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High prevalence of *Pneumocystis carinii* in *Sorex araneus* in Finland

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I determined the prevalence of *Pneumocystis carinii* in *Sorex araneus* ($N = 228$) and *Sorex minutus* ($N = 56$) at several locations in Finland, and in *Sorex caecutiens* ($N = 50$) in northern part of the country. The overall prevalence of this fungal pulmonary pathogen in *Sorex araneus* was 45% in southern Finland, 42% in the eastern, 58% in the western and 36% in the northern part of the country. The prevalence was high in every sex and age group, in all study areas, which represented different habitat types, in all seasons, and both in low and high host density populations. No clinically ill shrews were found. The prevalence of *P. carinii* was only 6% in a high density population of *S. caecutiens*. *Pneumocystis carinii* cysts were not detected in any of the *S. minutus* examined. The interspecific differences in the prevalence of *P. carinii* in shrews of the genus *Sorex* may be due to the species specific genotypic variation in the organism or due to differences in the biological characters (e.g. abundance, starvation time) of the host species. Analyses of faecal and histological samples of *S. araneus* revealed no sign of *Cryptosporidium* indicating that the high prevalence of *P. carinii* in *S. araneus* is not due to any general susceptibility to opportunistic parasites which usually are common only in immunocompromised hosts.

1. Introduction

Pneumocystis carinii is an opportunistic pulmonary pathogen, which is thought to be widespread in the environment. This fungal organism causes pneumonia in immunocompromised hosts, including humans, and the infection can be induced experimentally in laboratory animals by administration of immunosuppressive drugs (Boylan & Current 1992). The transmission is known to be air borne (Hughes 1982) but the infective form has not yet been identified. Two distinct developmental stages occur in the mammalian alveolus; trophozoite, which is the proliferating form of *P. carinii*, and cyst. It was originally thought that there was an animal reservoir of the human infection, but an increasing number of recent reports (Gigliotti 1992; Peters et al. 1994a) has shown antigenetic and genetic diversity among the organisms infecting different mammals and it is likely that each host species is infected with one (or more than one) distinct species of *P. carinii*.

Studies done with rodents and shrews show notable interspecific differences in the prevalence of *P. carinii* between small mammal species (Laakkonen et al. 1993; Laakkonen et al. 1995).Only one arvicoline species, *Microtus agrestis*, is known to be commonly infected with *P. carinii* in Finland (Laakkonen et al. 1995). The average prevalence in this host species varies from 6% to 17%, and the prevalence shows not only seasonal but also geographical variation (Laakkonen et al. 1995, Laakkonen et al., unpublished). In contrast, the highest prevalence of *P. carinii* so far reported in any wild animal population was 70% in common shrews, *Sorex araneus*, in northern Finland (Laakkonen et al. 1993). The fact that this prevalence value is exceptionally high compared to those found in other small mammals in Finland (Laakkonen et al. 1995), and to those reported in wild mammals in general (Settnes & Lodal 1980, Shiota et al. 1986), raises the question of whether the high prevalence in northern Finland is an isolated incident or whether high prevalence is a common character in the host-parasite relationship of *Sorex araneus* and *P. carinii*. To answer this question, I studied the geographical and spatial variation in the prevalence of *P. carinii* in *S. araneus* in Finland. I also aimed to determine whether interspecific differences in the prevalence of *P. carinii* exist between sympatric *Sorex araneus* and *Sorex minutus* similar to that seen in *Sorex araneus* and *Sorex caecutiens* in northern Finland (Laakkonen at al. 1993). Finally, I studied whether cryptosporidia, widespread coccidian protozoa causing diarrhoeal disease in various species of mammals (O'Donoghue 1995), occur in *S. araneus* in Finland. Due to their exceptionally high metabolic rate (Hanski 1984), *Sorex* shrews are likely to suffer from nutritional deficiencies and impaired immunity, and are thus especially susceptible to various opportunistic parasites. Since *S. araneus* is exceptionally susceptible to *P. carinii* compared to other small mammals (Laakkonen et al. 1993, Laakkonen et al.1995), I wanted to find out whether this shrew species is generally more susceptible to opportunistic parasites, which usually are common only in immunocompromised hosts, than other small mammals. Cryptosporidia were chosen for this investigation since they primarily infect immunocompromised, especially young hosts, and are known to be rare in subadult and adult arvicolines in Finland (Laakkonen et al. 1994). Also, they usually are enteric rather than pulmonary and are not phylogenetically related to *P. carinii*.

