at the height of the febrile reaction from Sheep 1, infected with the tick/mouse spleen homogenate, were added to 5 mℓ BLP and after storage in liquid nitrogen inoculated i.v. into Sheep 2 (Table 10). After 3 sheep passages, uninfected *A. hebraeum* larvae or nymphae were allowed to feed on the sheep as soon as a febrile reaction commenced. The ticks were left to moult and the ensuing infected stages allowed to feed on susceptible sheep. The sheep were challenged with either the Mara 87/7 isolate, the Ball 3, Welgevonden (Du Plessis, 1985b), Germishuys (Du Plessis *et al.*, 1989) or Breed (Du Plessis *et al.*, 1989) stocks of *C. ruminantium* 4–6 weeks after infection.

Incidence of heartwater and concurrent infections

The animals were observed daily and blood smears prepared from those that showed clinical signs of listlessness, inappetence and a raised body temperature. Those of which the Giemsa stained blood smears were negative for haemoparasites other than *Anaplasma* were treated with oxytetracyclines. If sero-conversion for heartwater was recorded in the absence of infections by haemopa-

[2] Miles-Yeda Ltd.

[3] Terramycin, Pfizer

TABLE 1 Persistence of IFA test antibody in cattle after withdrawal from exposure to ticks

Animal No.	Age at withdrawal	Reciprocal of IFA tire										
		Months prior to withdrawal			Months after withdrawal							1 month after challenge
		12	6	3	0	3	5	6	12	18	24	
1	7 months			80	80	80	–[1]					
2	7 months			20	320	80	20	20	–			
3	7 months			20	320	80	20	–				
4	7 months			80	80	80	20	–				
5	7 months			80	80	20	–					
6	7 months			20	320	80	20	20	–			
7	7 months			80	80	20	20	20	–			
8	7 months			320	320	320	80	20	–			
9	7 months			–	1 280	80	80	20	–			
10	7 months			–	1 280	80	–					
F233	5 years	80	80		20	20		20	20	20	–	–
D24	7 years	20	–		320	80		20	20	20	20	320
C158	8 years	20	20		80	80		320	320	80	–	320
A6	10 years	320	320		320	320		320	320	80	320	320

[1] –ive at a serum dilution of 1:20

rasites, the clinical signs were considered to have been due to heartwater. In the absence of sero-conversion the sera were also subjected to a card agglutination test for anaplasmosis (Potgieter & Van Rensburg, 1983). The sera of all the adult animals collected at the commencement of the experiments, those collected 6 months later and the sera of 16 bull calves born in 1989 were also tested for antibodies to *Anaplasma*.

Animals that died over the course of 6½ years were autopsied. Positive brain smears confirmed the diagnosis of heartwater.

RESULTS

IFA test and infection rate of cattle

On one hand antibody was no longer detectable with the IFA test in 5 out of 10 calves 6 months after withdrawal from tick exposure at the age of 7 months and by 12 months the other 5 were also seronegative (Table 1). Considering the antibody titres recorded 3 months prior to withdrawal, it would seem that Calves 2, 3 and 6 had been re-infected during the 3 months prior to withdrawal and that Calves 9 and 10 were probably infected for the first time.

On the other hand, 4 cows were still seropositive 18 months after withdrawal from tick exposure and in 2 of them (Cows D24 & A6), antibody was detectable in their serum 2 years after exposure (Table 1). Three of these animals (Cows C158, D24 & F233) had been re-infected at least twice before withdrawal (Table 2), D24 during the 6 months prior to withdrawal (Table 1). Although Cows C158 and A6 were seronegative at the commencement of the investigation when they were 4 and 6 years old, respectively (Table 2), it can be assumed that by then they had been infected at least once or twice. It is noteworthy that all 4 cows showed only a mild febrile response when they were challenged. It would seem therefore that contrary to the case in calves, antibody persists much longer in older animals reinfected several times.

The antibody response of 33 8-month-old calves and 11 cows, the majority of them heartwater immune through tick infection and subsequently challenged artificially, is shown in Table 3. It can be seen that in only 19 of these animals, 14 calves and 5 cows, was there a rise in antibody titre or a conversion from seronegative to seropositive. In the case of 18 animals the titre remained unchanged and in 7 of them there even was a drop in antibody levels. The reaction index of >10 to challenge shown by 2 of the calves and their sero-conversion prove that they had been fully susceptible. The sero-conversion of the 3 calves with 0 or <10 reaction indices, suggests that they too had not been infected through the tick.

The IFA test reactions of 47 cows at 6 month intervals over 2½ years are given in Table 2. The percentage serologically positive animals calculated from these reactions are also shown in Table 2 as well as in Fig. 1. Irrespective of whether Groups 1, 2 and 3 carried averages of, respectively, 2, 8 or 32 ticks before they were dipped, there was no significant difference between the percentages of seropositivity recorded in the 3 groups over this period.

TABLE 2 IFA test titres and averages of 10–20 tick counts (adult *A. hebraeum)* per 6-month period of 3 groups of animals dipped when they were infested with an average of 2, 8 or 32 ticks, respectively

