

## FATAL EXPERIMENTAL BABESIA MICROTI INFECTIONS IN THE NORWEGIAN LEMMING, LEMMUS LEMMUS (L.)

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**Abstract.** Experimental infections of *Babesia microti* in laboratory-reared *Clethrionomys glareolus* revealed that approximately 15 % of the erythrocytes were infected with single ring forms during peak parasitemia. Infected erythrocytes could be detected in blood smears up to one month post infection. *C. glareolus* treated with a single injection of Depo-Medrol i.m. two days prior to infection displayed a four-fold increase in number of infected erythrocytes at peak parasitemia, 35 % of which contained more than one *Babesia*, and a prolongation of the infection. *B. microti* infections in 35 laboratory-reared *Lemmus lemmus* were fatal. Multiple invasion of erythrocytes, anemia, icterus, hemoglobinuria, anorexia and weight loss, and adrenal and splenic hypertrophy were characteristic for *B. microti* infections in Norwegian lemmings.

*Babesia microti* has been isolated from a number of small rodents and insectivores. In Norway, it was first isolated from a wild bank vole, *Clethrionomys glareolus*, and was transmitted to two laboratory-reared *C. glareolus* in which the resulting infections had peak parasitemias 10–12 days post infection. After four weeks the parasites could not be detected in blood films. These results are similar to reports of other experimental *B. microti* infections which are seldom fatal (Cox and Turner 1970). Natural infections with *B. microti* have also been detected in several other small mammal species from southern Norway (unpublished data) but not in more than 550 wild Norwegian lemmings, *Lemmus lemmus*, examined (Wiger 1971, 1973). Consequently, when *B. microti* was isolated from *C. glareolus* it was injected into laboratory-reared *L. lemmus*, *Microtus oeconomus* and *Mus musculus* to test their susceptibility to this parasite. The results of these experimental infections are the basis for this report.

