Haemogregarines of the genus *Hepatozoon* (Apicomplexa: Adeleina) in rodents from northern Europe

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Abstract. We studied the prevalence and distribution of *Hepatozoon* infections in small rodents from Finland and other areas in northern Europe. *Hepatozoon* infections were more common in voles (Arvicolinae) than mice (Murinae) and more prevalent in voles of the genus *Clethrionomys* than in voles of the genus *Microtus*. Transmission electron microscopical examination of *Hepatozoon erhardovae* Krampitz, 1964 from bank voles *Clethrionomys glareolus* (Schreber) showed that intracellular lung meronts were located in alveolar septa. Meronts consisted of varying numbers of merozoites packed with amylopectin vacuoles inside electron-lucent parasitophorous vacuole. The size of the meronts was approximately $19 \times 14 \mu\text{m}$. Monozoic or dizoic cysts were frequent findings in the lung alveoles; the size of cysts was approximately $10 \times 6 \mu\text{m}$. Gametocytes were found inside eosinophilic granulocytes in the capillaries of lung tissue. Ultrastructurally, micronemes, microtubules, mitochondria, nuclei and lipid droplets were visible.

The genus *Hepatozoon* Miller, 1908 consists of apicomplexan blood parasites that have been described from a wide range of mammals and other vertebrates, especially reptilians and amphibians (Levine 1982, Smith 1996). Sexual stage with sporogony takes place in different blood-sucking arthropods, and asexual phase in endothelial cells of various organs or parenchymal cells of the liver, according to the species of parasite and the intermediate vertebrate host. Intracellular gametocytes are found in circulation either in erythrocytes or in leukocytes. Surveys of small mammals in Central Europe (Mahnert 1972, Frank 1977, Šebek 1978, Šebek et al. 1980, Walter and Liebis 1980, Healing 1981, Turner 1986) indicate that *Hepatozoon* infections are especially common in the bank vole, *Clethrionomys glareolus* (Schreber). One species, *Hepatozoon erhardovae* Krampitz, 1964, has the bank vole as the type host.

There are only a few reports of *Hepatozoon* infections in small mammals from northern Europe (Tadros, cited in Killick-Kendrick 1974, Wiger 1979, Soveri et al. 2000). In this paper we report prevalence and distribution data of *Hepatozoon* infections of small rodents from Finland and some other areas in northern Europe. Furthermore, we examine the morphology of different life-cycle forms of *H. erhardovae* found in bank voles from central Finland.

MATERIALS AND METHODS

The rodents analysed in this survey were captured using live traps (Ugglan special, Allan Ahlgren AB, Marieholm, Sweden) as part of studies investigating diseases of small mammals (Laakkonen et al. 1999, Nemirov et al. 1999, Soveri et al. 2000). The study areas, rodent species and number of individuals examined are shown in Table 1. Kilpisjärvi is in the subarctic birch forest zone, and Pallasjärvi in the northern taiga forest zone in Finnish Lapland. Heinola, Evo and Luhanka are in the boreal zone in southern and central Finland. In Russian Karelia, rodents were collected from several areas in the boreal forests in the eastern part of the republic. In Estonia, rodents were trapped in mixed deciduous forests and oak forests of the Saaremaa Island. The Evo material covers two peak density (in the autumn) phases, and the following crash phases (the next spring) of cyclic *Microtus agrestis* (Linnaeus) populations. The Luhanka material covers similar peak/crash phase (1991-1992), an autumn of an increasing population density (1993), and an autumn of a peak density (1994).

Animals were euthanised with ether and tissue samples from the lungs (right cranial and medial lobes), liver (near the edge of the largest lobe), stomach (fundus), intestines (mid-jejunum and on the top of the colonic spiral), and transversal sections from the middle of the heart, spleen, right kidney, and brain were fixed in 10% buffered formalin, and embedded in paraffin to make standard histological sections, which were stained with haematoxylin and eosin (H&E). Samples were screened for meronts (=schizonts) and other life-cycle forms

with a light microscope at $\times 200$ and $\times 400$ magnification. Sections of some paraffin-embedded *Hepatozoon*-positive lung tissue were also stained with PAS (periodic acid-Schiff) and GMS (Gomori's methenamine silver) stainings. For electron microscopical examination, lung samples were fixed with 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer, pH 7.2, for 1 h at 20°C. After being rinsed with several aliquots of phosphate buffer, the samples were post-fixed in phosphate buffered 1% osmium tetroxide for 1 h at 4°C and embedded in Epon after dehydration in ethanol. Ultrathin sections were stained with 3% uranyl acetate and 3% lead citrate. TEM observations were made with a JEOL 100 S electron microscope (JEOL Ltd., Tokyo, Japan).

