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Inverse Age Resistance to Experimental *Babesia Divergens* Infection in Cattle.

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Christensson, D. A.: Inverse age resistance to experimental Babesia divergens infection in cattle. Acta vet. scand. 1989, 30, 453-464. – Two groups of calves, 1.5-2 and 7-11 months old respectively, and dairy cows were inoculated i.v. with 3×10^7 erythrocytes infected with *Babesia divergens.*

High parasitaemia, fever and other clinical signs of babesiosis occurred among adult animals. A very low parasitaemia and a slightly increased body temperature but no other symptoms occurred in calves. These findings substantiate the conclusion that there exists an inverse age resistance against *Babesia divergens.* The kinetics of *B. divergens* IgG antibody formation were similar in all age groups. Consequently this antibody response was not the factor determining the development of the primary parasitaemia and thus the inverse age resistance phenomenon.

However, age is not necessarily the only factor involved in the clinical expression of babesiosis. The kinetics of antibody formation was not associated with the intensity of the parasitaemia. In fact only about half the animals had a demonstrable parasitaemia although the antibody responses were similar in all age groups.

clinical babesiosis; IgG.

Introduction

There is a plethora of field reports that adult cattle are more likely to fall ill with babesiosis than calves (*Smith & Kilborne* 1893, *Pound* 1897, *Kragerüd* 1901, *Bergman & Waxberg* 1915, *Klarin* 1925, *Hinderson* 1928, *Legg* 1933, *Riek* 1968, *Pipano* 1969, *Callow* 1977, *Callow & Dalgliesh* 1982, *Taylor et al.* 1983, *Gray et al.* 1983, *Gray & Murphy* 1985, *Christensson & Thorburn* 1987, *Christensson & Enfors* 1987, and others). The phenomenon of inverse age resistance has been associated with maternally transferred immunity (*Hall* 1960, 1963, *Hall et al.* 1968) and from observations reported by *Ross & Löhr* (1970) and *Weisman et al.* (1974). However, this explanation is not generally accepted (*Riek* 1968, *Callow & Dalgliesh* 1982).

There is a limited number of experimental studies on the inverse age resistance phenomenon of babesiosis, each elucidating only isolated parameters. *Trueman & Blight* (1978) showed that clinical symptoms and packed cell volume (PCV) values differed between 6-months-old calves, yearlings and adults after inoculation with *Babesia bovis. Latif et al.* (1979) found that yearlings, in contrast to calves, were highly susceptible to *B. bigemina* but they found no serological difference between groups. In other experimental studies, which primarily dealt with other problems of babesiosis, there is information showing that there may exist an inverse age resistance to *Babesia* in cattle (*Smith et al.* 1978, *Taylor et al.* 1983). However, *Löhr* (1969) and *Brocklesby et al.* (1971) who were working with *B. bigemina*

and B. divergens respectively, found no difference between clinical haematological responses in adults and calves.

Although there is conflicting information available, there is circumstantial evidence that there may exist an inverse age resistance to *B. divergens,* the only *Babesia* species demonstrated in Swedish cattle. However, this phenomenon is not completely explained.

The aim of the present study was to define the susceptibility of previously unexposed cattle of different ages to experimentally inoculated *B. divergens.* In the planning of the study parameters of relevance to the Swedish *Babesia* vaccination program were taken into consideration.

Materials and methods

Animals

Two groups of calves and 2 groups of cows were used for experimental inoculation of *B. divergens* as summarized in Table 1.

Group 1 comprised 5 calves, 1.5-2 months old, born and raised indoors at the University farm. Two calves were males and 3 were females.

Group 2 comprised 10 male castrates, 7-11 months old, born and raised indoors on a commercial farm (A). The stock had after end of grazing season moved from a *Babesia*-free area into an infected area. Experiment was made during the indoor season.