Animal No.	Year of birth	Group	6-month period					
			Dec '84 May '85	June '85 Nov '85	Dec '85 May '86	June '86 Nov '86	Dec '86 May '87	Times reinfected
U1	1973		2,8/320[1]	6/320	6,8/ 20	5,4/320[3]	5,2/ 80	1
Z22	1977		2,6/ 20	7/ 20	8,1/ –	Culled		0
A11	1978		4,2/ 80	1/320[3]	4,2/ 80	2,6/ 80	2,4/320[3]	2
B7	1979		1,4/ 20	5,7/ 80[3]	8/ –	3,3/ 20[3]	3,5/ 20	2
B12	1979		2,5/ –	2/ 20[3]	4,2/ –	2/ 20[3]	1,6/ –	2
C149	1980		0,8/ 20	4/ 20	3,5/ –	1,4/ –	1,9/20[3]	1
D30	1981	1	1,3/ –	1/ 80[3]	4,4/ 80	1,6/ 80	1/ 20	1
D53	1981		2,2/ 20	0,8/ 20	4,8/ 20	1,4/ 20	2,1/ 20	0
E58	1982		1/ –	1,3/ 80[3]	2/ 80	2,2/ 20	1,5/ 20	1
E132	1982		1,5/ –	2,3/ 80[3]	3,6/ 80	0,6/ 80	1/ 80	1
F9	1983		0,8/ 20	1/ 20	2/ 20	0,8/ 20	1,3/ 20	0
F64	1983		0,9/ –	1,8/ 20[3]	2,9/ –	1,4/ 20[3]	0,8/ –	2
F233	1983		1,3/ 20	1,3/320[3]	2,8/ –	0,3/ –	1,1/ –	1
G31	1984			1,4/320	2,4/ 80	0,6/ 80	1,6/ –	0
G59	1984			1,1/ 20	2/320[3]	0,3/ 80	0,4/ 80	1
G77	1984			0,6/320	2/ 20	1,1/ 80[3]	0,7/ –	1
			62 %[2]	100 %	63 %	87 %	67 %	
U160	1973		6,7/ –	1,1/320[3]	8,8/ 80	10,8/ 80	8/ –	1
W212	1974		4/ –	2,4/ 80[3]	12,6/ 80	12,6/320[3]	11,8/320	2
X103	1975		7,5/ 80	13,7/ 80	16,9/ 80	Culled		0
Y71	1976		5,3/ 80	8,7/320[3]	7,1/ 80	8,1/320[3]	16,3/ 20	2
B27	1979		4,5/ 80	9,9/ 80	6,4/ 80	8,4/ 20	7,5/ 20	0
C16	1980		2,1/ 20	4/ 20	7,7/320[3]	6,2/320	19,7/ 80	1
C108	1980		2,5/ –	6,4/320[3]	7,6/ 20	4,3/ 80[3]	16,5/ 20	2
D24	1981	2	7,2/ 20	5,8/ 80[3]	6,1/ 20	6,8/ 20	12,7/ 20	1
D74	1981		2,7/320	3/ 20	2,8/ 80[3]	4,3/320[3]	11/ 20	2
E2	1982		3,2/320	2,4/ 20	4,9/ –	2,6/ –	6/ –	0
E183	1982		3,8/ –	6,6/ 80[3]	6,3/ 80	7,6/ 20	26/ 20	1
F1	1983		1,7/ 80	4,3/ 80	4,9/ 80	5,3/ –	Culled	0
F14	1983		4,2/ –	4,6/320[3]	8,3/ 20	4,2/ 20	2,7/ 80[3]	2
F37	1983		2,4/ 80	4,1/ 20	7,2/ 20	5,8/ –	15/ –	0
G60	1984			2/ 20	2,7/320[3]	1,4/ 80	17/ 20	1
G102	1984			1,4/320	5,6/ 80	5,1/ 80	4,5/ 20	0
			64 %	100 %	94 %	80 %	79 %	
X1	1975		4,9/ 20	9,5/ 20	12,9/ –	27,7/320[3]	45/320	1
X43	1975		9,4/ 20	18,9/ –	31,4/ –	34/ 80[3]	75/320	1
Y92	1976		14,5/ –	21/320[3]	30/ 80	31,4/320[3]	13/320	2
A6	1978		0,7/ –	25,5/320[3]	28,9/ 80	31,8/320[3]	42/320	2
B3	1979		25,9/320	21,5/ 80	20,4/ 80	50/320[3]	73/ 80	1
B35	1979		7,9/ –	15/ 80[3]	11/ 80	30,7/320[3]	12,3/ 20	2
C14	1980	3	10,5/ –	8/320[3]	21,8/ 80	8,1/ 80	19/320[3]	2
C158	1980		16,5/ –	12,2/ 80[3]	20/ 20	31,4/ 80[3]	29/ –	2
C279	1980		10,8/ –	19/320[3]	11,9/ 20	10,1/ 20	13/ –	1
D93	1981		17,9/ –	24/ 80[3]	13/ 20	14/ 80[3]	23/ –	2
E61	1982		12,9/ –	19,5/ –	22,4/ 80[3]	7,4/ 20	7/ 20	1
F12	1983		7/ –	13,8/ 80[3]	16,9/ 20	25,7/ –	17/ 20[3]	2
F19	1983		1,4/320	7,6/ 80	11,9/ –	20,4/320[3]	29/320	1
F250	1983		6,4/320	12/20	18,7/ –	28,7/ –	13,5/ 20[3]	1
G3	1984			6,2/320	14,3/ –	14/ 20[3]	19,7/ 20	1
			36 %	87 %	67 %	87 %	80 %	

[1] 2,8/320 = tick count reciprocal of IFA titre
[2] % serologically positive
[3] Probable re-infection

Since it was shown above that animals infected through ticks several times can remain seropositive far in excess of a year in the absence of re-infection and that re-infection is not always followed by a rise in antibody titre, it is impossible to calculate from the data in Table 2 the rate at which the adult animals were infected or re-infected. It can be assumed with reasonable certainty though, that a conversion from a seronegative to a seropositive or a fourfold rise in the titre indicated re-infection (Tizard, 1987). Based on this assumption the minimal times that each animal was re-infected are shown in Table 2. It can be seen that many more re-infections were recorded in Group 3 that was dipped only if an average of 32 ticks were counted.

The IFA-titres of 35 calves from the age of 4–24 weeks are given in Table 4 and summarized in Talbe 5. In the case of 28 calves the time of first infection throught the tick, indicated by a distinct rise in antibody titre or a conversion from negative to positive, could with reasonable certainty be distinguished from persistant colostral antibody. At the age of 24 weeks 4 animals (Calves 10, 27, 31 and 33) had not yet been infected, one of which (Calf 10) succumbed to heartwater at an older age. Calves 26

