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THE NATURAL RESISTANCE OF CATTLE TO ARTIFICIAL INFECTION WITH *COWDRIA RUMINANTIUM*: THE ROLE PLAYED BY CONGLUTININ

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ABSTRACT

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The conglutinin titres of year-old Bonsmara-cross cattle infected with *Cowdria ruminantium* were inversely proportional to the severity of the reactions elicited by the infection. There was no correlation, however, between conglutinin levels of 8-month-old calves of the same breed, sex and origin and their susceptibility to heartwater. The role possibly played by conglutinin in the non-specific resistance of cattle to heartwater and in the epidemiology of the disease is 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; Uilenberg, 1971). The age up to which calves are resistant to artificial infection may vary considerably. Although it is generally accepted that the suckling-calf resistance lasts about 4 weeks, as originally stated by Neitz & Alexander (1941), this is not an absolute finding since, on one hand, calves infected at less than 3 weeks of age are known to have died (Uilenberg, 1971) and, on the other hand, calves up to the age of 6 months have been shown to exhibit a high degree of natural resistance (Du Plessis, Bezuidenhout & Lüdemann, 1984). Furthermore, 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 of cattle 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 a previous study (Du Plessis & Bezuidenhout, 1979), the susceptibility of 1- to 2-year-old Afrikander-Simmentaler-cross heifers to *Cowdria ruminantium*-infected sheep's blood appeared to be related to the pre-infection levels of conglutinin (K)* in their serum, those with higher levels being more resistant. Since K activity is unstable in stored serum and is lost after prolonged storage at −20 °C (Rose, Amerault & Roby, 1979), a final conclusion that serum K influences the susceptibility of cattle to HW could not be drawn in this study in which the sera had been stored for several months.

In a subsequent study (Du Plessis *et al.*, 1984), the difficulty of assessing the acquired specific immunity of cattle if non-specific factors, such as the innate resistance of the animals, are also involved, became evident. It was decided therefore to investigate more fully the role played by K in the natural resistance of cattle to heartwater.

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

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MATERIALS AND METHODS

Experimental animals

Bonsmara-cross bull-calves, bred and kept at pasture on a government experimental farm, Kaalplaas (Du Plessis *et al.*, 1984), were used in 2 separate experiments.

Experiment 1

Thirty calves were monitored for serum K from 3 months of age. To determine the influence of weaning on serum K levels, they were divided into 3 groups of 10 animals each 2 weeks prior to being infected at an average age of 8 months. One group was separated from their mothers and placed in a small camp with natural grazing and a salt-mineral lick containing urea and maize meal as supplement. The 2nd group was weaned in a harsher manner by separating them from their mothers and placing them in a small concrete-floored enclosure where they were fed on 0,7 kg of pelleted balanced ration per animal, supplemented with poor quality hay *ad libitum*. The 3rd group was left with their mothers until 6 weeks after they had been infected.

The K levels of 20 of these animals were assayed up to the age of 2 years.

Experiment 2

Twenty-four calves were weaned in a similar manner to those in the 2nd group of Exp. 1 and infected with *C. ruminantium*, when their average age was 12 months. Serum samples collected on the day of infection was assayed for K.

Assay of conglutinin

The test, as previously described (Du Plessis & Bezuidenhout, 1979), was slightly modified by the use of freezedried horse serum as the source of complement. Aliquots of 0,5 mℓ of fresh horse serum were freezedried and, after reconstitution, added to 3,5 mℓ of sensitized sheep red blood cells and 6 mℓ of diluent. For the rest, the test procedure was carried out as previously outlined. Twofold serial dilutions of the sera were tested within one week after sampling. The titre was expressed as the highest dilution of serum that agglutinated the 3-part complex.

Infection of calves

Because the 2 experiments were carried out 18 months apart, 2 different batches of infective sheep's blood were used. The calves in Exp. 1 were infected by inoculating them intravenously (i.v.) with 10 mℓ of sheep's blood infected with the Ball 3 strain of *C. ruminantium*, currently issued as a vaccine by the Onderstepoort Veterinary Research Institute.

