The impact of sharing a home with a pet on the physiological state of the human microbiome: a comprehensive study on the Czech population with a focus on filamentous fungi

Jan Wipler[1], Zuzana Čermáková[2], Vladimír Buchta[1], Pavel Žák[3], Markéta Vlčková[4]

[1]Charles University in Hradec Králové and Hradec Králové University Hospital, Faculty of Medicine, Institute of Clinical Microbiology, Hradec Králové, Czech Republic
[2]Lab Media Servis s.r.o., Jaroměř, Czech Republic
[3]Charles University in Hradec Králové and Hradec Králové University Hospital, Faculty of Medicine, 4th Internal Clinic-Haematology, Hradec Králové, Czech Republic
[4]Charles University and Motol University Hospital, 2nd Faculty of Medicine, Institute of Biology and Medical Genetics, Prague, Czech Republic

Received December 20, 2022
Accepted May 4, 2023

Abstract

Czechs commonly share their homes with pet animals. However, the likelihood of transmission of filamentous fungi (FF) between the pet and the owner is not well known. The aim of this study was to define the frequency of such transmission. At the same time, the degree of closeness of owner-animal cohabitation, the effect on the spectrum of shared FF and health risk assessment were defined. The effect of previous antibiotic therapy on fungal flora was also assessed. In total, 150 pet owners and 135 pet animals from 125 households provided 911 samples; 80 non-owners provided 320 samples. All owners completed a questionnaire focusing on the level of contact with the pet and information on previous antibiotic treatment. The relationship between the contact index (CI) and the effect of previous antibiotic treatment on the number of FF species shared was quantified. Results were compared with those of non-owners. The CI was very close (CI > 4) in 131 owners (87.3%). A total of 110 FF were isolated. Common FF were found in 42 households (33.6%); 65 FF were identified in the non-owners. In the last year, 46 pets, 43 owners and 25 non-owners used antimicrobial agents. *Aspergillus niger* was most prevalent in owners and pets and *Alternaria alternata* in non-owners. The degree of contact intimacy did not seem to have any effect on the joint abundance of FF, but antibiotic treatment had a significant effect on FF abundance in non-owners. This effect was not significant in either owners or pets.

Companion animals, hyphomycet es, shared mycobiota, contact index

Companion animals are considered members of the family by many owners. Close contact between the pet animal and its owner can lead to exchange and communication of the microbiome. Opportunistic pathogenic mycoorganisms such as filamentous fungi (FF) can pose a health risk to pets and their owners due to the long-term disruption of the ecological balance between bacterial and fungal microbiota in favour of fungi (Chomel and Sun 2011). The human body is populated by a complex and heterogeneous microbial ecosystem that plays an important role in human health. This ecosystem is composed of a fungal component (mycobiota) that can be competitively inhibited by a rapidly growing bacterial component (bacteriobiota). If this balance is disturbed, for example by the effect of broad-spectrum antibacterial drugs (ATB), the mycobiota overgrows and can cause mycosis (Sullivan et al. 2001). Human mycotic infections were not common in the past, but currently the number of cases is increasing, especially in immunodeficient patients (Garbee et al. 2017; Mercier and Maertens 2017).

The main objective of our study of randomly selected participants was to monitor the species diversity of FF (including subspecies and varieties) present in pets and their owners

Address for correspondence:
Jan Wipler
Department of Clinical Microbiology
Faculty of Medicine in Hradec Králové
Charles University and University Hospital Hradec Králové
Sokolská 581, 500 01 Hradec Králové

Phone: +420 777 884 895
E-mail: wolvariella@seznam.cz
http://actavet.vfu.cz/

with respect to opportunistic pathogens. Monitoring fungal biodiversity, transmission, and sharing with humans could highlight the potential risk of outbreaks of infection with opportunistic mycopathogens, as contact with pets is nowadays an integral part of most people's lives. To this end, we defined a contact index (CI) that determines the degree of intimacy between owners and their pets. To our knowledge, no such index has been defined to date. In this study, we compared fungal colonization between owners, pets, and a control group of non-pet owners. Furthermore, we studied the effect of ATB administration on the increasing number of fungal species and the recovery of mycobiont/bacteriobiont balance after ATB administration in our sample compared to the control group of non-owners.

Materials and Methods

For our study, we collected samples in the period of 2014–2017 and included 150 pet owners and 135 pets (110 dogs, 18 cats, 4 reptiles, 2 guinea pigs, and one small rabbit) from 125 households that provided a total of 911 different sample types. We analysed swabs from the nasal mucosa, the space between the fingers, the axillae, and the auditory canal. All types of the abovementioned samples were collected from all 150 owners. In the case of domestic animals, we obtained and analysed only nasal mucosa and ear canal swabs (mainly from the nostrils in reptiles). For some of the animals tested (38 animals and 9 owners) we also included samples from suspicious lesions (e.g. inflammatory lesions, etc.). Analysis of the nasal mucosa, ear canal, axillae and the space between the toes (320 swabs) was also performed on samples obtained from a control group of 80 humans who had not shared a household with any pet animal for more than one year. No suspicious lesions were found in any of them. All samples collected were numbered to preserve the anonymity of the person tested. All owners completed a questionnaire and provided an informed consent for the inclusion in the study, and the study was approved by the Ethics Committee of the University Hospital Hradec Králové.

The following materials were used for the cultivation and identification of microbes, following the manufacturer's instructions. Mycological solid media included SAB (Sabouraud's glucose agar; Lab Media Servis, Jaroměř, Czech Republic), SAB2 (Sabouraud's glucose agar with chloramphenicol; Lab Media Servis), SAB3 (Sabouraud's glucose agar with chloramphenicol and cycloheximide; Lab Media Servis), PDA (Potato Dextrose Agar; Sigma Aldrich, St. Louis, Missouri, USA;), MEA (Malt Extract Agar; Sigma Aldrich), CZA (Czapek Dox Agar; Lab Media Servis), CYA (Czapek Agar with Yeast Extract; Sigma Aldrich). FF detection equipment included a thermostat (Memmert, Schwabach, Germany), a microscope (Olympus BX 60, Tokyo, Japan), MALDI-TOF (Matrix Assisted Laser Desorption/Ionisation-Time of Flight, Bruker Daltonics GmbH, Hamburg, Germany), and a laminar flow cabinet EM 180 (MK Servis, Praha, Czech Republic).

The CI value was defined so that the questionnaire could measure the degree of intimacy between owners and pets. All owners reported activities (see Table 1) that indicate a higher possibility of microbiota exchange with their pets. The CI indicates their level of contact relevant to the transmission of microorganisms by values ranging within 1–8.