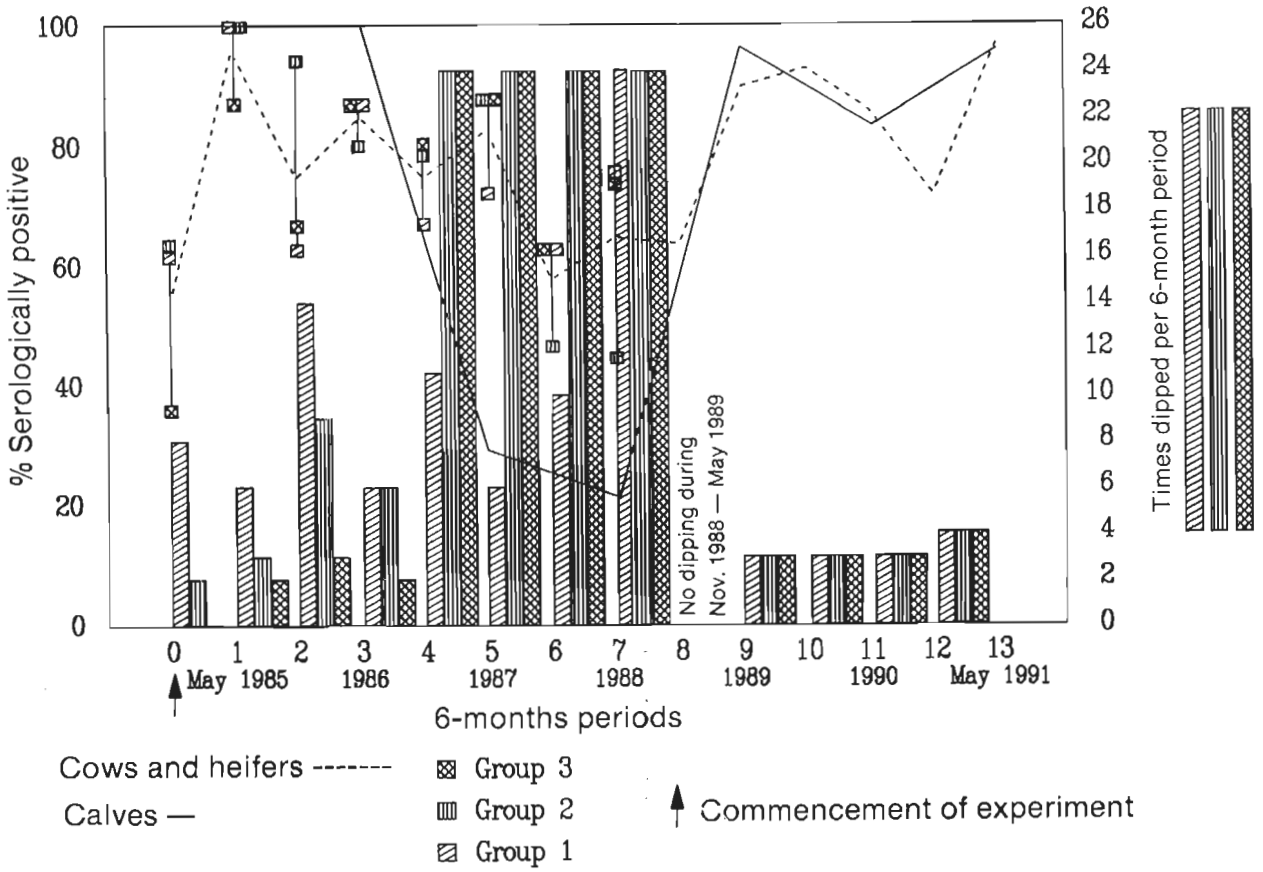

FIG. 1 Seropositivily of cows and heifers (....) and 6-month-old calves (—) and dipping frequency of experimental herd over 6 years

and 34 died of heartwater when they were infected for the first time at the age of 20 and 12 weeks, respectively. The rate at which the calves were infected is shown in Table 5.

The persistence of colostral antibody, indicated by low levels of antibody followed by a negative test at a serum dilution of 1:20 could be determined in the case of 21 calves (Tables 4 and 5). In the other 14 calves the overlap of colostral antibody and that resulting from infection made it impossible. It can be seen that in 2 calves colostral antibody was detectable for as long as 16 weeks after birth but had disappeared in the majority of cases by 12 weeks of age. It can be seen in Table 4 that with one exception (Calf 28), the dams of all the calves had variable levels of circulating antibody at the time of parturition.

Tick counts

The averages of 10–20 counts of adult *A. hebraeum* per animal per 6-month period of the 3 groups dipped according to their tick burdens, are given in Table 2. Only 6 out of 10 animals (D30, F9, F233, D74, E2, F1, G102, B35, C279 & F19) with

TABLE 3 IFA test antibody response to the artificial challenge of tick-infected immune animals

Age group	Interval in months between tick exposure and challenge	No. of animals according to reaction index			IFA test titre				
					Rise		Static at		Drop
		>10	<10	0	From sero −ive to sero +ive	1 or more 4-fold dilutions	Sero −ive	1/20 to 1/320	
Calves	1	2	11	20	2 1 2	0 7 2	0 0 5	0 2 6	0 1 5
Cows	1 24		4*	7	0 1	3 1	2 1	1 1	1 0
Total			44		6	13	8	10	7

* Cows A6, C158, D24 & F233 shown in Table 1

TABLE 4 IFA test titres of 1989 calf crop during the 1st 6 months after birth

Calf No.	Reciprocals of IFA test titres								
	Calves: weeks after birth								Dam of calf at parturition
	4	6	8	10	12	16	20	24	
1	20	–	80[1]	320	320	320	320	320	320
2	80	20	20	20	20	1 280[1]	1 280	–[2]	80
3	80	20	20	20	20	20	1 280[1]	1 280	320
4	80	20	20	20	20	20	–	80[1]	320
5	20	20	20	20	–	1 280[1]	1 280	80	80
6	20	20	20	20	80[1]	80	80	80	20
7	–[6]	20[1]	80	20	20	20	20	80	80
8	20	20	20	20	20	1 280[1]	1 280	320	80
9	–	–	–	–	80[1]	80	80	80	20
10	20	20	20	20	20	20	20	20[4]	320
11	20	20	20	–	–	–	–	80[1]	320
12	20	20	20	20	20	20	–	320[1]	320
13	80	20	80[1]	80	80	80	80	80	320
14	20	20	1 280[1]	1 280	320	320	320	80	320
15	20	20	20	20	320[1]	320	320	80	20
16	20	–	320[1]	320	320	320	80	80	80
17	20	20	20	20	–	80[1]	80	80	20
18	20	20	20	–	–	–	320[1]	320	80
19	20	20	20	–	–	1 280[1]	1 280[1]	320	320
20	20	20	–	–	–	80[1]	80	80	320
21	20	20	20	320[1]	320	320	80	80	320
22	20	20	20	–	–	1 280[1]	1 280	320	80
23	20	20	20	–	–	320[1]	320	320	20
24	20	20	20	1 280[1]	1 280	1 280	320	320	80
25	80	20	320[1]	320	320	320	320	80	20
26	80	80	20	20	20	20	–	–[3]	80
27	80	20	20	20	–	–	–	–	320
28	20	–	–	–[5]	–	–	–	–	–
29	80	80	20	20	20	80[1]	80	20	320
30	–	–	–	–	20[1]	20	20	20	20
31	80	80	80	20	20	–	–	–	80
32	20	20	20	20	20	1 280[1]	1 280	320	320
33	80	20	20	20	–	–	–	–	320
34	20	20	–	–	–	–	–	–[2]	80
35	80	20	20	20	–	1 280[1]	1 280	320	320

[1] 1st infection through tick

[2] Died of tick-acquired heartwater complicated by anaplasmosis at 18–24 weeks of age

[3] Died of tick-acquired heartwater at 20 weeks of age

[4] Died of tick-acquired heartwater at 13 months of age

[5] Died of black-quarter

[6] –ive at a serum dilution of 1:20

low average counts had serological evidence of re-infection and together they were serologically positive in 36 out of 48 tests, whereas out of 5 animals (U1, B7, C108, B3 & D93) with higher average counts, all the animals were re-infected once or twice and together they were serologically positive in 21 out of 25 tests. These data suggest that the tick burdens that animals carried appeared to be related to their antibody response.

