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THE PREVALENCE OF ADIASPIROMYCOSIS IN THREE SYMPATRIC SPECIES OF GROUND SQUIRRELS

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Abstract: Lungs from three species of ground squirrels collected in south central Saskatchewan were examined by histopathology and a digestion technique for adiaspores of *Emmonsia crescens*. Two of 81 (2.5%) *Citellus richardsoni*, 3 of 17 (17.6%) *C. tridecemlineatus* and 35 of 44 (79.5%) *C. franklini* were infected. Infection was more common in adults than in young-of-the-year. Tissue digestion was the more sensitive method for detecting adiaspores. Possible reasons for the difference in prevalence among the species are discussed.

INTRODUCTION

Adiaspiromycosis is a common, widespread, and apparently benign pulmonary mycosis of mammals caused by dimorphic fungi of the genus *Emmonsia*.⁷ *Emmonsia crescens* is the most frequently encountered member of the genus and is the only species known to occur in mammals in Canada. It has been reported from five continents⁷ and at least 124 species or subspecies of mammal.⁴ It was first reported in Canada in 1947⁵ and there have been a number of subsequent reports.^{1,2,7,10}

In 1970, a number of wild-trapped ground squirrels were submitted to the Department of Veterinary Pathology, Western College of Veterinary Medicine, for routine necropsy. Of these animals, none of five Richardson's ground squirrels (*Citellus richardsoni*) were infected with this fungus, while all of seven Franklin's ground squirrels (*C. franklini*) captured in the same general area were infected.

This apparent difference in prevalence of infection in two sympatric and closely related species appeared unusual, considering the extremely broad host range of the fungus. The present study was undertaken to determine if the prevalence of infection is truly different in these species.

MATERIALS AND METHODS

A total of 142 ground squirrels, comprised of 44 *C. franklini*, 81 *C. richardsoni* and 17 *C. tridecemlineatus* were collected by live-trapping or shooting near Saskatoon, Saskatchewan during May to August of 1976 and 1977. At necropsy the right lung was preserved in 10% buffered formalin, and subsequently one 6 μ m histologic section, stained with hematoxylin-eosin, was prepared from each lobe. The left lung was frozen, and later digested in 2% NaOH and examined for adiaspores with a dissecting microscope. Post-fixation of digested tissue in 70% ethanol greatly improved clarification of the tissue and preserved the digested tissue indefinitely. Identification of *E. crescens* was based upon morphology and size of the spherules.⁷

In the spring and early summer, adults and young-of-the-year could be distinguished easily on the basis of body size and weight; however, in late summer this distinction was more difficult. Eye lens weight data were collected from some animals as a possible method for age determination.⁹ For this purpose, eyes were fixed in formalin and the lenses were later removed, dried for 48 h at 37 C, and weighed. A plot of eye lens weight against date of capture proved sufficient to distinguish the two age classes.

In vitro culture of the fungus was not attempted routinely; however, isolations of fungi from several cases were made on Sabouraud's media. One isolate from a typically infected *C. franklini* lung (Fig. 1) was grown on Sabouraud's media at room temperature for 2 months. A saline suspension was prepared from the culture and 0.2 ml were inoculated subcutaneously into each of two rats, three *C. richardsoni* and three *C. franklini*.

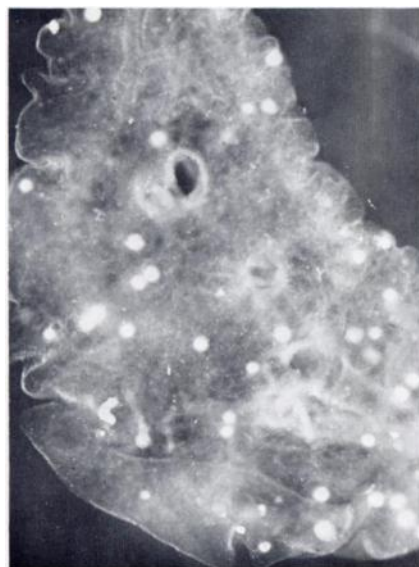


FIGURE 1. Adiaspores of *Emmonsia crescens* in lung tissue from *Citellus franklini*. Tissue was digested in 2% NaOH and post-fixed in 70% ethanol. $\times 10$

RESULTS

C. franklini had a much higher prevalence of infection than did either of the other species, and *C. tridecemlineatus* appeared to have an intermediate prevalence of infection between the other species (Table 1). The differences in prevalence of infection were significant in all cases (χ^2 , $P < 0.005$, $df=1$).

Although the age information on the squirrels is incomplete, infection was more common among those animals known to be adult than in those animals known to be young-of-the-year, with infection being almost universal in adult *C. franklini*.

Numerous adiaspores were found at the injection site in all experimentally infected animals at necropsy 4 months post-infection.