Table 1. Contact Index: Scoring the risk activities of owners, which could lead to microbiota exchange with their pets.

Activity	Score
Sharing the bed	1
Sharing the chair/sofa	1
Caressing and holding in arms	1
Hand licking	1
Face licking	1
Feet licking	1
Sharing the cutlery	1
Other activities (e.g. playing and direct contact with pet toys)	1
Total score	8

All collected samples were first inoculated for SAB and incubated at 25 °C for 7 days with periodic checks every 24 h. When colony growth was detectable macroscopically, FF colonies were inoculated onto selective media (e.g. CYA, MEA) to facilitate the assay. Presence of microscopic fungi was determined using microscopic observation techniques of native and cultured slides. These results were confirmed using MALDI-TOF identification system. Standard approaches such as chi-square test of independence (CHITEST function; MS Excel), linear regression model, and Mann-Whitney test were used for statistical analyses.

Throughout our study, we use the term ‘anamorph’, although it is not recognized by the recent taxonomic nomenclature (Turland et al. 2018; de Hoog 2015). In the taxonomic system set by The International Code of Botanical Nomenclature, ‘teleomorph’ is preferred to ‘anamorph’. However, for many fungal species, the growth phase from clinical material is expressed more specifically, and the names of anamorph are conventionally used in medicine (Otcenasek et al. 1990).

Results

Based on the questionnaire, we found that 43.2% (n = 54) of the owners live in a household with more pets. The CI value was > 4 for 87.3% (n = 131) of owners. The mean CI value equaled 6 for cats, 7 for dogs, and 5 for reptiles. The other mammals examined (2 guinea pigs and one rabbit) had the least close contact with humans on average (CI = 3.33) of the four pet animal species tested.

Analysis of all the samples collected (from owners, pets, and non-owners) resulted in the identification of a total of 110 fungal species (including lower taxa). A total of 108 fungal species (including lower taxa) were isolated from common households, of which 87% (n = 93) were identified in owner samples, 66.3% (n = 71) were isolated from both pet and human samples, and 23.7% (n = 22) were present exclusively in human samples. We identified 85 FF species in pet samples and 13.6% (n = 15) of the 110 species were isolated from pet samples only. In contrast, a total of only 65 FF species were isolated from the control group, but the spectrum of FF species was almost identical to that of the owner fungi, with the exception of two species (*Aspergillus lentulus* and *Aspergillus pseudotamari*) that were present only in the non-owner control group. The fungal species isolated in all three groups tested are summarized in Table 2; the fungal species isolated in particular pets are summarised in Table 3.

The most commonly shared species was *Aspergillus niger*. Only 14 of the identified shared species are proven to be pathogenic for humans, but the other isolated species with proven pathogenicity are only claimed to be pathogens in immunodeficient hosts (Fig. 1).

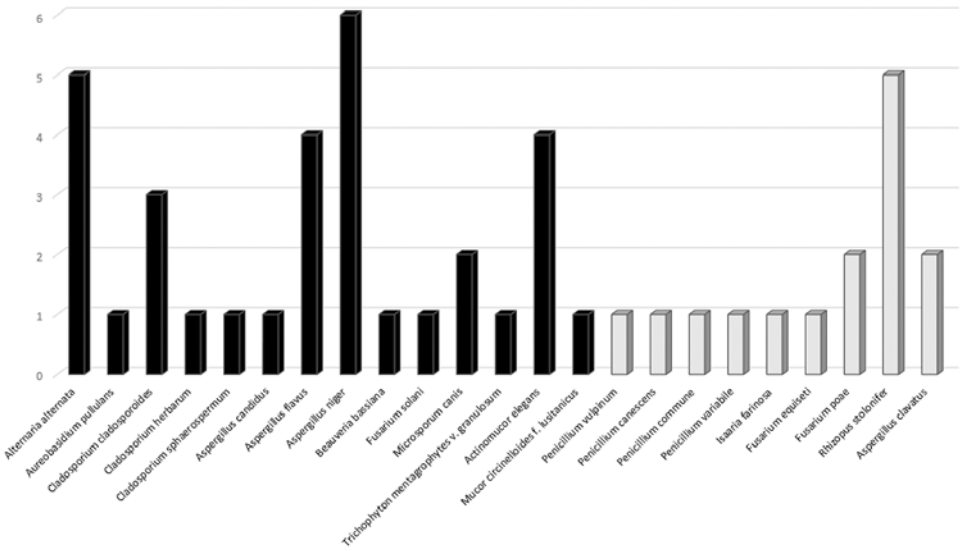

Fig. 1. The shared filamentous fungi species with proven/unproven zoopathogenicity. The shared fungal species pets/owners with respect to the mammalian pathogenicity. Pathogenic species are marked by black, non-pathogenic in grey.

Table 2. Summary of identified fungal species in our cohort.