### MATERIAL AND METHODS

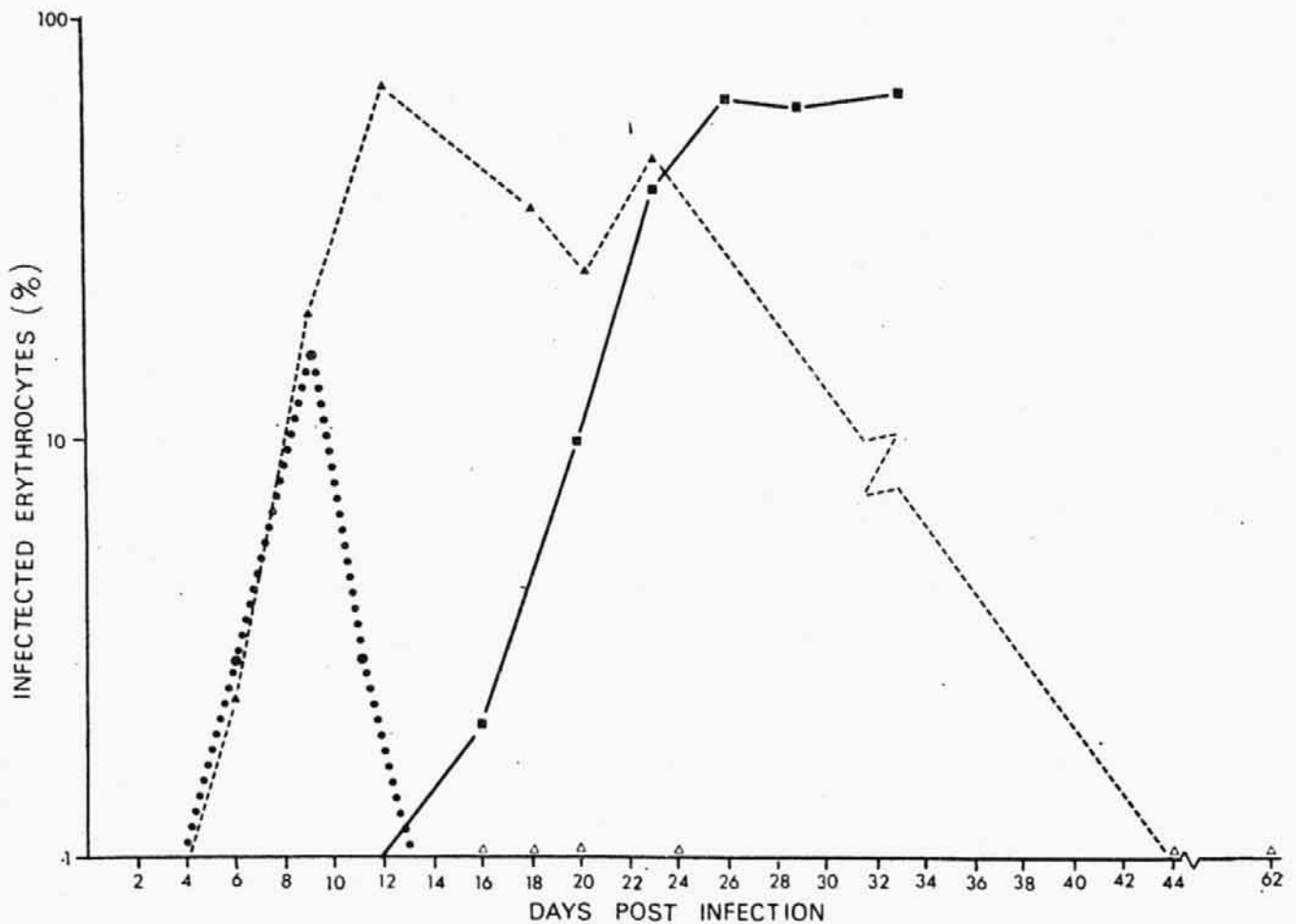
To test for host susceptibility, experimental *B. microti* infections were attempted in the following laboratory-reared species: 35 *L. lemmus*, 6 *C. glareolus*, 3 *M. musculus* and one *M. oeconomus*. All experimental infections were initiated with i.p. injections of infected blood. During the first two syringe passages the source of infected blood was *C. glareolus*. Thereafter, infected blood from *L. lemmus* was used. Usually undiluted blood from cardiac punctures was injected but occasionally small amounts of blood from orbital punctures diluted with saline served as the source. Hematocrits were taken periodically and blood glucose determinations (Glox, Kabi) were made for five *L. lemmus* during the terminal stage of infection. Thin smears were dried, fixed in methanol and stained with Giemsa. Two *C. glareolus* received 8 mg i.m. Depo-Medrol (Methylprednisolone acetate, Upjohn) two days prior to infection to see how the course of infection would be altered and if the infections would be fatal. Body weight and the general health of the animals were recorded periodically. Autopsies were performed at the time of sacrifice or within 24 hours after death. The spleens and adrenals of *L. lemmus* which were sacrificed during the terminal stage of infection were fixed in 10% formalin, cleaned of excess fat, blotted and weighed to the nearest 0.1 mg. Relative organ weights were ex-

pressed in mg per 100 g body weight, where the greatest body weight during the infection period, and not the final weight, was used in these calculations. Light infections were estimated by counting the number of erythrocytes per 100 fields, or approximately 25,000 erythrocytes, under oil immersion. The numbers of *Babesia* per infected cell were also determined.

**Table 1.** Changes in *Lemmus lemmus* associated with experimental *Babesia microti* infections

	N	X	S. D.	Range
Percent weight loss ( $100 - \frac{\text{final body weight}}{\text{greatest body weight}} \times 100$ )	16	21.7	10.7	7.5-45.0
Final hematocrit (%)	7	16.7	2.8	13-22
Final blood sugar (mg%)	5	81.0	20.7	55-110
Relative spleen weight ♂ (mg/100 g body weight)*	5	437.2	189.7	283.4-754.1
♀ (mg/100 g body weight)*	7	836.9	376.2	266.3-1068.0
Relative adrenal weight ♂ (mg/100 g body weight)*	5	21.1	7.3	10.6-26.8
♀ (mg/100 g body weight)*	7	26.0	8.2	14.0-35.7

\* In the calculation of these values the greatest body weight which was recorded during the infection period was used.



**Fig. 1.** Patterns of parasitemia in laboratory-reared *Clethrionomys glareolus* and *Lemmus lemmus* infected with *Babesia microti*. Untreated *C. glareolus* ●.....; Depo-Medrol treated *C. glareolus* ▲.....; *L. lemmus* ■——. (Δ = less than one percent of the erythrocytes were invaded.)