TABLE 5 Infection rate of new-born calves and persistence of colostral antibody

	Age in weeks								
	4	6	8	10	12	16	20	24	>24
No. of calves infected	0	1	5	2	4	11	2	3	4
Persistence of colostral antibody	3*	3	2	5	5	1	2	0	0

* Colostral antibody no longer detectable in 3 calves 4 weeks of age

Although superficial scanning of the data in Table 2 suggest that older animals carried more ticks, statistical evaluation showed no signs either of larger tick burdens or of immunity against *A. hebraeum* in the older animals.

It is noteworthy that during the last 6-month period of differentiated dipping the ratio of female to male ticks varied between 1:2 and 1:4 and rarely exceeded 1:6. During the succeeding 6 months, when the animals were never dipped once because an average of 4 females was never attained, however, the ratio varied between 1:6 and 1:30 and several times exceeded 1:100. During the period that followed on these 6 months, when an average of 10 ticks (males and females) served as guideline and the herd was dipped only 13 times during the course of 2 years (Fig. 1), the ratio once again varied between 1:2 and 1:6.

Relationship of tick control to antibody response and infection rate of cattle

The percentages of both cows and 6-month-old calves serologically positive to the IFA test and the

TABLE 6 *C. ruminantium* infection rate of adult *A. hebraeum* ticks collected from Mara cattle

6-month period	No. of ticks						% ticks infected
	Inoculated		That elicited clinical signs in mice		That elicited antibody response in mice		
	Males	Females	Males	Females	Males	Females	
1	20	20	0	0	0	1(1:100)[1]	2,5
2	10	10	0	0	0	1(1:1000)	5
3	10	10	0	0	0	0	<5
4	27[2]	13	0	2[3]	0	0	5
5	19	1	0	0	0	0	<5
6	20[2]	20	0	1[4]	0	0	2,5
7	ND[6]	–	–	–	–	–	–
8	ND	–	–	–	–	–	–
9	10	10	0	0	0	0	<5
10	3	17	0	0	0	1:(100)	5
11	6	14	1[5]	0	0	0	5
Total	125	115	1	3	0	3	2,9

[1] 1:100=IFA titre of mouse
[2] Ticks collected from cattle in camp adjacent to those of experimental herd
[3] Origin of Mara 87/7 isolate
[4] Origin of Mara 88/9 isolate
[5] Origin of Mara 90/20 isolate
[6] Not done

number of times per 6-month period that the animals were dipped are shown in Fig. 1. It is evident that whereas the percentage seropositivity of the adult animals dropped only moderately during the 19 months when stringent tick control was practised, that of the calves dropped as low as 30 and 21 % during the same period. Although the seropositivity of the older animals rose from 64 to 90 % during November 1988 to May 1989 when tick numbers unhampered by dipping increased, the rise from 21 to 97 % in the case of the calves was much more dramatic. During the next two years when an average of 10 ticks per animal determined the dipping frequency, the seropositivity of the adult animals, with the exception of the November 1990 serum sample, parallelled that of the calves. The seropositivity of the calves therefore followed the tick challenge much more closely than that of the older animals and for that reason reflected the *C. ruminantium* infection rate of the cattle much more accurately.

TABLE 7 Pathogenicity of 3 Mara isolates to mice, sheep and cattle

C. ruminantium isolate	Pathogenicity to				
	Mice			Sheep	Cattle
	1:2	1:20	1:200		
Mara 87/7	5*	5	2	High	High
Mara 88/9	2	0	0	Moderate	Very mild
Mara 90/20	5	0	0	High	Moderate

* 5 out of 5 BALB/c mice inoculated with a 1:2 dilution of sheep blood infected with the Mara 87/7 isolate died

C. ruminantium infection rate of ticks

The *C. ruminantium* infection rate of adult *A. hebraeum* ticks collected from the experimental Hereford herd and Bonsmara cows in a camp adjoining those in which the former rotated, never exceeded 5 % (Table 6). On 3 occasions not one out of 20 ticks inoculated individually into mice was

TABLE 8 Cross-immunity between 3 Mara isolates in sheep.

Sheep No.	Infection isolate	Reaction to infection			Challenge isolate	Reaction to challenge	
		Febrile response	Treatment	Reaction index		Febrile response	Reaction index
1	87/7	7/8/41,5[1]	10,12[2]	22,6	90/20	23/3/40	2
2	87/7	8/9/41,3	10,12	21,5	90/20	19/2/40	1,3
3	87/7	7/7/41,5	9,12	21,8	88/9	13/8/41,9	14,1
4	87/7	10/6/41	12,15	18,2	88/9	15/5/41,4	7,8
5	88/9	11/6/40,5	–	8,8	87/7	12/4/40,9	5,8
6	88/9	7/13/41,4	–	11,9	87/7	9/7/41,7	9,9
7	88/9	10/6/41,8	–	12,2	90/20	11/7/41,5	12,8
8	88/9	8/8/41,5	–	10,2	90/20	17/3/39,8	1,9
9	90/20	7/7/41,2	9,11	20,8	87/7	11/9/40,9	11,4
10	90/20	7/9/40,8	9	21,4	87/7	14/10/40,9	10,7
11	90/20	7/10/41,6	10	23,3	88/9	13/13/41,9	16,3
12	90/20	7/7/41,2	9	19,3	88/9	11/9/41,6	13,3

[1] 7/8/41,5=the febrile reaction of Sheep 1 commenced 7 days p.i., lasted for 8 days and attained a maximum of 41,5 °C.
[2] Sheep 1 was treated 10 and 12 days p.i.