1	*Absidia coerulea*	O, P	38	*Fusarium acuminatum*	O, P, C	75	*Paecilomyces marquandii*	O
2	*Acremonium berkeleyanum*	O, P, C	39	*Fusarium avenaceum*	P	76	*Paecilomyces variotii*	O, P, C
3	*Acremonium murorum*	O, P	40	*Fusarium culmorum*	O, P, C	77	*Penicillium canescens*	O, P, C
4	*Acremonium ochraceum*	O, P	41	*Fusarium dimerum*	O, P, C	78	*Penicillium citrinum*	O, P, C
5	*Acremonium potroni*	O, P, C	42	*Fusarium equiseti*	O, P	79	*Penicillium commune*	O, P, C
6	*Actinomucor elegans*	O, P, C	43	*Fusarium incarnatum*	O, P	80	*Penicillium crustosum*	O, P, C
7	*Alternaria alternata*	O, P, C	44	*Fusarium oxysporum*	P	81	*Penicillium digitatum*	O, P, C
8	*Alternaria tenuissima*	O	45	*Fusarium poae*	O, P, C	82	*Penicillium expansum*	O, P, C
9	*Aspegillus flavus*	O, P, C	46	*Fusarium solani*	O, P, C	83	*Penicillium griseofulvum*	P
10	*Aspergillus candidus*	O, P, C	47	*Fusarium verticilloides*	O, P, C	84	*Penicillium chrysogenum*	O, P, C
11	*Aspergillus carbonarius*	O, C	48	*Gongronella buttlerii*	P	85	*Penicillium italicum*	O, C
12	*Aspergillus clavatus*	O, P, C	49	*Chaetomium globosum*	O, P, C	86	*Penicillium lividum*	P
13	*Aspergillus fumigatus*	O, C	50	*Chrysolinia sitophila*	O, P, C	87	*Penicillium solitum*	O, P, C
14	*Aspergillus lentulus*	C	51	*Isaria farinosa*	O, P, C	88	*Penicillium thomii*	O, P, C
15	*Aspergillus nidulans*	O, P, C	52	*Isaria fumosorosea*	O, P	89	*Penicillium variabile*	O, P, C
16	*Aspergillus niger*	O, P, C	53	*Lichtheimia corymbifera*	O	90	*Penicillium viridicatum*	O
17	*Aspergillus ochraceus*	O	54	*Mariannaea elegans*	P	91	*Penicillium vulpinum*	O, P
18	*Aspergillus parasiticus*	O, P, C	55	*Microsporum canis*	O, P	92	*Phycomyces nitens*	O, P, C
19	*Aspergillus pseudotamari*	C	56	*Mortierella zychae*	P	93	*Rhizomucor pusillus*	O, P, C
20	*Aspergillus restrictus*	O, C	57	*Mucor hiemalis f. silvaticus*	P	94	*Rhizopus arrhizus*	O, P
21	*Aspergillus tamari*	O, C	58	*Mucor circinelloides f. circinelloides*	O, P, C	95	*Rhizopus microsporus*	O, P
22	*Aspergillus terreus*	O, P, C	59	*Mucor circinelloides f. griseocyanus*	O, P, C	96	*Rhizopus oryzae*	O, P
13	*Aspergillus ustus*	O, P, C	60	*Mucor circinelloides f. janssenii*	O, P	97	*Rhizopus stolonifer*	O, P, C
24	*Aspergillus versicolor*	O, P, C	61	*Mucor circinelloides f. lusitanicus*	O, P, C	98	*Scedosporium prolificans*	P
25	*Aureobasidium pullulans*	O, P, C	62	*Mucor dimorphosporus f. dimorphosporus*	O, P	99	*Scopulariopsis brevicaulis*	O, C
26	*Beauveria bassiana*	O, P	63	*Mucor dimorphosporus f. sphaerosporus*	O, P, C	100	*Schizophyllum commune*	P
27	*Beauveria brongniartii*	O, P	64	*Mucor genevensis*	O, P, C	101	*Stachybotrys chartarum*	O, P, C
28	*Beauveria felina*	P	65	*Mucor hiemalis f. corticolus*	O, P, C	102	*Syncephalastrum racemosum*	O
29	*Byssochlamys nivea*	P	66	*Mucor hiemalis f. hiemalis*	O, P, C	103	*Tolypocladium inflatum*	O
30	*Cladobotryum dendroides*	O	67	*Mucor hiemalis f. luteus*	O, C	104	*Trichoderma harzianum*	O, C
31	*Cladosporium cladosporioides*	O, P, C	68	*Mucor mucedo*	O	105	*Trichoderma viride*	O, P, C
32	*Cladosporium herbarum*	O, P, C	69	*Mucor piriformis*	O, P, C	106	*Trichophyton mentagrophytes v. granulosum*	O, P
33	*Cladosporium sphaerospermum*	O, P, C	70	*Mucor plumbeus*	O, P, C	107	*Trichophyton mentagrophytes v. interdigitale*	O, C
34	*Cunninghamella elegans*	O	71	*Mycotypha microspora*	P	108	*Trichophyton mentagrophytes v. mentagrophytes*	P
35	*Curvularia lunata*	O, P, C	72	*Myrothecium inundatum*	O	109	*Trichophyton rubrum*	O, C
36	*Doratomyces microsporus*	P	73	*Myrothecium roridum*	O	110	*Ulocladium atrum*	O, P
37	*Epidermophyton floccosum*	O	74	*Pacilomyces lilacinus*	O, P, C			

O - owners; P - pets; C - control group

Table 3. The fungal species isolated in particular pet animals.

No.	Species	Kind of pet	No.	Species	Kind of pet	No.	Species	Kind of pet
1	*Absidia coerulea*	D	31	*Fusarium culmorum*	D	61	*Paecilomyces lilacinus*	C, D
2	*Acremonium berkeleyanum*	D	32	*Fusarium dimerum*	D, DR	62	*Paecilomyces variotii*	D
3	*Acremonium murorum*	D	33	*Fusarium equiseti*	D	63	*Penicillium canescens*	D
4	*Acremonium ochraceum*	C, D	34	*Fusarium incarnatum*	D	64	*Penicillium citrinum*	C
5	*Acremonium potroni*	D	35	*Fusarium oxysporum*	D	65	*Penicillium citrinum*	C
6	*Actinomucor elegans*	C, D	36	*Fusarium poae*	C, D, GP	66	*Penicillium commune*	D, BC
7	*Alternaria alternata*	C, D, GP	37	*Fusarium solani*	C, D, GP	67	*Penicillium crustosum*	D, GP
8	*Aspegillus flavus*	D	38	*Fusarium verticilloides*	D	68	*Penicillium digitatum*	C, D
9	*Aspergillus candidus*	D	39	*Fusarium violaceum*	C, D	69	*Penicillium expansum*	C, D
10	*Aspergillus clavatus*	C, D	40	*Gongronella buttlerii*	D	70	*Penicillium griseofulvum*	C, D
11	*Aspergillus flavus*	C, D	41	*Chaetomium globosum*	D	71	*Penicillium chrysogenum*	C, D
12	*Aspergillus nidulans*	C, D, DR	42	*Chrysolinia sitophila*	D	72	*Penicillium lividum*	C, D
13	*Aspergillus niger*	C, D	43	*Isaria fumosorosea*	C	73	*Penicillium solitum*	D
14	*Aspergillus parasiticus*	D	44	*Isaria farinosa*	D	74	*Penicillium thomii*	D
15	*Aspergillus terreus*	C, D, DR, GP	45	*Mariannaea elegans*	C	75	*Penicillium variabile*	C, D, GP
16	*Aspergillus ustus*	C, D	46	*Microsporum canis*	C, D	76	*Penicillium vulpinum*	D
17	*Aspergillus ustus*	D	47	*Mortierella zychae*	C	77	*Phycomyces nitens*	D
18	*Aspergillus versicolor*	D	48	*Muc. hiemalis f. silvaticus*	D	78	*Rhizomucor pusillus*	C, D
19	*Aureobasidium pullulans*	D, BC	49	*Mucor circinelloides f. circinelloides*	D	79	*Rhizopus arrhizus*	D
20	*Beauveria bassiana*	BD, C	50	*Mucor circinelloides f. griseocyanus*	D	80	*Rhizopus microsporus*	D
21	*Beauveria brongniartii*	D	51	*Mucor circinelloides f. janssenii*	D	81	*Rhizopus oryzae*	C, D
22	*Beauveria felina*	BA	52	*Mucor circinelloides f. lusitaniae*	C, D	82	*Rhizopus stolonifer*	C, D
23	*Byssochlamys nivea*	D	53	*Mucor dimorphosporus f. dimorphosporus*	C, D	83	*Scedosporium prolificans*	D
24	*Cladosporium cladosporioides*	BD, C, D	54	*Mucor dimorphosporus f. sphaerosporus*	C, D	84	*Schizophyllum commune*	D
25	*Cladosporium herbarum*	C, D	55	*Mucor genevensis*	D	85	*Stachybotrys chartarum*	D
26	*Cladosporium sphaerospermum*	BD, C, D	56	*Mucor hiemalis f. corticolus*	D	86	*Trichoderma viride*	D
27	*Curvularia lunata*	C, D	57	*Mucor hiemalis f. hiemalis*	D	87	*Trichophyton mentagrophytes v. granulosum*	D
28	*Doratomyces microsporus*	D	58	*Mucor piriformis*	D	88	*Trichophyton mentagrophytes v. mentagrophytes*	D
29	*Fusarium acuminatum*	C, D	59	*Mucor plumbeus*	C, D	89	*Ulocladium atrum*	C, D
30	*Fusarium avenaceum*	C, D	60	*Mycotypha microspora*	D			

