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EMMONSIOSIS OF WILD RODENTS AND INSECTIVORES IN CZECHLAND

Zdeněk Hubálek

Department of Medical Zoology, Institute of Vertebrate Biology, Academy of Sciences, Květná 8, CZ-60365 Brno, Czech Republic (e-mail: zhubalek@brno.cas.cz)

ABSTRACT: Adiaspores of the fungus *Emmonsia crescens* were detected microscopically in the lung tissue of 13% of 10,081 small mammals belonging to 24 species examined in 14 areas of the Czech Republic between 1986 and 1997; 441/1,934 (23%) *Clethrionomys glareolus,* 1/6 (17%) *Arvicola terrestris,* 357/2,172 (16%) *Apodemus flavicollis,* 220/1,981 (11%) *A. sylvaticus,* 23/265 (9%) *A. microps,* 11/81 (14%) *Microtus subterraneus,* 93/1,275 (7%) *M. arvalis,* 98/1,439 (7%) *M. agrestis,* 1/3 (33%) *Ondatra zibethicus,* 1/1 *Cricetus cricetus,* 1/20 (5%) *Crocidura suaveolens,* 2/40 (5%) *Neomys fodiens,* and 13/529 (2%) *Sorex araneus* were infected. Emmonsiosis was not recorded among the species of rodents that do not build their nests in the soil (*Muscardinus avellanarius, Micromys minutus, Mus musculus, Rattus norvegicus*). The overall prevalence of emmonsiosis was significantly higher in adult (19%) than in juvenile (7%) mammals, and in rodents (13%, and 20% in adults) than in insectivores (2%, and 4% in adults). The frequency of infected mammals also varied according to geographic area, altitude, habitat, and season.

Key words: Adiaspores, *Emmonsia crescens,* fungi, insectivores, mycosis, rodents, survey.

INTRODUCTION

Emmonsiosis (adiasporomycosis, adiaspiromycosis) is a pulmonary infection of mammals (including man) caused by dimorphic fungi of the genus *Emmonsia* (Onygenaceae: Currah, 1985): *E. crescens* of Emmons and Jellison (1960) (=*E. parva* var. *crescens* of van Oorschot, 1980; teleomorph *Ajellomyces crescens* of Sigler, 1996) or, less often and in warm dry climates only, *E. parva* described by Ciferri et Montemartini (1959) (=*E. parva* var. *parva* of van Oorschot, 1980). Fungi of the genus *Emmonsia* are phylogenetically closely related to the causative agents of blastomycosis, histoplasmosis, and paracoccidioidomycosis (McGinnis et al., 1992; Leclerc et al., 1994). The infection by *E. crescens* is characterized by the development of large, thick-walled spherules called adiaspores, measuring as much as 700 μm, and originating from minute (2–4 μm) subglobose conidia after their inhalation in the lungs of mammalian hosts (Fig. 1). Expanding adiaspores, especially at their higher density in the tissue, lead to collapse of the adjacent alveoli and cause respiratory distress or even failure in the mammalian host. However, the infection with *Emmonsia* sp. is often symptomless. Emmonsiosis has been found widespread among small mammals in many parts of the world, including Czechland (Emmons and Jellison, 1960; Dvořák et al., 1973; Křivanec, 1977; Hubálek et al., 1991). The present paper summarizes the results of our long-term survey of small mammals for emmonsiosis. The extent of the material (>10,000 specimens) has made possible an evaluation of various ecological variables that could affect the distribution of emmonsiosis among small mammals.

MATERIALS AND METHODS

Small mammals were caught in snap-traps in 14 areas of Czechland (see Table 2 for map coordinates) during the years 1986 to 1997. Captured animals were identified to species, weighed, and sexed. For the purpose of this study, sexually active animals and those in sexual regression were considered as adults while all others were considered to be juveniles.

