CZECH MYCOL. 61(1): 99–106, 2009

Examination of rodents (*Rodentia*) for emmonsiosis in the Czech Republic, Israel and Africa

PETRA SVOBODOVÁ[1, 2], MILAN PEJČOCH[3], MARTA HEROLDOVÁ[1], TOMÁŠ PAVLÍČEK[4], EVIATAR NEVO[4], RADIM ŠUMBERA[5] and ZDENĚK HUBÁLEK[1, 2]

[1] Institute of Vertebrate Biology v. v. i., Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic;
svobodova@ivb.cz
[2] Department of Microbiology, Institute of Experimental Biology, Masaryk University, Brno, Czech Republic
[3] Institute of Public Health, Brno, Czech Republic
[4] Institute of Evolution, University of Haifa, Mount Carmel, Israel
[5] Department of Zoology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

Svobodová P., Pejčoch M., Heroldová M., Pavlíček T., Nevo E., Šumbera R. and Hubálek Z. (2009): Examination of rodents (Rodentia) for emmonsiosis in the Czech Republic, Israel and Africa. – Czech Mycol. 61(1): 99–106.

Lung tissue of 156 rodents (genera *Apodemus*, *Myodes*, *Microtus*, and *Muscardinus*) from the Czech Republic, 29 rodents of the species *Spalax ehrenbergi* from Israel and 106 rodent specimens from Africa (genera *Heliophobius*, *Mastomys*, *Acomys*, *Aethomys*, *Saccostomus*, *Tatera*, *Mus*, *Cryptomys*, *Dasymys*, *Dendromus*, *Grammomys*, and *Steatomys*) were examined for presence of adiaspores of the fungal genus *Emmonsia*. In the Czech Republic, nine (5.8 %) animals revealed adiaspores of *E. crescens*. The positive samples were found in rodents sampled in three of the five Czech areas studied. Out of six species of the Czech rodents, three species were positive: *Apodemus flavicollis*, *Myodes glareolus* and *Microtus agrestis*. *A. flavicollis* was the most frequently infected species, whereas *A. sylvaticus*, *Microtus subterraneus* and *Muscardinus avellanarius* were negative. Adiaspores were recorded in five females and four males. A significant preference of the fungus for the host sex was not observed. Tissue samples of rodents from Africa and Israel were all negative.

Key words: *Emmonsia*, adiaspiromycosis, adiasporomycosis, lung tissue.

Svobodová P., Pejčoch M., Heroldová M., Pavlíček T., Nevo E., Šumbera R. a Hubálek Z. (2009): Vyšetření hlodavců (Rodentia) z Česka, Izraele a Afriky na emmonsiózu. – Czech Mycol. 61(1): 99–106.

Byla vyšetřována plicní tkáň 156 hlodavců (rody *Apodemus*, *Myodes*, *Microtus*, *Muscardinus*) z České republiky, 29 hlodavců *Spalax ehrenbergi* z Izraele a 106 z Afriky (rody *Heliophobius*, *Mastomys*, *Acomys*, *Aethomys*, *Saccostomus*, *Tatera*, *Mus*, *Cryptomys*, *Dasymys*, *Dendromus*, *Grammomys*, *Steatomys*) na přítomnost adiaspor mikromycetů rodu *Emmonsia*. V Česku bylo pozitivních 9 (5,8 %) zvířat, u nichž byly prokázány adiaspory druhu *E. crescens*. Pozitivní vzorky byly zjištěny u hlodavců ve třech z pěti studovaných oblastí. Bylo vyšetřeno šest druhů hlodavců, tři druhy byly pozitivní: *Apodemus flavicollis*, *Myodes glareolus* a *Microtus agrestis*; největší prevalence infekce byla u druhu *A. flavicollis*. Druhy *Apodemus sylvaticus*, *Microtus subterraneus* a *Muscardinus avellanarius* byly

negativní. Adiaspory byly zaznamenány u pěti samečků a čtyř samiček hlodavců; průkazná preference houby k pohlaví hostitele nebyla zjištěna. Vzorky tkáně hlodavců z Afriky a Izraele byly negativní.