BC - boa constrictor; BD - bearded dragon; C - cat; D - dog; DR - dwarf rabbit; GP - Guinea pig

Of the owner samples, 54.8% (n = 51) of hyaline hyphomycetes, 28% (n = 26) of mucormycetes, 11.8% (n = 11) of pigmented hyphomycetes, and 5.4% (n = 5) of dermatophytes were isolated. The species spectrum of FF in pets was similar to that of owners. Hyaline hyphomycetes were the most common, accounting for 55.3% (n = 47) of the species. Less commonly isolated fungi were zygomycetes (28.2%; n = 24), pigmented hyphomycetes (10.6%; n = 9) and dermatophytes (4.7%; n = 4). Surprisingly, we isolated 1 (1.2%) basidiomycete from a pet. This species was absent in all human samples. It grew as sterile mycelium and was identified as a common lignicolous species *Schizophyllum commune,* which is a dangerous occasional pathogen. A total of 65 FF species were isolated from the control group. Hyaline hyphomycetes were the most common, representing 64.6% (n = 42) which is similar to the other groups (Fig. 2).

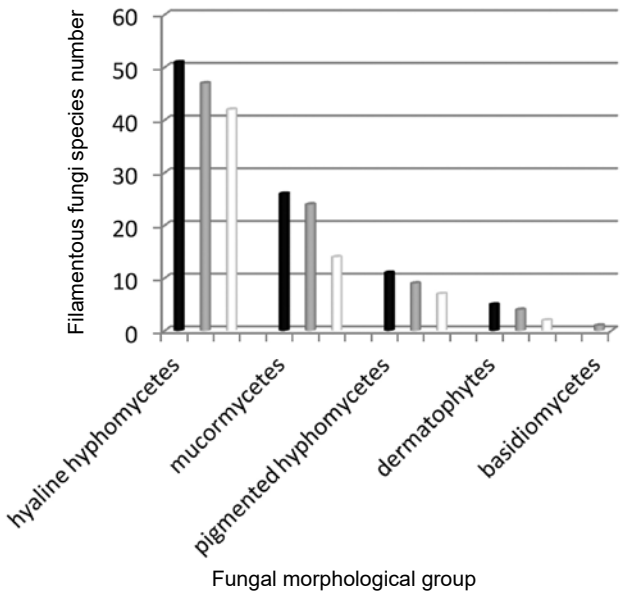

Fig. 2. The representation of the morphological groups of filamentous fungi (FF) isolated from samples. The quantity of representation of isolated FF taxonomical groups in owners (■), pets (■) controls (□).

In our cohort, 28.67% (n = 43) of owners and 34.8% (n = 46) of pets had received ATB treatment during the year preceding the sampling. In the control group, we identified 31.25% (n = 25) of ATB users. The number of FF species isolated from the samples of all three groups of ATB users studied (owners n = 43, pets n = 46, and control group n = 25), represents more than half of the FF species isolated (Fig. 3). The FF biodiversity of pets was minimally affected by ATB therapy, as in the control group. In owners, ATB treatment played a more important role.

From the owners, 36 FF species were isolated from the nasal mucosa, 43 from the ear canal, 39 from the axillae, 73 from the foot areas and 5 from suspicious lesions. The abundance of FF species isolated was the highest in samples from the toes in both groups (pets/owners) (Fig. 4).

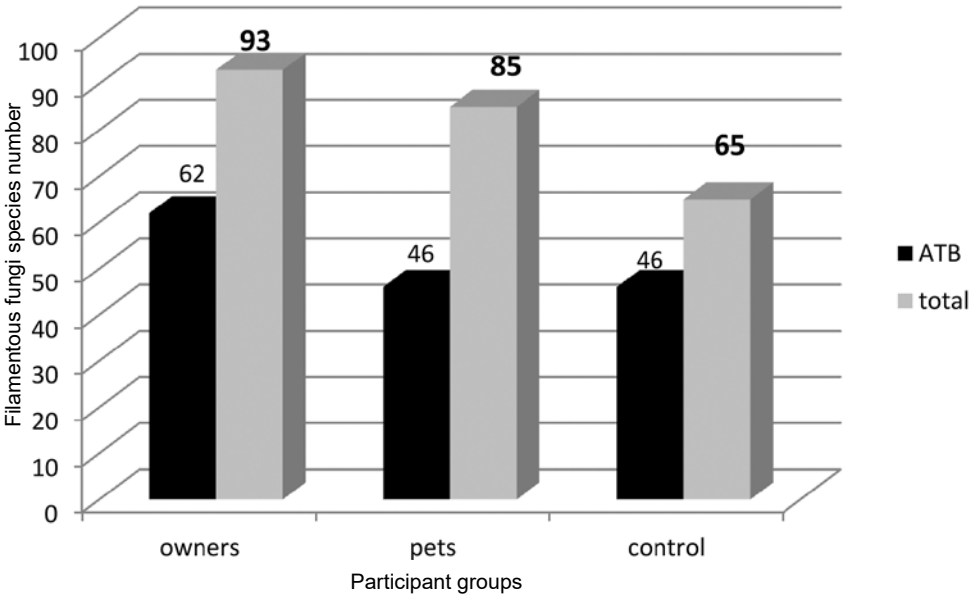

Fig. 3. The total counts in participant groups compared to antibiotics (ATB) users.