The calves in Exp. 2 were infected i.v. with 10 mℓ of sheep's blood infected with a field strain of the HW

TABLE 1 Reaction index points per kg loss in body mass

	Loss in body mass (kg)							
	1–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39
R.I. Points	1	3	5	7	9	11	13	15

agent isolated from a tick-infected sheep. A 10 % mass/volume suspension was prepared by homogenizing the prescapular and periportal lymph nodes of the sheep in buffered lactose peptone (BLP). The homogenate was centrifuged at 200 g for 10 min and 5 mℓ of the supernatant fluid was injected i.v. into a HW susceptible sheep. The sheep reacted 9 days after the inoculation. Its blood was collected in citrated BLP 3 days later at the height of the febrile reaction, dispensed in 10 mℓ volumes and stored in liquid nitrogen. Samples of the stored blood were tested for infectivity and absence of contaminating micro-organisms as previously described (Du Plessis, 1982 a).

The immunological identity of the strain to the Ball 3 strain was confirmed by challenging 5 sheep that had recovered from infection with the latter by injecting them i.v. with 5 mℓ of the field strain infected sheep's blood. None of the animals showed any febrile reaction.

Observations on calves

Early morning rectal temperatures of the calves were recorded. None of the animals in either of the 2 experiments were treated when they reacted. The mass of the animals was determined 8 days after having been infected, and every 3 days thereafter, until a week after their temperatures had returned to normal. The mass of the animals was determined in the early morning before being fed, water having been withheld for 15 h. Animals that died were autopsied and Giemsa-stained smears prepared from their brains were examined for the presence of colonies of the HW agent.

Reactions to infection

The severity of the reactions to the infection was evaluated by calculating the reaction index (R.I.) of each animal. The febrile reaction, loss in body mass and the eventual death or recovery of the animal were used as parameters to calculate the R.I.

The R.I. score of the febrile reaction was obtained by first determining for each animal the day of onset of the febrile reaction and the mean daily temperature from the day of infection to the day of onset of the reaction. The total rise in °C above the average daily pre-febrile temperature on each day of the ensuing reaction was recorded as the febrile reaction R.I. score.

The R.I. score attributable to the loss in body mass was calculated from the difference between the body mass of each animal before the onset of the febrile reaction and the minimum mass recorded during the succeeding days. The number of R.I. points allotted in relation to the loss in body mass is given in Table 1. In the case of animals that gained mass, the mass recorded 8 days after infection was subtracted from that recorded 12 days later.

The R.I. score of the animals that succumbed to the infection was calculated by allotting 36 points to the animal that survived for 7 days after the onset of the febrile reaction. For every day shorter than this period that the other animals with a fatal outcome lived, an additional 3 points were added, and for every day longer 3 points were subtracted.

The R.I. for each animal was expressed as the sum of the R.I. scores obtained for the 3 parameters.

Statistical evaluation

The Spearman rank correlation was used to evaluate the relationship between the K titres and the R.I.'s of the experimental animals. To compare the R.I.'s and the K titres of the 12-month-old animals that died with those of

TABLE 2 Reaction indices of 8-month-old calves and their serum conglutinin titres

