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From the Department of Veterinary Microbiology, Section of Parasitology, Swedish University of Agricultural Sciences and National Veterinary Institute, Uppsala, Sweden.

Clinical and Serological Response after Experimental Inoculation with Babesia Divergens of Newborn Calves with and without Maternal Antibodies

By *Dan A. Christensson*

Christensson, D. A: Clinical and serological response after experimental inoculation with Babesia divergens of newborn calves with and without maternal antibodies. Acta vet. scand. 1987, 28, 381-392. – The influence of maternal antibodies on clinical and serological response after experimental inoculation with Babesia divergens of newborn calves was studied. Five calves, born to dams seropositive for B.divergens, (Group 1) had specific maternal antibodies when tested 12 h after their first feeding of colostrum. At that point they were inoculated i.v. with B.divergens infected erythrocytes. Five other calves, born to dams seronegative for B.divergens, (Group 2) had no Babesia specific maternal antibodies when inoculated at the same age.

Babesia divergens organisms were demonstrated in blood smears from calves in both groups at some point 5 to 10 days p.i. All calves in both groups had B.divergens specific IgM antibodies at 7 to 17 days p.i. as shown by a modified IF-test. Specific IgG antibodies, transferred by colostrum, were found in all calves of Group 1 before inoculation of B.divergens. The IgG titre of these animals increased by a doubling dilution step at 11-25 days p.i. Among calves of Group 2 specific IgG antibodies were found at first between day 9 and 15 p.i. Both IgM and IgG antibody titres had to be investigated since demonstrated IgG antibodies can originate both from maternally transferred antibodies and from actively produced antibodies after an infection.

There was no difference in clinical parameters; parasitaemia, PCV, Hb, and rectal temperature between the groups.

This experiment gives evidence that there can be a resistance to bovine babesiosis in newborn calves independent of maternal antibodies.

inverse age resistance; IgM, IgG antibodies.

Introduction

»Resistance« to infection with a species of Babesia is often used to mean that no clinical symptoms of babesiosis are observed in the proper host animal (*Riek* 1968, *Zwart & Brocklesby* 1979, *Zwart* 1985). The animal, however, often gets a subclinical infection and becomes a chronic carrier (*Joyner & Donnelly* 1979). It is well substantiated that pastured calves are less frequently ill with clinical babesiosis than adult cattle, a phenomenon often designed as »inverse age resistance« (*Smith & Kilborne* 1893, *Legg* 1933, *Riek* 1968, *Pipano* 1969, *Uilenberg* 1971, *Christensson & Thorburn* 1987). Experimental studies by *Trueman & Blight* (1978), *Latif et al.* (1979) and *Taylor et al.* (1983) support these observations. Some authors have suggested that inverse age resistance can be explained as being a result of passive-

ly transmitted antibodies from an infected dam (*Ross & Löhr* 1970, *Weisman et al.* 1974, *Hopps* 1977, *Berry et al.* 1981). Support for this suggestion comes from experiments in which maternal antibodies against Babesia bovis and B.bigemina have been found in calves up to an age of 5 months (*Ross & Löhr* 1970, *Weisman et al.* 1974, *Hopps* 1977). Furthermore, plasma from infected calves or mice were shown to protect other calves or mice against clinical babesiosis when injected i.v. (*Mahoney* 1967, *Phillips* 1969, *Mahoney & Goodger* 1972, *Mahoney* (1983). *Hall* (1960, 1963) and *Hall et al.* (1968) were of the opinion that calves are protected against babesiosis for their first 1-2 months of life by passively acquired resistance transferred by colostrum. *Callow & Dalgliesh* (1982) working with B.bovis came to the conclusion that up to 2 months old calves may be at least as susceptible as their dams, if the latter were not immunized during pregnancy. What appeared to be a non-specific resistance which protected the calves developed then during the next 5 months of life.

The general opinion today is that maternal antibodies are of significance for the inverse age resistance phenomenon. However, specific information on this item concerning B.divergens, the only bovine species found in Swedish cattle, is lacking.

The aim of the present investigation was to study the reactions of newborn calves to experimental infection with B.divergens.

