

Adiasporomycosis of rodents inhabiting the shores of fishponds

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The lung tissue of 180 rodents belonging to five species, trapped at 17 fishponds in the Studenec area (district of Třebíč) in 1991-1992, was examined for the presence of adiaspores of *Emmonsia parva* var. *crescens* (Emmons et Jellison) van Oorschot. The overall prevalence of adiasporomycosis was high, 30%, but its distribution varied markedly according to rodent species (*Clethrionomys glareolus* 45%, *Apodemus flavicollis* 56%, *A. sylvaticus* 26%, *Microtus agrestis* 13%, *M. arvalis* 9%) and fishpond ('Donát' 61%, 'Čikovec' 28%, 'Štičí' 27%, 'Hlad' 19%, other ponds 14%). During a year, the highest infection rate (53%) was found in the rodents captured in March and April, compared with 21% to 25% of infected animals in the other seasons of the year.

Key words: Adiasporomycosis, *Emmonsia*, *Clethrionomys*, *Apodemus*, *Microtus*

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Plicní tkáň 180 hlodavců 5 druhů, odchycených v letech 1991-1992 na březích 17 rybníků v okolí Studence (okres Třebíč), byla vyšetřena na přítomnost adiaspor houby *Emmonsia parva* var. *crescens* (Emmons et Jellison) van Oorschot. Celková prevalence adiasporomykózy byla vysoká (30%), avšak lišila se značně podle druhu hlodavce (norník rudý 45%, myšice lesní 56%, myšice křovinná 26%, hraboš mokřadní 13%, hraboš polní 9%) a rybníka (Donát 61%, Čikovec 28%, Štičí 27%, Hlad 19%, ostatní 14%). V průběhu roku byl vrchol promořenosti (53%) hlodavců adiasporomykózou zaznamenán v březnu až dubnu; v jiných měsících byla zřetelně nižší (21-25%).

INTRODUCTION

Adiasporomycosis (adiaspiromycosis) is a pulmonary mammalian (human inclusive) infection caused by fungi of the genus *Emmonsia* Ciferri et Montemartini, largely by *E. parva* var. *crescens* (Emmons et Jellison) van Oorschot (Emmons and Jellison 1960, Dvořák et al. 1973, van Oorschot 1980). This dimorphic fungus produces conspicuously large spherules ('adiaspores': Fig. 1) in the vertebrate host tissue. *Emmonsia* is closely related to two other human pathogenic dimorphic fungi, viz *Blastomyces dermatitidis* Gilchrist et Stokes and *Histoplasma capsulatum* Darling, as demonstrated by their 18S ribosomal DNA sequences (McGinnis et al. 1992). Teleomorphs of the latter two fungi belong to the ascomycetous genus *Ajellomyces* McDonough et Lewis, *Onygenales*.

A few parasitologists and mycologists studied adiasporomycosis of free-living mammals in the Czech Republic (Otčenášek et al. 1965, Prokopič et al. 1965,

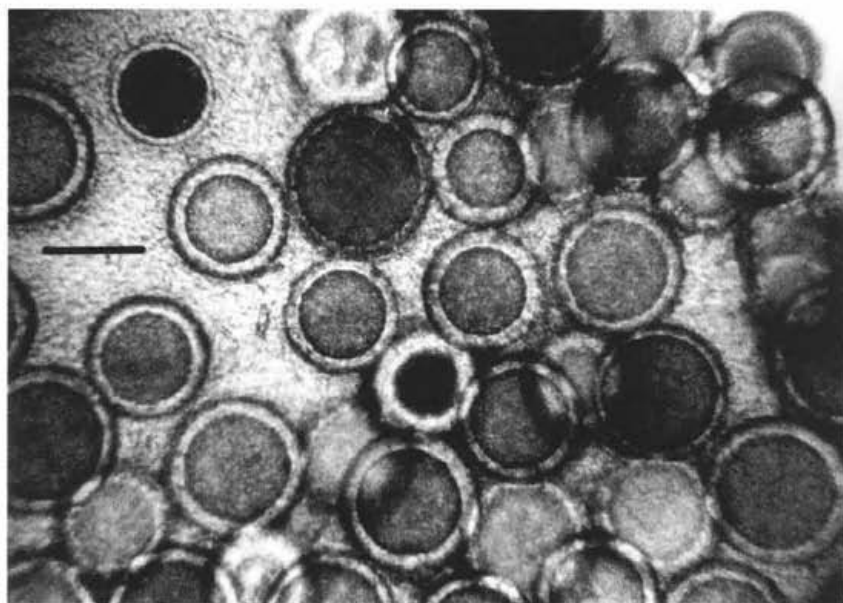


Fig. 1. Adiaspores of *Emmonsia parva* var. *crescens* in the lung tissue of a wood mouse (*Apodemus sylvaticus*). Bar = 100 μ m.