2. Materials and methods

The material was collected at the following five locations and consisted of:

a) 28 *Sorex araneus*, and 2 *Sorex minutus* trapped at Helsinki (60°10′N, 24°58′E), in southern Finland from June to September 1991. Shrews were trapped in a small grove dominated by deciduous trees, and surrounded by a small road and a river.

b) 67 *S. araneus* and 24 *S. minutus* collected at Espoo (60°14′N, 24°46′E) in southern Finland during several trapping periods from July 1992 to September 1993. The study area was located in a small forest dominated by spruce, *Picea abies* and blueberry, *Vaccinium myrtillus*, and surrounded by old fields. The distance between the two southern study areas (a and b) is about 10 km.

c) 45 *S. araneus* and 20 *S. minutus* trapped at Ilomantsi (65°25′N, 25°36′E) in the eastern part of central Finland in September 1993. The trapping was done in old forest patches dominated by pine, *Pinus sylvestris*, and surrounded by bogs and clear-cuts.

d) 24 *S. araneus* were collected at Virrat (62°14′N, 23°47′E) in the western part of central Finland in July 1994. Shrews were trapped in a small old fields and in a nearby forest edge. No *S. minutus* specimens were caught in this study area.

e) 64 *S. araneus*, 50 *S. caecutiens* and 10 *S. minutus* trapped at Pallasjärvi (68°03′N, 24°09′E) northern Finland in September 1994. This study area, which was used also in the earlier study (Laakkonen et al. 1993), was located in old taiga forest including also a few old fields. Compared to the shrew densities during the earlier study (28 *S. araneus* and 21 *S. caecutiens* caught in September, average of the 3-year study, 1988–1990), the shrew densities were high in 1994 (72 *S. araneus* and 49 *S. caecutiens* caught in September). The shrew densities at Helsinki and Virrat were low compared to the other three study areas.

Shrews were caught with snap-traps baited with cheese and checked with 8 hr intervals. At Ilomantsi, both pitfalls and snap-traps were used. At Pallasjärvi, shrews were found dead in livetraps used for monitoring vole populations (see Laakkonen et al. 1993). Shrews examined histologically for cryptosporidia (see below) were live-trapped (Ugglan Special) and checked every four hours. Shrews were classified as adults (overwintered) and juveniles according to the tooth wear and condition of the pelage (Crowcroft 1957).

Pneumocystis carinii. Histological samples of the right cranial and caudal lung lobes of each shrew were prepared

according to the standard methods and stained with Methenamine silver (for details see Laakkonen et al. 1993).

Samples were rated as follows: 1) no cysts found, 2) several individual cysts or a cluster of cysts per section found, 3) cysts were found throughout the sections. Differences in prevalence between years at Espoo were analyzed with Pearson's Chi-square test (Sokal & Rohlf 1981), and log-linear model analysis (Fienberg 1970) was used to determine the dependence between sex (S) and age (A) of *S. araneus*, and the occurrence of *P. carinii* (P) (model accepted if $P > 0.05$).