Material and methods

Preliminaries for the investigation

Ten cows, 5 from tick infested areas (*Wahlgren et al.* 1984) (Group 1 calves) and 5 cows from tick free areas (Group 2 calves) of Sweden were accommodated since at least 1 month at the National Veterinary Institute or at the Department of Cattle and Sheep Diseases in the winters of 1977, 1979, and 1981. They were kept indoors for at least 1 week after calving and were then excluded from this study. The calves, all singles, were born between March 1 and May 15. Immediately after calving the calves were allocated individually to separate boxes and colostrum for the calves was taken from the dams. There was no further contacts between the dam and the offspring. The calves were kept indoors throughout the study.

Experimental calves

Group 1. The dams had been vaccinated with B.divergens or been clinically infected with babesiosis. Accordingly they were all serologically positive for antibodies against B.divergens, when tested about 1 week before and after calving. The IgM and IgG titres ranged 1/40-1/80 except for the dam of calf no. 7, which was negative for IgM. These cows gave birth to 3 heifer and 2 bull calves of the Swedish Red and White (SRB) breed. IgG antibodies against B.divergens were found in the calves when tested 12 h after their first uptake of colostrum.

Group 2. The dams originated from farms where clinical cases of babesiosis had not been reported, animals had never been vaccinated against babesiosis and they were accordingly serologically negative for antibodies (IgM and IgG) against B.divergens. They gave birth to 1 heifer and 4 bull calves of the SRB breed (n = 3) or SRB × Swedish Friesian breed (n = 2).

Feeding schedule of calves

Colostrum was given to the calves within 6 h after birth, each having about 1 l. The calves were then fed colostrum for 4 consecutive days 4 times a day, each time having approximately 1 l. Thereafter they were fed milk and milk substitute according to the feeding routines of the clinic.

Inoculation of calves
The calves in both groups were inoculated i.v. with $3 \cdot 10^7$ B.divergens infected erythrocytes at 12-18 h after birth, corresponding to approximately 12 h after the very first feeding of colostrum.

Inoculates of Babesia divergens
The inoculates consisted of different B.divergens strains, originally isolated for the vaccine production. They were passaged through splenectomized calves and stored in liquid nitrogen as described by *Dalgliesh* (1972) and *Dalgliesh & Mellors* (1974). Before use, strains were re-passaged through splenectomized calves and blood for inoculation was taken when about 5% of the erythrocytes were infected with B.divergens. This blood was stored 1-3 days at about +6°C and a live inoculate was prepared by a method similar to that described by *Callow* (1977) for B.bovis and B.bigemina vaccine in Australia. Hank's solution added with Na-citrate 0.3 mol/l, 0.5% laceral albumin, 500 IE penicillin/mg and 100 ug streptomycin/ml were used to dilute the blood. Adjustment to compensate for losses of infectivity during storage was made according to *Callow* (1977) and the calves were infected i.v. with a fresh prepared inoculate. All strains (2/76, 1/78, and 4/80) caused fever and parasitaemia in splenectomized calves.

Blood samples
Blood samples were drawn from the calves: approximately 12 h after birth, daily for the next 14 days, every other day thereafter until day 21 p.i., on day 25 p.i., on day 30 p.i., and finally 2 months after birth. Blood samples from the dams were drawn about a week before and after calving.
Sera from these blood samples were separated by centrifugation and stored at -20°C until used.

Parasitaemia
The parasitaemia was investigated on thin film smears from all blood samples and stained with acridin-orange (*Winter* 1967, *Trees* 1974). The number of infected *rbc* per 10^4 rbc was counted using an ocular square net with 10×10 squares. If less than 2 parasites were found 10^5 rbc were investigated and if no parasites were found the slide was examined for 3 more min. An infected rbc found at this point was counted as 1 per 10^6 and if no parasite was detected the sample was recorded as negative.

Haematological examination
Packed cell volume (PCV) was determined from the blood samples at the National Veterinary Institute's unit of Vaccine research. The PCV was established with a microhaematocentrifuge. Haemoglobulin (Hb) was measured colorimetrically using the cyanmethaemoglobulin test read at 540 u.

Clinical observations
Rectal temperatures were taken whenever blood samples were drawn. The animals were also observed on a daily basis for other clinical symptoms as haemoglobinuria during the first 4 weeks after inoculation.

IF-test
Serological examination was performed using a modified IF-test as described previously (*Christensson* 1987). The rabbit anti immunoglobulins used were specific for bovine IgM and IgG respectively (Miles Ltd). Sera were diluted in doubling dilutions starting at 1/40, which previously had been found to be the lowest positive titre of the test (*Christensson* 1987). End point titres were established in all serum samples and presented as $\log^{10}$ of the reciprocal titre values.