1971, 1981, Dvořák et al. 1967, 1969, 1973, Křivanec 1977, Křivanec and Otčenášek 1977, Ječný and Vojtěchová 1984, Hubálek et al. 1988, 1991, 1993, 1995). However, a systematic investigation of the infection in mammals inhabiting the fishpond ecosystem has not yet been carried out.

MATERIAL AND METHODS

Small mammals were caught using standard snap-traps and live-traps during all seasons of the years 1991 and 1992. The traps were laid on the shores of 17 small (2 to 30 hectares) fishponds with stands of *Glyceria*, *Phragmites*, *Typha*, less *Carex*, *Juncus* and *Baldingera*, situated between 390 and 475 m a.s.l. in the area of Studenec (district of Třebíč, Moravia, Czech Republic). In addition to rodents, one *Mustela nivalis* and several insectivores (14 *Sorex araneus*, 11 *S. minutus* and 3 *Neomys fodiens*) were captured and examined for adiasporomycosis. They all were negative and have not been included in this survey, similarly as 3 specimens of *Micromys minutus*. For the purpose of this survey, sexually mature animals and those in sexual regression have been considered as adults, while all other specimens as juveniles.

The lungs of identified, aged and formalinized mammals were removed 'in toto', and placed in a 2% solution of potassium hydroxide at room temperature for two days. The whole lung tissue of each animal was then examined microscopically at 32x magnification. Only typical adiaspores (at least 70 μm in diameter, with a thick spherule wall) of *E. parva* var. *crecens* were counted and their diameter measured.

Distribution of the prevalence rates in different groups of rodents was evaluated statistically by the chi-square goodness of fit test, while t-test was used to evaluate differences in the intensity of infection (number of adiaspores per infected animal) and in the adiaspore mean size (Snedecor and Cochran 1967).

RESULTS

A total of 180 rodents of five species, inhabiting the shores of local ponds, were examined microscopically (Table 1). The overall infection rate of adiasporomycosis was high (30.0%), but the prevalence in individual rodent species varied greatly: Common Vole, *Microtus arvalis* (Pall.) 9%; Short-tailed Vole, *M. agrestis* (L.) 13%; Bank Vole, *Clethrionomys glareolus* (Schreb.) 45%; Yellow-necked Mouse, *Apodemus flavicollis* (Melchior) 56%; Wood Mouse, *A. sylvaticus* (L.) 26%. The rodents of the genera *Clethrionomys* and *Apodemus* were significantly (chi-square 13.33; $P < 0.001$) more frequently infected with *E. parva* var. *crecens* than the members of the genus *Microtus*. Young rodents ($n=62$) were generally less often infected (17.7%) than adults (36.4%; $n=118$; chi-square 4.74; $P < 0.05$).

The highest prevalence rate was found in animals caught at the Donát fishpond (Table 1): 61% of local rodents (and as much as 78% of local adult rodents) were infected with *E. parva* var. *crecens*, which represents a significantly (chi-square 15.84; $P < 0.001$) greater proportion than that at the fishponds Štičí and Čikovec (27-28%) or at the remaining ponds (14-19%).

Table 2 shows the seasonal distribution of rodent adiasporomycosis in the area studied. The significantly (chi-square 10.51; $P < 0.05$) highest prevalence (53%) was found in the spring (March to April) while the lowest rate (21%) in November to December. Rodents of the genera *Clethrionomys* and *Apodemus*, taken collectively, were found to be infected significantly (chi-square 7.08; $P < 0.01$) more frequently between January and April (60%) than between May and December (26%); an analogical difference is also significant (chi-square 4.02; $P < 0.05$) for the adults of these genera (63% vs. 22%, resp.).

The mean intensity of infection with *E. parva* var. *crecens* was 25.3 adiaspores per infected animal, with a maximum of 388 adiaspores and a minimum (in 10 cases) of only one adiaspore. The mean intensity in adult rodents was 28.4 while in the juveniles it was 17.5 adiaspores per rodent; the difference was statistically insignificant. The infection intensity values were 19.9, 20.3, 60.6, 18.5 and 22.7 in the rodents caught at the ponds Donát, Štičí, Čikovec, Hlad and others, respectively.

