SUSCEPTIBILITY TO HEARTWATER OF CALVES BORN TO NON-IMMUNE COWS

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ABSTRACT

DU PLESSIS, J. L. & MALAN, LETITIA, 1988. Susceptibility to heartwater of calves born to non-immune cows. *Onderstepoort Journal of Veterinary Research*, 55, 235–237 (1988)

The resistance to artificial infection with *Cowdria ruminantium* of calves born to cows fully susceptible to heartwater is no different from that of calves bred in heartwater endemic areas where the tick challenge is negligible to considerable. The sub-inoculation into mice of blood collected 14–26 days after infection proved the presence of the heartwater agent in the blood of 8 out of 10 calves with no other clinical signs than mild to moderate fever. The combined use of a mouse model and the indirect fluorescent antibody test revealed considerable variation in the degrees to which calves become infected and react to artifical infection.

INTRODUCTION

Although Neitz & Alexander (1941) stated that the innate resistance to artificially induced heartwater of calves up to the age of 4 weeks was independent of the immune status of the dams, they admitted that no definite opinion could be expressed as to the susceptibility or immunity of the dams, since their experimental calves were the progeny of cows running on a farm in a heartwater endemic area where the disease had been controlled by systematic dipping over a number of years. In a subsequent experiment on the same farm 30 Bonsmara calves under 4 weeks of age were found to be resistant to artificial infection with *C. ruminantium*. In the same experiment an equal number of calves of the same breed and age, but in this case born to cows exposed to appreciable numbers of the tick vector, were also resistant (Du Plessis, Bezuidenhout & Lüdemann, 1984).

It was therefore decided to infect calves born to cows known to be susceptible to heartwater in an area where the disease does not occur. Since a stock of the heartwater agent pathogenic to mice was employed, the opportunity was also used to titrate the infectivity of the blood of the infected calves in mice.

MATERIALS AND METHODS

Infection of calves

Ten Friesland calves, 11–49 days old, born from cows raised and kept in an area free from heartwater, were artificially reared and housed under tick-free conditions. They were inoculated intravenously (i.v.) with 5 mℓ of sheep's blood infected with the Welgevonden stock of *C. ruminantium* (Du Plessis, 1985a). At the same time, 10 mℓ of blood was collected and the serum stored for serology. Early morning rectal temperatures were recorded daily and the reaction index of each calf calculated by first ascertaining the day of onset of the febrile reaction. The mean daily temperature from the day of infection to the day of onset of the reaction was then determined. The total rise in °C above the average daily, pre-febrile temperature on each day of the ensuing reaction was recorded as the reaction index.

The sera of the calves collected on the day of infection and 33–47 days thereafter were subjected to the indirect fluorescent antibody (IFA) test (Du Plessis & Malan, 1987a). A 1:20 and 3 further fourfold dilutions were tested.

Calf blood sub-inoculation into mice

Five mℓ of blood for sub-inoculation into mice was collected in heparin on the day the first distinct rise in temperature occurred and thereafter on every alternative day until the end of the febrile reaction. In the case of Calf 2 that failed to show any febrile reaction, blood was collected on Days 13 and 15 after infection and a pool of the 2 collections inoculated into mice. Immediately after collection, suitable amounts of heparinized blood were added to equal volumes of buffered lactose peptone (BLP) and stored in liquid nitrogen. This sample constituted the 1:2 dilution inoculated into mice.

Since it was impossible to know beforehand when the febrile reaction would reach its peak and how long it would last, it was decided to select retrospectively the deep frozen specimens that corresponded with the height of the febrile reaction. In the case of some of the calves 2 specimens collected during the most prominent peaks of the reaction were pooled and, in the case of a single peak the specimen collected on or near that peak was injected into mice. Two further serial tenfold dilutions were made in BLP from the 1:2 dilution and 3 groups of 5 6-week-old conventional, outbred mice were inoculated i.v. with 0,3 mℓ per mouse of each of the 3 dilutions. The mortalities of the mice were recorded and the titre of infectivity of the blood specimens, collected from the calves, was calculated according to the method of Reed & Muench (1938). The specificity of the mouse mortalities, 7 days and later post-infection, was ascertained by verifying the presence of hydrothorax, a pathognomonic lesion in mice dying from infection with the murinotropic stocks of *C. ruminantium* (Du Plessis, 1975; Prozesky & Du Plessis, 1985). Earlier mortalities very rarely occurred and were considered non-specific. Infection with the heartwater agent as the cause of death was also confirmed in a sizeable proportion of the mortalities by the demonstration of colonies of *C. ruminantium* in the capillary endothelial cells of the lungs in conventionally prepared histological sections.