Complete lungs of the captured mammals were placed in 2% potassium hydroxide solution in 10-ml glass tubes overnight, and then the whole lung tissue (usually 7 to 20 compression slides) was examined for typical adiaspores of *E. crescens* microscopically at ×32 magnification. Adiaspores were counted, and their diameter measured at ×150 magnification. The intensity of infection was classified as either low (1 to 9 adiaspores), moderate (10 to

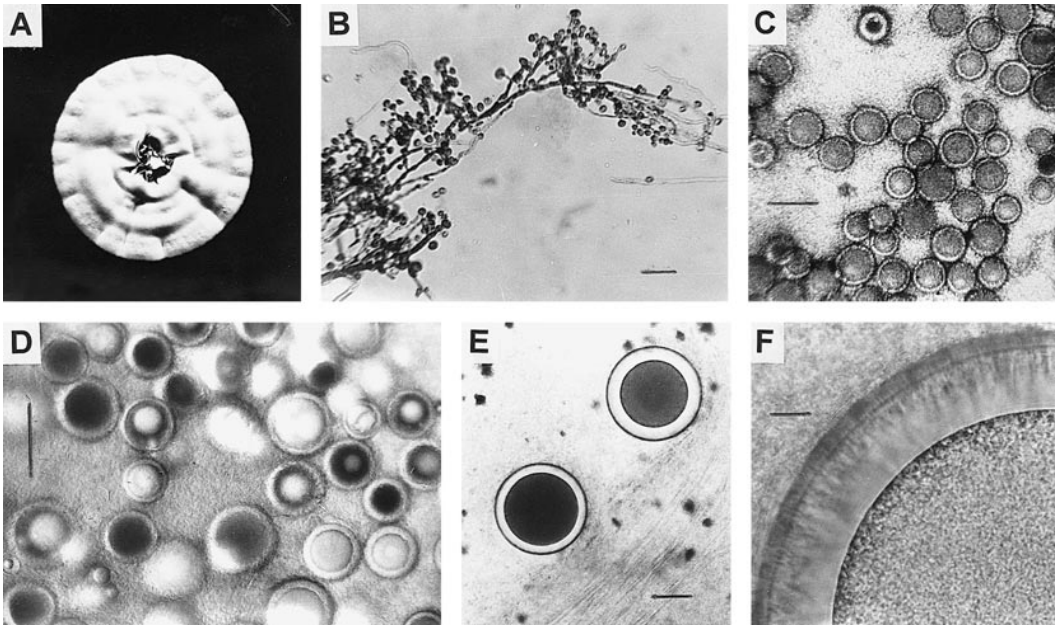

FIGURE 1. Growth characteristics of *Emmonsia crescens*. A. Colony (diameter 49 mm) grown on Sabouraud glucose agar plate at 28 C for 14 days. B. Hyphae with conidia stained with Lugol's iodine (bar = 20 μm). C, D. Adiaspores in the lung tissue, treated with 2% KOH, in two heavily infected female wood mice, *Apodemus sylvaticus* (bar = 200 μm). E. Typical adiaspores in the lung tissue of a male *A. sylvaticus* (2% KOH; bar = 100 μm). F. Detail of an adiaspore wall in the lungs of the same animal (2% KOH; bar = 10 μm).

99 adiaspores), high (100 to 999 adiaspores), and very high (≥1,000 adiaspores per animal).

All data were stored in a database (dBASE III Plus, Ashton-Tate, California, USA) as records including the following fields: protocol number, mammalian species, sex, age category, weight, collection date, site, altitude, habitat type, number of adiaspores, and their average, maximum and minimum size. The data were exported in, and evaluated statistically with, the SOLO package (BMDP Statistical Software, Los Angeles, California, USA) using chi-square and Fisher's exact 2 × 2 tests for prevalence rates (homogeneity of proportions in contingency tables), *t*-tests for adiaspore size differences, and three nonparametric tests (Mann-Whitney, Kolmogorov-Smirnov, Kruskal-Wallis) to compare the intensity of infection (the number of adiaspores per infected animal) among host species. The level of significance was set at $P = 0.01$ of the null hypothesis.