INTRODUCTION

Emmonsiosis (adiaspiromycosis, adiasporomycosis) is a pulmonary disease caused by microscopic fungi of the genus *Emmonsia* Ciferri et Montemartini. Infections were recorded in rodents (Rodentia), insectivores (Insectivora) and carnivorous animals (Carnivores) (Emmons and Jellison 1960, Dvořák et al. 1973, Křivanec and Otčenášek 1977). The disease was observed rarely also in humans in the Czech Republic (Dvořák et al. 1970; Koďousek et al. 1970a, b, 1971) and in the world, especially in South America, including several fatal cases (Moraes et al. 1989, Moraes and Gomes 2004, Sun et al. 2007). Emmonsiosis is caused by two species, *Emmonsia crescens* Emmons et Jellison and *E. parva* (Emmons et Ashburn) Ciferri et Montemartini. Sigler (1996) described the sexual stage of *E. crescens* as *Ajellomyces crescens* (order *Onygenales*, Ascomycetes). A teleomorph of *E. parva* has not yet been reported.

These dimorphic fungi produce spherules called adiaspores in the lungs of the affected host. Only exceptionally adiaspores can disseminate into other organs such as the brain (Van Oorschot 1980) and the peritoneal cavity (Drápela et al. 1980). The adiaspores of both fungal species are morphologically very similar but differ markedly in size (Dvořák et al. 1973). Those of *E. crescens* measure up to 500 (700) µm in diameter. This species occurs across the Holarctic region (Dvořák 1965) including the Czech Republic (Otčenášek et al. 1965, Prokopič et al. 1965, Prokopič 1971, Ječný and Vojtěchová 1984, Hubálek 1999, Fischer 2001). On the contrary, *E. parva* occurs in arid zones of North America (Carmichael 1951, Sigler 1998), Africa and Israel (Hubálek et al. 2005), and on the Arabian peninsula (Al-Musallam 1989). The adiaspores of the last species are very small, up to 40 (50) µm. The diagnosis of emmonsiosis is mainly based on the microscopy of lung tissue post mortem or in bioptic samples. Cultivation of the fungus is uneasy and the sensitivity of this diagnostic method is low. Specific serodiagnostic procedures have not yet been developed (Nuorva et al. 1997).

MATERIALS AND METHODS

Rodents were caught in the Czech Republic (Tab. 1), Israel and in Malawi (Africa). Snap-traps were used to catch rodents on the Czech territory, whereas most of the rodents from Malawi and Israel were excavated and captured manually. The trapped rodents were identified, weighed and sexed. The lung tissue of rodents

from Malawi and Israel were fixed in 95 % alcohol, while the Czech samples were frozen at –20 °C. Before examination, the lungs were submersed in a 2 % potassium hydroxide solution at room temperature for several hours. Only half of the lung tissue was used for emmonsiosis examination in the Czech samples, while the second half was kept for hantavirus examination. Compression slide preparations were made from the cut small pieces of the tissue, and examined microscopically at 160× magnification. Adiaspores (Fig. 1) were counted and measured (diameter and wall thickness). Later, positive samples were photographed. Differences in prevalence of infection between different categories of animals were evaluated with the chi-square test (Snedecor and Cochran 1967).

Tab. 1. Rodents examined in the Czech Republic.

Species/ Locality	*Microtus agrestis*		*Microtus subterraneus*		*Myodes glareolus*		*Apodemus flavicollis*		*Apodemus sylvaticus*		*Muscardinus avellanarius*		Σ
Sex	M	F	M	F	M	F	M	F	M	F	M	F	
Beskydy – Kněhyně	5	6		2	9	8	29	20	2	3		3	87
Rájec					5	4	6	7					22
Vranovice – Hájek							3	9		1			13
Lednice – Horní les					6	1	5	6		1			19
Blučina					1	2	5	7					15
Σ	11		2		36		97		7		3		156

Abbreviations: M – male, F – female.
Beskydy – Kněhyně 49° 27' N, 18° 18' E
Blučina – Rumunská 49° 30' N, 16° 38' E
Lednice – Horní les 48° 47' N, 16° 48' E
Rájec 49° 24' N, 16° 38' E
Vranovice – Hájek 48° 58' N, 16° 36' E

RESULTS

Czech Republic

A total of 156 rodents representing six species were examined (Tab. 1). Adiaspores of *Emmonsia crescens* were recorded in nine cases (5.8 %). Detailed data on positive animals are given in Tab. 2. The recorded adiaspores were typical of the species *E. crescens* in that they measured more than 40 µm (in the range of 113–677 µm). Infections were recorded in males in four cases (5.3 % of all males examined) and in females in five cases (6.2 % of all females tested). No significant

intersexual difference in infection prevalence was found. Adults were infected more often than juveniles (but the difference was statistically insignificant). The frequency of emmonsiosis was significantly (P = 0.0025) higher in spring (March to May; 14 % infected animals) than in summer and autumn (June to October; 2 %).

