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THE NON-SPECIFIC RESISTANCE OF CATTLE TO HEARTWATER

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ABSTRACT

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A distinction is drawn between the non-specific resistance to heartwater of very young calves and that of cattle older than a year. The suckling-calf resistance is at its greatest during the first few weeks after birth and then gradually declines during the next 6–8 months. Aspects of its possible mechanism and significance are discussed. The role played by conglutinin in the natural resistance of older cattle to heartwater and in the epidemiology of the disease in discussed.

INTRODUCTION

The variation in the susceptibility to heartwater (HW) of cattle of different breeds and ages has been known for a long time. Young calves have an innate resistance independent of the immune status of their mothers (Neitz & Alexander, 1941). It is well known that adult cattle also vary markedly in their susceptibility to artificially induced HW (Uilenberg, 1983).

Reports on the role played by different breeds are conflicting. Van der Merwe (1979) found *Bos indicus* breeds more resistant and therefore relatively easier to immunize than the *Bos taurus* breeds, whereas Uilenberg (1983) concluded that, although the great differences in susceptibility between local and exotic breeds in Africa have often been confirmed, the innate resistance has nothing to do with Zebu influence. According to preliminary findings, Neitz & Alexander (1941) suggested that pure-bred individuals of the exotic breeds (Aberdeen Angus and Hereford) were more susceptible than grades, but subsequently (Neitz & Alexander, 1945) concluded that the pure-bred Afrikander is no more resistant than the exotic breeds mentioned.

In this paper some of the non-specific factors that play a role in the natural resistance of cattle to HW will be discussed. Evidence will be presented that apart from the innate resistance of young calves, at least one other factor, the serum protein conglutinin, also plays a role. This contention is based mainly on two earlier studies (Du Plessis & Bezuidenhout, 1979; Du Plessis, 1985), but additional data to support the hypothesis will also be presented.

MATERIALS AND METHODS

Susceptibility to artificial infection according to age

To obtain an overview of the variation in the susceptibility of cattle of different ages, the reactions of animals inoculated with sheep blood infected with the Ball 3 strain of *Cowdria ruminantium* in earlier experiments, as well as hitherto unpublished results, are recorded. The reactions of 10 6-month-old and 10 12-month-old Afrikander-cross cattle infected as controls in a vaccination trial [(Du Plessis, Bezuidenhout & Lüdemann, 1984), (Experiment 1)] and those of 30 8-month-old Bonsamara calves and 24 year-old animals of the same breed used to study the relationship between susceptibility and conglutinin levels [(Du Plessis, 1985) (Experiment 2)] are included. In addition, 53 Bonsmara calves from 1–4 weeks to 6 months of age were infected intravenously with 5 mℓ of *C. ruminantium* infected sheep blood (experiment 3). Early morning rectal temperatures were recorded and the severity of the reactions shown by the calves and the older animals classified into 4 categories as previously described (Du Plessis & Bezuidenhout, 1979). Animals in Category I exhibited a severe febrile reaction of at least 40,5 °C for 3 consecutive days accompanied by clinical signs of anorexia and depression or followed by death. Those in category II showed a marked febrile response unaccompanied by clinical signs, those in category III only a mild transient febrile reaction and those in category IV failed to react at all.

To determine the infectivity of blood drawn from some of the calves in Exp. 3 at the time during the course of the infection when cattle usually react, sub-inoculations were carried out in sheep. Ten mℓ of heparinized blood were collected from 26 of them on days 12 and 15 after infection and stored in liquid nitrogen. The pooled samples of each calf were injected intravenously into a HW susceptible sheep. The temperatures of the sheep were recorded daily and those that survived were challenged with Ball 3 strain infected sheep blood one month later. The results obtained with the sub-inoculation of blood in a similar manner from 30 calves used in Exp. 1 (Du Plessis *et al.*, 1984), are also given.

The calves used in this trial, as well as the older animals used in the earlier studies, were born and kept at pasture on a government experimental farm situated in a HW endemic area where the vector, *Amblyomma hebraeum* can for all practical purposes be considered to be absent, as a result of an intensive dipping programme over several years prior to the experiments. According to official records, no clinical cases of or mortalities due to HW have been recorded on this farm for the past 7 years.