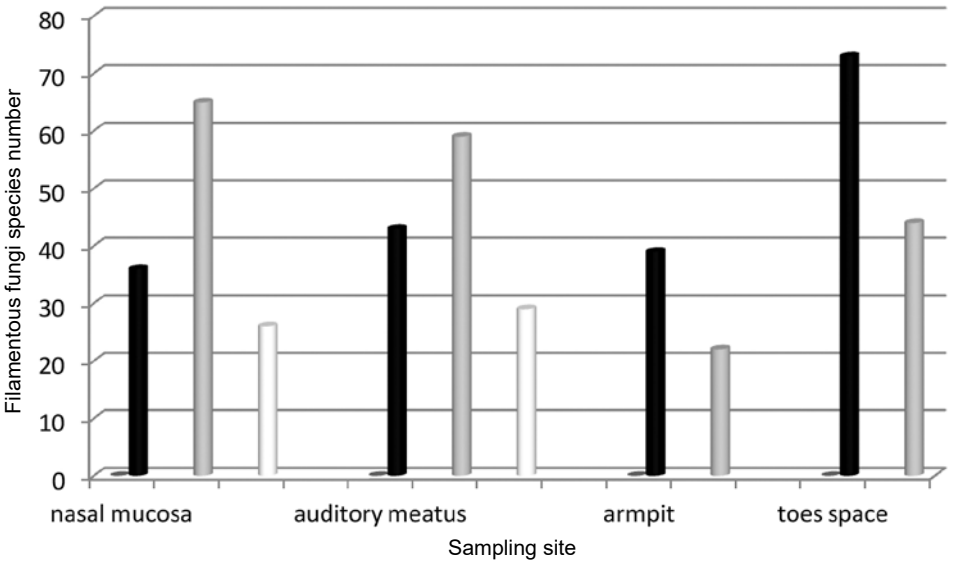

Fig. 4. The comparison of filamentous fungi species abundance in all participant groups regarding the type of tested samples in owners (■), pets (■) controls (□).

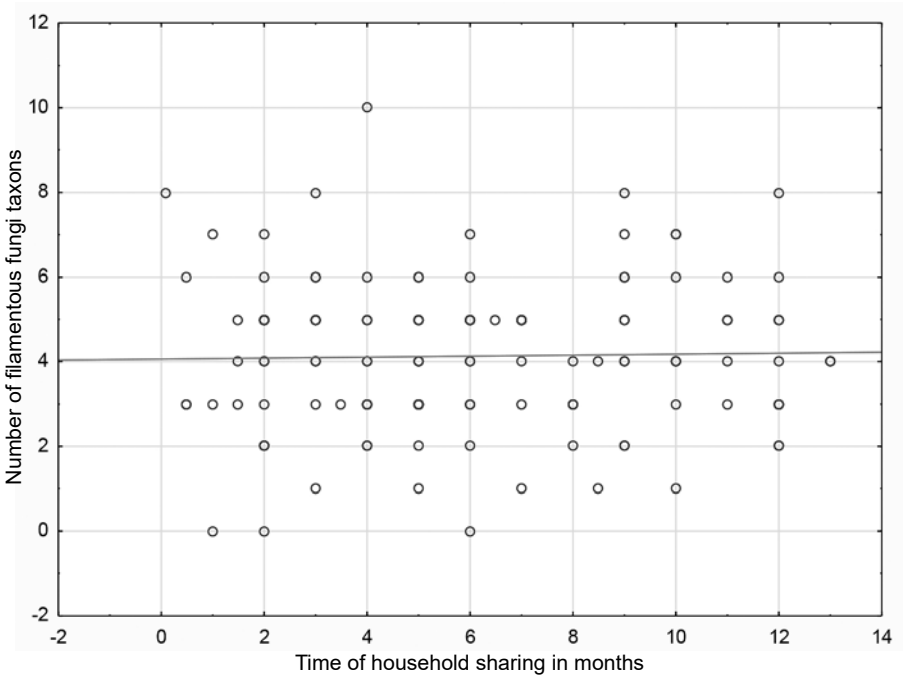

Fig. 5. Linear regression describing the dependence of the number of species on the time of household sharing with pets.

Using chi-square tests of independence (CHITEST function; MS Excel), we tested whether CI influenced the number of FF species colonizing the owners. We did not demonstrate a significant relationship between CI and the number of FF species ($P = 0.244$), and there was no effect of CI on the number of FF species shared ($P = 0.608$). Similarly, we did not demonstrate a significant relationship between the number of FF species in the owners and control groups ($P = 0.465$). Using a linear regression model, there was no relationship between the number of FF species and the time of sharing the household with pets ($P = 0.805$) (Fig. 5). This suggests our assumption that FF are not very effective colonizers of mucous membranes.

Subsequently, we sought to analyse whether the time of sharing a household with a pet affects the amount of fungal species shared. The dependent variable took only three values of 0 (94 ×), 1 (26 ×) or 2 (5 ×). Therefore, the data were combined into two categories: 1) the amount of FF species shared equals 0, versus 2) the amount of FF species shared equals 1 or 2. Mann-Whitney test was performed for these two categories ($P = 0.810$). There was no significant difference in sharing time between the two categories.

Finally, we analysed how ATB treatment affects the species abundance of FF in humans (Fig. 6). The difference in abundance between participants taking ATB and not taking ATB was again compared by Mann-Whitney test with a determined significance of $P = 0.0017$. The same analysis was therefore performed for owners (Fig. 7) and separately for non-owners control group (Fig. 8). The difference in the number of owners with and without ATB was compared by Mann-Whitney test and found to be non-significant ($P = 0.854905$). Differences in the number of FF types in the control group between ATB users and non-users were analysed by Mann-Whitney test which yielded a significant result ($P = 5 \cdot 10^{-6}$).