Statistix for Windows 2.0 statistical software package was used in all analyses (Analytical Software, Tallahassee, FL, USA).

RESULTS

Forms of *Hepatozoon* haemogregarines were only found in the lungs of the host animals, meronts specifically in the alveolar septa and also close to it free in the alveolus space. Empty looking halo (parasitophorous vacuole) surrounded the meronts. Observed tissue reactions in infected lungs were minimal (Fig. 1). The meronts showed reactivity with both PAS and GMS staining.

In bank voles, the parasite was identified as *H. erhardovae*, based on morphology (Frank 1977, Krampitz 1981) and the previous reports associating this parasite with the bank vole (Šebek et al. 1980, Walter and Liebesch 1980, Turner and Cox 1985). In transmission electron microscopy intracellular meronts were found located in alveolar septa. Inside electron-lucent parasitophorous vacuole, varying numbers of merozoites were packed together with amylopectin granules in meronts. The size of the parasitophorous vacuoles was approximately $22 \times 18 \mu\text{m}$, and that of the meronts $19 \times 14 \mu\text{m}$. The different consistency of meronts compared to lung tissue was demonstrated by their tendency to rip off during preparation of ultrathin sections (Fig. 2). Gametocytes were found inside eosinophilic granulocytes in the capillaries of lung tissue. Ultrastructurally, micronemes, microtubules, mitochondria, nuclei and lipid droplets were seen (Fig. 3). The granulocytes appeared to have suffered from being parasite-laden, karyopyknosis was often evident. Intra-alveolar monozyotic or dizoic cysts, approximately $10 \times 6 \mu\text{m}$ in size, were also commonly seen (Fig. 4).

The prevalence of *Hepatozoon* meronts in different host species is shown in Table 1. Forms of *Hepatozoon* were prevalent in all three *Clethrionomys* species occurring in the study areas but infection was found only occasionally in *Microtus* species. *Hepatozoon*-infected mice were not found. In *C. glareolus*, no association between sex and age of the animal and occurrence of *Hepatozoon* was found. In that species, infections were observed in all seasons.

DISCUSSION

In the present study, the predilection site of *Hepatozoon* meronts was the lungs, and that of gametocytes was leukocytes, intriguingly especially eosinophilic granulocytes. We did not investigate blood smears; that would have revealed if gametocyte-laden eosinophilic granulocytes were in the general circulation. The tissue tropism varies greatly between animal species, also within mammals (Yanai et al. 1995, Smith 1996, Panciera et al. 2000). Only minimal tissue reactions were detected, and no clinically ill rodents were observed, but in experimental infections, mortality due to *H. erhardovae* has been reported (Krampitz and Haberkorn 1988). Monozyotic or dizoic tissue cysts rather similar but sturdier ($10 \times 6 \mu\text{m}$ compared to $9 \times 2 \mu\text{m}$) than those observed in squirrels (Desser 1990) were commonly seen in lung alveoles of *C. glareolus*. In squirrels, all cysts were monozyotic, but in reptile species dizoic cysts have been reported (Landau et al. 1972).

Compared to host cells, developing meronts are large (Fig. 2). It is of interest that gametocytes appeared to be ultrastructurally intact (Fig. 3) inside the eosinophilic granulocytes, which are thought to act in the anti-parasitic defence. On the other hand, the eosinophiles appeared to have suffered. Pyknotic nuclei of granulocytes were also observed in Japanese martens in phagocytes containing merozoites (Yanai et al. 1995).

Results of this study indicate that *Hepatozoon* haemogregarines are more common in voles (Arvicolinae) than mice (Murinae). Furthermore, *Hepatozoon* infection is more prevalent in voles of the genus *Clethrionomys* than in voles of the genus *Microtus*. Šebek (1975) reported similar results from small mammals in Czechoslovakia. The difference between *Clethrionomys* and *Microtus* species does not appear to be associated with definitive flea hosts; our own observations (H. Henttonen, unpublished) support those by Savitskii and Kulnazarov (1988), who found no *Microtus oeconomus*-specific fleas or other ectoparasites, but fleas were shared with other vole species, including bank voles.

