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LUNG PARASITES OF SHREWS FROM PENNSYLVANIA

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ABSTRACT: We examined lung parasites of three species of soricids, *Sorex cinereus* (n = 58), *Sorex fumeus* (n = 23) and *Blarina brevicauda* (n = 45) collected from Pennsylvania (USA), from 1990 to 1995. Yeast-like cells of *Histoplasma capsulatum* var. *capsulatum* were found in lung sections stained with Grocott's modification of Gomori's methenamine silver, periodic acid-Schiff, Giemsa, and hematoxylin-eosin in two (3%) *S. cinereus*, eight (35%) *S. fumeus* and two (4%) *B. brevicauda*. The number of spores of *H. capsulatum* in the lungs was low and no inflammatory reaction was evident. The infection was not disseminated to other organs. This is the first report of *H. capsulatum* infection in any species of shrews of the genus *Sorex* and the prevalence in *S. fumeus* was remarkably high compared to those reported for other wild mammals. A nematode, possibly *Angiostrongylus michiganensis*, was found in the lungs of one *S. fumeus* on necropsy and in a stained lung section of one *S. cinereus*. In both cases the host was also infected with the fungus. *Pneumocystis carinii*, which is the most common lung parasite in *Sorex araneus* (the numerically dominant Eurasian species of shrew), was not found in any of the North American species of shrew examined in this study.

Key words: *Angiostrongylus michiganensis*, *Blarina*, *Histoplasma capsulatum*, *Pneumocystis carinii*, *Sorex*.

INTRODUCTION

Shrews of the genus *Sorex* are widely distributed and abundant in most parts of the northern hemisphere. Local communities of *Sorex* spp. usually consist of many similar species differing mainly in size and abundance. In Europe and western Siberia, the numerically dominant species is the relatively large *Sorex araneus*, whereas the most widely distributed and abundant shrew in North America is a small species, *Sorex cinereus* (Hall, 1981).

Based on results of parasite studies of shrew communities in Finland (Haukisalmi, 1989; Laakkonen, 1995), host abundance or body size appear to affect the species diversity and abundance of endoparasites in shrews: *S. araneus* is more heavily infected by both macro- and microparasites than the smaller species *Sorex caecutiensis* and *Sorex minutus*. The most common lung parasite in *Sorex* spp. shrews, especially in *S. araneus*, is *Pneumocystis carinii* (Laakkonen, 1995).

In the present study we examined three species of shrews, *S. cinereus*, *B. brevicauda*, and *S. fumeus*, for lung parasites. This paper is a part of a larger, comparative study, designed to delineate which biological characteristics of soricid hosts determine the occurrence of parasites in species of *Sorex* by comparing shrew communities between Europe and North America. As in shrews in Eurasia, we expected the infection levels to vary according to the body size or abundance of the host, and we hypothesized that if infection levels were higher in *S. cinereus* than in other species of *Sorex*, host abundance was the key character determining the occurrence of the parasites in these hosts. The concentration of parasites in the larger host species, on the other hand, would support the view that size of the host is the most important factor influencing the composition of the parasite community.

MATERIAL AND METHODS

Animals were trapped on several locations at the Biological Station of the Carnegie Museum of Natural History (Powdermill Biological Station (40°10′N, 79°16′W), southeastern Westmoreland County, Pennsylvania, USA), located

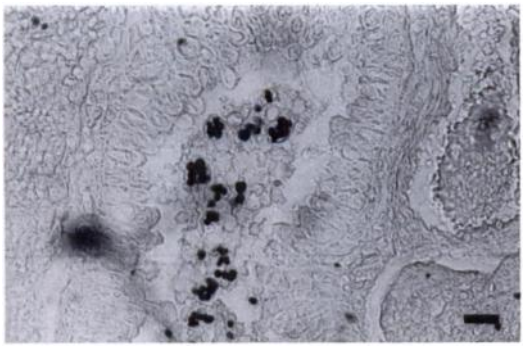

FIGURE 1. Lung section of the *Sorex fumeus* containing *Histoplasma capsulatum* var. *capsulatum* yeast-form cells in the bronchial area. GMS-stain. Bar = 10 μm.