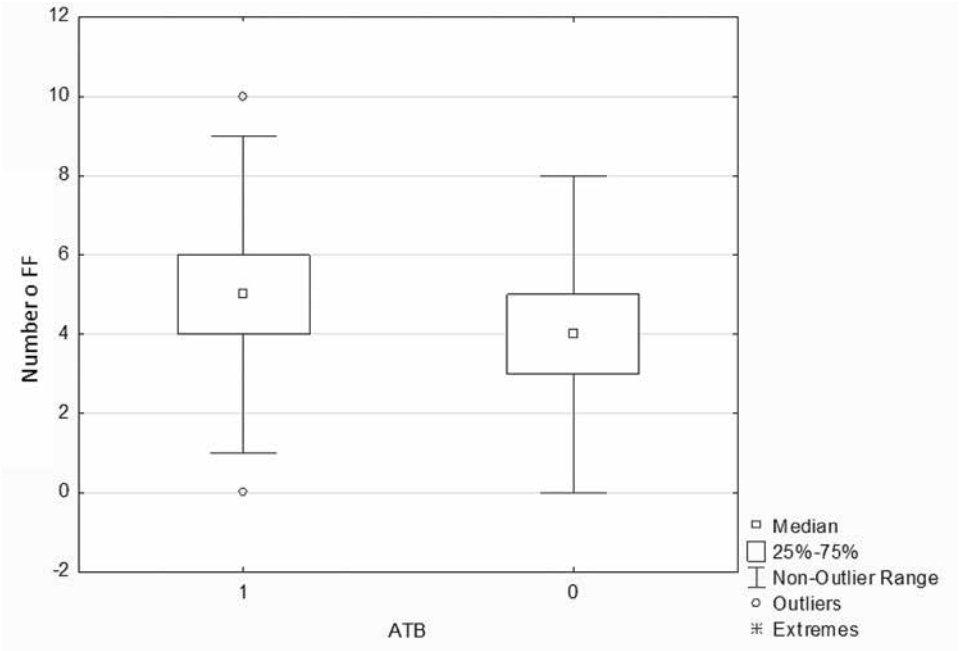

Fig. 6. Number of filamentous fungi (FF) species in humans using (1) and not using (0) antibiotics (ATB) (pet owners and petless controls together).

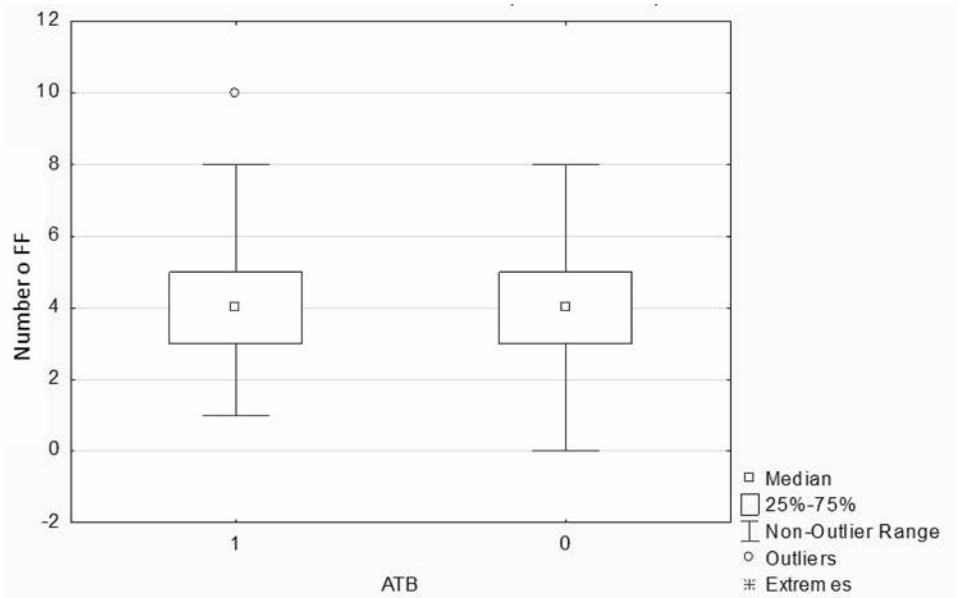

Fig. 7. Number of filamentous fungi (FF) species in pet owners using (1) and not using (0) human antibiotics (ATB). X axis: ATB users (1), non-users (0); Y axis: number of FF taxons

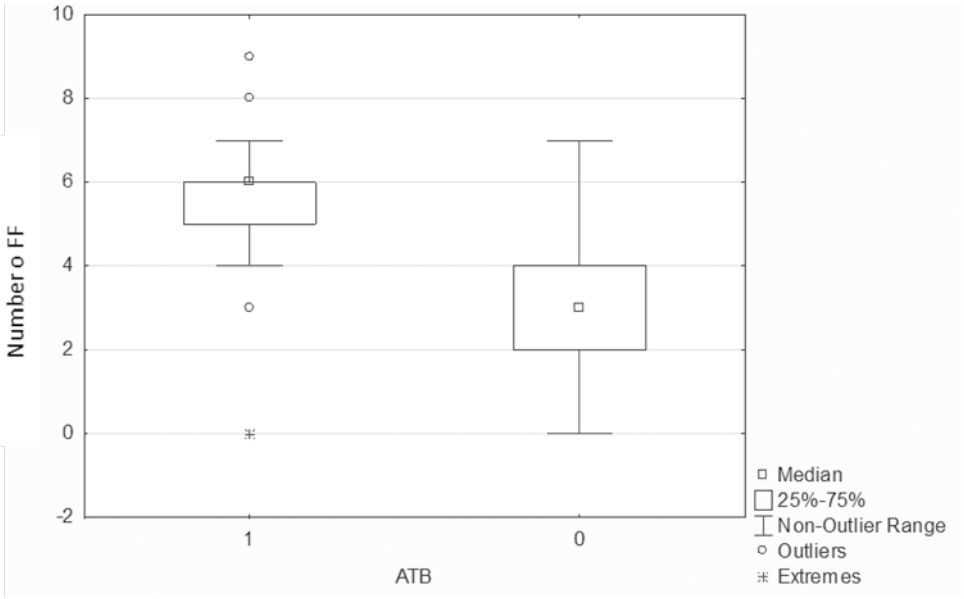

Fig. 8. Number of filamentous fungi (FF) species in control petless humans using (1) and not using (0) antibiotics (ATB). X axis: ATB users (1), non-users (0); Y axis: number of FF taxons

Discussion

The aim of our study was to investigate the level of health risk arising from pet ownership with respect to the prevalence of FF and microbiota balance compared to a control group. Furthermore, we set out to identify the mycobiota present in domestic animals in the Czech Republic. Finally, we investigated the effect of ATB treatment on the microbial ecosystem in all three groups. To the best of our knowledge, no similar large-scale study has been conducted in the Czech Republic.

Statistics (2022) confirm that 42% of all households had at least one dog by 2021. The Czech Republic ranks third in the EU in dog breeding, after Romania and Poland (Statista 2022).

We followed 125 households of 150 owners, shared by 135 pets (81.48%; n = 110 dogs, 13.33%; n = 18 cats and 5.19% n = 7 other pets), with the most common pet being a dog.

