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Source: Journal of Wildlife Diseases, 37(2) : 408

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-37.2.408

Journal of Wildlife Diseases, 37(2), 2001, pp. 408–412

Pneumocystosis in Wild Small Mammals from California

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ABSTRACT: Cyst forms of the opportunistic fungal parasite *Pneumocystis carinii* were found in the lungs of 34% of the desert shrew, *Notiosorex crawfordi* ($n = 59$), 13% of the ornate shrew, *Sorex ornatus* ($n = 55$), 6% of the dusky-footed wood rat, *Neotoma fuscipes* ($n = 16$), 2.5% of the California meadow vole, *Microtus californicus* ($n = 40$), and 50% of the California pocket mouse, *Chaetodipus californicus* ($n = 2$) caught from southern California between February 1998 and February 2000. Cysts were not found in any of the harvest mouse, *Reithrodontomys megalotis* ($n = 21$), California mouse, *Peromyscus californicus* ($n = 20$), brush mouse, *Peromyscus boylii* ($n = 7$) or deer mouse, *Peromyscus maniculatus* ($n = 4$) examined. All infections were mild; extrapulmonary infections were not observed. Other lung parasites detected were *Hepatozoon* sp./spp. from *M. californicus* and *Notiosorex crawfordi, Chrysosporium* sp. (Emmonsia) from *M. californicus,* and a nematode from *S. ornatus.*

Key words: Lung parasites, pneumocystosis, rodents, shrews.

Pneumocystis carinii is an opportunistic pulmonary pathogen capable of causing fatal pneumonia in immunocompromised hosts. The antigenic and genetic diversity observed among organisms infecting different hosts (Gigliotti et al., 1993; Wakefield et al., 1992) suggests that each species may be infected with one or more distinct strains (or species) of *P. carinii.* The transmission of this fungus is known to be airborne (Hughes, 1982), but the infective form has not yet been identified. Results of a recent study (Kaneshiro and Maiorano, 1996) indicate that the fungus has a dormant form that remains infective outside the mammalian host for at least several months. In Europe, *P. carinii* has been reported to be more common in shrews than in sympatric rodents (Laakkonen, 1998) but in a previous survey of shrews from North America *P. carinii* was not detected in any of the three shrew species examined from Pennsylvania (USA) (Laakkonen et al., 1997). Although characterization of *P. carinii* infection in wild animal populations may help to elucidate the life cycle and transmission of this elusive organism, information on the occurrence and nature of the infection in wild mammals is still scarce, especially from Africa, Australia and North America (Laakkonen, 1998). The purpose of this study was to investigate the occurrence of *P. carinii* cysts in small mammals in southern California (USA).

The animals were caught with arrays of 20 l plastic buckets used to capture reptiles for a long-term reptile monitoring project (Fisher and Case, 2000; Case and Fisher, 2000) in several localities (between 32°55′N, 117°13′W and 33°86′N, 117°19′W) in San Diego and Riverside counties (California) during several trapping periods between February and May in 1998 and 1999, and in February 2000. The main habitat types of the study areas are coastal sage scrub and chaparral (Fisher and Case, 2000; Case and Fisher, 2000). After recording the species, sex and age of the animals, live animals were released, and those found dead were frozen until necropsy in spring 2000 (for species and number of animals examined see Table 1). About 50% of shrews caught died in traps but more than 90% of rodents trapped were released alive. All species caught were also represented in the material examined for this study. No animals were sacrificed for this study because many of the mammal species caught are listed as an endangered or threatened in California. Since many more shrews died in pitfalls than rodents, a few animals found dead in Sherman traps of a separate rodent monitoring study in the same areas were analyzed in this study in an effort to

TABLE 1. Occurrence of *Pneumocystis carinii* in trapped wild small mammals in southern California from 1998 to 2000 (Pooled data from all study areas arranged according to the subfamily of the host species).