The intensity of infection was low to moderate: the number of adiaspores per sample varied from 1 to 36.

Tab. 2. List of rodents infected with *Emmonsia crescens*, Czech Republic.

Number	Species	Locality	Date	Sex	Weight (g)	Age	Number of adiaspores
11255	*Microtus agrestis*	Beskydy – Kněhyně	5 Oct 2005	M	28	Ad.	1
11317	*Myodes glareolus*	Beskydy – Kněhyně	5 Sept 2006	F	24	Juv.	15
11374	*Apodemus flavicollis*	Blučina – Rumunská	6 Mar 2007	F	41	Ad.	2
11395	*Apodemus flavicollis*	Blučina – Rumunská	7 Mar 2007	M	37	Ad.	36
11396	*Apodemus flavicollis*	Blučina – Rumunská	6 Mar 2007	F	33	Ad.	27
11397	*Apodemus flavicollis*	Blučina – Rumunská	6 Mar 2007	F	27	Juv.	34
11402	*Apodemus flavicollis*	Lednice – Horní les	7 Mar 2007	M	42	Ad.	5
11404	*Myodes glareolus*	Lednice – Horní les	7 Mar 2007	F	18	Juv.	6
11406	*Apodemus flavicollis*	Blučina – Rumunská	7 Mar 2007	M	40	Ad.	1

Abbreviations: M – male, F – female, Ad. – adult, Juv. – juvenile.

Israel

Lung tissue samples of 29 rodents belonging to the genus *Spalax* (blind mole rats), namely to the species *Spalax ehrenbergi* complex represented by three genomic groups: *S. galili* (5), *S. carmeli* (14), and *S. judaei* (10), came from seven localities in the northwest of the country (Anza, Muchraka, Dalton, Lahav, Atlit, Sasa, and Nahal Oren). Many of the Israeli rodents were held captive for several years. All samples of the examined rodents were negative.

Malawi

The African collection involved 106 rodents of 12 species: *Dendromus* sp. (1), *Mastomys natalensis* (28), *Aethomys chrysophilus* (14), *Saccostomus campestris* (5), *Heliophobius argenteocinereus* (30), *Mus triton* (3), *Tatera leucogaster* (4), *Acomys spinossisimus* (16), *Dasymys incomtus* (1), *Grammomys dolichurus* (1), *Cryptomys hottentotus* (2), and *Steatomys pratensis* (1). The rodents were caught in the southern part of Malawi in different ecological habitats, such as dry grassland areas, arable fields, pasturelands. No animal revealed presence of adiaspores in the lungs.