Statistical analysis
Statistical analysis of days to seroconversion between groups were made using Wilcoxon's (Mann-Whitney) rank test. (*Hyrenius* 1962).

Results

Parasitaemia
Babesia organisms 0.1-1 per 10^4 erythrocytes were recorded in 5 calves out of 5 in Group 1 and in 3 out of 5 calves in Group 2 at some point day 5-10 p.i. No Babesia organisms were seen in any sample taken at any other occasion during this study. The 2 negative calves were inoculated with strain 1/78.

Haematological observations
The PCV and Hb values are shown in Tables 1 and 2. They reached a peak immediately after birth and fell gradually in both groups during the first month of life. Considering each group as a whole, there were no significant differences between the 2 groups. There were , however, great individual variations within each group.

Rectal temperature
The individual rectal temperatures varied from 38.9-40.2°C and the daily group mean values from 38.1-39.2°C (Tables 1 and 2), but there was no correlation between increased temperatures and parasitological findings.
There was no other clinical symptom of babesiosis observed in any of the calves.

Serology
Group 1: Calves born to seropositive dams. The IgM antibody titre values are summarized in Fig. 1. The IgM antibodies first appeared 9 to 17 days p.i. About 6 days after their first appearance, the IgM titres reached a plateau at a level individual for each calf. The titres then remained at that level for the next 3-4 weeks and thereafter dec-

Table 1. PCV, Hb and rectal temperature of 5 newborn calves with maternal antibodies specific for Babesia divergens (Group 1) after inoculation with B.divergens 12-18 h after birth.

	PCV %		Hb g/100 ml		Temp.°C	
Days after infection	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	41.8	4.1	12.1	1.0	39.0	0.5
1	42.0	6.3	11.6	1.8	38.7	0.5
3	40.6	4.5	11.2	0.8	39.0	0.3
5	40.8	3.1	10.9	0.5	39.1	0.2
7	40.2	2.4	11.2	1.1	39.1	0.3
9	40.2	2.7	11.5	0.7	39.0	0.2
11	40.0	1.6	10.9	0.6	39.2	0.4
13	38.6	0.9	10.9	0.6	39.2	0.3
15	39.6	1.5	11.0	0.3	38.9	0.2
17	38.7	0.6	11.1	0.5	38.5	0.1
19	38.4	0.9	10.4	0.6	38.9	0.4
21	37.8	1.5	11.0	0.5	38.8	0.1
25	36.8	1.0	10.5	0.5	38.6	0.2
1 month	36.6	1.7	10.4	0.4	38.4	0.3
2 months	36.6	1.7	9.9	0.7	nd	nd

nd = not done

Table 2. PCV, Hb and rectal temperature of 5 newborn calves without maternal antibodies specific for Babesia divergens (Group 2) after inoculation with B.divergens 12-18 h after birth.

Days after infection	PCV %		Hb g/100 ml		Temp.°C	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	38.6	3.1	10.3	0.4	39.3	0.2
1	41.6	6.8	11.6	1.9	39.0	0.3
3	38.2	6.5	10.7	1.0	38.8	0.3
5	37.2	5.8	10.5	1.2	39.1	0.5
7	36.2	3.9	10.7	1.4	38.9	0.3
9	37.8	4.8	10.9	1.6	38.8	0.2
11	37.3	5.4	11.0	2.0	39.0	0.1
13	35.2	4.0	10.3	1.1	38.8	0.3
15	36.2	3.1	10.4	1.2	39.0	0.3
17	35.4	3.4	10.3	1.0	38.9	0.2
19	35.0	3.2	10.3	1.0	38.8	0.3
21	34.2	3.3	10.1	0.9	38.8	0.1
25	33.2	3.4	9.8	1.0	nd	nd
1 month	33.4	3.1	9.7	0.9	38.1	0.1
2 months	35.4	2.6	10.0	0.6	nd	nd

nd = not done

lined. The highest IgM titres were obtained from those animals, which had an early IgM response. Three weeks p.i. the calves' IgM titres ranged between 1/80 and 1/640, while 2 months p.i. the lowest titre was 1/40 and the highest was 1/320. The maximum IgM titre reached in this group was 1/1280 on day 19 p.i. (Fig. 1).