Animal No.	Febrile reaction				Mass in kg				Reaction index	Reciprocal of conglutinin titre
	Day of onset	Duration in days	Max temp. °C	Score	Prior to febrile reaction	Minimum after onset of feb. reac.	Loss/ gain	Score		
1	13	7	41,2	10,3	277	265	−12	5	15,3	320
2	11	8	40,9	7,2	248	236	−12	5	12,2	320
3	10	9	40,3	8,2	277	272	−5	3	11,2	320
4	9	10	40,5	9,2	252	251	−1	1	10,2	80
5	10	6	41,0	7,0	240	234	−6	3	10,0	160
6	9	9	40,3	8,9	278	274	−4	1	9,9	160
7	11	8	40,4	6,8	238	230	−8	3	9,8	640
8	9	8	40,4	6,1	260	254	−6	3	9,1	160
9	10	6	40,1	3,6	275	262	−13	5	8,6	320
10	11	6	40,3	4,9	282	273	−9	3	7,9	320
11	11	8	40,0	6,5	227	223	−4	1	7,5	40
12	11	7	40,1	3,6	281	274	−7	3	6,6	320
13	14	5	39,8	3,5	229	222	−7	3	6,5	160
14	12	4	40,1	3,4	233	227	−6	3	6,4	160
15	11	6	39,8	3,3	234	228	−6	3	6,3	160
16	11	6	39,4	4,5	209	206	−3	1	5,5	160
17	10	5	39,8	4,2	297	293	−4	1	5,2	320
18	10	2	39,8	1,9	265	260	−5	3	4,9	320
19	10	8	39,6	4,1	224	225	+1	0	4,1	160
20	15	2	39,6	2,5	257	257	0	0	2,5	160
21	16	2	39,7	1,6	251	253	+2	0	1,6	1280
22	16	1	39,9	1,0	298	302	+4	0	0,9	320
23	16	1	39,9	0,9	298	302	+4	0	0,9	320
24	—	—	—	0	260	267	+7	0	0	80
25	—	—	—	0	231	233	+2	0	0	80
26	—	—	—	0	215	217	+2	0	0	320
27	—	—	—	0	257	260	+3	0	0	640
28	—	—	—	0	225	228	+3	0	0	160
29	—	—	—	0	258	259	+1	0	0	160
30	—	—	—	0	227	229	+2	0	0	320

TABLE 3 Correlation between the reaction indices of 12-month-old animals and their serum conglutinin titres

Animal No.	Febrile reaction				Outcome	Score	Mass in kg				Reaction index	Reciprocal of conglutinin titre
	Day of onset	Duration in days	Max temp. °C	Score			Prior to febrile reaction	Minimum after onset of feb. reac.	Loss/ gain	Score		
1	13	5	41,5	7,9	Died	42	259	247	−12	5	54,9	80
2	13	3	41,1	5,0	Died	48	263	260	−3	1	54,0	160
3	13	5	41,2	8,6	Died	42	285	276	−9	3	53,6	80
4	13	5	41,5	6,9	Died	42	296	288	−8	3	51,9	80
5	11	5	41,5	7,9	Died	42	240	238	−2	1	50,9	20
6	12	7	41,3	11,2	Died	36	208	203	−5	3	50,2	80
7	12	7	41,4	12,5	Died	36	275	273	−2	1	49,5	80
8	13	8	41,5	11,4	Died	33	308	296	−12	5	49,4	320
9	13	6	41,0	10,1	Died	39	250	250	0	0	49,1	20
10	13	7	41,4	9,7	Died	36	266	261	−5	3	48,7	160
11	13	6	41,2	8,7	Died	39	249	246	−3	1	48,7	320
12	13	5	40,8	6,5	Died	42	244	244	0	0	48,5	80
13	13	9	41,0	11,3	Died	30	269	255	−14	5	46,3	80
14	15	9	41,2	15,5	Recovered	0	318	282	−36	15	30,5	80
15	11	10	41,5	17,3	Recovered	0	230	198	−32	13	30,3	40
16	15	12	41,0	17,6	Recovered	0	234	207	−27	11	28,6	160
17	12	11	41,3	16,5	Recovered	0	270	246	−24	9	25,5	320
18	14	11	40,8	12,8	Recovered	0	300	275	−25	11	23,8	640
19	11	14	40,9	20,5	Recovered	0	278	272	−6	3	23,5	320
20	13	9	41,4	14,7	Recovered	0	249	238	−11	5	19,7	320
21	13	9	41,3	13,7	Recovered	0	242	240	−2	1	14,7	320
22	13	8	41,2	9,7	Recovered	0	272	266	−6	3	12,7	320
23	—	—	—	0	Recovered	0	280	291	+11	0	0	1280
24	—	—	—	0	Recovered	0	295	298	+3	0	0	1280