## RESULTS

In the present study all 35 *L. lemmus* died of overwhelming *B. microti* infections. Some hematological and morphological changes which accompanied the infections are presented in Table 1. The course of *B. microti* infections in laboratory-reared *L. lemmus* and *C. glareolus* are shown in Fig. 1. The intensity of infection at peak parasitemia in *C. glareolus*, untreated and treated with Depo-Medrol, and *L. lemmus* are shown in Fig. 2.

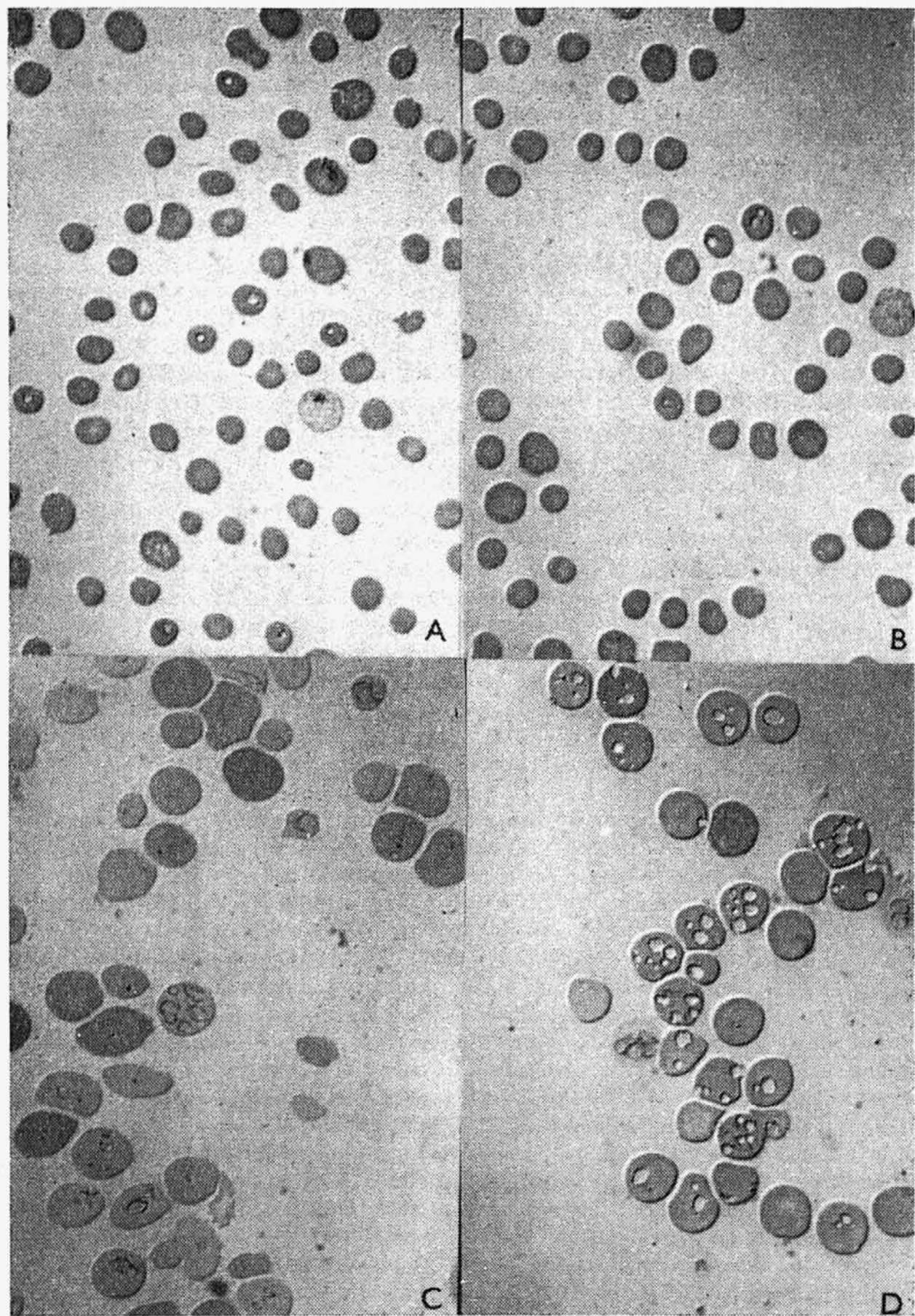
## DISCUSSION

In natural infections of *B. microti* in *C. glareolus*, the parasites are present in successive blood films for at least 11 weeks (Baker et al. 1963). The Kings strain of *B. microti* established in TO mice disappears from the blood, in most cases, by the 30th day (Cox and Young 1969) but continuous high parasitemias in BSVS mice with the same strain of *B. microti* last well over a year (Irvin and Brocklesby 1969). In the present study, no *Babesia* could be detected in the blood films from experimentally infected *C. glareolus* after four weeks (Fig. 1). Latent infections are not ruled out however, inasmuch as recrudescence has been reported after 42 days using betamethasone treatment (Young and Cox 1971). The duration of the infection is obviously dependent upon both strain of the parasite and the host species. The course of the infection in laboratory-reared *M. oeconomus* was similar to that of *C. glareolus* but with a low parasitemia which remained under one percent infected erythrocytes. The laboratory mice in the present study also had low parasitemias. On the contrary, all 35 *L. lemmus* died of overwhelming *B. microti* infections. The course of the infections had a pattern similar to *L. lemmus* in Fig. 1, but showed certain variations, such as length of prepatent period and duration of infection. The duration of the infection varied between 9—34 days, with an average of approximately 22 days. Fay and Rausch (1969) found that the majority of voles and lemmings from nonenzootic areas died before the 15th day post infection.

The mortality of infected *L. lemmus* was apparently not due to an excessively great number of *B. microti* in the inoculum. This opinion is supported by the following evidence: Firstly, *C. glareolus*, *M. oeconomus* and *L. lemmus* all received similar doses but only the lemmings died. Secondly, the number of *Babesia* in the inocula used in other experiments were considerably greater than the approximately  $5 \times 10^7$  which was lethal in *L. lemmus*. Nowell (1969) reported that an increased inoculum with *B. microti* or *B. rodhaini* caused the peak parasitemia to be both earlier and higher. Finally, some of the infections in *L. lemmus*, as seen in Fig. 1, had prepatent periods which were two to three times longer than typical infections in *C. glareolus*, and this would apparently be ample time to allow the immune system to respond to the parasites. Nevertheless, the infections terminated fatally.

