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EXPERIMENTAL *BABESIA DIVERGENS* INFECTION IN REINDEER *(RANGIFER TARANDUS)*

By

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The various Babesia species have hitherto been believed to be host-specific with *Babesia divergens* representing the species infecting cattle. *Enigk & Friedhoff* (1962), however, have illustrated how strict host specificity is no longer a valid assumption. In their experiments they were able to induce *Babesia divergens* infection in wild sheep (*Ovis musoni*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*), and roe-deer (*Capreolus capreolus*), although only after splenectomy.

The present study is concerned with the induction of *Babesia divergens* infection in reindeer. *Babesia divergens* is the only Babesia species known to occur in Sweden.

MATERIAL AND METHODS

Blood samples for transmission.

The experimental animals were injected intravenously with 10 ml citrate blood (one part 3.8 per cent sodium citrate solution, nine parts blood). The blood was obtained from two cattle (N1 and N2) which had naturally acquired babesiosis. *Babesia divergens* could be demonstrated in some 20 per cent of the erythrocytes in blood smears from the two cattle.

A control blood sample was taken from a cow (N3), about four years old, which had never been at pasture and never shown any signs of babesiosis. No Babesia organisms could be demonstrated in thick or thin blood smears.

Experimental animals.

Two intact reindeer males and a splenectomized bull calf were used in the transmission experiments. The two intact

animals (R1, 5 years old, from Jämtland and R2, 8 years old, from Härjedalen) had been kept at the institute since one year of age. They had been observed daily and had never shown any signs suggesting babesiosis.

The 6-weeks-old bull calf (N4) came from a cattle herd known to be free from babesiosis. After splenectomy, 3 weeks before being inoculated, the calf was kept in a stable with no opportunity of acquiring natural *Babesia divergens* infection.

An intact reindeer male (R3, about 9 months old, from Lappland) served as a control.

Several blood smears from each animal were examined before the beginning of the experiment. No Babesia were demonstrated.

Blood smears.

Samples of capillary blood were taken from the ears. Smears were made from both citrate blood and blood without added anticoagulant. The smears were fixed in methyl alcohol and stained with Giemsa's. Microscopical fields ($\times$ 1250) with 200 to 300 erythrocytes were chosen for examination.

Histological methods.

Tissue samples were fixed in Carnoy's fluid, embedded in paraffin and the sections taken through the conventional alcohol and xylol series. After being washed in distilled water, the sections were stained for 20 minutes in a solution of two drops Giemsa stock solution per ml distilled water. The sections were then dried in air, transferred to xylol, and mounted in neutral Canada balsam under a cover glass. Air drying is an important step since we have often observed that alcohol and acetone decolourize the sections to a degree which makes it difficult to detect Babesia organisms.

RESULTS

Clinical pattern of experimental babesiosis.

Reindeer R1 (not splenectomized) was inoculated with cattle blood from N1. Babesia in the peripheral blood could first be demonstrated on the tenth day — three Babesia-containing erythrocytes in two smears. The number of Babesia-containing erythrocytes steadily increased to give 8 per microscopical field on day 14. Agglutination (see below) occurred in the blood sample taken the afternoon the same day. The reindeer was now anorexic

and polypnoeic with pale mucos membranes and haemoglobinuria. The temperature was 39.4°C. The condition of the animal became worse during the day and in the evening he was treated with Acaprin (Bayer). Recovery was rapid.

Blood from this reindeer (R1) was injected into the splenectomized calf N4. Six days after this injection *Babesia divergens* first appeared in blood smears from the calf followed on the eighth day by haemoglobinuria and other clinical signs of babesiosis. Some 30 per cent of the erythrocytes contained *B. divergens*. After treatment with Acaprin the calf rapidly recovered.