TABLE 4 Reciprocals of conglutinin titres of animals in Exp. 1 before, at the time of, and after weaning

No. allotted to animal in Table 2	Reaction index	Method of weaning	Reciprocal of K titre: Age in months						
			3	5	7½[1]	8[2]	10	13	24
4	10,2	On concrete	<20	20	160	80			
6	9,9		<20	40	320	160			
7	9,8		80	320	640	640			
8	9,1		<20	20	160	160			
17	5,2		<20	320	640	320	160	2 560	2 560
19	4,1		<20	20	80	160	40	320	1 280
20	2,5		20	160	320	160	40	640	2 560
21	1,6		<20	320	640	1 280	160	2 560	2 560
22	0,9		20	40	640	320	320	320	640
24	0		20	40	160	80	40	40	160
3	11,2	On veld	<20	160	320	320			
5	10,0		<20	20	640	160			
9	8,6		<20	320	320	320	320	640	640
11	7,5		<20	40	80	40			
12	6,6		<20	40	640	320	40	320	320
13	6,5		<20	40	160	160	40	40	160
15	6,3		<20	40	160	160	160	320	320
16	5,5		<20	320	320	160	80	320	320
25	0		<20	20	40	80	40	80	80
26	0		<20	40	320	320	320	1 280	2 560
1	15,3	Unweaned controls	20	160	320	320			
2	12,2		<20	80	320	320			
10	7,9		80	320	320	320			
14	6,4		20	20	160	160	160	160	160
18	4,9		<20	20	320	320	160	160	320
23	0,9		<20	160	320	320	160	320	1 280
27	0		<20	20	640	640	80	160	640
28	0		40	40	160	160	80	320	640
29	0		<20	160	160	160	40	320	320
30	0		<20	80	320	320	320	320	1 280

[1] Calves weaned here

[2] Calves infected here

the animals that survived, the U-test of Mann-Whitney was used (Van Ark, 1981).

Serology

To ascertain whether the calves were free from specific immunity to HW as a result of the remote possibility of tick infection, the serum of the experimental animals collected at 4 months of age and on the day of infection was submitted to the indirect fluorescent antibody test. The sera diluted to 1:20 were subjected to the test as previously described and modified (Du Plessis, 1981, 1982 b, 1985).

RESULTS

Reactions and conglutinin levels

The correlation between the R.I. of the animals in the 2 age-groups and the levels of serum K on the day when they were infected are reflected in Tables 2 and 3. According to the Spearman rank correlation coefficient

there was no correlation (r_s=0,0224) between the R.I.'s of the 8-month-old calves and K levels in their sera. Although some of the calves developed mild to moderate febrile reactions and lost as much as 13 kg body mass, the levels of K in their sera were not related to their R.I.'s and showed the same variation between low and high titres as in the case of the 7 animals that showed no febrile reaction at all. There was also no difference in susceptibility between the weaned and unweaned groups (Table 4).

By contrast, there was a highly significant negative correlation (r_s = −0,676) between the R.I.'s and the K titres of the 12-month-old animals. Although only 45,7 % of the variation can be attributed to a negative relationship between the rank orders of the R.I.'s and the K titres, these findings nevertheless indicate a reasonable agreement between the 2 variables.

Furthermore, according to the Mann-Whitney U-test, there were significant differences between the K titres of the animals that died and those of the animals that survived. The R.I. ranks of those that died were significantly greater than those of the surviving animals, and the ranks of the K titres contrarily significantly smaller. It must also be pointed out that the only 2 animals (No. 23 & 24) that failed to react and that had gained rather than lost mass, had the highest levels of K recorded in this age-group. Although 3 animals (No. 14, 15 & 16) with titres of 1:160 and less survived, they suffered severe loss of mass and had prolonged severe febrile reactions.

The Giemsa-stained brain smears of all 13 animals that died were positive for HW.

Serology

Both serum samples of each of the 54 experimental animals were negative for antibodies to *C. ruminantium*.