The IgG antibodies were found in the calves after they had been fed colostrum. Their titres then ranged from 1/40 to 1/160. A rise of 2 doubling dilution steps occurred in all of the calves at some time 11 to 25 days p.i. Thereafter, the titre values gradually approached and then reached a level at 1/320-1/1280. Three weeks after being infected, the calves' IgG titre values varied from 1/40 to 1/1280. Two months after infection titre values varied from 1/320 to 1/1280, the latter being a maximum value (Fig. 2).

Group 2: Calves born to seronegative dams. The titres for B.divergens specific IgM antibodies of the calves are shown in Fig. 1. IgM antibodies first appeared 7 to 11 days p.i. and a plateau in the titre value was reached at a level specific for each animal within a week. The titre then remained at that level for the remaining observation period. Three weeks p.i. the titre values varied from 1/160 to 1/640 and 2 months p.i. the values also varied from 1/160 to 1/640 (Fig. 1). A titre of 1/1280 was the maximum value observed on day 15-21 p.i. and 2 months p.i.

B.divergens specific IgG antibodies were found in the calves in Group 2, 9 to 15 days p.i. (Fig. 2). The individual plateau was reached about 8 days after IgG antibodies first appeared. Three weeks after being infected, the calves' titres ranged from 1/160 to 1/1280 and 2 months p.i. they ranged

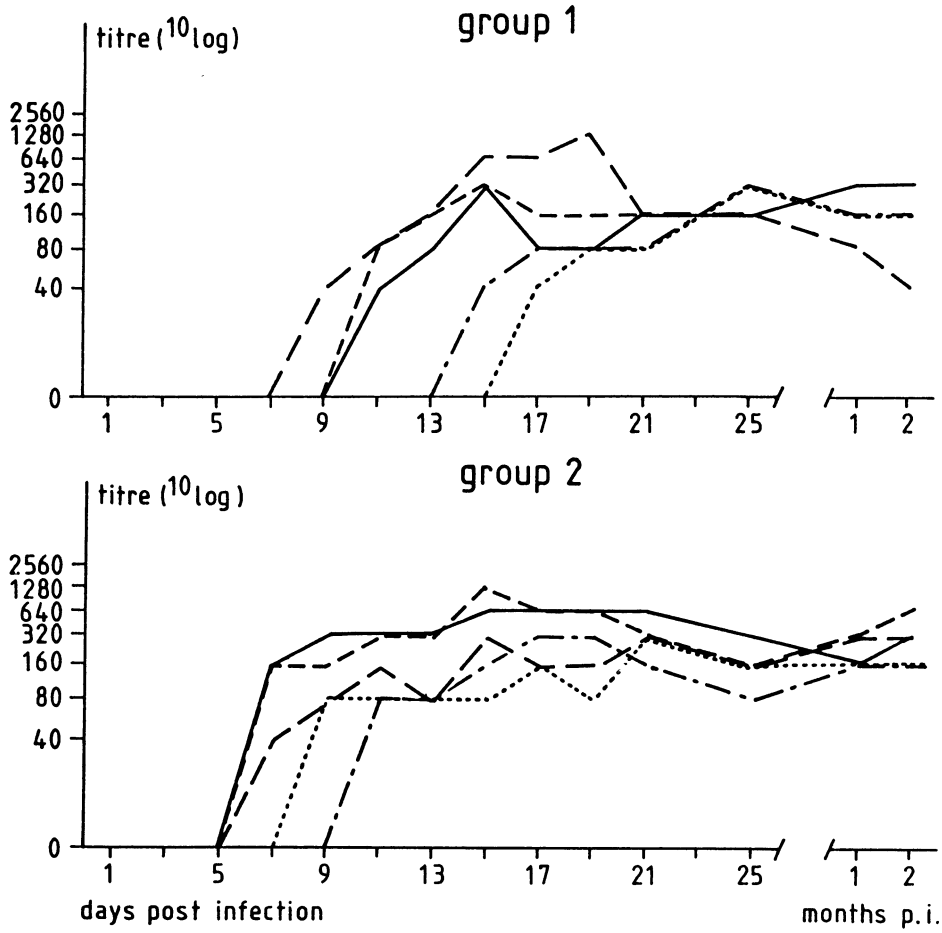

Figure 1. IgM antibody levels of 10 newborn calves which had been inoculated i.v. with Babesia divergens 12-18 h after their first feeding of colostrum. The reciprocal titre values are presented on a 10-logarithmic scale. *Group 1:* Calves fed colostrum 1-6 h after birth from dams which had IgM and IgG antibodies specific for B.divergens. *Groups 2:* Calves fed colostrum from dams which did not have antibodies specific for B.divergens. Animals: No 1: ....., no 2: - . -, no 3: - -, no 4: - - - and no 5: ____. of group 1 resp. group 2.