It is a well known phenomenon that the pathogenicity of certain parasitic protozoa can be altered when passaged and maintained in animals which are not natural hosts. For example, *B. microti* was maintained in laboratory rats by fortnightly syringe passages for 18 months during which time the maximum parasitemias increased from a very low to a measurable value (Nowell 1969). Did *B. microti* undergo an alteration in pathogenicity when syringe passaged 20 times through *L. lemmus*? Apparently very little, if any. During the course of the experiment two *C. glareolus* were infected during syringe passages 10 and 21, respectively. Both the intensity and duration of these infections were quite similar to untreated *C. glareolus* in Fig. 1.

*B. microti* infections could be prolonged and heightened in laboratory-reared *C. glareolus* after a single injection with 8 mg Depo-Medrol (methylprednisolone



**Fig. 2.** Intensity of invasion of erythrocytes with *Babesia microti* during peak parasitemia in untreated *Clethrionomys glareolus* A and B; Depo-Medrol treated *C. glareolus* C; and *Lemmus lemmus* D, viewed through Nomarski Differential Interference Contrast microscopy. (Magnification 1250 times.)

acetate) i.m. two days prior to infection. During peak parasitemia 70 % of the erythrocytes were infected compared with 16 % during typical infections (Figs. 1, 2). During peak parasitemia in untreated *C. glareolus* five to six percent of the infected erythrocytes contained more than one *Babesia* per cell, whereas in Depo-Medrol treated animals this figure rose to 35 % (Fig. 2). The behaviour of the parasite, as judged by the percentage of infected erythrocytes and number of multiply-infected erythrocytes at peak parasitemia, was similar for Depo-Medrol treated *C. glareolus* and untreated *L. lemmus* (Fig. 2). The number of *Babesia* per infected erythrocyte on the day of death for one *L. lemmus* was approximately 2.9 compared with 1.8 for Depo-Medrol treated *C. glareolus* during peak parasitemia. Nevertheless, the *C. glareolus* survived. Infected erythrocytes could still be detected in the blood 62 days post infection in the hormone treated animals. Young and Cox (1971) described similar effects on *B. microti* infections in mice which had been treated with betamethasone.