Influence of weaning and infection on K levels

The K levels of the sera sampled from the animals in Exp. 1 at 3–24 months of age and the influence of weaning on these levels are reflected in Table 4. It can be seen that only small amounts were present at 3 months of age and that levels commenced to rise at 5 months. By 7½–8 months, the K titres of almost 50 % of the 20 animals whose K levels were followed for 2 years had reached their peak. At 2 years of age, titres of 1:320 and higher were recorded in all except 4 animals (No. 13, 14, 24 & 25).

It can also be seen from Table 4 that in 50 % of the calves that were weaned at 7 ½ months of age, a twofold and in case of one calf (5) a fourfold drop was recorded within 14 days after weaning, whereas none of the unweaned controls showed any fluctuation at this period. There was no appreciable difference between the K levels of the 2 groups of calves that were differently weaned.

It is also evident from Table 4 that, compared with titres recorded at infection, there was a distinct drop in the K levels of 70 % of calves 2 months later. As many as eightfold decreases were recorded in 3 of the calves (12, 21 & 27). Samples were not available to determine at which stage after infection the drop occurred.

Discussion

The statistically significant correlation between the susceptibility to heartwater and the K titres of the year-old cattle as well as the significant differences between the K titres of the animals in this group that died and those of the animals that survived, add support to the contention of Du Plessis & Bezuidenhout (1979) that K influences the pathogenesis of the disease and that high enough concentrations protect cattle against a fatal infection. The recent finding (R. E. J. Burroughs, L. P. Colley, J. L. du Plessis & H. Ebedes, unpulished data, 1985) that 5 out of 8 antelopes (born in captivity and therefore potentially susceptible to heartwater) with K titres of 1:80 and lower died from typical heartwater after having been inoculated with *C. ruminantium*-infected sheep blood, whereas the other 3 with K titres of 1:2560 and higher showed no clinical signs, is yet further support. There is therefore mounting evidence that K influences the susceptibility of ruminants to heartwater, even though the correlation between K levels and the susceptibility of the year-old animals in the present study does not necessarily reflect a cause and effect relationship.

There was a marked difference in the susceptibility to HW of the 2 age groups of cattle that were of the same breed, sex and origin. The reactions of the 8-month-old calves, reflected by low degree R.I.'s, were only mild to moderate, and none of them succumbed to the infection. On the other hand, the 12-month-old animals reacted severely with R.I.'s significantly higher than those of the younger calves, and more than 50 % of them died from the infection.

The mild reactions generally shown by the 8-month-old calves may well have been partly due to K. Since it is as yet impossible to determine the relative importance of the suckling-calf resistance and that attributable to K in the younger calves, the year-old animals were considered more appropriate for studying the relationship between K levels and susceptibility to heartwater. Although the duration of the suckling-calf resistance is not known and may vary considerably between individuals and breeds, its effect in the older animals is expected to be minimal or absent.

Furthermore, the K levels recorded during the calfhood of the 8-month-old animals suggest that K associated HW resistance in the type of cattle used in these experiments takes effect at a much later stage than the suckling-calf resistance, since at 3 months of age only low levels of K were recorded. Adult levels were only attained by 8 months and older, and not by 6 months of age as reported elsewhere (Kakoma & Kinyanjui, 1974).

These findings therefore suggest that the suckling-calf resistance, which decreases as calves become older, must be differentiated from K-associated resistance which in calves increases with advancing age. Since the one strengthens as the other wanes, these 2 non-specific resistance factors are probably complementary in their protective action against *C. ruminantium* infection. At times they may have a combined effect. The resultant fluctuation in the resistance of young calves may well explain the sporadic losses from HW amongst calves, particularly in the absence of artificial or tick-mediated specific immunity. In this respect, it would be important to study the stabilizing effect of calf vaccination.

The interplay between the suckling-calf and the K-associated resistance of the 8-month-old calves in the present study is compatible with the lack of correlation between their K titres and the severity of the reactions that they showed. On the other hand, the correlation between these factors in the 12-month-old animals suggests that their resistance is probably almost exclusively dependent on K levels.

