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PNEUMOCYSTIS CARINII AND HELMINTH PARASITISM IN SHREWS *SOREX ARANEUS* AND *SOREX CAECUTIENS*

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ABSTRACT: The prevalence of *Pneumocystis carinii* was compared in two species of shrews, *Sorex araneus* and *Sorex caecutiens* in Finnish Lapland. The overall prevalence of *P. carinii* in *S. araneus* was 70% and in *S. caecutiens* was 17%. The prevalence for *S. araneus* is the highest reported for wild animals. The interspecific differences in prevalence were significant for all sex and age groups except for adult males. Based on multiway contingency tables (log-linear models), there was no dependence between sex or age of *S. araneus* and occurrence of *P. carinii*. In individual *S. araneus* the intensity of *P. carinii* was not related to the total number of helminths or the number of helminth species, and no dependence was observed between the presence of *P. carinii* and various helminth species.

Key words: *Pneumocystis carinii*, *Sorex araneus*, *Sorex caecutiens*, prevalence, helminths.

INTRODUCTION

Pneumocystis carinii is an opportunistic pulmonary pathogen first seen in guinea pigs (*Cavia porcellus*) experimentally infected with *Trypanosoma cruzi* (Chagas, 1909). Since then *Pneumocystis carinii* has been found in many species of mammals, especially rodents (Settnes and Lodal, 1980; Shimizu et al., 1985; Settnes et al., 1986; Shiota et al., 1986). However, extensive investigations into the prevalence of *P. carinii* in natural populations are rare (Poelma, 1972; Settnes and Lodal, 1980; Shiota et al., 1986).

In rats, experimental immunosuppression with corticosteroids elicits *P. carinii* pneumonia in 6 to 8 wk (Walzer et al., 1988). *Pneumocystis carinii* infection has been provoked in rats also by dietary protein deprivation alone (Hughes et al., 1974).

Sorex shrews are assumed to suffer from energy constraints leading to a high risk of starvation (Vogel, 1980; Hanski, 1984). An additional nutritional shortage could be caused by gastrointestinal parasites which may decrease the efficiency of absorption and digestion of food (Munger and Karasov, 1989). Thus high intensities of gastrointestinal parasites may be associated with high prevalences of organisms like *P. carinii*, which are manifested when the host falls into a immunosuppressive condition. This most likely would happen during high host population density.

In this study we compared the prevalence of *P. carinii* in different sexes and age groups of two syntopic species of shrews, *Sorex araneus* and *Sorex caecutiens*, in western Finnish Lapland. To determine whether *P. carinii* infection had any interaction with gastrointestinal parasites, we compared the prevalence and intensity of *P. carinii* in individual shrews to the intensity of helminths and number of helminth species.

MATERIALS AND METHODS

The *P. carinii* information was selected from a larger data set originally collected for a study on gastrointestinal parasites of shrews (Haukisalmi et al., 1992), and consisted of 63 common shrews (*Sorex araneus*) and 60 masked shrews (*Sorex caecutiens*), collected from April 1988 to June 1990 at Pallasjärvi (68°03′N, 24°09′E), northern Finland. Most of the material (*S. araneus*, 71%; *S. caecutiens*, 60%) was obtained between June and October. Shrews were found dead in livetraps used for monitoring vole populations. Traps were checked at 6- to 8-hr intervals. Despite the potentially long postmortem interval, shrews were not decomposed because of the low temperatures in northern Finland during the summer. The trapping was done in old taiga forests characterized by a thick moss layer and dominance of spruce (*Picea abies*) and blueberry (*Vaccinium myrtillus*). A more de-

TABLE 1. Interspecific comparison of number of *Pneumocystis carinii* (PC) positive[a] shrews in different sexes and age groups in *Sorex araneus* and *S. caecutiens*.

	S. araneus			*S. caecutiens*			
	Number examined	PC-positive	Percent positive	Number examined	PC-positive	Percent positive	*P* value
Males							
Adult	19	11	58	12	5	42	0.3785
Juvenile	20	15	75	24	4	17	<0.001
Male total	39	26	67	36	9	25	0.0003
Females							
Adult	6	5	83	8	0	0	0.003
Juvenile	18	13	72	16	1	6	<0.001
Female total	24	18	75	24	1	4	<0.001
Grand total	63	44	70	60	10	17	<0.001

[a] The maximum number of cysts seen in one sample varied from 25 to 30.

tailed description of the study area is given by Henttonen et al. (1987).