from 1/320 to 1/2560, the latter being the maximum value.

The mean number of days until seroconversion for IgM was 12.5 ± 2.9 days of calves in Group 1 and 8.6 ± 1.5 days in Group 2. This difference was, however, not significant ($p > 0.05$).

Discussion

Resistance to infection with Babesia in cattle may be the result of more than one process influencing the degree to which the parasite can develop in the proper host. The native animal is here the parasites' physical environment. Its characteristics and the presence

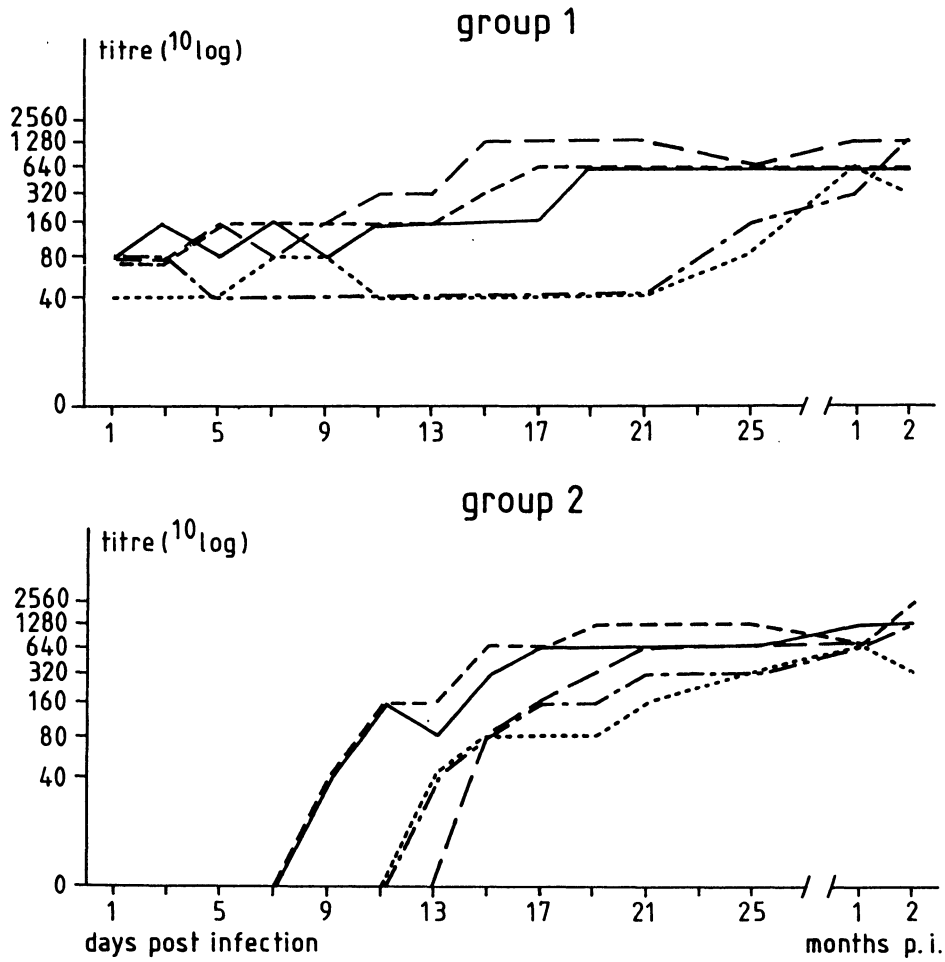

Figure 2. IgM antibody levels of 10 newborn calves which have been inoculated i.v. with Babesia divergens 12-18 h after their first feeding of colostrum. The reciprocal titre values are presented on a 10-logarithmic scale. *Group 1:* Calves fed colostrum 1-6 hafer birth from dams which had IgM and IgG antibodies specific for B.divergens. *Groups 2:* Calves fed colostrum from dams which did not have antibodies specific for B.divergens. Animals: No 1: ....., no 2: - . -, no 3: - -, no 4: - - - and no 5: ____. of group 1 resp. group 2.

or absence of passively acquired substances, may influence the development of the parasite.