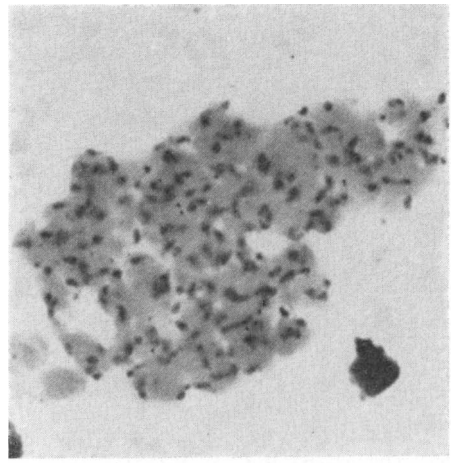
The male reindeer R2 was injected with blood from cow N2. Babesia were first identified in blood smears on the eighth day after injection. By the twelfth day one to three erythrocytes per microscopical field were affected. Fifteen days after injection, clinical signs were evident — anorexia, icteric conjunctivae, temperature 39°C. Satisfactory blood samples could not be obtained from the ears so smears of jugular vein were made directly on slides without an anticoagulant. The number of Babesia-carrying erythrocytes was the same as on the twelfth day, one to three per field, with a few scattered clumps of agglutinated Babesia-carrying erythrocytes. On day 16 this reindeer died.

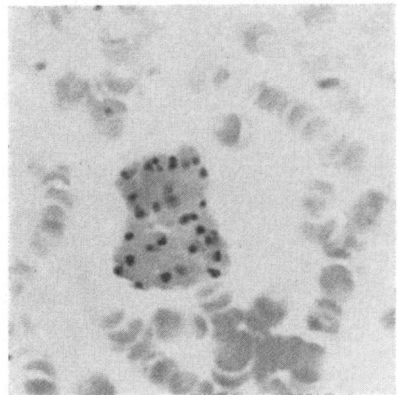
Agglutination of Babesia-carrying erythrocytes.

In smears of citrate blood taken from reindeer R1 in the afternoon of the fourteenth day the number of Babesia-carrying erythrocytes was lower than in smears made earlier the same day. Clumps of agglutinated Babesia-carrying erythrocytes, however, were present in every fifth or sixth microscopical field (Figs. 1 and 2). Some of these clumps were large enough to fill out the whole field. Babesia-free erythrocytes had not agglutinated at all.

Similar agglutination was apparent in blood taken from reindeer R2 on the fifteenth day but the clumps were much smaller, including up to eight erythrocytes at the most. All the agglutinated erythrocytes contained Babesia. Similar clumps of Babesia-containing erythrocytes were also encountered in microscopical sections from various organs, particularly the spleen, of this animal.

A control animal, the non-splenectomized reindeer R3, was injected in a similar manner with Babesia-free cattle blood (from





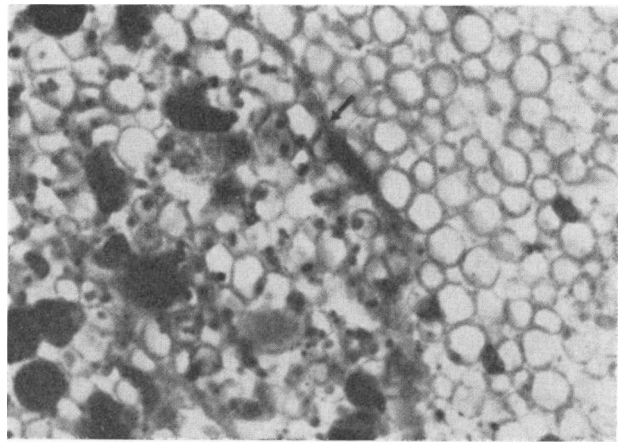
Figs. 1 and 2. Agglutinated Babesia-containing erythrocytes in blood smears from reindeer R1, 14 days after inoculation. Giemsa × 875.

N3). Blood smears were made daily for seven days after injection and then three times daily until the 21st day. No Babesia and no erythrocyte agglutination were observed.

Pathological observations.

Reindeer R2 was in a poor nutritional state at death. The spleen was enlarged and congested. The contents of the rumen were watery and foul-smelling, the abomasal folds oedematous and hyperaemic. The lungs were oedematous and patchily congested. All the joints examined had an oedematous, hyperaemic synovial membrane and an increased amount of synovial fluid.

In microscopical sections stained with Giemsa's, only a few Babesia-containing erythrocytes and a few clumps of agglutinated erythrocytes were present in the liver, kidney, lung, myocardium and brain. The sinusoids of the spleen, however, were packed with large numbers of Babesia-containing erythrocytes with numerous agglutinated clumps. Only a few Babesia-containing erythrocytes were present in the veins of the spleen (Fig. 3).



F i g. 3. Microscopical section of the spleen from reindeer R2, 15 days after inoculation. Numerous Babesia-containing erythrocytes in the sinusoides but only a few in the vein (arrow indicates borderline). Giemsa × 875.