Group 3 comprised 13 dairy cows in their first lactating period, having had their calf 4-6 months before. They were 34-38 months old. These animals were also part of farm A. They were born and raised on the original farm. There were 11 more cows on the farm, not participating in the experiments beyond a serum test for *Babesia.*

Group 4 comprised 11 dairy cows belonging to another commercial farm (B) which also moved their stock from a *Babesia*-free area to an infected farm. The experiment was performed indoors before animals were put on the new, infected pasture. Besides a primary serological test for *Babesia,* these animals were investigated only with regard to body temperature and other clinical observations.

All of the animals used were of the Swedish Red and White breed except in Group 1, where the 3 female calves were cross breed Swedish Red and White and Swedish Frisian breed.

All animals, including the other 11 cows on farm A, were *B. divergens*-seronegative when tested a week before inoculation. They were clinically healthy for at least 1 months before inoculation. Throughout an observation period of at least 2 months, only symptoms of babesiosis following the inoculations were observed.

With regard to the risk of animals contracting uncontrolled babesiosis on grass, the experimental inoculations were sanctioned by

Table 1. Data of experimental animals

Group no.	Exp. sites	No. of animals	Age range (months)	Sex	Remarks
Calves					
1	Univ. farm	5	1.5-2	2 m, 3 f	Born and raised on camp.
2	Herd A	10	7-11	m	Born and raised on the farm (indoor).
Cows					
3	Herd A	13	34-38	f	First lactation period. Pastured in *Babesia*-free area.
4	Herd B	11	>30	f	Lactating, pastured in *Babesia*-free area.

f = females, m = males

the farmers, provided that animals falling ill with babesiosis were treated.

Inoculation

All of the animals were inoculated intravenously (i.v.) with 3×10^7 erythrocytes (rbc) infected with *Babesia divergens* parasites. The inoculum had a volume of 3 ml. It consisted of a fresh prepared live vaccine for babesiosis as described by *Callow* (1977) and modified as previously described by *Christensson & Morén* (1987).

The infective dose of *Babesia* organisms was that of the vaccine dose which is given subcutaneously.

Group 2 and 3 were inoculated on the same day using the same passage of isolate 1/78. Group 1 and Group 4 respectively were inoculated with other passages of the same isolate. All animals were kept indoors for a subsequent observation period of 3-4 weeks.

Blood samples

Jugular blood samples were collected on Day 0 (day of inoculation), Day 3 and thereafter daily to Day 11 p.i. Subsequently, samples were collected every other day until Day 21 p.i. with some irregularities. From Group 1 there was also a sample on Days 23 and 30 p.i.

Blood for haematological examination was drawn into heparinized test tubes, while blood for serological tests was drawn into non-heparinized test tubes. Serum was separated by centrifugation and store at -20°C until used.

Parasitaemia

The parasitaemia was investigated on thin films stained with acridine-orange (*Winter* 1967, *Trees* 1974). The number of red blood corpuscles (rbc) was counted using an ocular grid with 10×10 squares. One infected rbc per 10^6 rbc was the minimum count for a sample to be designated positive and consequently the limit for the designation parasitaemia.

Haematological parameter

The PCV established on p.i. Day 0 and 3 and then alternate days with a Linson microhaematocrit centrifuge.

Body temperature

Rectal body temperatures were measured daily from Day 0 until Day 13 p.i. and then every other day until Day 21 p.i., on Day 30 in Group 1. In Group 4 the temperature was recorded from Day 5 p.i. until Day 11 p.i. The lowest temperature for febrile reaction was stipulated to be 39.4°C as previously suggested (*Purnell & Brocklesby* 1977, *Purnell & Lewis* 1981, *Taylor et al.* 1983).

Other clinical observations

Clinical observations were made daily with regard to appetite, listlessness, consistency of faeces, and color of the urine. For the animals in Group 3 the farmer also estimated the daily individual drop of milk production in 4 steps, 25%, 50%, 75% and 100% of normal yield (scored 25% = 1 to 100% = 4).