The results of the present experiment, however, show that the inoculation of B.divergens in newborn calves is taken a subclinical course which is uninfluenced by maternal antibodies. The exposure of the dam to Babesia infection apparently has no influence on the course of the infection in her offspring.

In the experiments made by *Hall* (1960, 1963) and *Hall et al.* (1968), working with B.bigemina and B.bovis, the calves were

protected by colostrum from their dams to a challenge infection with the homologous strain of the Babesia used, but not to a heterologous strain of the same species. Their results may also have been influenced by the large infective dose of tick transmitted origin given to the calves, which might have overcome any non-immunological resistance. The existence of such a non-immunological resistance factor has recently been demonstrated by *Levy et al.* (1982), who by in vitro cultivation of B.bovis in blood from young calves, found a factor, other than antibody, responsible for the resistance of erythrocytes to the growth of Babesia organisms.

In the study reported here IgM antibodies were found in calves with and without maternal antibodies (Fig. 1). The period of time which elapsed between inoculation and the first appearance of IgM antibodies was approximately the same for calves in both groups. Furthermore, the titre levels were similar in both groups. The presence of these IgM antibodies can reasonably be attributed to the subclinical infection following the inoculation of B.divergens infected erythrocytes. This suggestion is supported by the fact that B.divergens specific antibodies were not found after feeding colostrum and prior to the experimental inoculation.

The IgG antibody titres in sera from newborn calves in Group 1 (calves with maternal antibodies) increased approximately at the same time as the IgM titres, and reached almost the same levels as in Group 2 (Figs. 1 and 2). This antibody response was probably caused by the experimental infection. The calves in Group 1 did, however, tend to have lower IgG titres than calves in Group 2. This tendency may be duo to an inhibiting effect which the passively acquired IgG antibodies can have on the host animal IgG antibody production as described by *Salomon* (1971). The period of time which elapsed from the inoculation of B.divergens to the detection of actively acquired antibodies is in accordance with other investigations on time to seroconversion in older calves after infection with B.divergens (*Purnell et al.* 1976, *Bidwell et al.* 1978) and on B.bigemina (*O'Donoghue et al.* 1985).

There were great individual variations of IgM and IgG responses of the newborn calves. The IgM and the IgG titres reached their peaks approximately 2-3 weeks after the calves were inoculated. After a month the IgM titres showed a tendency to decline. The IgG titres, on the other hand, remained at the same high level, and even continued to rise in Group 2.

The kinetic of the antibody response of the newborn calves shown in this study is in agreement with that described by *O'Donoghue et al.* (1985). These authors found that 10-month-old splenectomized calves which were infected with B.bigemina, had IgM titre peaks at some point between 12 and 22 days p.i. the antibody titres then persisted at these levels until the end of an observation period of 49 days. *Cox & Turner* (1970) found a similar kinetic of the specific IgM and IgG response in young mice infected with B.microti. *Goff et al.* (1982) demonstrated specific IgM activity in sera from cows after inoculation with B.bovis by using immunoelectrophoresis of seroglobulins. Both IgM and IgG antibodies were then observed from day 18 p.i. until the end of the observation period 35 days p.i. *Mahoney* (1972) noted the presence of IgM antibodies in serumglobulins that had been taken from cows infected with B.bovis and then been purified.

The great individual variations in titre values found among calves from both groups and variations in reaction time may be due to differences in the individual uptake of maternal

globulin as previously shown by *Klaus et al.* (1969) and *Salomon* (1971).

It was possible to find B.divergens specific IgG antibodies but not IgM antibodies, in serum samples exclusively from calves which had been fed colostrum from B.divergens seropositive cows (Group 2, Figs. 1 and 2). It is, however, possible for newborn calves to absorb both maternal IgM and IgG antibodies (*Pierce & Feinstein* 1965, *Klaus et al.* 1969, *Staley et al.* 1971), but the major immunoglobulin selectively transferred is IgG (*Murphy et al.* 1964, *Pierce & Feinstein* 1965, *Aalund* 1968, *Klaus et al.* 1969, *Butler* 1974). The serum level of any maternal transferred B.divergens specific IgM antibodies was too low during the present experimental period to be detected by the modified IF-test.