After trapping, shrews were frozen (−20 C) until dissection. After thawing, shrews were classified as adults and juveniles according to tooth wear and condition of the pelage. Lung samples were fixed in 10% neutral buffered formalin. Fixed tissues were dehydrated, embedded in paraffin and sectioned at 5 μm. For each sample, one section each was cut from the middle part of the right cranial and caudal lobes. Both sections from one animal were placed on one slide which then was stained with Grocott's modification of Gomori's Methenamine Silver (GMS) stain (Grocott, 1955). The slides were examined by microscope at ×200 and ×400. Samples with *P. carinii* cysts were rated as follows: 1 = one cyst/slide, 2 = 2 to 10 cysts/slide, 3 = >10 cysts/slide. Identification of *P. carinii* was based on typical morphological structure and staining characteristics with GMS staining (Hopkin, 1991). The occurrence of *P. carinii* in shrews was characterized by prevalence (percent of shrews infected) and intensity. Intensity was defined as the number of cysts per slide. Interspecific differences in prevalence in different sex and age groups were analyzed with Pearson's Chi-square (χ^2) test (if the sample size was large enough) or with Fisher's exact test (Sokal and Rohlf, 1981). Since we found no dependence between the sex of *S. araneus* and occurrence of *P. carinii*, data on sex were combined. Multiway contingency tables (log-linear models) (Fienberg, 1970) were used to further analyze dependence between sex and age of the shrews and occurrence of *P. carinii*.

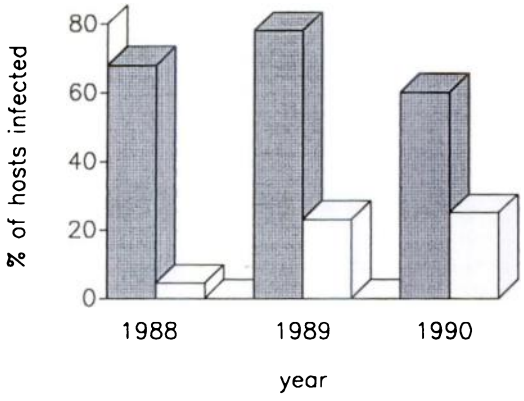

FIGURE 1. Prevalence of *Pneumocystis carinii* in shrews *Sorex araneus* (stippled bars) and *Sorex caecutiens* (empty bars), 1988 to 1990.

Because the gastrointestinal parasites were not normally distributed in the shrews (Haukisalmi et al., 1992), the relation of intensity of *P. carinii* to total number of gastrointestinal parasites and to the number of gastrointestinal parasites species was evaluated using the Kruskal-Wallis test (Sokal and Rohlf, 1981). Log-linear model analyses were used to determined the dependence between the occurrence of individual helminth species (H), *P. carinii* (P) and age of the shrew (A). The simplest model that fit the observed data was selected for each parasite species (χ^2-test, $P > 0.05$; see Fienberg, 1970). A higher-order interaction term in the model also included all lower-order interactions and interactive terms (H, P, A).

RESULTS

We found *Pneumocystis carinii* in 44 (70%) of 63 *Sorex araneus* and in 190 (17%) of 60 *S. caecutiens*. The interspecific dif-

TABLE 2. Best log-linear models for dependence of age (A) of *S. araneus*, occurrence of *Pneumocystis carinii* (P) and of various helminth species (H).

Helminth species	Variables included in the model	df	Chi-square value	*P*
Cestoda				
Choanotaenia crassiscolex	AP, AH[a]	2	0.8	0.66
Hymenolepis schaldybini	A, P, H	4	4.5	0.34
H. singularis	A, P, H	4	3.4	0.50
H. scutigera	A, P, H	4	3.3	0.50
H. diaphana	AP, AH	2	1.6	0.45
H. infirma	AH, PH	2	2.1	0.36
Nematoda				
Longistriata depressa	A, P, H	4	1.6	0.81
L. pseudodidas	A, P, H	4	1.3	0.85
Parastrongyloides winchesi	A, P, H	4	1.5	0.82

[a] Independence of variables is indicated by separating the symbols with a comma (e.g., A, P, H), and interactions by a lack of comma (e.g., AH = AGE-HELMINTH species interaction).

ference in prevalence was significant in every sex and age group of shrews except for adult males (Table 1). In *S. araneus* prevalence was constantly high (Fig. 1), and there were no significant differences between years when the sex and age groups were pooled. In *S. caecutiens*, prevalence was remarkably lower in 1988 than in the following two years (Fig. 1).