Cryptosporidium. Faecal samples of 56 ($N = 20$ in 1991 at Helsinki; $N = 8$ in 1992 and $N = 13$ in 1993 at Espoo; $N = 15$ in 1994 at Virrat) *S. araneus* were obtained from the colon and rectum to make faecal smears, which were stained with Ziehl-Neelsen method (for details see Laakkonen et al. 1994). Since none of the commonly used diagnostic methods is able to detect all specimens positive for *Cryptosporidium* (MacPherson & McQueen 1993), histological sections of the small intestine were prepared to study in detail the most susceptible host cohort (young shrews, $N = 18$), as well as three adult males and one adult pregnant female. These shrews were live-trapped at Virrat (2 of the 24 died in traps), euthanized with ether, and one 2 cm piece of the distal part of the small intestine and cranial part of colon from each sample were fixed in 10% buffered formalin, cut into 1 mm segments and embedded in paraffin. Two 5 µm sections were placed on one slide which then was stained with haematoxylin and eosin. Cryptosporidia samples were examined by microscope at ×1000.

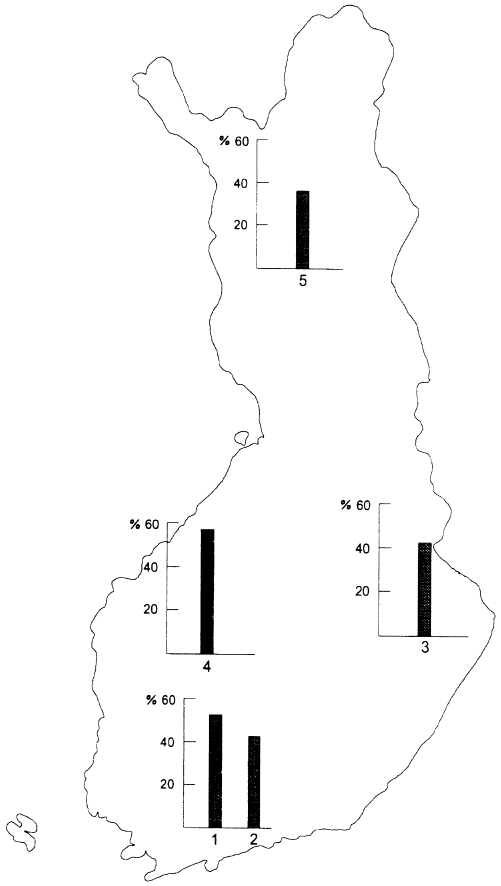

Fig. 1. The prevalence of *P. carinii* in *S. araneus* in southern (1 = Helsinki, 1991; 2 = Espoo, 1992–1993), eastern (3 = Ilomantsi, 1993), western (4 = Virrat, 1994) and northern (5 = Pallasjärvi, 1994) Finland.

3. Results

The prevalence of *P. carinii* in *S. araneus* was 45% in southern Finland (study areas 1 and 2 combined), 42% in the eastern, 58% in the western and 36% in the northern part of the country (Fig.1). The prevalences did not differ significantly between the study areas (not shown). At Espoo, there was no significant difference in prevalence between years when the sex and age groups were pooled ($P = 0.31$). In the pooled data, the prevalence in winter and spring (from January to May) was 43% (6/14), in summer (from June to August) 48% (40/84), and in autumn (from September to November) 41% (53/130). The number of *P. carinii* infected *S. araneus* in different sexes and age groups is compared in Table 1 (pooled data). According to the best log-linear model (S, AP; interaction indicated by combined variables) the occurrence of *P. carinii* (P) depended on the age (A) of the host ($P = 0.09$, $df = 3$, $\chi^2 = 6.41$), adults shrews being more often infected than juvenile ones. None of the models, however, fitted well with the data.

Pneumocystis carinii cysts were found in 3 of the 50 (6%) *S. caecutiens* examined from northern Finland but none were found in *S. minutus* ($N = 56$) in southern, central or northern Finland.

No *Cryptosporidium* spp. oocysts were detected in the 56 faecal samples examined; 35 of the examined shrews were juveniles. The histological examination of the ileum and colon of 22 of the 56 shrews did not reveal any sign of cryptosporidia. Variable degree of stunting of the villi, probably because of rapid self-diffusion, was found frequently.