DISCUSSION

This survey clearly shows that the prevalence of infection with *E. crescens* differs significantly among the three ground squirrel species in one area of Saskatchewan. Although a direct species comparison has not been made previously, the difference in prevalence of infection among these species likely exists in other areas as well. For example, the prevalence of infection in *C. richardsoni* has been reported as 0 of 75 in Alberta⁵ and 1 of 10 in North Dakota;⁷ while that in *C. franklini* has been reported as 3 of 6 and 0 of 6 in two different years in central Alberta¹⁰ and 15 of 16 in North Dakota.⁷

TABLE 1. Prevalence of infection with *Emmonsia crescens* in the lungs of three species of ground squirrels of the genus *Citellus*.

	<i>C. richardsoni</i>	<i>C. tridecemlineatus</i>	<i>C. franklini</i>
Adults (> 1 yr)	1/28 ^a = 3.6%	3/9 = 33.3%	32/33 = 96.9%
Young of the year	1/32 = 3.1%	0/4	2/10 = 20.0%
All classes ^b	2/81 = 2.5%	3/17 = 17.6%	35/44 = 79.5%

^anumber infected/number examined

^bincludes animals of unknown age

Infection in young-of-the-year was less common than that in adult *C. franklini*. This might be due to difficulties in detection since adiaspores may require several months to reach a large size⁶ and small adiaspores might have been missed. Both of the infected young-of-the-year *C. franklini* were captured late in the summer, perhaps supporting this hypothesis. Alternatively, the intensity of exposure may be seasonal, so that infection is uncommon during the first summer.

Of the two techniques used for adiaspore detection, NaOH digestion was much the more sensitive method. In several cases, light infections not detected by histopathologic examination were identified in digested tissue. Detection of a single adiaspore in an entire right lung was possible with this technique. Where doubt existed as to the identity of a suspected adiaspore, the individual spores were teased from the digested tissue and examined for characteristic morphology with a compound microscope (Fig. 2).

The observed differences in prevalence of infection could result from differences in either exposure, or susceptibility, of the squirrel species to the fungus. *C. richardsoni* and *C. tridecemlineatus* may be more "resistant" to the fungus and thus do not become infected as readily as does *C. franklini*. Alternatively, they could be very susceptible, with most infected individuals dying of the disease and thus disappearing from the population. From what is known of the benign nature of the disease,⁷ the latter hypothesis appears unlikely.

A low prevalence of infection in nature does not necessarily mean a low susceptibility to the disease. For example, the red-cheeked ground squirrel (*C. erythrogenes*) has not been found infected in nature (219 specimens examined),⁸ but is susceptible to experimental infection and has been used for studies of experimental adiaspiromycosis.⁸ Two *C. richardsoni*

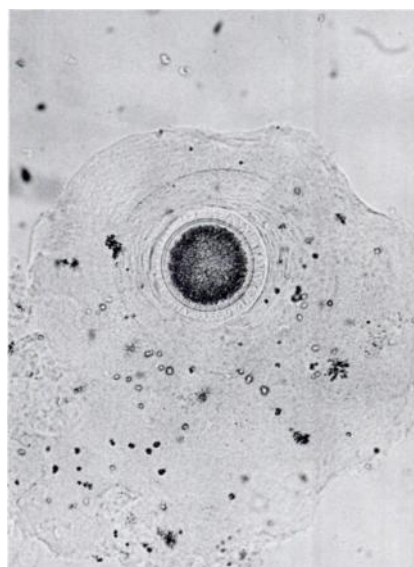


FIGURE 2. Wet mount of adiaspore of *Emmonsia crescens* teased from NaOH digest of formalin-fixed lung tissue from *Citellus tridecemlineatus*. $\times 88$

experimentally inoculated subcutaneously with spores from an *in vitro* culture of *E. crescens* developed adiaspores at the injection site, indicating that the organism can persist and form adiaspores in this species.

The habitat requirements of *E. crescens* are poorly understood, but those of the three squirrel species have been defined.³ *C. richardsoni* inhabits open grassland and avoids woody vegetation, remaining in the open areas among stands of trees and brush. In contrast, *C. franklini* inhabits wooded areas and brushland bordering the prairie and its range in Canada coincides closely with that of the aspen parkland. *C. tridecemlineatus* is not an open grassland species, but inhabits tall grass areas, hedgerows on prairie agricultural land, prairie margins, brushland and aspen bluffs. Thus this species occupies an intermediate position in both habitat requirements and prevalence of

adiaspiromycosis between the other two species.

Sharapov⁸ concluded, after examining 5647 individuals of 45 mammalian species in the USSR, that the optimal habitat for *E. crescens* was the forest-steppe interface, since the greatest prevalence of infection was found in specimens from this zone. He suggested

that this species is part of the rhizospheric root-associated mycoflora of certain shrubby and herbaceous plants and that the best conditions for the fungus occur in thickets of woody and herbaceous plants. Our results suggest a similar association between prevalence of infection and use of deciduous wooded habitat at the prairie margins.

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