Tab. 1. Distribution of adiasporomycosis according to locality (fishpond) and rodent species. (No. infected/no. examined).

Fishpond	Donát	Štičí	Čikovec	Hlad	Other*	Total	%
<i>M. agrestis</i>	2/8	0/5	1/8	2/21	3/21	8/63	12.7
<i>M. arvalis</i>	1/1	0/0	0/6	0/0	0/4	1/11	9.1
<i>C. glareolus</i>	17/22	8/27	3/7	6/18	1/4	35/78	44.9
<i>A. flavicollis</i>	1/1	1/2	3/4	0/2	0/0	5/9	55.6
<i>A. sylvaticus</i>	1/4	4/14	0/0	0/1	0/0	5/19	26.3
Total	22/36	13/48	7/25	8/42	4/29	54/180	
%	61.1	27.1	28.0	19.0	13.8		30.0

* Vrbinec 2/6, Rathan 0/5, Poulik 0/3, Štěpánek 1/3, Podhorník 0/3, Příbyl 0/2, Hranečník 1/1, Maršovec 0/1, Čepička 0/1, Ostrovec 0/1, Kacíř 0/1, Netušil 0/1, Novostudenecký 0/1.

Tab. 2. Seasonal distribution of infection in the rodent genera. (No. infected/no. examined).

Months	I-II	III-IV	V-VIII	XI-XII
<i>Microtus</i>	3/37	2/10	2/11	2/16
<i>Clethrionomys</i>	9/17	13/20	5/14	8/27
<i>Apodemus</i>	1/2	8/13	0/3	1/10
Total	13/56	23/43	7/28	11/53
%	23.2	53.5	25.0	20.8

Analogical values for the species *M. agrestis*, *C. glareolus*, *A. flavicollis* and *A. sylvaticus* were 23.2, 12.4, 112.0 and 16.6, respectively. However, all these differences among either ponds or rodent species were insignificant ($P > 0.10$).

The diameter of adiaspores detected in the rodent lung tissue varied between 101 μm and 658 μm (the latter size in an adult female of *M. agrestis* caught at the 'Hranečník' pond in January 1992), with an arithmetic mean of 336.7 μm . The differences in the mean adiaspore size (in μm) between adults (349.8) and juveniles (298.0) were insignificant ($P > 0.10$), as were those amongst individual rodent species (*M. agrestis* 325.9, *C. glareolus* 367.0, *A. flavicollis* 273.8, *A. sylvaticus* 236.6) and the rodents caught at particular fishponds (Donát 332.1, Štičí 320.5, Čikovec 348.9, Hlad 377.9, others 311.0).

DISCUSSION

The overall prevalence of rodent adiasporomycosis at the fishponds in the Studenec area was high, 30% (and 36% in adults). However, the distribution of this infection was heterogeneous, and it varied significantly among the ponds. The

highest prevalence was found at the Donát pond, where as much as 61% of local rodents (and 78% of the adults) were infected. The principal trapping site at this pond was a ca. 40 x 17 m area covered by a terrestrial stand of *Phragmites australis* [syn. *P. communis*] with a solitary willow shrub, situated down the pond dam. The bank vole, the yellow-necked mouse and the wood mouse were significantly more often infected with *E. parva* var. *crescens* than *Microtus* spp., similarly as observed in southern Moravia previously (Hubálek et al. 1991, 1995). Cumulative proportions of non-*Microtus* rodents out of all rodents captured were significantly (chi-square 14.29; $P < 0.01$) heterogeneous among the ponds: Donát 75.0%, Štičí 89.6%, Čikovec 44.0%, Hlad 50.0% and the rest 13.8%. The higher prevalence rate of adiasporomycosis in rodents caught at some ponds might be therefore explained by the greater proportions of non-microtine rodents among trapped mammals. However, when the prevalence rates in only non-*Microtus* rodents were compared among the ponds Donát (70.4%), Štičí (30.2%) and the rest (36.1%), the differences have remained significant (chi-square 6.81; $P < 0.05$) and indicative of the most common infection of rodents with *E. parva* var. *crescens* at the Donát fishpond. The shores of this pond might thus be regarded as a natural focus of adiasporomycosis.

The higher frequency of adiasporomycosis in adult mammals than in the young ones is a well-known phenomenon (Prokopič 1971, Ječný and Vojtěchová 1984, Hubálek et al. 1988, 1991), as well as the spring seasonal peak of the infection in rodents (Dvořák et al. 1967, 1969, Hubálek et al. 1993). This peak means that most of the positive rodents have contracted the infection during the winter or early spring.

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