To determine whether infection occurred in the case of specimens where no mice mortalities occurred, the sera of the 5 mice inoculated with the lowest dilution of calf blood were collected a month later, pooled and subjected at a dilution of 1:10 to the IFA test.

Effect of storage on infectivity of blood

In a preliminary experiment to determine the effect of storage in liquid nitrogen on the infectivity of *C. ruminantium*-infected blood, a heartwater susceptible sheep was inoculated i.v. with 10 mℓ of the sheep's blood used to infect the calves. The febrile reaction of the sheep commenced on Day 7, lasted for 7 days and attained a maximum temperature of 42 °C on Day 11. Blood was collected in heparin on Days 9, 10 and 11 and each sample divided into 2 aliquots. After the addition of BLP 1 aliquot was immediately titrated in mice, as described, and the other stored in liquid nitrogen. The deep-frozen specimens were withdrawn 14 days later and likewise titrated in mice. The titre of infectivity on the 3 consecutive days was found respectively to be $10^{1,8}$, $10^{2,5}$ and $10^{2,1}$ in the case of the fresh specimens, and $10^{1,1}$, $10^{1,7}$ and $10^{1,4}$ in the case of the stored samples. There was therefore an average of 0,73 log loss of infectivity after storage.

TABLE 1 Reaction indices, serum antibody levels and blood infectivity titres of calves infected with the Welgevonden stock of *C. ruminantium*

Calf No.	Age in days	Febrile reaction			Reaction index	Reciprocals of calf blood infectivity titres	Reciprocals of post-infection IFA test titres
		Day of onset	Duration in days	Maximum temp. °C			
1	11	12	8	40,2	6,4	0,9 (17)[1]	ND[2]
2	14	—	—	—	0	(13 & 15)[3]	80
3	16	19	8	39,7	5,2	1,7 (26)	320
4	20	16	12	40,2	10,1	(17 & 20)[4]	80
5	20	15	10	39,8	6,8	0,6 (17 & 21)	320
6	25	15	6	39,7	5,2	1,0 (17)	320
7	26	10	6	40,6	9,4	0,8 (14)	80
8	30	17	7	39,9	8,1	(17 & 20)[5]	320
9	42	9	11	39,7	9,4	(9)[3]	80
10	49	12	6	40,4	10	(15 & 17)[5]	ND

[1] (17) = The blood collected on Day 17 after infection was inoculated into mice
[2] ND = Not done
[3] = None of the mice inoculated with the 1:2 dilution died and they were serologically negative a month later
[4] = None of the mice inoculated with the 1:2 dilution died, but they were serologically positive a month later
[5] = Only 2 out of 5 mice inoculated with the 1:2 dilution died and an infectivity titre was not calculated

RESULTS

It can be seen from Table 1 that 9 out of 10 calves showed mild to moderate febrile reactions to the i.v. inoculation of the Welgevonden stock of *C. ruminantium,* as reflected by reaction indices that varied between 5,2 and 10,1. None of the calves showed any other clinical signs. There was no loss of appetite and no apparent loss in body mass. The reaction indices show there was no correlation between the severity of the febrile reactions and the ages of the calves.

The sera of the calves collected on the day that they were infected were all negative in the IFA test. All 8 sera collected 33–47 days after infection were positive to titres of 1:80 and 1:320 (Table 1).

The infectivity of the blood of the calves collected during the febrile reaction varied considerably as shown by the mortality titres recorded in the mice. In Calf 3 a titre as high as the maximum titre found in the sheep used to determine the influence of storage on the infectivity of blood, was recorded. Although none of the mice inoculated with the blood of Calf 4 died, the 5 mice injected with the 1:2 blood dilution were serologically positive. The blood of Calves 2 and 9, however, failed to elicit detectable antibodies in the mice. The demonstration of antibody to titres of 1:80 in the serum of these calves prove that although the heartwater agent could not be detected in their blood through sub-inoculation into mice, infection had nevertheless been established. Replication of the heartwater agent had presumably therefore taken place in all 10 animals, and *C. ruminantium* had circulated in the blood of 8 of them over the course of a total period of 12 days, from Day 14 in Calf 7 to Day 26 in Calf 3. There was once again no correlation between the concentration of *Cowdria* circulating in the blood of the calves and the severity of the febrile reactions that they showed or their age.