Serology

The serum antibodies to *B. divergens* were established by a modified IF-technique (*Christensson* 1987a) using FITC-protein A on rabbit anti-bovine IgG (Miles, Ltd). The IgG titres were established on Day 0, 3 and every other day, as described above. The lowest positive titre was stipulated to 1/40.

Sera giving an unspecific fluorescence at 1/40 were not found to have any stronger fluorescence at 1/20 when retested.

Statistical calculations

The Mann & Whitney (Wilcoxon) rank-sum test, and X^2-test with Yates correction were used in the statistical calculations.

Table 2. Parasitaemia: Number of animals with a demonstrable parasitaemia and the number of infected erythrocytes per 10^6 rbc in cattle experimentally inoculated with *B. divergens*.

Group	No. of animals	*Babesia divergens* positive animals		Average no. of inf. rbc per animal	Individual maximal no inf. rbc
		n	%		
Calves					
1	5	2	40	1	3, 1
2	0	4	40	1	6, 2, 1, 1
Cows					
3	13	10	77	7054	26000, 12000, 10000, 10000, 5100, 5000, 2100, 10, 10, 1

Treatment

In Groups 2 and 3 the indication to treat was a rectal temperature of ≥40°C. In Group 4 the indication to treat was haematuria. The chemotherapeutic used was chinuridmethylsulphate (Acaprin®, Bayer).

Because some cows were treated and some were not, the results from these are shown separately.

Results

Babesiosis

A. Clinical babesiosis. Eleven out of 24 adult cattle inoculated with *B. divergens* fell ill in clinical babesiosis and they had to be treated. Seven of the 13 animals of Group 3 showed a moderate to high parasitaemia at the day of treatment, 0.2-2.6% of the erythrocytes being infected.

B. Subclinical babesiosis. Thirteen adult cattle and all 15 calves developed a subclinical babesiosis, only detectable serologically in some of them. The number of animals reacting clinically was significantly different ($p < 0.01$).

Parasitaemia

Sixteen (57%) of the 28 animals responded with a parasitaemia, i.e. at least 1 infected rbc/10^6 were detected in the blood smears (Table 2). Six (40%) of the calves and 10 (77%) of the adult were positive. The parasitaemia was very mild in calves and significantly pronounced in a high proportion of adult animals.

The time which elapsed from inoculation to the first appearance of *B. divergens* in blood films (incubation period) varied from 4 to 11 days, the average time ± SD being 6.8±1.8 days. There was no significant difference between age categories (Table 3).

The duration of the first parasitaemia in individual animals lasted 1 to 3 days. Duration

Table 3. Parasitaemia: Distribution of animals on no. days p.i. when parasites first were detected in cattle experimentally inoculated with *B. divergens*.

Group	No. of animals with a parasitaemia	No. of anim. showing their first parasitaemia on Day p.i.								Average duration of parasitaemia, days
		4	5	6	7	8	9	10	11	
Calves										
1	2	1		1						0.4
2	4				2	2				0.4
Cows										
3	10	1	2	1	3	1	1		1	1.8*

*Seven animals treated when body temperature ≥40°C. The untreated adults had a parasitaemia lasting 1 day.

Table 4. Sequential development of parasitaemia and other clinical parameters in individual adult cattle (Group 3) after experimental inoculation of *Babesia divergens*.

Animal no	Parasitaemia no of inf rbc/10^4 rbc								Body temperature increse above 39 3°C								Loose fecal consistency						Milk loss estimate 1-4 (25-100%)								Day p i of sero-conv	Day p i of treat
	Day p i								*Day p i*								*Day p i*						*Day p i*									
	4	5	6	7	8	9	10	11	5	6	7	8	9	10	11	12	6	7	8	9	10	11	5	6	7	8	9	10	11	12		
1	1	9	100		01				6	1							x							3							7	6
2				1	100						2	1	0					x	x							3					9	8
3				1	51						2	1	4		5			x	x							3	2				7	8
4		5	50						1	1	5	3					x						1	4	2						7	6
5			2	120						2	7							x						2	3						9	7
6		1	21						1	5							x							1	2						7	6
7							2	260						5	1	8	9				x	x						2	3	4	7	11
8					01						1	3							x						2	1					7	
9										1																					9	
10											1	5							x							1					9	
11												3							x							1	1				9	
12						1							6							x							2				7	
13				1							1	4							x							1					7	

of more than 1 days was only observed among cows which had clinical symptoms. In some animals parasites reappeared within 2 days, but then lasted just for 1 day (Table 4).