Host species		Number infected	Number examined	%	*P* versus *Notiosorex*
Soricinae					
Ornate shrew	*Sorex ornatus*	7	55	13	0.008
Desert shrew	*Notiosorex crawfordi*	20	59	34	
Arvicolinae					
California meadow vole	*Microtus californicus*	1	40	2.5	<0.001
Sigmodoninae					
Harvest mouse	*Reithrodontomys megalotis*	0	21	0	0.002
Dusky-footed wood rat	*Neotoma fuscipes*	1	16	6	0.03
California mouse	*Peromyscus californicus*	0	20	0	0.003
Brush mouse	*Peromyscus boylii*	0	7	0	NT[a]
Deer mouse	*Peromyscus maniculatus*	0	4	0	NT[a]
Perognathinae					
California pocket mouse	*Chaetodipus californicus*	1	2	50	NT[a]

[a] NT = not tested due to the small sample size.

increase the sample size of rodents. On necropsy pieces of lung, heart, liver, spleen and kidney were fixed in 10% buffered formalin to produce standard histological sections which were stained with hematoxylin-eosin (H&E) and Grocott's modification of Gomori's methenamine silver (GMS; Grocott, 1955). The slides were examined by a light microscope at 400× and 1000×. The size of the parasites found was measured with an ocular micrometer. The intensity of infection was measured as cysts found per lung sections (for details see Laakkonen and Soveri, 1995). Chi-square tests were used to analyze the interspecific differences in parasite occurrence, and differences between age and sex groups. Statistix® for Windows statistical software package (Analytical Software, Tallahassee, Florida, USA) was used in all analyses.

All animals examined were normal on gross pathologic examination. Cyst forms of *P. carinii* were found only in the lungs of the infected hosts. The cysts were of an oval form with a "parenthesis-like" thickening of the cyst wall similar to those seen in other wild mammal species (Laakkonen and Sukura, 1997). The diameter of the cysts varied between 3.5 and 3.9 μm ($\bar{X} \pm SE = 3.7 \pm 0.03$ μm). No histopathological changes were seen around the cysts. A GMS-stained lung section sample was deposited in the U.S. National Parasite Collection (Beltsville, Maryland, USA; USNPC No. 90033).

The percentage of individuals of each species found dead in traps that had cysts of *P. carinii* in their lungs is shown in Table 1 (Pooled data from all study areas). The percentage positive was significantly higher in *Notiosorex crawfordi* than in the other small mammals examined (Table 1). None of the other interspecific differences in the percentage of infected animals were significant (not shown). There was no difference in the percentage positive between study areas, or between sexes and age groups in either shrew species (not shown).

Of the 20 infected *N. crawfordi,* six had more than 20, and three had over 50 cysts per lung section. Of the seven infected *Sorex ornatus,* four had more than 20 cysts per lung section. The rest of the infected shrews, and the three infected rodents had less than 20 cysts per lung section.

As in previous studies with Palearctic small mammal communities (Laakkonen, 1998; but see also Laakkonen et al., 1997) the prevalence of *P. carinii* was significantly higher in one shrew species compared to other sympatric shrew or rodent species. We consider it unlikely that the

sample in this study was biased due to mainly infected shrews dying overnight in traps. Shrews are well known to die easily in any kind of trap even without any apparent infection or disease and if such a bias existed, it would show up in the rodents as well. Furthermore, *P. carinii* cysts are commonly found also in live caught shrews but rarely found in live-trapped rodents elsewhere (Laakkonen, 1995, 1998).