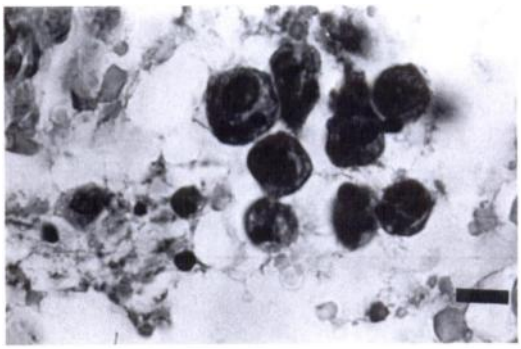

FIGURE 2. *Histoplasma capsulatum* var. *capsulatum* infection in *Sorex fumeus*. Parasitized macrophages in the PAS-stained lung sections. Bar = 10 μm.

within the physiographic division referred to as the Allegany Mountains section of the Appalachian Plateaus Province (Merritt, 1986, 1987). Shrews were caught with pitfalls or Sherman live-traps (51 by 64 by 165 mm, H. B. Sherman Traps, Inc., Tallahassee, Florida, USA) checked early in the morning and just before the sunset between 28 August and 6 September 1995. Forty-six masked shrews (*S. cinereus*) five smoky shrews (*Sorex fumeus*) and 24 short-tailed shrews (*Blarina brevicauda*) were caught and taken immediately into laboratory for necropsy. The sample was supplemented with 12 *S. cinereus*, 18 *S. fumeus* and 21 *B. brevicauda* caught by J. F. Merritt between November 1990 and August 1995 in the same area and stored at −20 C until necropsy in September 1995. The shrews were classified as mature (usually over-wintered) and immature according to the tooth wear and condition of the pelage (Crowcroft, 1957).

On necropsy, the lungs were examined macroscopically for parasites and anomalies. The lobes of the right lung of all the above shrews were fixed in 10% buffered formalin, embedded in paraffin and sectioned at 5 μm. From the shrews processed immediately after trapping, samples of tissue were also taken from the liver, kidneys, spleen, and heart for histological examination. Two to four sections of each sample were placed on one slide, and stained with hematoxylin-eosin (H&E), Grocott's modification of Gomori's methenamine silver (GMS), periodic acid-Schiff (PAS) and Giemsa stains (Bancroft et al., 1990). Most fungi can be seen with GMS and PAS stains, and Giemsa's stain is useful for the demonstration of most protozoans. The identification of *Histoplasma capsulatum* var. *capsulatum* was based on the demonstration of unicellular (2 to 4 μm) yeast-like form of the fungus morphologically compatible with *Histoplasma capsulatum* var. *capsulatum* (Chandler et al., 1980). No material for tissue cultures was obtained.

RESULTS

Yeast-like cells morphologically compatible with *Histoplasma capsulatum* var. *capsulatum* were found in lung sections stained with GMS in eight (35%) of 23 *S. fumeus*, two (3%) of 58 *S. cinereus*, and two (4%) of 45 *B. brevicauda*. Shrews possessing *H. capsulatum* were found in an area used for long-term monitoring of small mammal populations as well as in randomly chosen areas where animals were trapped for this particular study only. In *S. fumeus*, the fungus was found in all months from which samples were available (from May to September) representing three different years (1991, 1993, 1995). Five of the infected *S. fumeus* were males (two mature, three immature) and three were females (two pregnant, one immature). The infected *S. cinereus* and *B. brevicauda* were males caught in August or September 1995.

The yeast-like cells stained more intensively in GMS than the simultaneously stained *P. carinii* cysts of rats used as positive control of the GMS staining, and unlike *P. carinii*, the *Histoplasma* sp. cells were concentrated in the bronchial area (Fig. 1). Based on replicate sections stained with PAS, the organisms were largely intracellular (Fig. 2). In Giemsa-

FIGURE 3. A nematode, apparently *Angiostrongylus michiganensis*, in the H & E stained lung section of *Sorex cinereus*. Bar = 100 μm.

stained sections, the cells appeared as light or dark blue whereas the organisms stained very moderately with H&E. The number of yeast cells in the lungs was low and no inflammatory reaction associated with the fungus was evident. The infection was not disseminated to other organs and no sign of clinical disease was observed.

A single female nematode, possibly *Angiostrongylus michiganensis* was seen at necropsy of one over-wintered pregnant *S. fumeus* caught in July 1995, which was also infected with the *H. capsulatum*. Also, in one of the two fungus infected *S. cinereus* a similar nematode was found located in bronchioles in stained lung sections (Fig. 3); a lung section sample was deposited in the U.S. National Parasite Collection, Beltsville, Maryland (USNPC No. 86742). This adult male shrew was caught in August 1995. A thickening of the bronchiolar epithelium and a mild fibrous reaction with mononuclear cell infiltration in bronchiolar walls were seen surrounding the worm.