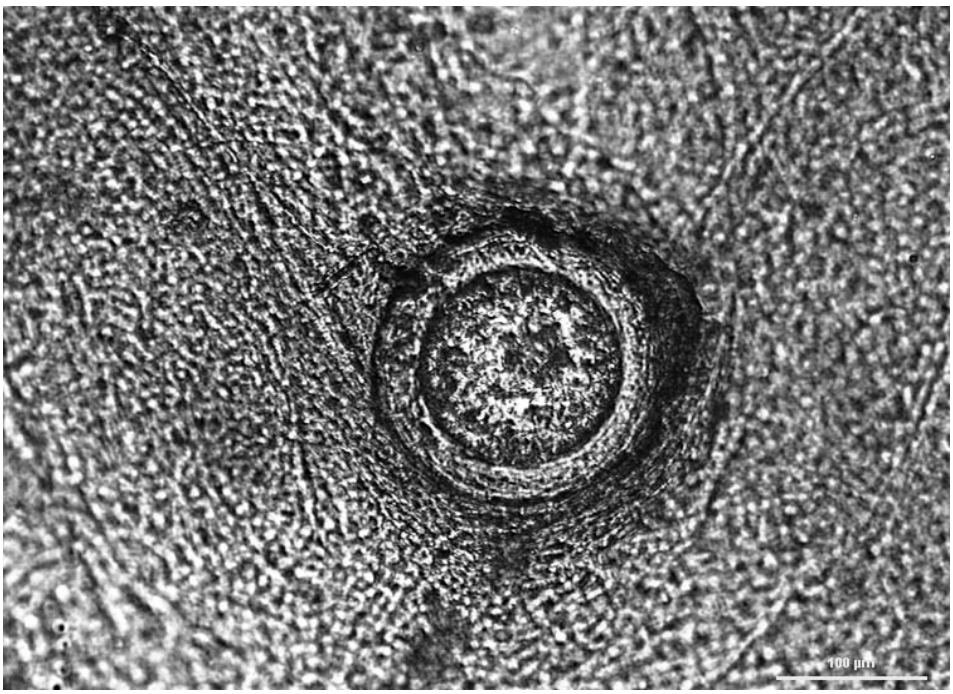

Fig. 1. An adiaspore of *Emmonsia crescens* in the lungs of *Apodemus flavicollis*. Bar 100 µm. Photo P. Svobodová.

DISCUSSION

Rodent emmonsiosis in the Czech Republic

The prevalence of emmonsiosis of examined rodents in the Czech Republic was 5.8 %. This proportion is quite low in comparison with other related studies. For instance Hubálek et al. (1991) observed 16.2 % rodents to be infected. Later, Hubálek et al. (1995a) recorded a similar positivity (16.6 %) in rodents from South Moravia. The bulk of examined rodents in the present study were sampled in the Beskydy Mountains at 900–1200 m above sea level. According to earlier records, prevalence of emmonsiosis in rodents is generally low at higher elevations (Hubálek et al. 1995b, Hubálek 1999). On the contrary, as many as 28 % of rodents sampled in a lowland floodplain forest at Blučina were infected. The prevalence of infected rodents of the species *A. flavicollis* and *M. glareolus* correlates well with papers by Hubálek et al. (1998a,b) and Hubálek (1999). In general, the fungus occurs very often especially in lowland forests, in habitats such as windbreaks and natural forests (Hubálek et al. 1995a, Hubálek 1999).

We found that prevalence of emmonsiosis does not depend on the host's sex. This is in accordance with a number of previous studies (Prokopič 1971, Hubálek 1999, Fischer 2001).

Adults were infected more often than juveniles in this study. Studies by Prokopič (1971) and Hubálek et al. (1991) observed a similar age-related pattern.

The frequency of emmonsiosis was higher in spring than in summer and autumn. This fact was already acknowledged in studies by Dvořák et al. (1967) and Hubálek et al. (1993).

The number of adiaspores per sample varied from 1 to 36. For comparison, Hubálek (1999) recorded a case of a massive infection with the number of adiaspores in the lungs of *A. flavicollis* reaching several thousands.

Occurrence of emmonsiosis in Israel and Malawi

Lung tissues of Israeli rodents tested negatively for presence of *E. crescens* and *E. parva* adiaspores. But positivity was expected based on a previous study (Hubálek et al. 2005) that revealed 28 % positive rodents of the species *Spalax galili* in Israel, where males, females, and juveniles were infected. Why the present samples were negative is unclear. Almost one half of the presently examined animals lived in captivity and they were exposed to a natural environment before capture for a year or longer. Examined Israeli rodents (blind mole rats) are subterranean animals, therefore the real percentage of infected rodents is expected to be higher. Ecological conditions play an important role in the development of the fungus. The previous study (Hubálek et al. 2005) dealt with the occurrence of emmonsiosis in rodents from Malawi and Zambia. They found 100 % positivity for emmonsiosis in *Cryptomys anselli* but complete negativity in *Heliophobius argenteocinereus*. Both species are strictly subterranean, but individuals of *Cryptomys* live in communities, build their burrows deeply in the soil, with places for food and with latrines, whereas *H. argenteocinereus* is a solitarily living species with no deep burrows (Hubálek et al. 2005). Behavioural factors of the host are probably important for the development of *Emmonsia* infection in the host. In our study, however, the two tested individuals of *Cryptomys* were negative. The negativity of 30 *H. argenteocinereus* specimens is in accordance with the study by Hubálek et al. (2005).

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