4. Discussion

The most interesting result of this study was the total absence of *P. carinii* cysts in *S. minutus*. Also the prevalence of *P. carinii* in *S. caecutiens* was surprisingly low (6%) compared to that found in the earlier study (mean 17%; Laakkonen et al. 1993). In contrast, the prevalence in S. araneus, although lower than in the earlier study (see above), was high in all areas studied. The interspecific difference in the prevalence of *P. carinii* between *Sorex* shrews may be due to host species specific genotypic variation in the organism. Although it is not possible to distinguish *P. carinii* organisms originating from different *Sorex* species, *P. carinii* of *S. araneus* is known to be genetically distinct from isolates of other hosts (e.g. rat, mouse, rabbit etc., Peters et al. 1994b). It is not yet known, however, whether *P. carinii* of *S. araneus* also differs genetically from the one found in *S. caecutiens*. Even though RNA analyses have shown that one host species can harbour genetically different *P. carinii* strains (Lu et al. 1994), the results of the studies done with arvicolines (Laakkonen et al. 1995), and those of this study indicate that cross-species transmission does not occur easily even in sympatric species.

Even if *P. carinii* isolates from different *Sorex* species were genetically distinct, the differences in abundance (Haukisalmi 1989) or starvation time (which is related to the size of the shrew; Hanski 1984) between *Sorex* species may also contribute to the interspecific differences in prevalence of *P. carinii* in these host species. Furthermore, behavioral differences of the hosts may affect the transmission efficiency of this airborne parasite. *S. araneus*, for example, stays underground for a considerable time in autumn and winter while *S. minutus* remains epigeal at least half the time (Croin Michielsen 1966).

The prevalence of *P. carinii* in *S. araneus* was similar in all study areas (representing different habitat types) in southern and central Finland. Prevalence of *P. carinii* was high also in *S. araneus* caught at Helsinki and at Virrat although population densities of *S. araneus* were very low in those areas during the study. Surprisingly, at Pallasjärvi where *Sorex* densities were very high during the study, the prevalence was lower (but still as high as 36%) than those found in the other study areas. In contrast, during the earlier study (Laakkonen et al. 1993), *Sorex* densities were low but the prevalence of *P. carinii* in *S. araneus* remained very high (> 60%) during the three year study.

Nutritional stress and impaired immunity can be expected to occur especially in high density populations (see however, Lochmiller et al. 1994), which also favour the transmission of infectious agents. However, no heavy infections similar to those seen in rats were detected in this study in any sites during low or high population densities. Since no clear relationship was found between population density of the host and prevalence of the parasite, it is possible that the annual variation in prevalence may depend mainly on factors affecting the parasite rather than those affecting the host. Unfortunately the life-cycle of *P. carinii* outside the mammalian lung is not complete known, and the existence of a possible environmental form remains to be solved. I conclude that mild, probably non-pathogenic *P. carinii* infections are common in all sex and age groups of *S. araneus*, in all seasons throughout the country.

In this study I did not find any sign of cryptosporidia in *S. araneus* either in faecal or histological samples. However, in a study done in northern Poland (Siński et al. 1993) 5 of the 16 *S. araneus* examined were naturally infected with

Table 1. Comparison of the number of *P. carinii* infected *S. araneus* in different sexes and age groups (pooled data). The number in parentheses indicates the number of the more heavily infected shrews (category 3, see text).

	Number examined	PC positive	Percent positive
Males			
Adult	38	24 (8)	63%
Juvenile	83	37 (10)	45%
Male total	121	61 (18)	50%
Females			
Adult	24	12 (1)	50%
Juvenile	83	26 (6)	31%
Female total	107	38 (7)	36%
Grand total	228	99 (25)	43%

Cryptosporidium parvum, and the prevalence was higher (31.2) than those found in other small mammals (from 0% to 20%).Based on these results, it is impossible to say whether the constant high prevalence of *P. carinii* in *S. araneus* is a character of this particular host-parasite relationship rather than indication of general susceptibility of *S. araneus* to opportunistic parasites which usually are common only in immunocompromised hosts.

Clearly, however, *S. araneus* is much more susceptible to many different kinds of parasites than most other small mammals.

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