DISCUSSION

Histoplasma capsulatum var. *capsulatum* has a worldwide distribution and is enzootic in the central United States (Selby, 1975). Infection usually follows respiratory exposure (Chandler et al., 1980) to air-borne infective spores originating in the soil. This dimorphic fungus exists both in mycelial form in soil and in yeast form in mammals (Chandler et al., 1980).

Most infections in wildlife are based on incidental findings during necropsy and without evidence of clinical disease. The prevalence of infection is reported to be low in wildlife (Menges et al., 1967a; Sanger, 1981). In contrast, the prevalence of infections in *S. fumeus* in this study was remarkably high (35%). Sex or age did not seem to have any effect on the occurrence of the fungus in *S. fumeus*. *Histoplasma capsulatum* is reported to be more common in older animals (Emmons, 1949), but the effect of the age of the host has not been studied in detail.

The fungus was more often found in the larger *S. fumeus* than in the smaller, but much more numerous and widely distributed *S. cinereus*, supporting the body-size hypothesis. However, the observed differences in infection levels could be explained by interspecific microhabitat differences as well, causing *S. fumeus* to encounter the fungus more often than *S. cinereus* (Owen, 1984; Merritt, 1987). To our knowledge histoplasmosis has not been studied in any species of shrews of the genus *Sorex* before. In contrast, *B. brevicauda* has been specifically examined for histoplasmosis (Emmons et al., 1947) and the first isolation was made in 1964 (Menges and Weeks, 1965). However, based on Menges et al. (1967a), the prevalence of the fungus is low in *B. brevicauda*. The much lower prevalence of this organism in *B. brevicauda* compared to the *S. fumeus* could be due to the differences between species or genera of shrews in susceptibility to histoplasmosis. Helminths of shrews, for example, are specific at the level of host genus (Vaucher, 1971).

Small mammals may be good indicators of the distribution of the histoplasmosis within small areas because their home ranges usually are small (Menges et al., 1967a). Whether the fungus exists commonly in the soil in our study area is not known. Histoplasmosis is not considered to be enzootic in Pennsylvania (Selby,

1975), but as Menges et al. (1967b) have pointed out, the fungus may be more widely distributed in nature than generally believed and not all foci will produce detectable infections.

The distribution of *H. capsulatum* in the soil is determined by specific nutritional, microclimatic, and structural properties (Taylor et al., 1962; Selby, 1975). Propagation of the fungus is favored in habitats where the soil is enriched with feces of birds and bats (Hoff and Bigler, 1981). Bats and birds also disseminate the fungus and may introduce it to new areas (Hoff and Bigler, 1981). Bats and birds are numerous in the area of this study (Leberman, 1976; Merritt, 1987), as well as decayed wood and hollow trees which also harbor *H. capsulatum* (Davies and Colbert, 1990).

Seasonal variation in temperature and moisture most likely affect the proliferation of the fungus but little seasonal data on *H. capsulatum* exists (Selby, 1975). The study site we used for monitoring small mammal populations is mesic with high annual precipitation values (Merritt and Zegers, 1991). Since most shrews in our study were collected in late August and early September in 1995, we could not evaluate the possible seasonal or annual variation of the infection in shrews. At least in *S. fumeus,* however, the infection was present in different years.

Pneumocystis carinii may be closely related to the ustomycetous red yeast fungi (Wakefield et al., 1992) and to ascomycetes (Eriksson, 1994), and this opportunistic pathogen causes clinical disease only in immunocompromised hosts. Despite the constantly high prevalence throughout Finland, no clinically ill *S. araneus* have been caught (Laakkonen, 1995). Similarly, immunosuppression seems to be required for the clinical histoplasmosis to appear (Quandt and Nesbit, 1992). Further interspecific comparisons are needed to clarify whether asymptomatic histoplasmosis, like mild *P. carinii* infection, is common in some species of *Sorex* while other wild mammals rarely are infected.

The female nematode specimen found in the lung sections of one of the *S. cinereus* matches Ash's (1967) decription of *Angiostrongylus michiganensis* in lung sections of *S. cinereus.*

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