The observations made on the year-old animals in the present study suggest that the cut-off point, above which cattle will be protected from a fatal infection, lies above a titre of 1:320. Animals with K titres of 1:320 either succumbed to the infection or recovered after having shown severe febrile reactions, anorexia and mass loss. This conclusion is compatible with observations made, but not published, in a previous study (Du Plessis *et al.* 1984).

The use of a reaction index to evaluate the severity of the reactions in the present study has advantages above the system of categories previously employed (Du Plessis & Bezuidenhout, 1979; Du Plessis *et al.*, 1984). It is more objective than the latter method and takes the loss in body mass into consideration. A marked loss of mass was recorded in several year-old animals with low K titres in contrast to 2 animals with high titres that failed to react, and gained rather than lost mass. Some of the calves infected at 8 months of age also lost mass, but they showed no clinical signs and had R.I.'s well below the average of the year-old animals.

Loss of condition in cattle suffering from HW is not mentioned in the literature, but the anorexia that sets in is well known and partially accounts for the rapid emaciation. That is why anorexia as a clinical sign was not included as a parameter to calculate the R.I. All the animals in the present study that manifested other clinical signs, such as central nervous disturbances and dyspnoea, eventually died and these signs were therefore accounted for by the R.I. points allotted to a fatal outcome of the disease.

The findings in this study suggest that serum K levels should 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. A conclusion that these animals are resistant to subsequent challenge at the age of one year or older, can therefore only be drawn if it has been established that their K titres did not exceed 1:320 on the day of challenge.

Furthermore, K no doubt plays a role in the epidemiology of HW. On one hand, cattle with high K levels in HW endemic areas should be well protected against the natural disease, particularly in the presence of specific immunity and possibly even when immunity wanes. High level K animals would thus have a better chance of either being infected or re-infected through ticks, 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 parallels the ability of an infective inoculum to elicit a reaction (unpublished observation, 1983), it stands to reason that a factor capable of influencing the severity of the reaction will also affect the eventual immunity. The effect of K therefore appears to be twofold, because an animal with low K will develop a stronger immunity of longer duration, but another with high K will possibly develop a poorer immunity of shorter duration, but will be better protected against re-infection.

While it seems highly probable that K plays a role in the protection of cattle against HW, it must be borne in mind that not only do fluctuations in K levels related to the season and the nutritional state of animals occur (Ingram & Mitchell, 1971), but also that a marked decrease in K activity is associated with parturition (Ingram & Mitchell, 1970). The degree of K-associated protection is therefore not necessarily stable and consistent.

The effect of weaning on the K activity of the younger age-group in the present study was neither marked nor consistent, since only 50 % of the calves weaned showed a decrease. It is therefore unlikely that the weaning of calves constitutes a weak spot in their resistance to HW, particularly in view of the fact that the suckling-calf resistance in most cases persists up to and even beyond the usual weaning age of 6–7 months.

There is evidence, though, that the drop in K levels subsequent to *C. ruminantium* infection is more marked. This phenomenon was noted previously (Du Plessis & Bezuidenhout, 1979) and also occurs in anaplasmosis (Rose, Amerault, Roby & Martin, 1978), but its significance is at present unknown.

At the present state of our knowledge and on the findings in this study, it can be stated in conclusion that the HW resistance of cattle from calf- to adulthood appears to have both a specific and a non-specific component. The suckling-calf resistance, which declines with advancing age and starts disappearing by 6–8 months of age, and K, which takes effect from about 5 months of age and persists lifelong, are the non-specific components. The role played by acquired immunity as the specific component is determined by the time of primary infection and the frequency of re-infection. The effects of these factors obviously overlap, and a better knowledge of their interrelationship will facilitate their manipulation in order to improve their concerted protective action. In this respect, the finding that the value of suckling-calf vaccination is questionable (Du Plessis *et al.*, 1984) must be studied more fully. A much better understanding of the mechanism of the suckling-calf resistance is required, however, before this can be done.

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