Ehrlichia phagocytophilia was not found in any thin film examined.

Packed cell volume

The mean PCV values are shown in Fig. 1. The mean PCV values of Groups 2 and 3 gradually decreased to 5-10% of the preinfection values until Days 11 to 15 p.i. and then it began to return to the preinfection level. There was no difference in the PCV decrease between these calves and untreated cows. The PCV values of treated adults also decreased about 10%. They were then affected by treatment, but returned to the preinfection levels within the same period of time as the untreated animals. The mean PCV values of the very young calves in Group 1 responded within 3 days p.i. and remained low for 2 weeks before approaching preinfaction values.

Body temperature

All adult animals (Groups 3 and 4) and 10 of the calves responded with fever (≥39.4°C), cows significantly more than calves (p

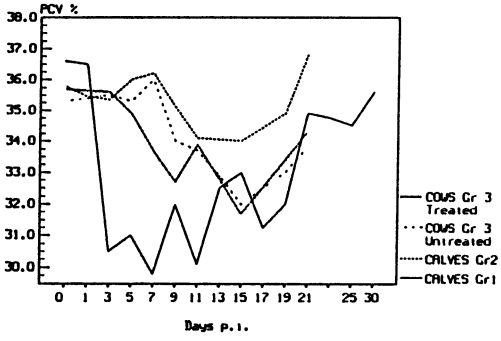

Figure 1. Mean PCV of calves 1.5-2 months old (Group 1), calves 7-11 months old (Group 2) and treated and untreated diary cows (Group 3) after inoculation with *Babesia divergens*.

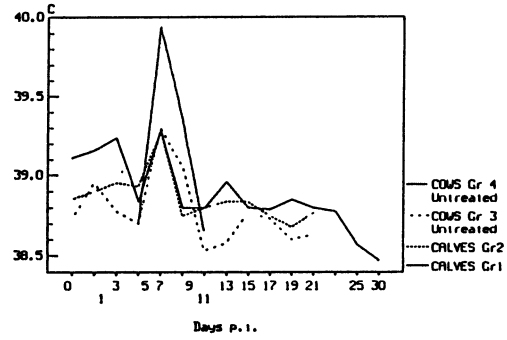

Figure 2. Mean body temperature C°, of calves 1.5-2 months old (Group 1), calves 7-11 months old (Group 2) and untreated diary cows (Group 3 and Group 4) after inoculation with *Babesia divergens*.

<0.05). Eleven of the cows had high fever ($\geq 40.0°C$) but just 2 of the calves ($p < 0.05$). The daily average fever values are shown in Fig. 2. The average fever values peaked on Day 7 p.i. except for Group 1. The average sum of the daily febrile response ($>39.3°C$) is summarized in Table 5. It was significantly higher for cows than for calves although about every other adult was treated. Of the 16 animals which showed a parasitaemia at the day of maximum temperature coincided with the day of maximum parasitaemia with one exception.

Table 5. Body temperature: sum of individual daily febrile response $\geq 39.4°C$ after experimental inoculation with *B. divergens*.

Group	No. of animals	Mean temperature $\geq 39.4°C$
Calves		
1	5	0.3
2	10	0.4
Cows		
3 (untreated)	6	0.4
4 (untreated)	7	1.6
3 (treated)*	7	1.5
4 (treated)**	4	3.4

*treated at a temperature of $\geq 40°C$
**treated when haematuria was observed

Other clinical signs

Haemoglobinuria was recorded only in adult animals. Four of 11 animals of Group 4 displayed this reaction.