The reasons for the similar interspecific differences observed in this and previous studies elsewhere are not known. The high metabolic rate of *Sorex* spp. shrews, which may be suspected to lead to energy constraints, has previously been suggested (Laakkonen, 1998) to be a possible factor contributing to the high prevalence of *P. carinii* in *Sorex araneus.* The metabolic rate of *N. crawfordii,* however, is lower than that of *S. ornatus* and other *Sorex* spp. shrews (Taylor, 1998), suggesting that factors other than the high metabolic rate, may be more important in contributing to the observed interspecific differences at least between different shrew species. A previous study indicates (Bishop et al., 1997) that *P. carinii* from one shrew species is genetically distinct from isolates of other shrew (and small mammal) species. Host specific differences in infectivity and pathogenicity between the isolates may be a reason for the observed interspecific differences in the prevalence of *P. carinii.* It is of interest that *P. carinii* seems to be more prevalent among shrews than rodents (Table 1, Shiota et al., 1986; Laakkonen, 1998) in all geographic areas studied so far despite significant differences in habitats of these areas. This would indicate that the occurrence and transmission of *P. carinii* is not related to any particular habitat.

The percentage of *P. carinii* infected shrews found in this study were similar to those (usually between 15 to 50%) found in Palearctic shrews (Laakkonen, 1998). Among shrews, the percentage of infected was higher in *N. crawfordi* than *S. ornatus.* The reason for this difference is not known.

Results of a previous study (Laakkonen et al., 1999) indicate that there is seasonal variation in *P. carinii* infections both in shrews and rodents. It was not possible to study possible seasonal variation in this study because very few animals were caught during the hot months during summer and fall.

The percentage of infected animals recorded using the histological diagnostic method are likely to be underestimates (Laakkonen and Sukura, 1997). We chose to use this method, however, because it allows accurate comparisons both in percentage infected and intensity of the infection between the previous studies done with the same method in different geographic areas (see Laakkonen, 1998). Also, several immunohistochemical stains differing in developmental-form specificity are commercially available, but due to the antigenic differences between *P. carinii* originating from various host species, commercial immunofluorescence kits may produce false-negative results (Laakkonen and Sukura, 1997).

The intensity of the infections was similar to those seen in previous studies in shrews (Laakkonen, 1998). None of the animals were heavily infected or apparently ill, and none of the species showed any extrapulmonary dissemination or histopathological changes typical for *P. carinii* pneumonia. It is not known from this or other studies, whether such mild *P. carinii* infections are harmful to their hosts. At least in voles, such infections do not easily develop into a clinical disease (Laakkonen et al., 1995). The present knowledge on the occurrence of different species/strains of *P. carinii* in various host species (Wakefield et al., 1992; Gigliotti et al., 1993) indicates that *P. carinii* is not a zoonosis.

Of the other lung parasites, schizonts of *Hepatozoon* sp. were detected in three *Microtus californicus* (Voucher specimen, USNPC No. 90030) and one *N. crawfordi, Chrysosporium* (*Emmonsia*) sp. in two *M.*

californicus (USNPC No. 90031), and a nematode from one *S. ornatus* (USNPC No. 90032). Schizonts of *Hepatozoon* spp. and adiaspores of *Chrysosporium* spp. are common findings in many small mammal species but have rarely been found in small mammals from the western part of the USA (Jellison, 1969; Smith, 1996). Lung nematodes of shrews from North America have previously been reported from Michigan (Ash, 1967) and Pennsylvania (Laakkonen et al., 1997).

We gratefully acknowledge C. Brown, E. Erwin, B. Haase, A. Backlin, and the many volunteers for the sampling, and many land owners and agencies for use of their properties for our study. We are particularly indebted to B. Collins, J. Opdycke, P. Stine, S. Weber and M. Wells. This work is supported by funding from the California Department of Fish and Game, Biological Resources Division of the USGS, MCB Camp Pendleton, Metropolitan Water District, California State Parks, United States Forest Service, Motte Rimrock Reserve, United States Fish and Wildlife Service, and the Research Council for Natural Resources and Environment of the Academy of Finland (project no. 43542). This work was carried out under the Californian Department of Fish and Game permit number 801133-01 for Ted Case and permit number 801048-05 for Juha Laakkonen, and UCSD Animal Subjects Committee approved protocol number S99045 for Ted Case and Juha Laakkonen.

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Received for publication 16 May 2000.