RESULTS

Of the 10,081 animals examined, 1,262 (13%) were found infected. All adiaspores were typical of *E. crescens*, distinguishable from those of *E. parva* by their size. Overall, emmonsiosis was significantly more frequent in adult (19%) than in juvenile (7%) animals, but it was uniformly distributed between males (12%, adult males 20%) and females (13%, adult females 19%). Therefore, the age effect has been taken into consideration during all other analyses.

Three insectivore and 10 rodent species were infected of 24 small mammalian species examined (Table 1). These involved the common shrew (*Sorex araneus*), water shrew (*Neomys fodiens*), lesser white-toothed shrew (*Crocidura suaveolens*), common hamster (*Cricetus cricetus*), muskrat (*Ondatra zibethicus*), water vole (*Arvicola terrestris*), field vole (*Microtus agrestis*), common vole (*M. arvalis*), pine vole (*M. subterraneus*), bank vole (*Clethrionomys glareolus*), yellow-necked mouse (*Apodemus flavicollis*), wood mouse (*A. sylvaticus*) and pygmy field mouse (*A. microps*). Interestingly, the infection was not

TABLE 1. Prevalence (number infected/number examined) of emmonsiosis according to the mammalian host species in Czechland.

Mammalian species	Prevalence (%)	
	All animals	Adults
INSECTIVORA		
Sorex araneus	13/529 (2)	4/124 (3)
S. minutus	0/170 (0)	0/35 (0)
S. alpinus	0/7 (0)	0/3 (0)
Neomys fodiens	2/40 (5)	2/18 (11)
N. anomalus	0/3 (0)	0/0 —
Crocidura suaveolens	1/20 (5)	1/8 (12)
C. leucodon	0/13 (0)	0/7 (0)
Talpa europaea	0/10 (0)	0/3 (0)
RODENTIA		
Cricetus cricetus	1/1	1/1
Ondatra zibethicus	1/3 (33)	1/1
Arvicola terrestris	1/6 (17)	1/1
Microtus agrestis	98/1,439 (7)	82/899 (9)
M. arvalis	93/1,275 (7)	63/755 (12)
M. subterraneus	11/81 (14)	8/39 (21)
Clethrionomys glareolus	441/1,934 (23)	307/853 (36)
Apodemus flavicollis	357/2,172 (16)	278/1,116 (25)
A. sylvaticus	220/1,981 (11)	146/765 (19)
A. microps	23/265 (9)	18/164 (11)
A. agrarius	0/1	0/0 —
Micromys minutus	0/63 (0)	0/18 (0)
Mus musculus	0/54 (0)	0/26 (0)
Rattus norvegicus	0/4 (0)	0/0 —
Muscardinus avellanarius	0/3 (0)	0/0 —
Sicista betulina	0/7 (0)	0/6 (0)
Total	1,262/10,081 (13)	912/4,842 (19)

recorded among the species of rodents that do not build their nests in the soil such as the hazel dormouse (*Muscardinus avellanarius*), harvest mouse (*Micromys minutus*), house mouse (*Mus musculus*) and black rat (*Rattus norvegicus*). The overall prevalence of emmonsiosis was significantly higher in rodents (13%, and 20% in adults) than in insectivores (2%, and 4% in adults). The prevalence rates also differed significantly between the genera *Clethrionomys* (23%, and 36% in adults), *Apodemus* (14%, and 22% in adults), *Microtus* (7%, and 9% in adults) and *Sorex* (2%, and 3% in adults). Within the genus *Apodemus, A. flavicollis* was infected significantly more frequently than either *A. sylvaticus* or *A. microps*; and adult *A. sylvaticus* significantly more often than adult *A. microps*. Alternatively, the prevalence rate of emmonsiosis did not differ between *M. agrestis* and *M. arvalis*, while in *M. subterraneus* it seemed to surpass that of the latter two species ($P < 0.05$). For the following analyses of ecological variables, seven common species of rodents with prevalence >5% have only been included (*A. flavicollis, A. sylvaticus, A. microps, C. glareolus, M. agrestis, M. arvalis, M. subterraneus*).