Other changes noted were haemosiderosis in the liver, spleen and lungs, acute splenitis, and in the liver, dissociation of the liver cells and degenerative changes in the nuclei, sometimes with cytoplasmic vacuolization. *Salmonella typhi murium* phage type NS (23) was isolated from samples of the liver incubated in a selective medium.

DISCUSSION

These experiments have demonstrated that *Babesia divergens* can induce disease associated with haemoglobinuria in non-splenectomized reindeer. Re-inoculation of cattle, as in the first experiment, suggests that the Babesia organisms in the original blood sample, in the reindeer and finally again in the calf blood belonged to the same species.

The Salmonella infection in R2 can be assumed to have been latent and exacerbated in conjunction with experimental babesiosis. *S. typhi murium* of the same phage type had been isolated from gulls (*Laurus ridibundus*) found dead a few months previously near the reindeer paddock.

Babesiosis has long been known to occur in reindeer. *Kerzelli* (1909, cit. *Chambers* 1921) described "Milzkrankheit", a disease in reindeer associated with the presence in the erythrocytes of organisms which he named *Piroplasma tarandi rangiferi.* The organism was studied in reindeer in North Siberia by *Yakimoff & Kolmakoff* (1929) who re-named it *Francaiella tarandi rangiferi Kerzelli.* It remains uncertain whether or not their organism is identical with *Babesia divergens.*

No disease suggestive of babesiosis is known to have occurred in Sweden. In this connection it can be noted that the natural distribution of reindeer does not coincide with the areas where bovine babesiosis is enzootic and, furthermore, that *Ixodes ricinus* has never been recovered from Swedish reindeer. Exposure of reindeer to ticks carrying *Babesia divergens* is the only way of determining whether or not these geographical and ecological factors are the full explanation why babesiosis does not occur naturally among reindeer in Sweden.

A feature which seems to be peculiar to babesiosis in reindeer is the tendency for Babesia-carrying erythrocytes to agglutinate.

The long interval between inoculation and the occurrence of agglutination, the involvement of only Babesia-carrying erythrocytes, and the failure to induce agglutination by injecting reindeer with normal cattle blood make it unlikely that agglutination is merely a reaction to blood from another species.

Agglutination of Babesia-containing erythrocytes has not apparently been described before. Its mechanism is unknown as yet.

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SUMMARY

Two non-splenectomized reindeer developed fever, icterus and haemoglobinuria after inoculation intravenously with cattle blood containing *Babesia divergens*.

14 and 15 days after inoculation agglutination of Babesia-containing erythrocytes from the two reindeer was observed in blood films. In one reindeer there was also microscopical evidence of intravascular agglutination in the sinusoids of the spleen.

As a control, a non-splenectomized reindeer calf was inoculated with normal cattle blood; there were no signs of agglutination.

ZUSAMMENFASSUNG

Experimentelle Infektion von Renntieren (Rangifer tarandus) mit Babesia divergens.

In zwei Versuchen lassen sich Renntiere, an denen keine Milzextirpation vorgenommen worden war, durch intravenöse Injektion von Babesia-haltigem Rinderblut mit *Babesia divergens* infizieren. Auch bei dem Renntiere verläuft die Krankheit mit Fieber, Ikterus und Haemoglobinurie.

Am 14. bzw. 15. Tage nach der Impfung wurden in beiden Versuchsställen in Ausstrichpräparaten Agglutinate von infizierten roten Blutkörperchen und in einem Fall auch histologisch in den Sinusoiden der Milz nachgewiesen.

In einem Kontrollversuch wurde normales Rinderblut an ein Renntierkalb mit intakter Milz verimpft. Agglutinate roter Blutkörperchen konnten in diesem Fall nicht festgestellt werden.

SAMMANFATTNING

Experimentell babesios hos ren (Rangifer tarandus) orsakad av Babesia divergens.

I två försök har visats, att icke splenektomerade renar kunna experimentellt infekteras med *Babesia divergens* genom intravenös ympning med babesiahaltigt nötblod. Den framkallade sjukdomen förlöper även hos ren med feber, ikterus och haemoglobinuri.

På 14:de resp. 15:de dagen efter ympningen kunde i båda försöken agglutinat av infekterade röda blodkroppar påvisas i blodutstryk samt i det ena fallet även histologiskt i mjältens sinusoider.

I ett kontrollförsök ympades normalt nötblod på en icke splenektomerad renkalv utan att agglutination kunde påvisas.

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