TABLE 9 Reactions of cattle infected with 3 Mara stocks and subsequently challenged with either the Ball 3 or the Germishuys stock

Animal No.	Infection			Challenge		
	Stock	Reaction	IFA/K[2]	Stock	Reaction	IFA/K
1	Mara 87/7	12/7/41,3; 18,2; T4[1]	[3]–/160	Ball 3	16/3/39,4; 2,3	1280/320
2	Mara 87/7	15/5/40,2; 16,4; T5	–/320	Ball 3	No reaction	1280/640
3	Mara 87/7	14/8/40; 21,2; T7	–/320	Germishuys	No reaction	320/160
4	Mara 87/7	11/5/40,4; 16,7; T4	–/160	Germishuys	No reaction	1280/160
5	Mara 88/9	15/1/39,4; 1,1	–/160	Ball 3	13/8/41; 18,4; T4	–/320
6	Mara 88/9	No reaction	–/160	Ball 3	16/6/41; 17,8; T3	–/320
7	Mara 88/9	No reaction	–/80	Germishuys	16/3/39; 2,2	–/320
8	Mara 88/9	15/2/39,4; 1,3	–/320	Germishuys	No reaction	–/320
9	Mara 90/20	14/5/40,4; 19,3; T5	20/320	Ball 3	No reaction	1280/320
10	Mara 90/20	14/3/40; 4,6	–/320	Ball 3	No reaction	320/640
11	Mara 90/20	15/5/41,2; 18,6; T4	20/160	Germishuys	No reaction	1280/320
12	Mara 90/20	15/1/40,7; 2,2	–/80	Germishuys	No reaction	320/640
13	Control			Ball 3	14/8/40; 20,3; T6	–/160
14	Control			Ball 3	16/4/39,8; 7,6	–/1280
15	Control			Germishuys	18/5/39,5; 2,8	–/1280
16	Control			Germishuys	15/5/39,2; 1,8	–/160

[1] 12/7/41,3; 18,2; T4 = the febrile reaction of Animal 1 commenced 12 days p.i., lasted for 7 days, attained a maximum of 41,3 °C and gave a reaction index of 18,2; it was treated on Day 4 of the febrile reaction

[2] reciprocals of IFA and conglutinin (K) titres at infection

[3] –ive at a serum dilution of 1:20

TABLE 10 Passage of Mara 88/9 isolate through sheep and *Amblyomma* ticks

Sheep No.	Mode of infection	Reaction	Challenge stock	Reaction to challenge
1	Tick/mouse spleen homogenate	11/6/40,5; 8,8	Mara 87/7	12/4/40,9; 5,8
2	Blood from Sheep 1	8/8/41,4; 11,1	Ball 3	11/6/41,2; 7,8
3	Blood from Sheep 2	9/9/42; 16,6	Welgevonden	10/6/41,4; died
4	Blood from Sheep 3	8/8/41,6; 10,8	Germishuys	No reaction
5	*Amblyomma* adults fed on Sheep 4	18/7/41,6; 14	Ball 3	13/6/41,9; died
6	*Amblyomma* nymphae fed on Sheep 5	29/6/39,6; 2,2	Ball 3	10/5/41; died
7	Blood from Sheep 3	10/8/41,4; 23[1]	—	
8	*Amblyomma* adults fed on Sheep 7	15/14/41,5; 14,1	Ball 3	11/9/42; died
9	*Amblyomma* adults fed on Sheep 8	12/8/41,5; 12,4	Mara 87/7	9/7/41,7; 9,9
10	*Amblyomma* adults fed on Sheep 8	20/8/41,2; 12,6	Breed	27/4/40,7; 4,8

[1] Died from heartwater complicated by pasteurellosis

found to be infected. Out of a total of 240 ticks examined, one male and 6 females were infected. It can be seen that in the case of 4 of the ticks the mice showed clinical signs, whereas the other 3 elicited an antibody response but no clinical signs. The ticks from which the 3 isolates of *C. ruminantium* were recovered are indicated in Table 6.

TABLE 11 Conglutinin levels in serum samples collected in May over the course of 6 years from animals older then 2½ years

Year	No. of animals	Reciprocals of conglutinin titres					
		160	320	640	1 280	>1 280	%>320
1985	40	5	3	4	12	16	80
1986	41	0	6	7	20	8	85
1987	44	4	4	12	22	2	82
1988	41	4	4	14	15	4	80
1989	38	0	2	21	11	4	95
1990	40	2	2	5	16	15	90

Isolates of C. ruminantium recovered from ticks

The pathogenicity of the 3 Mara isolates to mice, sheep and cattle differed (Table 7). Whereas Mara 87/7 was highly pathogenic to mice, sheep and cattle, Mara 90/20 was only moderately pathogenic to mice and cattle and Mara 88/9 only moderately pathogenic to mice and sheep but almost non-pathogenic to cattle. In sheep (Tables 8 & 10) Mara 88/9 was distinctly less pathogenic and out of 14 sheep infected either with sheep blood or through *A. hebraeum* ticks, only one animal (Sheep 7, Table 10) died and that while suffering from pasteurellosis as a complication. Throughout the study this was the

TABLE 12 Conglutinin levels of experimental animals under 3 years of age

Age-group (months)	No of animals	Reciprocals of conglutinin titres							%>320
		<80	80	160	320	640	1280	>1280	
4–6	15	0	4	9	1	1	0		6,7
8–10	16	0	1	4	6	3	2	0	31
16–18	14	0	0	2	2	7	3	0	71
28–30	10	0	0	0	2	1	4	3	80

TABLE 13 Correlation between seropositivity of 8-month-old calves and their reactions to challenge

Year of birth	No. of calves	Serology		Reaction to challenge		Challange stock
		+ive	–ive	Resistant	Susceptible	
1984[1]	17	16	1	16 1		Ball 3
1985	19	14	5	14 3	2	Ball 3
1986	19	10	9	10 7	2	Mara 87/7
1987	17	1	16	1 10	6	Mara 87/7
1988	16	16	0	16[2]	0	Mara 87/7
1989	16	14	2	14 1	1	Mara 87/7
Total	104	71	33	93	11	

[1] Born October–December, 1984, and challenged June, 1985
[2] 10 of these 16 calves were resistant to challenge with the Welgevonden and 6 to challenge with the Mali stock 6 weeks after the Mara 87/7 challenge

only sheep infected with Mara 88/9 from which a positive diagnosis of heartwater could be made from a brain smear. Futhermore, whereas the sheep infected with Mara 87/7 and 90/20 had to be treated, the 4 animals inoculated with 88/9 showed no clinical signs other than a febrile reaction and recovered without treatment (Table 8).

The difference between Mara 87/7 and 90/20 on one hand and Mara 88/9 on the other was even more striking in cattle (Table 9). Mara 87/7 seemed somewhat more pathogenic than 90/20, since all 4 Friesland cows infected with the former had to be treated, whereas 2 out of 4 cows infected with 90/20 had only mild reactions and recovered without treatment. Two out of 4 cows infected with 88/9, however, failed to react altogether in spite of low conglutinin titres, while the other 2 had only mild febrile reactions. With the exception of 2 control animals (Cows 14 & 15) that duely had only mild reactions, the conglutinin levels of the group did not exceed a titre of 1:320 and were therefore comparable from a susceptibility point of view.

The 2 cows that developed severe reactions to Mara 90/20 were the only 2 out of the group of 16 that had low levels of antibody in the IFA test before being infected, in all probability due to *Ehrlichia*.