We were the first author group to define the CI as part of the study, which was used for subsequent statistical analyses. The CI has an indicative value of probability with which microbiota communication between owner and animal occurs. This value is based on the repeated activities that we assessed as most likely to transmit and colonize the microorganisms that were subsequently isolated from the samples.

Similar studies were carried out before. In 260 households in the UK, only 14% owners were found to share their bed with their dog, but overall, 45% of cats slept in the bed next to their owner (Westgarth et al. 2008). A study conducted in the Netherlands indicates that 50% of owners allow the animal to lick their face, 45% of dogs and 62% of cats can climb on the owner's bed but no more than 30% of animals can sleep in the same bed with the owner and lick their face, 45% of dogs and 62% of cats can climb on the owner's bed but no more than 30% of animals can sleep in the same bed with the owner (Overgaauw

et al. 2009; data summarized in Chomel and Sun 2011). In our sample, the CI was > 4 for 87.33% (n = 131) of owners, indicating a very close relationship between the owners tested and their pets. In 61.33% (n = 92) of owners, the pet licks their face, 66.67% (n = 100) of owners let their pet sleep in their bed, 43.33% (n = 65) of owners use the same utensils and sometimes a spoon to share meals with their pet. The higher level of contact can be attributed to the strong emotionality of Czech owners and their close relationship with pets as family members. Our work builds on previous studies and contributes to expanding and refining knowledge about the nature of mycobiota in pet owners and their possible influence on health status.

In our study, we further investigated the prevalence of FF and other microorganisms in domestic animals and compared our results with previous studies. Meason-Smith et al. (2015) characterized the cutaneous mycobiota of 18 dogs. Using whole-genome sequencing methods, they investigated the effect of the sampling site and dog health status on the distribution of fungal species. Analyses showed that the sampling site was not a factor affecting the abundance or structure of the mycobiota of healthy skin, but mucous membranes were colonized by a narrower species spectrum. The mycobiota of allergic skin was significantly poorer than that of healthy skin. An interesting finding was that the genera *Alternaria* and *Cladosporium,* two of the most common human allergens, were most frequently isolated from the skin of the dogs included in the study, regardless of their health status (Meason-Smith et al. 2015). The species spectrum of FF recovered from the skin of our canine population was similar. However, only a small number of samples were collected from affected skin areas, which is a limitation of our study. The most common fungal species isolated from skin samples in our study were *Cladosporium herbarum*, *Actinomucor elegans*, *Alternaria alternata* and two species of the genus *Penicillium* (*P. thomii* and *P. commune)*. The dermatophyte *Microsporum canis* was isolated in only one case from a dog. The other 3 isolates of *M. canis* were isolated from the ears and nasal mucosa of asymptomatic cats.

Mycobiota of cats was also monitored by the same authors (Meason-Smith et al. 2017) using the same methods. They collected 132 samples from 11 healthy cats and 54 samples from 9 allergic cats. They sampled healthy cats at twelve body sites, allergic cats at six sites. The most abundant FF sequences from the skin of all cats were identified as *Cladosporium* spp. and *Alternaria* spp. Findings from mucous membranes, including the nostrils, conjunctivae, and reproductive tract, were the least mycologically rich, whereas the external ear canal was the most mycologically rich. Significantly higher numbers of representatives from the classes Agaricomycetes and Sordariomycetes, but significantly fewer representatives of *Epicoccum* spp. were found on the skin of allergic cats compared to healthy cats (Meason-Smith et al 2017). In the cats in our cohort, 22 FF species were isolated from the ear canal. The most common finding was *Aspergillus niger* (44.44%; n = 8), which is also commonly found in human earwax. *Aspergillus niger* also dominated the total number of the pets' ear canal swabs in our study (almost 30%). The genus *Penicillium* was isolated from half of the feline ear canal samples, with *P. chrysogenum* being the most common. Zygomycetes were isolated in 33.33% (n = 6) of the ear canal swabs. The nasal mucosa of the cats differed in FF composition. Zygomycetes were not as frequently isolated here, but the pigmented hyphomycete *Cladosporium herbarum* was the most frequently isolated. This is probably due to the fact that cats do not use their sense of smell as much and do not inhale as many spores, especially those of coprophilic zygomycetes, as dogs do. Moreover, indoor cats never leave their home, unlike dogs that are taken for walks outside by their owner.

Sedlák and Tomšíčková (2006) reported that *M. canis* causes dermatomycosis in up to 90% of cats and dogs. This zoophilic species also causes dermatomycosis in humans (Sedlák and Tomšíčková 2006). In the cases of two households in our study, these

keratinophilic fungi were shared by both the owner and the cat. We conclude that this is likely to be a zoonotic transmission from the pet to the owner, so far without clinical cutaneous manifestations. Anthropozoophilic transmission of this highly contagious dermatophyte from an asymptomatic dog to a human was described in a review article by Katoh et al. (1991). Asymptomatic animal reservoirs of *M. canis* are considered a critical epidemiological factor for human dermatomycosis (Katoh et al. 1991).

Cafarchia et al. (2006) studied a total of 136 dogs and 248 cats. Of these, 78 animals (22 dogs and 56 cats) were individuals affected by tinea corporis caused by *M. canis* and 306 (114 dogs and 192 cats) were individuals without dermatophytosis. Age, sex, breed, location and sampling period were recorded for each animal. Dermatophytes were isolated from 20.5% of dogs and 28.2% of cats. *Microsporum canis* was isolated from 36.4% dogs living in the same household with owners diagnosed with tinea corporis, but was never isolated from dogs whose owners were asymptomatic. In contrast, *M. canis* was isolated from 53.6% of cats living in the same household with owners diagnosed with tinea corporis and from 14.6% of cats whose owners had no symptoms. Their results clearly show that both cats and dogs are considered to be a major source of pathogenic dermatophytes in humans, even when they are asymptomatic (Cafarchia et al. 2006). A Japanese study by Kano (2012) lists *M. canis* as the most common zoophilic agent of DM-to-human-transmitted dermatomycosis (Kano 2012).

Focusing on fungi shared betwen owners and pets, we found that *Aspergillus niger* was the most commonly shared species. In terms of potential health risk, we identified 14 shared species with proven pathogenicity. Dermatophytes have an affinity for the skin adnexa and epidermis of mammals, therefore, their pathogenicity is primary. The other isolated species with proven pathogenicity are opportunistic (Fig. 1).