*Assay of conglutinin (K)**

In addition to the K levels recorded in Exp. 2, twofold serial dilutions of sera from a further 10 year-old cattle and 8 game animals infected with the HW agent as described, were also subjected to the K test (Experiment 4). The game animals listed in Table 3 had either been born in captivity or introduced into the Johannesburg and Pretoria Zoological Gardens several years prior to infection. The K test was carried out as previously described (Du Plessis, 1985). Body temperatures of the game animals were not taken. Their reaction categories were determined according to daily clinical observations and those that died were autopsied and their brain smears examined.

Significance of conglutinin levels

To illustrate the significance of high levels of K in the interpretation of the immune status of cattle, the K titres of cattle challenged at varying intervals after being infected and re-infected are presented. Twenty of the 30 8-month-old calves used in Exp. 2 were re-infected with the homologous strain of *C. ruminantium* 4 months after the primary infection and subsequently challenged 94–323 days after re-infection, again with the homologous strain. Sera collected on the day of callenge were tested to determine their K titres.

* Abbreviated K after Lachmann (1967) to avoid confusion with complement (C)

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TABLE 1 Susceptibility of cattle to heartwater according to age; results from Experiments 1, 2 and 3

Age in months	No. of animals	Reaction category				% animals in categories I & II
		I	II	III	IV	
1	18	2	2	5	9	22
2–3	15	0	5	4	6	33
4–6	20	4	7	3	6	55
5–8	40	0	4	24	12	10
9–12	34	21	7	4	2	82

TABLE 2 Reactions of sheep inoculated with blood from 56 calves infected with *C. ruminantium*; results from Experiments 1 and 3

Reaction category of							
Donor calves				Recipient sheep			
I	II	III	IV	I	II	III	IV
1				1			
	2			2			
		19		7		3	9
			34	12		2	20
Total			56	22	0	5	29

TABLE 3 Correlation between conglutinin levels and the susceptibility of cattle and game to heartwater (Exp. 4)

Animal species	No. of animals	Age	Reaction category	Reciprocal of conglutinin titre
Bovine (Simmentaler)	5	12 months	I (2 out of 5 died of HW)	80–160
Bovine (Simmentaler)	2	12 months	II	80–320
Bovine (Simmentaler)	3	12 months	III	640–1 280
Black wildebeest *(Connochaetes gnou)*	2	12 months	I (died of HW 21 days after infection)	80
Black wildebeest *(Connochaetes gnou)*	1	Aged	IV	2 560
Red hartebeest *(Alcelaphus buselaphus)*	1	Adult	IV	5 120
Springbuck *(Antidorcas marsupialis)*	1	Adult	I (died of HW 14 days after infection)	40
Scimitar horned oryx *(Oryx dammah)*	1	Adult	IV	5 120
Water buffalo *(Bubalus bubalus)*	1	Adult	I (died of HW 19 days after infection)	160
Mouflon *(Ovis musimon)*	1	Adult	I (died of HW 15 days after infection)	20

RESULTS

Age and susceptibility of cattle

It can be seen from Table 1 that the resistance to HW of the calves used in these experiments was inversely proportional to their age. Animals that showed category I and II reactions were considered susceptible and those in categories III and IV resistant. It is evident that whereas only 22 % calves under one month of age were susceptible, 33 %, 55 % and 82 % of 2–3–, 4–6– and 9–12-month-old animals respectively were susceptible to HW. In the age-group 6–8 months though, only 10 % of calves were susceptible.

C. ruminantium *in the blood of infected calves*

The reactions of sheep inoculated with the blood collected from calves on day 12 and 15 after infection are given in Table 2. It can be seen that the sheep inoculated with blood from 22 out of 56 calves fell in category I and either died from HW or developed severe febrile reactions and clinical signs of the disease. The sheep that reacted and survived were found to be solidly immune to challenge. It is also evident that the sheep inoculated with blood from all 3 calves that were fully susceptible, and 7 out of 19 sheep injected with blood from calves with category III reactions, either died or reacted severely, whereas only 12 out of 34 sheep inoculated with blood from calves that failed to react fell in this category. The higher resistance of calves would therefore appear to be associated with a lower rate of replication of the HW agent.