The results of the immunity tests of sheep and cattle infected with the 3 Mara isolates are shown in Tables 8 and 9. If it is accepted that sheep with a reaction index of 10 or higher are considered non-immune or only partially immune (Du Plessis *et al*, 1989), it can be seen that one out of 4 sheep immune to each of Mara 87/7 and 88/9 and all 4 immune to 90/20 were only partially immune to challenge with the heterologous isolates. It is interesting to note that 3 of these 6 sheep were only partially immune to challenge with Mara 88/9, which otherwise appears less pathogenic than the other 2 isolates. From a cross-challenge point of view the 3 Mara isolates therefore also differ.

All the Friesland cows immune to either the 87/7 or the 90/20 isolates were immune to challenge with both the Ball 3 and the Germishuys stocks and all 8 had antibody titres of 1:320 and higher (Table 9). Two out of 4 cows immune to 88/9 were however, fully susceptible to challenge with Ball 3 and the other 2 immune against the Germishuys stock. No antibody could be detected in their sera on the day of challenge. The 2 control animals infected with the latter stock, however, also had only mild febrile reactions, even so in the case of Cow 16 with a low level of conglutinin.

An attempt to enhance the pathogenicity of Mara 88/9 by passaging the isolate through *A. hebraeum* was unsuccessful (Table 10). Not one of the 5 sheep infected with either nymphal or adult ticks that had fed on sheep infected with sheep blood or through ticks, developed severe reactions and 3 of them were fully susceptible and died when they were challenged with the Ball 3 stock. Although 2 of them (Sheep 9 & 10) were partially immune against Mara 87/7 and the Breed stock, they had reaction indices of only 12,4 and 12,6 in response to infected ticks allowed to feed on them.

Conglutinin levels

Eighty to 95 % of the serum samples collected in May over the course of 6 years from cows older than 2½ years had conglutinin titres higher than 1:320 (Table 11). Only 5 to 20 % of non-immune older animals were therefore at risk during this period (Du Plessis, 1985a). The younger the animals were, however, the lower the percentages of animals with conglutinin levels higher than 1:320 (Table 12). The titres of the 10 cows 28–30 months old (Table 12) were comparable with those of the older animals shown in Table 11.

Challenge of tick-exposed cattle

The reactions of 104 tick-exposed 8-month-old calves and 30 cows and heifers subsequently chal-

TABLE 14 IFA test and conglutinin titres and reactions of tick-exposed cows and heifers challenged with Mara 87/7

Animal No.	Age (years)	Reciprocal of IFA titre				Reaction index to challenge	Reciprocal of conglutinin titre at challenge
		3rd last SMP [1]	2nd last SMP	Last SMP	At challenge		
1	15	80	80	80	20	0	160
2	14	320	–[2]	320	80	0	640
3	11	320	20	80	20	0	640
4	11	320	320	80	20	0	640
5	9	320	320	320	80	0	640
6	8	20	–	–	–	0	1 280
7	8	20	20	–	–	0	1 280
8	8	20	80	–	20	0	640
9	7	320	80	320	20	0	80
10	7	80	20	–	–	0	1 280
11	7	20	320	–	–	0	1 280
12	5	80	80	20	–	0	320
13	5	320	20	80	80	0	160
14	5	–	–	20	20	0	320
15	4	80	80	20	–	0	320
16	3	80	–	–	–	0	1 280
17	3	80	20	20	–	0	320
18	3	80	–	–	–	0	160
19	3	20	20	–	–	0	640
20	2	80	20	20	20	0	160
21	2	320	80	20	80	0	160
22	2	80	–	–	–	0	320
23	1½	Not born yet	–	20	80	0	320
24	1½	Not born yet	–	–	–	0	640
25	1½	Not born yet	–	–	320	8	160
26	1½	Not born yet	20	320	–	0	1 280
27	1½	Not born yet	–	–	–	20,3	320
28	1½	Not born yet	–	–	–	4,7	1 280
29	1½	Not born yet	–	20	–	7,2	160
30	1½	Not born yet	–	–	–	8,3	320

[1] SMP = 6-month period
[2] –ive at serum dilution of 1:20

lenged either with the Ball 3 stock or the Mara 87/7 isolate are given in Tables 13 and 14. It can be seen that only 11 of the calves (Table 13) and 3 of the heifers (Nos. 27, 29 & 30, Table 14) were susceptible to the challenge. It is important to note that all these animals were serologicaly negative when they were challenged and that not a single serologically positive animal, possibly due to infection by *Ehrlichia,* reacted to challenge.

Although 12 other animals that were serologically negative at the time when they were challenged, were also resistant to challenge (Table 14), they had tested positive 6–18 months prior to challenge or in addition had conglutinin titres of 1:640 or 1:1280 (Nos. 6, 7, 10, 11, 16, 19 & 26). The only 2 animals that had been seronegative in 3 consecutive IFA tests (Nos. 24 & 28) and that were resistant to challenge, had high levels of conglutinin.

TABLE 15 Incidence of heartwater over 6½ years: deaths and clinical cases followed by sero-conversion

Year	Animal No.	Age (months)	Died	Clinical cases treated	Total per year
1985	1	1½	+		
1985	2	2	+		
1985	3	2½		+	
1985	4	4	+		1985:7
1985	5	6		+	
1985	6	6½		+	
1985	7	27		+	
1986	8	3		+	
1986	9	5		+	1986:2
1987	10	2½	+		1987:1
1988	11	12	+		
1988	12	12		+	1988:3
1988	13	12		+	
1989	14	4		+	
1989	15	4		+	1989:4
1989	16	5	+		
1989	17	13	+		
1990	18	3		+	
1990	19	4	+		
1990	20	4½	+		1990:5
1990	21	13	+		
1990	22	4	+		
1991	23	4		+	1991:1

Incidence of heartwater

Over the course of 6½ years 11 animals died from heartwater and 12 recovered and sero-converted after having been treated for the clinical disease (Table 15). With an average herd size of 90 animals, including an average of 35 calves for 6 months of the year, this represents a disease incidence of 3,9 % per year. Out of this total of 25 cases, 16 (70 %) were 6 months old or younger. It is noteworthy that the majority of cases were recorded during the first year of the investigation when tick control was reduced and from 1989 onwards when a drasticaly reduced dipping frequency was aimed at establishing endemic stability. During the latter part of 1986, 1987 and the beginning of 1988 when intensive tick control was practiced, only 6 cases were recorded.

Intercurrent infections

At the commencement of the experiments in November 1984, 36 out of 68 (53 %) animals were

positive in the card agglutination test for anaplasmosis, against 69 out of 78 (88 %) 6 months later. Furthermore, 15 out of 16 bull calves (94 %) born in 1989 and transferred to the laboratory for the heartwater immunity test, were serologically positive for anaplasmosis.