Soft faecal consistency was seen in 12 of 13 cows of Group 3 and all animals of Group 4. All other animals had normal faeces. Loose stools occurred simultaneously with fever (Table 4).

The dairy cows in Group 3 had the maximum depression of milk yield simultaneously with the increased body temperature (Table 4). There was also close association between fever and lowered milk yield.

The appetite of the calves was uninfluenced by the subclinical babesiosis. Among the animals in Group 3, a reduced appetite was reported by the farmer when milk production was depressed.

Serology

B. divergens-specific antibodies appeared in all of the animals, the titre rise beginning between Days 5 and 9 p.i. There was no significant difference between young and adult animals. Already on Day 7 p.i. 16 out of 28 (71%) of the animals were seropositive.

The daily average antibody titre responses during the first 21 days p.i. followed a similar pattern in young and adult animals (Fig. 3). The changes in average antibody titres of treated and untreated adults were almost parallel (Fig. 3). Specific antibodies were established whether a parasitaemia was demonstrable or not. The serological response occurred in most cases within 1 to 2 days after the parasitaemia but in some cases antibodies were demonstrated before the parasites (Table 4). The minimum antibody titre value on Day 21 p.i. was 1/1280.

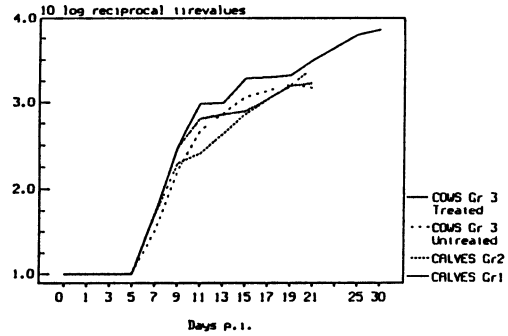

Figure 3. Mean and point IF-titre (IgG) values as ^{10}log of the reciprocal titre value of calves 1.5-2 months old (Group 1), calves 7-11 months old (Group 2) and untreated and treated diary cows (Group 3) after inoculation with *Babesia divergens*.

Treatment

Seven animals in Group 3 and 4 animals in Group 4, which responded with clinical babesiosis, were successfully treated.

Discussion

This investigation shows that dairy cows are more susceptible than calves to experimentally inoculated *B. divergens* organisms. The inoculate generated a high parasitaemia, fever, haemoglobinuria, inappetence and reduced milk production in a high proportion of the dairy cows. In contrast no calves showed evidence of clinical babesiosis.

This is in agreement with own field studies (*Christensson & Morén* 1987, *Christensson & Thorburn* 1987, *Christensson & Enfors* 1987). A similar age dependent pattern was reported previously for *B. bovis* and *B. bigemina* (*Smith et al.* 1978, *Trueman & Blight* 1978, *Latif et al.* 1979).

There were great within-age-group variations in parasitaemia (Table 2). This seems to be a natural individual response of cattle to *B. divergens,* which has been observed previously in experimental studies (*Davies et al.* 1958, *Trees* 1978, *Purnell* 1981, *Taylor et al.* 1986b) but not to *B. bovis* or *B. bigemina* (*Trueman & Blight* 1978, *Smith et al.* 1978, *Latif et al.* 1979). An explanation for this difference may be that these two *Babesia* species are more virulent and they may cause severe disease and high mortality even among yearlings. Only about half of the *B. divergens*-inoculated adults in this study developed a clinical babesiosis, a result not always observed in small groups of animals (*Brockesly et al.* 1971).