The general health and condition of the Norwegian lemmings showed no appreciable changes until that point in the infection where approximately 60 % of the erythrocytes were infected. After this stage, anorexia, weight loss and general debilitation became obvious. The mean body weight of 16 lemmings at time of death was 78 % of the greatest weight during the infection, with a range of 55—92.5 %. Autopsies revealed empty stomach and intestines, enlarged spleens, pale liver and a red urinary bladder.

There are a number of hematological changes which accompany *B. microti* infections. Typical infections are accompanied by an anemia which is proportional to the number of infected erythrocytes. Nowell (1969) found that the number of erythrocytes decreased by about 30 % at the peak of *B. microti* infections in laboratory rats. This pattern is similar for *B. microti* infections in *C. glareolus* in the present study. The hematological alterations in *L. lemmus* are similar but of a more drastic and irreversible nature. The final hematological picture of the infected *L. lemmus* include anemia which is characterized by a mean hematocrit of 16.7 % in comparison with 42 % for laboratoryreared lemmings (Wiger 1977). Thin blood smears revealed reticulocytosis, anisocytosis, and occasional circulating normoblasts. Icterus and hemoglobinuria were also marked. Blood glucose concentrations were determined for several *L. lemmus* in the terminal stage of the infections and revealed a hypoglycemic state (Table 1). The average for five animals was 81 mg% compared with 131 mg% for laboratoryreared *L. lemmus* (Wiger 1977).

Splenic hypertrophy accompanied *B. microti* infections in *L. lemmus*. During the terminal stage, the infected males had relative spleen weights of 437 mg/100 g body weight, females had 837 mg (Table 1) in comparison to 53 mg for laboratory-reared male lemmings. The spleens were often light red, similar to the color of arterial blood, and not a dark mahogany color. Fay and Rausch (1969) reported splenomegaly in *Microtus oeconomus operarius* with piroplasm infections. It is well known that the spleen is active in controlling blood-borne protozoal infections.

The relative adrenal weight of five males which were sacrificed during the terminal stage of the infection was 21.1 mg/100 g body weight in comparison to 13.5 mg for laboratory-reared males. The figure for seven infected females was 26.0 mg (Table 1). The parasites in themselves act as stressing agents which lead to adrenal hypertrophy. Adrenal hypertrophy has also been observed in experimental infections of *Trypanosoma lewisi* in laboratory rats (Woldow 1969) and in *L. lemmus* which were naturally infected with *Trypanosoma lemmi* and/or *Grahamella* sp. (Wiger 1978). It appears that adrenal involvement with blood parasite infections may be a common phenomenon.

The present results are similar to those of Fay and Rausch (1969) from Alaska, inasmuch as experimental *B. microti* infections were fatal in microtine rodents which originated from non-enzootic areas. The lemming and vole species from Alaska which died as a result of experimental infections originated from areas which were far removed from the enzootic areas. In southern Norway, on the other hand, the distances which lemmings live from enzootic areas are rather small and can be less than a few kilometers. The Norwegian lemmings are, however, ecologically isolated from the small rodent tick, *Ixodes trianguliceps*, which are the intermediate hosts of *B. microti*. In southern Norway *L. lemmus* live in the mountains above the tree line, approximately 900—1000 meters above sea level, in habitats where *I. trianguliceps* do not occur. In both Alaska and Norway selection towards tolerance to *B. microti* might be a process which occurs in the enzootic areas, whereby the infections are limited in duration and ultimately confer immunity and/or premunity in the infected individuals. On the other hand, related species from ecological situations where natural *B. microti* infections are absent cannot select against this parasite. Consequently a lack of innate and natural resistance render the host highly susceptible to *B. microti*, and fatal infections result.

During years of great abundance, commonly known as lemming years, many *L. lemmus* emigrate from the mountains down to the valleys and fjords where *I. trianguliceps* occur. The chance of becoming infected with *B. microti* probably increases with decrease in elevation. If natural infections in *L. lemmus* are as fatal as experimental infections, then this could be one of the contributing factors as to why Norwegian lemmings do not become established in the lowland habitats into which they have wandered.

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