Aragon (1976) rejected the use of the IF-technique for seroepidemiological studies of Babesia because of the possible transfer of maternal Babesia-specific antibodies to the calves which made it impossible to evaluate a seropositive finding.

Aragon's opinion was, however, based upon investigations using anti bovine Ig or IgG antiglobulin in the IF-test. The problem seems to be overcome by a combined analysis of specific IgM and IgG antibodies.

In the present study the developed parasitaemia was small in both groups and in Group 2 no parasites were found in blood smears of calf no. 3 and no. 5. this actually might happen as previously shown by *Goff et al.* (1982) when parasites are present only in very low numbers. In the IF-tests these calves performed in the same way as the other calves in Group 2 and most probably they have had a subclinical infection. Calf no. 5 was for other reasons splenectomized at the age of 3 months after it had been stabled indoors all the time. A B.divergens parasitaemia was then demonstrated.

The PCV- and Hb-values were similar to those described as normal for newborn calves by *Schalm* (1970). These high values might possibly have concealed any reaction due to the Babesia infection. However, there was no reaction at all to be compared with that of older animals with a subclinical babesiosis (*Callow & Pepper* 1974).

In order to infect every calf with B.divergens immediately after birth, different strains and passages had to be used. Differences between strains and passages (*Callow* 1964, 1967, 1968, *Uilenberg* 1970, *Phillips* 1971, *Purnell et al.* 1976, *Taylor et al.* 1983) and antigenic variations of the same strain (*Curnow* 1968, 1973), have been demonstrated but these differences between Babesia populations do not appear to be of importance as a cause of disease as proposed by *Callow* (1977).

In conclusion, there is no difference in clinical and serological response after inoculation of B.divergens organisms of newborn calves with and without maternal antibodies. This observation may be taken into consideration for Swedish Babesia vaccine program.

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Sammanfattning

Experimentell infektion med Babesia divergens av nyfödda kalvar med och utan maternala antikroppar.

Omvänd åldersresistens hos nötkreatur mot infektion med Babesia-arter är ett sedan länge observerat fenomen vars natur är oklar. Maternalt överförda antikroppar har av några författare ansetts kunna vara orsak till resistensen hos kalvar, åtminstone under de 2 första levnadsmånaderna. För att studera betydelsen av maternalt överförda specifika antikroppar infekterades 2 grupper nyfödda kalvar om 5 djur i vardera gruppen med B.divergens. Djuren i båda grupperna infekterades av en Babesiastam som visats kunna ge klinisk babesios hos splenektomerade kalvar. Den ena gruppen hade maternala, med råmjölken överförda, specifika antikroppar. Den andra gruppen fick råmjölk från kor utan antikroppar mot B.divergens. Parasitemi, hematokrit, Hb och rektaltemperatur mättes. Serologiskt undersöktes förekomst av specifika IgM och IgG antikroppar med en modifierad IF-teknik. Med hjälp av den tidpunkt då IgM antikroppar började uppträda kunde antikroppsförekomst orsakad av Babesia infektion hos kalven särskiljas från maternala, passivt överförda specifika IgG antikroppar.

Kalvarna i båda grupperna blev infekterade, men inga djur reagerade kliniskt efter infektionen. Specifika IgM antikroppar påvisades hos kalvar från båda grupperna 7-17 dagar efter infektion. Specifika IgG antikroppar påvisades från början hos kalvar med maternala antikroppar och 9-15 dagar efter infektion hos kalvar utan maternala antikroppar. Vid motsvarande tidpunkt sågs en titerstegring av IgG hos kalvar med maternala antikroppar. Inga andra skillnader mellan grupperna iakttogs. Infekterade nyfödda kalvar kan bli kroniska smittbärare av B.divergens, vilket visades genom splenektomi av en kalv 5 månader efter infektionen.

Denna undersökning visar att det finns en resistens mot B.divergens hos späda kalvar, vilken är oberoende av specifika maternala antikroppar.

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Reprint may be requested from: Dan Christensson, Dept. of Parasitology, National Veterinary Institute, P. O. Box 7073, S-75007 Uppsala, Sweden.