The PCV values showed great individual variations but there was no difference between age groups (Fig. 1), probably because of treatment of animals already at the point of a high febrile reaction. The mean percentage reduction of PCV was limited to 5-10% within 2 weeks as in subclinically affected cows and calves (Fig. 1). it is obvious that the parasitaemia was too low and of too short a duration to cause a significant drop in the PCV-values of these animals compared to the reports of *Smith et al.* (1978), *Trueman & Blight* (1978) and *Taylor et al.* (1983). The drop in PCV-values occurred in every animal independent of age and in calves was often the only subclinical reaction to the inoculation besides a seroresponse. A drop of the PCV-values even in animals reacting subclinically to *B. divergens* was reported earlier by *Brocklesby et al.* (1971) and *Taylor et al.* (1983).

The average temperature of cows and calves peaked on Day 7 p.i. simultaneously in most animals with a parasitaemia (Fig. 2) except for the group of the youngest calves (Group 1). This probably because the two calves having a parasitaemia and a small febrile response reacted earlier (Tables 2 and 3). Dairy cows were more sensitive to the inoculated *B. divergens* organisms as shown by the elevated body temperature which was recorded at some time in every adult, even in those with an undetectable parasitaemia (Table 4). In contrast, an incresed body temperature was recorded only in those calves with a parasitaemia. The incidence of temperature $\geq 39.4°C$ after infection was significantly more common in cows than in calves. There was a close correlation between the day(s) of parasitaemia and the day(s) of elevated temperature. However, the animals had too low a parasitaemia of too short a duration to allow any correlations with the values of the parasitaemia and PCV or body temperature-values as previously reported by *Callow & Pepper* (1974) for *B. bovis* and by *Purnell et al.* (1977b) for *B. divergens* in splenectomized calves.

Most dairy cows also showed depressed milk production and loose stools coinciding with the maximum body temperature (Table 4). These symptoms are, beside fever, the first signs of acute babesiosis in adults (*Bergman & Waxberg* 1915). Haemoglobinuria occurred only in animals in Group 4 after 1 or 2 days of febrile response.

All animals responded with antibody formation (IgG). This response was independent of age and the degree of parasitaemia. The kinetics of the antibody response were similar in cows and calves (Fig. 3). The clinical manifestation in a high proportion of cows and the severity of the parasitaemia in subclinically affected cows in comparison with these parameters in calves demonstrates that primary IgG antibody formation is not the responsible factor involved in the inverse age resistance phenomenon. The results reported in newborn calves (*Christensson* 1987b) and in older calves, yearlings and adults (*Latif* 1979, *Taylor et al.* 1983) supports this conclusion. The antibody system responded even to an undetectable parasitaemia. In fact antibodies could be demonstrated before infected erythrocytes appeared in blood smears (Table 4). Consequently these antibodies had no inhibitory effect on the multiplication of *Babesia* organisms in the primary infection. This finding is somewhat in contrast to the results reported by *Mahoney & Goodger* (1972), *Todorovic et al.* (1973), *Purnell & Brocklesby* (1977). These authors demonstrated a protective effect of hyperimmune serum in primary *Babesia* infections. Specific immunity to reinfection with *Babesia* is also reported to be antibody-mediated (*Callow & Dalgliesh* 1982).

The first IgG antibodies were demonstrated in every animal at some point between 5 and 9 days after inoculation of *B. divergens*. The end point titre values on Day 21 p.i. were 1/1280-5120. The same pattern of the humoral antibody response after infection with *Babesia* organisms was observed previously when the IF-technique or other tests to demonstrate specific IgG or Ig were applied (*Ross & Löhr* 1968, *Purnell et al.* 1976b, *Todorovic & Long* 1976, *Bidwell et al.* 1978, *Trees* 1978, *Latif et al.* 1979, *Rief* 1980, *Weiland et al.* 1980, *Dwivedi & Gautam* 1982, *O'Donoghue et al.* 1985). Another interesting observation was that cows treated in the early phase of parasitaemia and fever had an IgG antibody response comparable to that of subclinically affected cows which were untreated (Fig. 3).