The prevalence of rodent emmonsiosis differed significantly among geographic areas (Table 2). The highest rate was recorded in the lowland river valleys (Mikulov, Břeclav, Vnorovy) whereas the lowest prevalence was in the mountainous areas (Šumava, Krušné Hory, Jeseníky, Beskydy, Žďárské vrchy). Examined animals were

TABLE 2. Geographic distribution of emmonsiosis in seven common species[a] of rodents in Czechland.

Geographic area (coordinates)	Prevalence (%)	
	All animals	Adults
Šumava Mountains (48°54′N, 13°55′E)	0/19 (0)	0/7 (0)
Krušné hory Mountains (50°41′N, 13°35′E)	92/1,074 (9)	84/691 (12)
Jeseniky Mountains (50°05′N, 17°16′E)	12/185 (6)	9/104 (9)
Beskydy Mountains (49°31′N, 18°32′E)	11/439 (3)	9/251 (4)
Žďárské vrchy hills (49°42′N, 16°05′E)	20/352 (6)	14/215 (7)
Velké Meziříčí-Tišnov (49°21′N, 16°15′E)	29/353 (8)	19/185 (10)
Moravský Kras (49°29′N, 16°45′E)	12/101 (12)	12/63 (19)
Třebíč (49°43′N, 15°55′E)	77/404 (19)	58/251 (23)
Moravský Krumlov (49°03′N, 16°22′E)	123/957 (13)	83/411 (20)
Drnholec (48°51′N, 16°29′E)	200/1,239 (16)	130/663 (20)
Mikulov (48°50′N, 16°40′E)	367/2,295 (16)	232/826 (28)
Valtice (48°44′N, 16°46′E)	15/116 (13)	13/58 (22)
Břeclav (48°48′N, 16°50′E)	232/1,421 (16)	197/759 (26)
Vnorovy (48°56′N, 17°22′E)	53/192 (28)	42/107 (39)

[a] *A. flavicollis, A. sylvaticus, A. microps, C. glareolus, M. agrestis, M. arvalis, M. subterraneus.*

caught at altitudes between 153 and 1,470 ($\bar{x}$ = 386) m above sea level. The altitude affected the distribution of emmonsiosis among rodents significantly; they were most often infected at 400 to 599 m, whereas altitudes >800 m seem to be less favorable for the fungus (Table 3). Very similar results were obtained when the analysis was done for adult animals only (data not shown here).

Emmonsiosis also varied significantly according to habitat (Table 4). The rodents caught in farmland windbreaks and small coppices with deciduous trees were significantly more often infected (31%, and 49% of adults) than those from most of the other habitats. Very high prevalences also were found in rodents from other uncultivated openland habitats such as balks, shrubby and/or grassland rocky slopes, and fishpond banks. Conversely, the lowest infection rate of mammals was recorded on small (new-built and virtually treeless) islets on a lake (2%, and 3% in adults), in arable fields (cereals, root crops, oil plants, lucerne, or clover), meadows, and coniferous (spruce, pine) forests.

The prevalence of emmonsiosis was markedly higher in winter and spring than in either summer or autumn in seven common rodent species tested (Table 5). All differences among seasons were significant except for those between spring and winter, and summer and autumn. In adult mammals, the prevalence rates differed significantly even between summer and autumn.

Overall, 2% of mammals (and 3% of

TABLE 3. Prevalence of emmonsiosis (%) in common species of rodents according to altitude (metres above sea level) in Czechland.

Metres:	<200	200–399	400–599	600–799	800–999	≥1,000
7 spp.[a]	13.8	16.1	19.4	8.2	6.8	2.3
A. flavicollis	15.3	18.4	28.8	5.0	1.5	2.4
A. sylvaticus	6.8	16.2	14.0	6.3	—[b]	—
C. glareolus	19.7	27.4	32.6	14.5	12.8	7.9
M. arvalis	9.0	5.5	12.2	3.1	4.8	—
M. agrestis	—	—	8.3	8.8	7.3	1.4

[a] Sum of *A. flavicollis, A. sylvaticus, A. microps, C. glareolus, M. agrestis, M. arvalis,* and *M. subterraneus.*
[b] No data.