DISCUSSION

Although the calves in this experiment sowed mild to moderate febrile reactions when they were infected with the Welgevonden stock of *C. ruminantium,* highly pathogenic to 15-month-old cattle (Du Plessis, 1985a), they showed no other clinical signs of disease. The reaction indices used to measure the severity of these febrile reactions were no more severe than those of 8-month-old calves that reacted lightly to infection with the Ball 3 stock and markedly inferior to those of the majority of a group of 12-month-old animals that reacted severely to the latter stock (Du Plessis, 1985b). There were also no severe reactions or clinical disease in the case of 60 1-month-old calves, the moiety of which had been born to cows exposed to moderate numbers of the vector of heartwater, *Amblyomma hebraeum* (Du Plessis *et al.,* 1984). According to another report, however, 4 out of 22 1-month-old Bonsmara calves showed severe reactions (Du Plessis & Malan, 1987b). This is not unprecedented, since Neitz & Alexander (1941) found that 10,5 % of calves in this age group were susceptible and calves even under 3 weeks of age were known to have died (Uilenberg, 1971).

Most of the calves referred to in these reports had been born to cows on farms in heartwater endemic areas, and although one of these farms was practically free from the tick vector (Du Plessis *et al.,* 1984), it cannot be excluded beyond all doubt that the dams of some of these calves had been immune. The absence of any contact between the heartwater vector and the dams of the calves used in the present study, therefore, justify the conclusion that there can be no maternal influence in the resistance of new-born calves to heartwater. Although antibodies of colostral origin are demonstrable in the serum of calves up to the age of 4 weeks (Du Plessis, 1984), the innate resistance of the calves in the present study cannot be ascribed to a passive humoral immunity.

By means of subinoculations into mice and the application of the IFA test, the heartwater agent was detectable in the blood of 8 out of the 10 calves (80 %) 14–26 days after infection. Since there is an appreciable loss of infectivity after storage in liquid nitrogen, the number of calves with a parasitaemia was probably underestimated. In an earlier study, the blood from only 12 out of 30 calves (40 %), drawn 13 and 15 days after infection, was infective to sheep (Du Plessis *et al.,* 1984), and in another study only 22 out of 56 sheep (39,3 %), inoculated with the blood of 1 to 6-month-old calves, either died from heartwater or developed severe febrile reactions and clinical signs of disease (Du Plessis & Malan, 1987b).

The finding in the present study that as many as 80 % of the calves had a parasitaemia can probably be ascribed to the combined use of the mouse model and the IFA test and not to a difference in the susceptibility of the experimental animals. Infectivity titrations in mice showed that the concentration of infective organisms in the blood of the calves varied from numbers too small to produce clinical disease and death but enough to elicit an antibody response in mice to titres of infectivity comparable with that recorded in a sheep reacting to this particular stock of *C. ruminantium.* In 2 of the calves, not even a trace of circulating heartwater agent could be demonstrated when the sera of the mice inoculated with their

blood were subjected to the IFA test. The detection of antibody in the sera of these calves proved, however, that they too had become infected, since, unless replication of *Cowdria* takes place, there is no antibody response detectable with the IFA test (Du Plessis & Malan, 1987a).

C. ruminantium circulating in the blood of calves infected either artifically or through the tick are relevant, firstly, to the pathogenesis of heartwater, as yet poorly understood (Du Plessis, Malan & Kowalski, 1987c), and, secondly, to the epidemiology of the disease. The parasitaemia suggests that the absence of clinical signs and death cannot be attributed to an absence of replicating agent or to insufficient numbers thereof, but to other causes, such as, for example, the inadequate development of 1 or more cellular or humoral component playing an important role in the pathological process. The innate resistance of new-born calves would therefore appear to be rather an impediment to the unfolding of the pathogenic process than the inhibition of the establishment of the infection.

From an epidemiological point of view, these findings show that calves, infected either artificially or through the tick, can serve as a source of infection to uninfected ticks feeding on them and thereby form an important link in the epidemiological chain. The importance of the role that calves might play in this respect can be ascertained by determining for how long after infection the heartwater agent circulates in the blood of infected calves. Here again, the mouse model, infected with the Welgevonden stock, combined with the IFA test, can serve a useful purpose. The inoculation of fresh instead of deep-frozen blood into mice, should reflect more truly the duration of parasitaemia after infection.

Although cross-reactions with *Ehrlichia* spp. (Du Plessis, Camus, Oberem & Malan, 1987; Holland, Logan, Mebus & Ristic, 1987) may complicate the interpretation of IFA test results in studies on the epidemiology of heartwater, this study has once again shown the value of the test under experimental conditions.

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