There are conflicting opinions on the role of antibodies in inverse age resistance. *Hall* (1963), *Hall et al.* (1968), *Löhr* (1969), *Ross & Löhr* (1970) and *Weisman et al.* (1974) explained the phenomenon as a result of maternally transferred immunity followed by an early, natural infection.

The present study shows that this is not necessarily the only explanation. Support for this statement comes from reports that calves and yearlings beyond the reach of maternally acquired resistance fall ill in babesiosis in a lower frequency than adult cattle (*Riek* 1968, *Trueman & Blight* 1978, *Callow & Dalgliesh* 1982).

It may be speculated that the inverse age resistance and also the different individual response to *B. divergens* are related to a non-antibody factor in blood of young calves as previously suggested by *Levy et al.* (1982). An individual variation of the susceptibility has also been demonstrated among Trypanotolerant cattle (*Murray et al.* 1982, *Murray & Trail* 1986), a phenomena which also is not completely explained.

Immunosuppression is another factor which may be age-related in *Babesia* infection in mice (*Habicht et al.* 1983). Concomitant infections with bacteria, e.g. *Corynebacterium parvum,* may inhibit the development of babesiosis in mice by stimulating a non-specific immunity (*Clark et al.* 1976, *Clark* 1979, *Cox* 1980). This, however, failed to be proved for cattle (*Brocklesby & Purnell* 1977) or was not statistically significant (*Corrier & Wagner* 1984). Interactions between *Babesia spp.* and other parasites have been described. *Millot & Cox* (1985) found a

resistance to *Babesia* after a chronic *Trypanosoma brucei* infection. Suppressive effects on *Babesia* infection have been reported in rodents infected with *Schistosoma mansoni* (*Fagbemi et al.* 1985) and *Taenia* metacestodes (*Nichol & Sewell* 1984).

The animals in this study showed no symptoms of any specific disease to indicate an interaction with the *Babesia* experiments. *Ehrlichia phagocytophilia* may interfere with the clinical symptoms of a simultaneous *Babesia* infection (*Purnell et al.* 1976a, 1977a, *Taylor et al.* 1986a) This rickettsia has been found in Swedish cattle and sheep (*Christensson,* unpublished). However, any interaction with this pathogen may be excluded because *E. phagocytophilia* was not demonstrated.

In conclusion there exists an inverse age resistance to experimental *B. divergens* infection in cattle. Calves and cows become equally infected with *B. divergens* but a high proportion of dairy cows responds with clinical symptoms, while calves are affected to minor extent. The difference in the clinical responses of young and adult animals is not related to the kinetics of the IgG antibody response.

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Sammanfattning

Omvand åldersresistens hos notkreatur vid experimentell infektion med Babesia divergens.

Kalvar och mjolkkor inokulerades i.v. med 3×10^7 erythrocyter infekterade med *Babesia divergens*, dvs. samma dos som i SVA's vaccin mot babesios. Halften av de vuxna djuren svarade med hog parasitemi, hos en grupp aven med hemoglobinuri. Flertalet vuxna djur hade feber i samband med en parasitemi samt los avforing och minskad mjolkproduktion. Kalvarna visade inga kliniska symtom men en laggradig parasitemi kunde påvisas hos knappt halften av djuren. Dessa skillnader visar på en omvand åldersresistens mot *B. divergens*.

Kinetik och titernivå i det uppmatta humorala antikroppssvaret (IgG) var daremot likartade oavsett åldersgrupp. Det ar darfor osannolikt att produktionen av dessa IgG-antikroppar har en avgorande betydelse for det primara infektionsforloppet och darmed aven for den omvanda åldersresistensen. Antikroppssvaret var ej beroende av parasitemins storlek eller om varddjuret behandlats i akut sjukdomsskede

Endast hos ca halften av djuren i respektive åldersgrupp utvecklades en påvisbar parasitemi. Detta visar att andra faktorer an åldern som sådan inverkar på babesiosens kliniska forlopp.

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