According to the best log-linear model there was no dependence between the sex and age of *S. araneus* and occurrence of *P. carinii* when we used a model assuming no interactions. In *S. caecutiens*, no corresponding analysis was done due to the small number of infected shrews.

Neither the intensity of helminths nor the number of helminth species showed a significant relationship to the intensity of *P. carinii* in different sexes or age groups. Based on the best log-linear models, occurrence of *P. carinii* did not depend on any of the helminth species with the exception of *H. infirma*, which had a negative dependence on *P. carinii* (Table 2).

DISCUSSION

The prevalence of *P. carinii* in *S. araneus* at Pallasjärvi (70%) was surprisingly high. To our knowledge, this is the highest prevalence reported for wild mammals. Sebek and Rosicky (1967) reported pneumocysts in 16% of 25 *S. araneus* in Czechoslovakia. The prevalence in *S. caecutiens* at Pallasjärvi (17%) was similar to findings for other species of mammals (Settnes and Lodal, 1980; Settnes et al., 1986; Shiota et al., 1986). Although the shrew densities at Pallasjärvi were lower in 1989 than during the other years studied, no significant difference in prevalence of *P. carinii* in *S. araenus* was observed between years. In *S. caecutiens*, prevalence was remarkably lower in 1988 than in the following two years. However, statistical comparisons were not done because significant differences were difficult to detect due to the low overall prevalence of *P. carinii* in *S. caecutiens*, and relatively low sample size in each year. Thus only *E. araneus* was used in the analyses involving gastrointestinal parasites.

The interspecific difference of *P. carinii* was similar to differences in helminth intensities of these shrew species (Haukisalmi et al., 1992). However, among individual hosts, *P. carinii* was not related to any of the helminth infection parameters studied. Haukisalmi et al. (1992) recently have shown that gastrointestinal helminths do not have detectable effects on the condition of the shrews. Thus high numbers of helminths do not indicate poor condition which is prerequisite for opportunistic in-

fection like *P. carinii* to become manifested.

Hanski (1989) and Price et al. (1988) suggested that parasites might promote the coexistence of competing shrew species. Our data and those of Haukisalmi et al. (1992) support the idea that coexistence of competing species of shrews is promoted by the larger and more abundant species (*S. araneus*) being more severely infected by parasites. The higher susceptibility of *S. araneus* to parasites including *P. carinii* could be due to its larger size, more generalized diet or higher abundance (Haukisalmi, 1989), compared to *S. caecutiens*.

Clinical *P. carinii* infection previously was found to be most common in animals with insufficient immunological defenses, namely young animals and possibly the very old ones (Poelma, 1972; Richter et al., 1978; Matsumoto et al., 1987; Soulez et al., 1989). In contrast, we detected that infection was equally common in all age groups of *S. araneus*. The most probable reason for this is that, despite the high prevalence, infections remained subclinical. The number of cysts was much smaller than seen in experimentally immunosuppressed rats with *P. carinii* pneumonia (Sukura et al., 1991). Rats or other laboratory animals have been used in most animal models describing the biology of *P. carinii*. We conclude that there is a need for comparisons of this pathogen in various host species before generalizations are made.

The prevalence and intensity of *P. carinii* in shrews differed from our findings in voles (Sukura et al., 1992). In voles, we usually found only a few cysts on a single slide, and the overall prevalence was low. In the present study we often found shrews to be more heavily infected than voles. Although histologic techniques correlate well with quantitative ones (Kim et al., 1987), differences in prevalence and density of *P. carinii* between voles and shrews should be evaluated by digesting the lungs and counting the total number of cysts. Thus we might gain more accurate information concerning the infections of low intensity, and be able to compare infections of wild animals to those seen in immunosuppressed rats.

Based on our results, we believe that the high prevalence and intensity of *P. carinii* in *S. araneus* are signs of good adaptation of the host to its parasite. Sebek and Rosicky (1967, 1968) have pointed out the association of *P. carinii* with evolutionarily ancient mammals such as soricid shrews (George, 1986; Reumer, 1989) and voles of the genus *Pitymys*. *Pneumocystis carinii* may have originally evolved in association with species suffering from immunodeficiency, probably due to energy constraints.

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