TABLE 4. Emmonsiosis in seven common species of rodents according to habitat in Czechland.

Habitat groups	Prevalence (%)	
	All animals	Adults
Coniferous forests	107/1,580 (7)	77/897 (9)
Broad-leaved deciduous forests	512/3,201 (16)	398/1,528 (26)
Mixed forests	22/159 (14)	21/89 (24)
Windbreaks & coppices in cropland	151/489 (31)	90/185 (49)
Arable fields	57/882 (6)	47/592 (8)
Orchards & vegetable plantations	8/56 (14)	6/24 (25)
Cultivated meadows	29/373 (8)	17/219 (8)
Natural humid meadows	9/184 (5)	5/95 (5)
Bogs (peat-moors)	17/125 (14)	15/73 (21)
Grasslands on rocky slopes	64/291 (22)	46/124 (37)
Shrubby balks and slopes	96/356 (27)	55/123 (45)
Fishpond littoral	56/230 (24)	37/116 (32)
River and lake banks and dikes	88/639 (14)	62/246 (25)
New islets under succession	6/383 (2)	5/166 (3)
Mountain brooks	21/199 (11)	21/114 (18)

adult mammals) had a high or very high intensity of infection (≥100 adiaspores). The highest proportion of these heavily infected animals was found in *A. flavicollis* (3%, and 5% in adults), *M. subterraneus* (2%, and 5% in adults) and *A. sylvaticus* (2%, and 4% in adults). In 6% of all mammals (8% of adult mammals), ≥10 adiaspores were detected. The average intensity of *Emmonsia crescens* infection was 122 adiaspores per infected animal (135 in adults), with a maximum of 22,030 adiaspores in an *A. sylvaticus* and a minimum of only one adiaspore in 266 cases. The mean infection intensity values varied among the mammalian species (Table 6), but they did not differ significantly between adult and young animals. However, the arithmetic average of the mean number of adiaspores per infected animal is not a relevant measure, because distribution of the infection intensity deviates greatly from the normal distribution. Therefore median (*M*) represents a much better index for evaluation of infection intensity among animals (Table 6); it was estimated by three nonparametric tests that infected *A. flavicollis* and *A. sylvaticus* harbored significantly more adiaspores than *C. glareolus* and *M. agrestis*. All other pair-wise species comparisons were statistically insignificant. The 75th percentile in Table 6 means that 75% of infected animals had the indicated or a lower number of adiaspores.

Reinfection, as determined by adiaspores of two considerably distinct size classes present in the lungs of an animal (Hubálek et al., 1993), was found in 12% of all infected mammals and did not differ significantly between adult and young animals. The reinfection rate fluctuated slightly among individual species, it was 16% in *A. sylvaticus*, 17% in *A. microps*,

TABLE 5. Emmonsiosis of seven common species of rodents according to season in Czechland.

Season	Prevalence (%)	
	All animals	Adults
Spring (March to May)	267/1,153 (23)	263/969 (27)
Summer (June to August)	238/2,363 (10)	225/1,585 (14)
Autumn (September to November)	621/5,196 (12)	317/1,755 (18)
Winter (December to February)	117/435 (27)	97/282 (34)

TABLE 6. Intensity of *Emmonsia crescens* infection in rodents and insectivores in Czechland.