Seasonal (Wiger 1979, Turner 1986) and annual (Healing 1981) differences in the occurrence of blood forms of *Hepatozoon* have previously been reported from rodents in northern and Central Europe. In the present study, lung meronts were common in *C. glareolus* in all seasons (Table 1). A possible explanation to this difference is that the blood forms are associated to the transmission to definitive arthropod hosts, and therefore only are present when blood-sucking arthropods are active, but meronts represent a more slowly reacting stage of the parasite. In *M. agrestis*, most infected individuals were found during the autumn. However, the prevalence was low even when highest.

Fig. 1. *Hepatozoon erhardovae*-infected lung of *Clethrionomys glareolus*. Meront in alveolar septum. Parasitophorous vacuole surrounds the meront inside the host cell. Histological section, H&E. **Figs. 2-4.** Life-cycle stages of *Hepatozoon erhardovae* in pulmonary tissue of *Clethrionomys glareolus*. Transmission electron micrographs. **Fig. 2.** Meront. Numerous merozoites (M) and amylopectin vacuoles (A) are inside electron-lucent parasitophorous vacuole (PV); the nucleus of host cell (HN) is compressed to periphery of the cell. **Fig. 3.** Gametocyte inside the circulating eosinophilic granulocyte. Note typical eosinophilic granules with electron-dense crystals. Nucleus of the parasite (N). **Fig. 4.** Monozytic cyst form. Nucleus (N), amylopectin (A). Scale bars: Fig. 1 = 5 µm; Fig. 2 = 2 µm; Figs. 3, 4 = 1 µm.

Table 1. Lung meronts of *Hepatozoon* sp./spp. in voles of the genus *Clethrionomys* and *Microtus* and in mice of the genus *Apodemus* from Finland and some other locations in northern Europe. + – number of infected; n – number of examined; % – prevalence.

Host species	Locality	Coordinates	Month	Year	+ / n	%
<i>Clethrionomys rufocanus</i> (Sundevall)	Kilpisjärvi	69°03'N, 20°49'E	September	1989	7 / 30	23
<i>Clethrionomys rutilus</i> (Pallas)	Eastern Karelia, Russia	62°N, 34°E	August	1996	2 / 3	67
<i>Clethrionomys glareolus</i> (Schreber)	Pallasjärvi	68°03'N, 24°09'E	June	1988	20 / 45	44
			September	1988	14 / 41	34
			November	1988	23 / 47	49
			February	1989	24 / 44	55
			June–Dec	1988	2 / 22	18
	Heinola Eastern Karelia, Russia Saaremaa, Estonia	61°14'N, 26°02'E	August	1996	18 / 43	42
			November	1997	16 / 28	57
			Total		117 / 270	43
<i>Microtus agrestis</i> (Linnaeus)	Heinola Evo	61°10'N, 25°03'E	June	1988	1 / 39	3
			September	1989	0 / 13	-
			November	1989	1 / 46	2
			February	1990	0 / 18	-
			March	1990	0 / 24	-
	Luhanka	61°48'N, 25°45'E	September	1992	1 / 51	2
			November	1992	2 / 50	4
			January	1993	2 / 49	4
			September	1991	3 / 45	7
			November	1991	0 / 50	-
			January	1992	0 / 49	-
			March	1992	0 / 44	-
			September	1993	0 / 24	-
			November	1993	1 / 36	3
			November	1994	0 / 96	-
	Eastern Karelia, Russia Saaremaa, Estonia		August	1996	1 / 6	17
			November	1997	0 / 7	-
			Total		11 / 647	2
<i>Microtus arvalis</i> (Pallas)	Saaremaa, Estonia		October	1996	0 / 69	-
<i>Apodemus agrarius</i> (Pallas)	Saaremaa, Estonia		October	1996	0 / 111	-
			November	1997	0 / 63	-
<i>Apodemus flavicollis</i> (Melchior)	Saaremaa, Estonia		October	1996	0 / 7	-
			November	1997	0 / 45	-

Only one haemogregarine species, *Hepatozoon erhardovae*, has commonly been described from *C. glareolus*. Other species names have been occasionally used (see Šebek 1975) but while *C. glareolus* is the specific host for *H. erhardovae* other, non-specific host mammals may be infected with it sometimes (Frank 1977). Molecular studies on the intermediate host-

species specificity of *Hepatozoon* spp. in rodents are warranted.

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