During 1991 a blood smear from a 3-month-old calf with clinical signs of listlessness, inappetence and raised body temperature revealed 15–20 % of red blood cells parasitized by *Anaplasma marginale*. The calf recovered after tetracycline treatment. During the preceding 4 years 5 other 4–6 month-old-calves and 2 one-year-old heifers showed similar clinical signs and recovery after treatment. Serum samples collected 3–6 weeks after treatment were negative for heartwater and positive for anaplasmosis. Furthermore, in additiona to the fact that the brain smears of 2 calves born in 1989 (Calves 2 & 24, Table 4) were positive for heartwater, their blood smears were also positive for *A. marginale*. Perhaps in both cases, but particularly in the case of Calf 2, anaplasmosis may well have contributed to its death because it was serologically positive for heartwater to a high titre immediately prior to death.

It would therefore seem that there was an increase in the prevalence of anaplasmosis from the commencement of the study, with high seropositivity and clinical manifestation of the disease towards the end of the 6½-year period.

Tick associated abscessation

Abscesses, particularly in the udder but also on other parts of the body, were a regular finding associated with infestation by adult *A. hebraeum* ticks. Not exclusively so, because there was also an increase in the numbers of *Hyalomma marginatum rufipes* during the periods of less stringent tick control. Towards the end of the 2-year period during which the dipping frequency of the 3 groups of animals was different, subacute to chronic abscesses were palpable in the udders of 14 out of 50 (28 %) cows and heifers in the order of 4, 5 and 10 animals in the groups that were dipped when an average of 2, 8 or 32 ticks, respectively, were counted. During the last 3 months of the 6-month period when the herd was not dipped, abscesses were recorded in 14 cows and calves out of a total of 90 animals. The prevalence of abscessation therefore appeared to be related to the severity of the adult *A. hebraeum* infestation.

Game and the epidemiology of heartwater at Mara

Eleven out of 20 impala and 3 out of 5 scrub hares were positive to titres of 1:20–1:320 in the IFA test. The sera of the mice inoculated with blood collected from the impala and scrub hares were negative at a dilution of 1:10 in the IFA test. It would seem that although at least some of these animals had at some stage been infected with *C. ruminantium*, the parasite on the day of blood collection was no longer circulating in numbers capable of eliciting an antibody response detectable with the IFA test in mice inoculated with the blood.

Discussion

By manipulating tick numbers an attempt was made to determine the approximate number of adult *A. hebraeum* ticks that would ensure endemic stability in the heartwater immune status of a closed herd of Hereford cattle. By intensive tick control an intentional effort was subsequently made to create a situation of endemic instability, followed once again by cessation and limitation of dipping to move from the unstable to a stable situation.

Three parameters were used to assess the effects of variations in the tick numbers to which the cattle were exposed. The IFA test, in which the peritoneal macrophages of mice infected with the Kümm stock of *C. ruminantium* are used as antigen, was used to monitor the immune status of the herd. In addition the incidence of heartwater was taken into consideration and from time to time both calves and adult animals were challenged to determine their immunity.

For several reasons the IFA test failed to give a true reflection of the rate at which adult animals became infected and re-infected through the tick. Whereas in tick-infected calves antibodies do not persist for much longer than 6 months after their withdrawal from tick exposure, appreciable levels of antibody were still detectable in 2 out of 4 cows 2 years after withdrawal. Since there was serological evidence that older animals had been re-infected several times and the calves not, the persistence of antibody can be attributed to repeated tick infection.

Furthermore, the antibody response of artificially reinfected, tick-exposed calves and adult animals was inconsistent and variable. Although sero-conversion and a rise in antibody titres were recorded in some animals, particularly the calves, this was not the case with all the adult animals. In some of the cattle, both young and old, the IFA titre remained static or even dropped. It was therefore clear that IFA test antibody levels could not be used to determine the rate at which re-infection through the tick occurred.

The infection rate of calves from birth to 6 months of age can be determined with much greater reliability, but also not with absolute certainty early in this period. Up to the age of 3–4 months, the interference by colostral antibody makes it difficult to pinpoint the time of the first tick infection. In this study colostral antibody persisted for at least 12 weeks after birth or even up to 16 weeks in the case of 2 calves. This differs considerably from an earlier finding in calves born from Afrikaner-Simmentaler heifers that had recovered from artificial infection in which colostral antibody was no longer detectable 8 weeks after birth (Du Plessis, 1984). This discrepancy can possibly be ascribed to the fact that in the present study older animals of a different breed were repeatedly infected before the birth of the calves.

It can nevertheless be concluded without any doubt that in this study the seropositivity of the calves at 6 months of age can be attributed to tick infection and is a true reflection of the rate of infection. The marked drop in the percentage seroposi-

tivity of the calves during the period of instability and intensive tick control was followed by a dramatic rise in the percentage seropositivity when tick numbers increased rapidly as a result of minimal tick control. The percentage seropositivity of adult animals, on the other hand, did not fluctuate nearly as much as that of the calves during this period.

Since there is therefore a good correlation between the seropositivity of 6-month-old calves and their infection rate through ticks, antibody in calves was used to relate tick numbers with the degree of immunity conferred by the ticks during the last 2 years of the investigation. Using an average of 10 adult *A. hebraeum* ticks as a guideline to decide when to dip, 83 % of calves had been infected through the tick by the age of 6 months. To improve on this, an average of 15 ticks as guideline during the calving period until the youngest calf was 3 months old, resulted in 97 % of the calves being serologically positive. although at this infection rate there was still one calf with clinical heartwater, control of tick numbers at this level seems to be close to the ideal. It must be borne in mind that throughout this study calves were only dipped from the age of 8 weeks.

The relationship between tick numbers and the rate at which they infect cattle depends on the *C. ruminantium* infection rate of the ticks (Uilenberg, 1983). In this study an average infection rate of only 2,9 % was recorded and never exceeded 5 %. This is comparable with the rates on some of the farms but lower than the average of 7 % recorded in an earlier study (Du Plessis & Malan, 1987a). It is, however, very much lower than the infection rates of 0,0–44,9 % for males and 20,0–36 % for females reported by Norval, Andrew & Yunker (1990). In our study the ratio of infected female to male ticks also differed from the above finding. Only one of 7 infected ticks out of a total of 240 inoculated individually into mice was a male.

Our finding that an average of 10 ticks per animal throughout the year and 15 for 3 months during calving ensured a nearly 100 % infection rate in calves before they were 6 months old, is therefore conditional to a tick *C. ruminantium* rate of 3–5 %. With higher tick infection rates, lower tick numbers should obviously suffice.