TABLE 4 Conglutinin levels of animals in Exp. 2 and the interpretation of resistance to challenge

Bovine No.	Reaction category to challenge	Interval in days between re-infection and challenge	Reciprocals of K titres at challenge
1	IV	229	1 620
2	IV	260	1 620
3	III	94	1 620
4	IV	260	540
5	IV	229	180
6	IV	229	540
7	IV	260	1 620
8	IV	292	180
9	III	292	180
10	IV	292	540
11	IV	323	180
12	IV	323	180
13	IV	210	4 860
14	IV	292	180
15	IV	260	1 620
16	IV	260	180
17	IV	323	540
18	IV	323	1 620
19	IV	260	540
20	IV	260	4 860

Conglutinin and non-specific resistance

The K titres of 10 cattle and 8 antelope are given in Table 3. It is evident that 7 out of 10 year-old cattle with titres of 1:320 or lower were susceptible, whereas the 3 with higher titres showed only mild febrile reactions. Likewise, the 3 game animals that were fully resistant had extremely high levels of K in their sera at the time when they were infected, whereas, in the case of the other 5 that died, only low levels were recorded.

Conglutinin levels and the interpretation of immunity tests

The K levels of 20 cattle challenged at various intervals after re-infection are given in Table 4. It can be seen that all the animals were solidly immune 3–11 months after re-infection. The K titre of only 7 of them, however, were below 1:320, the cut-off point above which cattle will presumably be protected from a fatal infection (Du Plessis, 1985), so that the resistance to challenge of the other 13 may not necessarily have been attributable to specific immunity.

DISCUSSION

Available evidence at present suggests that the non-specific resistance of calves to HW can be distinguished from that of older cattle. The suckling-calf resistance is at its strongest within the first month after birth, a phenomenon known for a long time and used to immunize calves without the risk of fatal reactions to the vaccine (Neitz & Alexander, 1941 & 1945). Even at this young age the resistance is not absolute since in this study 22 % were found to be susceptible and in the 2–3 month age-group, 33 %. These findings are largely in agreement with those of Neitz & Alexander (1941) who found that 10,5 % and 40 % of calves respectively, in these age-groups, reacted, but differ from those of Uilenberg (1971) who found that all 13 Friesland-Zebu-cross calves of less than a month to 5 months of age either reacted or died from artificial infection.

The suckling-calf resistance diminishes with advancing age and appears to be at its lowest at about 9–12 months. As reported earlier (Du Plessis *et al.*, 1984) calves in the 6–8 months age-group were markedly less susceptible than animals just before or after this age. The apparently higher resistance of this age-group can possibly be ascribed to the combined effect of a waning suckling-calf resistance and increasingly higher levels of K, since at this age K levels are already appreciable (Du Plessis, 1985).

The mechanism of the suckling-calf resistance is poorly known. Although Neitz & Alexander (1941) state, rather categorically, that it bears no relationship to the susceptibility or immunity of the dam and must not be confused with passive immunity transmitted through the colostral milk, the artificially infected calves on which their opinion was largely based had been born on HW endemic veld where the disease had been controlled by systematic dipping over a number of years. The same applies to the calves used in the present study. Although it cannot be stated beyond all doubt that not a single calf had been born from a dam immunized by the tick, the possibility that the immune status of the dam may have influenced the resistance of the suckling calves used in these experiments, is remote. Furthermore, there was no difference between the susceptibility of 30 calves from cows exposed to tick infection and 30 calves from cows not so exposed (Du Plessis *et al.*, 1984).

There is no evidence that the breed of calves plays a role. Van der Merwe found that irrespective of their breed a high percentage of purebred calves under a month old were susceptible (personal communication, 1986). Neitz & Alexander (1941) also found purebred Aberdeen Angus and Hereford calves less resistant than grade calves.

Since levels of K are almost not detectable in newborn calves and only increase slightly during the first few months of their lives (Du Plessis, 1985), it is unlikely that this serum protein plays a role in suckling-calf resistance. Other factors may, however, play a role. Preliminary findings suggest that complement, for example, may be involved in the susceptibilty of calves (Du Plessis, Malan & Kowalski, 1987).