	Number of adiaspores					Percentiles	
	1–9	10–99	100–999	≥1,000	Mean	50 (*M*)[b]	75
S. araneus	8	3	2	0	43	6	31
N. fodiens	1	1	0	0	NT[a]	NT	NT
C. suaveolens	1	0	0	0	NT	NT	NT
C. cricetus	0	1	0	0	NT	NT	NT
O. zibethicus	0	1	0	0	NT	NT	NT
A. terrestris	0	0	0	1	NT	NT	NT
M. agrestis	62	29	6	1	32	4	18
M. arvalis	50	27	10	6	158	8	53
M. subterraneus	8	1	2	0	50	4	26
C. glareolus	281	125	31	4	74	4	20
A. flavicollis	175	111	63	8	106	11	61
A. sylvaticus	108	68	36	8	281	10	62
A. microps	12	8	3	0	57	9	32
Total	706	375	153	28	122	7	36

[a] Not tested.
[b] Median.

13% in *A. flavicollis,* 10% in *C. glareolus,* 4% in *M. agrestis,* 15% in *M. arvalis* and 15% in *S. araneus.* The only significant differences in the reinfection rate among the species were those of *M. agrestis* versus *A. sylvaticus, A. flavicollis* and *M. arvalis.*

The mean diameter of adiaspores in mammals varied between 34 and 677 μm, with an arithmetic mean of 254 μm (276 μm in adults). Average adiaspore sizes were 277 μm in *S. araneus* (adults 299 μm), 351 μm in *M. agrestis* (adults 364 μm), 185 μm in *M. arvalis* (adults 201 μm), 253 μm in *M. subterraneus* (adults 298 μm), 334 μm in *C. glareolus* (adults 369 μm), 186 μm in *A. flavicollis* (adults 201 μm), 192 μm in *A. sylvaticus* (adults 215 μm) and 218 μm in *A. microps* (adults 226 μm). The average adiaspore size was significantly greater (*t*-test) in *M. agrestis* and *C. glareolus* than in *A. flavicollis, A. sylvaticus, A. microps,* and *M. arvalis.* The adiaspores in *S. araneus* also were, on average, significantly bigger than those in *A. flavicollis, A. sylvaticus,* and *M. arvalis,* but smaller than those in *M. agrestis.*

DISCUSSION

The prevalence rate of rodent emmonsiosis found in this survey (13%, and 20% in adults) is very similar to the previously published data from South Moravia, Czech Republic (14 to 16%, and 20 to 21% in adults: Hubálek et al., 1991, 1997). In other Czech studies, Prokopič (1971) found only 2% of 6,506 rodents infected, whereas Křivanec (1977) detected emmonsiosis in 12% of 1,174 rodents examined. The differences in the mean prevalence of emmonsiosis in small mammals can be affected by the method used. In this survey, the whole lung tissue was examined microscopically which ensures detection of even small-sized adiaspores not identifiable under low magnification used, e.g., with compressors for trichinoscopy. Table 6 shows that of the 1,262 infected animals, as much as 706 (i.e., 56%) had <10 adiaspores in their lungs. Therefore, many of these animals would be missed as infected if a less sensitive method of detection had been used or only part of the lungs had been inspected.

The present results confirm previous studies (Prokopič, 1971; Dvořák et al., 1973; Hubálek et al., 1991, 1993) that host sex does not play a role in the distribution of emmonsiosis, while the pronounced age effect reflects the prolonged exposure of

the host to the fungal agent in the environment. Emmonsiosis was more frequent in the genus *Clethrionomys* than in the two other rodent genera, *Apodemus* and *Microtus*; similar data were published by Doby et al. (1971). The distribution of emmonsiosis also fluctuated between seasons (Dvořák et al., 1969; Hubálek et al., 1993) and among habitats. The results confirmed our previous data that, within an agroecosystem, emmonsiosis is much more frequent in rodents from windbreaks and field coppices than in those from the adjacent arable fields (Hubálek et al., 1995).

The mean intensity of infection (mean and median number of adiaspores per infected animal) was higher in the genus *Apodemus* than in *Clethrionomys.* Conversely, the mean diameter of adiaspores was greater in the genus *Clethrionomys* than in *Apodemus*; very similar results were obtained by Boisseau-Lebreuil (1970) and Hubálek et al. (1991).

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