Although there was no substantial evidence that impala and scrub hares act as a source of infection to the immature stages of *A. hebraeum,* they may play a role in the epidemiology of heartwater at Mara. This possibility cannot be ruled out as 55 % of the impala sampled and 60 % of the scrub hares had antibody to *C. ruminantium,* proving that these game species do become infected. On the day of blood sampling, however, none of these animals had circulating heartwater agent in numbers sufficient to elicit an antibody response in mice. The probability that ticks infected with small numbers of *C. ruminantium* could concentrate and amplify the infection (Andrew & Norval, 1989) should, however, be borne in mind in this respect.

The 3 isolates of *C. ruminantium* recovered from ticks collected at Mara differed to such an extent that it cannot be accepted that a homogenic stock of the heartwater agent was involved throughout this study. They not only differed in their pathogenicity to mice, sheep and cattle but also in the cross-immunity conferred between one another. The Mara 87/7 isolate, which is highly pathogenic to mice, sheep and cattle, contrasted sharply with the Mara 88/9 isolate which caused mortality in mice but which was only moderately pathogenic to sheep and almost non-pathogenic to cattle. With the exception of the Kümm stock (Du Plessis, 1982) this is the only isolate thus far known to be almost non-pathogenic to cattle. This isolate was also not able to elicit an antibody response in or confer any degree of immunity to Friesland cows challenged with the Ball 3 stock. Sheep, on the other hand, that were immune to Mara 88/9 were almost fully or at least partially resistant to challenge with either Mara 87/7 or Mara 90/20, but susceptible to challenge with the Ball 3 and Welgevonden stocks.

Only Mara 90/20 was isolated from a tick collected from the experimental Hereford herd. It is difficult to say whether the other 2 isolates played a role in the epidemiology of heartwater in the herd. If Mara 87/7, isolated from a tick in an adjoining camp, had been present in the ticks infesting the experimental herd, there must have been adequate cross-protection, judging from the low incidence of disease during the $6\frac{1}{2}$ years and the high degree of immunity of tick-infected animals to artificial challenge with the Mara 87/7 isolate.

The question arises how stable the pathogenicity of a particular isolate is. The increased pathogenicity after passage through *A. hebraeum* of an *Ehrlichia*-like agent, isolated from a *Hyalomma truncatum* tick, raised the possibility that the 2 parasites are very closely related or even identical (Du Plessis, 1990). In the present study, however, the passage of the Mara 88/9 isolate through *A. hebraeum* did not alter its pathogenicity to sheep in any way.

Tick counts were carried out to determine the dipping frequency and thereby manipulating the tick numbers to which the cattle were exposed. The interesting observation was, however, also made that the Hereford cattle used in this study did not appear to develop an immunity against *A. hebraeum.*

The epidemiology of heartwater at Mara was characterized by a multiplicity of *C. ruminantium* isolates, a tick *C. ruminantium* infection rate of less than 5 % and a cattle population with a high non-specific resistance against the disease. Not taking into consideration the role played by infected *A. hebrauem* nymphae and considering the relatively low *C. ruminantium* infection rate of the adult ticks, 83–97 % of calves became infected before the age of 6 months during the last 2 years of the investigation when the dipping frequency was reduced to only 3 or 4 times in 6 months. Despite this high calf infection rate, an overall disease incidence of 3,9 % was recorded.

This is perhaps somewhat higher than the incidence recorded on 2 farms in an earlier study (Du Plessis & Malan, 1987), but it is nevertheless considered low and can be attributed to a high degree of

specific tick-acquired immunity at an early age in a large proportion of the herd, complemented by a high degree of natural resistance due to high levels of conglutinin. Since on one hand conglutinin titres at levels that would protect were only recorded in animals older than a year and if on the other it is borne in mind that albeit a small percentage of calves will not have acquired a tick-mediated immunity by 6 months of age, it is not surprising that 72 % of deaths and clinical cases were recorded in calves.

It is interesting to note that only 6 cases of heartwater were recorded during the period of intensive tick control, whereas the majority occurred during the first year of the trial when tick control was reduced and from 1989 onwards when a drastically reduced dipping frequency was aimed at establishing endemic stability. This observation suggests that when advocating minimal tick control to ensure endemic stability, it would be wise to vaccinate particularly the young stock. It must nevertheless be noted that in this investigation it was possible to move out of an endemically unstable situation into a stable situation within a period of 6 months during which no tick control was practised. It is important to note that during this period the decision whether the animals should be dipped was based on the numbers of female ticks counted. Although there was a slight increase in the number of udder abscesses associated with heavy tick infestation, there was no difference between the average weaning masses of the calves born during this period and those recorded earlier or subsequently.

Should a drastic reduction of tick control to obtain endemic stability be recommended, it must also be borne in mind that an increase in the numbers, not only of *A. hebraeum*, but also in those of other tick species can be expected. The clinical manifestation of other tick-borne diseases would therefore not be unexpected. Towards the end of the present study several animals with clinical anaplasmosis were distinguishable from cases of heartwater by blood smears, recovery after tetracycline treatment and sero-conversion. At autopsy a diagnosis of heartwater complicated by anaplasmosis in 2 calves was further evidence. An increase in the anaplasmosis seropositivity of the experimental herd was also evident.

The fact that not a single seropositive animal that had been exposed to ticks reacted to the artificial challenge has another important implication. The cross-reaction between *C. ruminantium* as antigen in the IFA test and antibodies to *Ehrlichia* (Du Plessis *et al.*, 1987; Holland *et al.*, 1987) may complicate studies on the epidemiology of heartwater in which this test is used. Had some of the positive serological reactions in the challenged cattle been due to infection by *Ehrlichia*, they should have reacted to the challenge, because cattle immune to *Ehrlichia* remain susceptible to challenge with *C. ruminantium* (Donatien & Lestoquard, 1936; Girard & Rousselot, 1945; Morzaria, Irvin, Kocan & Voigt, 1985). Since this did not happen, one could assume that either ticks at Mara are not infected with *Ehrlichia* ot that according to a recent hypothesis it undergoes a transformation and becomes more pathogenic when taken up by *A. hebraeum* (Du Plessis, 1990).

The effective control of heartwater depends on several factors, one of which is vaccination. The shortcomings of the present infected sheep blood vacine are well known: a live vaccine eliciting fatal reactions in highly susceptible stock if not controlled by treatment, a highly vulnerable infectivity which is indispensable to immunogenicity and the necessity of administering the vaccine intravenously. It is therefore understandable that if natural infection through the tick can be judiciously implemented to establish and maintain immunity in a situation of endemic stability, considerable benefit can be derived from such a situation. In view of the mounting evidence that immunologically different stocks of *C. ruminantium* may be responsible for losses in vaccinated stock (Du Plessis *et al.*, 1989; Jongejan, 1990), an additional advantage would be that the animals on a farm would be immune to the *C. ruminantium* stock prevailing on the particular farm.

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