The question arises whether the replication rate of the HW agent in the resistant calf differs from that in a susceptible animal that reacts. The observation in the present study that the sheep inoculated with blood from only 22 out of 56 infected calves developed HW, and that the infectivity of the blood was related to the severity of the reactions shown by the calves, suggests that inhibited replication of the HW agent is associated with the resistance of very young calves.

At the age of 1–2 years and older, the natural resistance of cattle to HW is distinctly greater than at 9–12 months of age. While 12-month-old cattle were found to be more susceptible than 6-month-old animals in 2 separate experiments (Du Plessis, 1985; Bezuidenhout & Spickett, 1985), older animals of the same breed in 2 earlier trials displayed a high degree of resistance. Thus only 31 out of 60 1–2-year-old heifers developed category I and II reactions to artificial infection, whereas the other 29 only showed mild febrile reactions or no reaction at all (Du Plessis & Bezuidenhout, 1979). In the same way, only 3 out of 9 2-year-old cattle, serving as controls in a vaccination trial (Du Plessis *et al.*, 1984), were susceptible while the other 6 were resistant.

Referring to differences in susceptibility, Uilenberg (1983) states that these differences probably can be ascribed to inherited resistance acquired by local livestock through long, natural selection. There is as yet no experimental data to substantiate this hypothesis or characterize its mechanism.

In 2 separate studies K has been suggested as a serum factor that plays a role in the non-specific resistance of older cattle. The statistically significant correlation between the susceptibility and the K titres of year-old cattle and the significant differences between the K titres of the animals in this age-group that died, and those of the animals that survived (Du Plessis, 1985), added support to the findings in the earlier study (Du Plessis & Bezuidenhout, 1979) that K influences the susceptiblity to the disease and that high enough concentrations protect cattle against a fatal infection.

Additional data on cattle and antelope reported in the present study add further support to this contention. Although statistically inadequate in numbers, the correlation between the marked susceptibility of 5 out of 8 game animals and low K levels on one hand, and, on the other, absolute resistance and extremely high K titres in the other 3, is noteworthy. These were the only game animals in which susceptibility could be compared with K levels, but K assays on a large number of sera of several species of antelope have revealed considerable variations according to species and age in the levels of this serum protein (J. L. du Plessis, unpublished data).

K associated resistance to HW takes effect at a much later stage than the suckling-calf resistance. Since the former strengthens as the latter wanes, these 2 non-specific resistance factors are probably complementary in their protective action against *C. ruminantium* infection. The resultant fluctuation in the resistance of young calves may well explain the sporadic losses from HW among calves, particularly in the absence of artificial or tick-mediated specific immunity.

K no doubt also plays a role in the epidemiology of HW in older cattle. On one hand animals with high K levels in HW endemic areas should be well protected against the natural disease, particularly in the presence of specific immunity and probably when immunity wanes.

High level K animals would thus have a better chance of either being infected or re-infected by ticks without developing a severe reaction, particularly in a situation of insufficient tick numbers that would otherwise lead to enzootic instability and losses in susceptible animals. On the other hand, the influence of high K on the degree and duration of immunity to primary infection, either through ticks or artificially, must be borne in mind. Since in HW immunogenicity appears to parallel the ability of an infective inoculum to elicit a reaction (Du Plessis & Malan, 1987), it stands to reason that a factor like K, capable of influencing the severity of the reaction, might also have an effect on the eventual immunity. The mechanism of this effect is at present unkown.

K levels must be taken into consideration in the evaluation of the immunity of cattle that have either been vaccinated or that have been exposed to natural tick infection. In the present study, for example, it cannot be stated unequivocally that all 20 cattle were specifically immune when they were challenged 3–11 months after being re-infected, since K titres in excess of 1:320, the cut-off point above which cattle will be protected from a fatal infection, in the case of 13 of them may have accounted for their resistance to challenge.

In conclusion it can be stated that the HW resistance of cattle from calf- to adulthood has both a specific and a non-specific component. The effects of the two obviously overlap and a better knowledge of their interrelationship will facilitate their manipulation in order to improve their concerted protective action.

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