One of the aims of our study was to assess the extent of the effect of ATB treatment on mycobiota and bacteriobiota in owners. Noverr et al. (2004) observed an association between ATB-induced gut dysbiosis and allergic asthma in mice, which they had previously observed in patients after long-term ATB administration. The experimental animals showed a steady increase in gastrointestinal *Enterobacteriaceae* and *Candida* spp. without introduction of microbes into the lungs. Mice were treated with cefoperazone for 5 days. They were then given a single oral gastric probe containing *C. albicans*. This was followed by changes in the gastrointestinal bacterial population and an increase in yeast numbers for at least 2 to 3 weeks, resulting in the development of a CD4 cell-mediated allergic airway reaction after subsequent exposure to an aerosol of *Aspergillus fumigatus* conidia. Mice not treated with ATB did not develop an allergic reaction after exposure to the conidia. That study provided the first experimental evidence of the effect of ATB on intestinal mycobiota while promoting the development of allergic airway disease (Noverr et al. 2004). Our recent pilot study on the same issue, although showing a strong correlation between the use of ATB therapy in the last year and the species abundance of isolated fungi, also involved a small number of measurements. In fact, only 5 (25%) of the pets and 5 (25%) of the farmers surveyed used ATB (Wipler et al. 2018).

In the current study, 34.05% (n = 46) of the tested animals and 28.67% (n = 43) of the tested owners used ATB treatment in the last year. For both owners and pets, ATB therapy showed no significant effect on either the number of FF species isolated or the number of common FF species compared to the owners and pets who had not received any ATB therapy during the year preceding the sampling. In contrast, a significant effect on the abundance of FF species was demonstrated in the control group of 31.25% (n = 25) ATB users. An explanation may be the prolonged period in non-owners when the disturbed ecosystem returns to its original equilibrium. For owners, the bacterial species comprising the microbiota of their animal are the reservoir during ATB treatment. Therefore, after ATB treatment, a balanced ecosystem is restored more quickly than in non-owners.

In conclusion, this study makes an important contribution to the understanding of the risks and benefits of pet farming in terms of sharing FF and maintaining microbial balance in humans. The main objective of the study was to test whether fungal isolates can be transmitted from pets to their owners. There appears to be a relatively stable ecological balance between fungal and bacterial microbiota.

We examined several factors that may influence communication, such as previous ATB treatment, household sharing time, and the CI value. The results suggest that a close relationship between owner and pet does not increase the risk of a negative health outcome caused by mycopathogens or mycoallergens in a healthy immunocompetent macroorganism, with the exception of dermatophytes causing skin infections, where pets can be asymptomatic carriers. To avoid this, screening may be recommended before welcoming a new pet as a family member.

The balance of common bacterial and fungal microbiota does not appear to be affected by the length of pet ownership or CI. However, the results suggest that the equilibrium of both components may be temporarily disturbed by the administration of inhibitory agents such as ATB. Interestingly, our results also suggest that microbial equilibrium is restored more rapidly in the pet owner group (for both owners and pets) than in the control petless group.

Sharing a home with a companion animal and developing a close relationship with it may be a bigger boost to the owner's immune system, supported by their psychological well-being, than living in a disinfected home with minimal contact with outside microorganisms. We believe that only by re-monitoring the FF species spectrum in pets can we obtain more indicative information on which species are colonizers and which are only occasional skin and mucous membrane contaminants.

A limitation of our study is the situation in patients with impaired immune mechanisms (e.g. diabetes mellitus, users of immunosuppressive therapy, and haematological malignancies). In a case of inadequate immune response, especially when accompanied with prolonged neutropaenia, some FF species may be opportunistic pathogens and lead to serious infectious complications. When such individuals get a new pet, they may be at risk especially if they develop a very close relationship with it. Further studies are needed to clarify the level of such risk and to discover the clinical impact of sharing a household with pets in more detail, shedding light on how it may influence immunopathologies, such as immunodeficiency or allergy.

Acknowledgements

We thank all the pet owner families for their cooperation. This work was supported by Cooperative Research Area Oncology and by the internal grant project SVV 260 398 of the Faculty of Medicine in Hradec Králové of the Charles University in Prague.

References

Cafarchia C, Romito D, Capelli G, Guillot J, Otranto D 2006: Isolation of *Microsporum canis* from the hair coat of pet dogs and cats belonging to owners diagnosed with *M. canis* tinea corporis. Vet Dermatol **17**: 327-331

Chomel BB, Sun B 2011: Zoonoses in the bedroom. Emerg Infect Dis **17**: 167-172

de Hoog GS, Chaturvedi V, Denning DW, Dyer PS, Frisvad JC, Geiser D, Gräser Y, Guarro J, Haase G, Kwon-Chung KJ, Meis JF, Meyer W, Pitt JI, Samson RA, Taylor JW, Tintelnot K, Vitale RG, Walsh TJ, Lackner M; ISHAM Working Group on Nomenclature of Medical Fungi 2015: Changes in the names of medically important fungi and their implications for clinical practice. J Clin Microbiol **53**: 1056-1062

Garbee DD, Pierce SS, Manning J 2017: Opportunistic fungal infections in critical care units. Crit Care Nurs Clin North Am **29**: 67-79

Kano R 2012: Cutaneous mycoses in Japan originating from animals. Med Mycol J **53**: 19-23

Katoh T, Maruyama R, Nishioka K, Sano T 1991: Tinea corporis due to *Microsporum canis* from an asymptomatic dog. J Dermatol **18**: 356-359

Meason-Smith C, Diesel A, Patterson AP, Older CE, Mansell JM, Suchodolski JS, Rodrigues Hoffmann A 2015: What is living on your dog's skin? Characterization of the canine cutaneous mycobiota and fungal dysbiosis in canine allergic dermatitis. FEMS Microbiol Ecol **91**: fi139

Meason-Smith C, Diesel A, Patterson AP, Older CE, Johnson TJ, Mansell JM, Suchodolski JS, Rodrigues Hoffmann A 2017: Characterization of the cutaneous mycobiota in healthy and allergic cats using next generation sequencing. Vet Dermatol **28**: 71-e17

Mercier T, Maertens J 2017: Clinical considerations in the early treatment of invasive mould infections and disease. J Antimicrob Chemother **72**: 29-38

Noverr MC, Noggle RM, Toews GB, Huffnagle GB 2004: Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. Infect Immun **72**: 4996-5003

Otčenášek M, Hejtmánek M 1990: Identifikace izolátů (in Czech, Isolate Identification). In: Otčenášek M (Ed.): Vyšetřovací metody při mykotických onemocněních. Avicenum, Prague, 152 p.

Overgaauw PA, van Zutphen L, Hoek D, Yaya FO, Roelfsema J, Pinelli E, van Knapen F, Kortbeek LM 2009: Zoonotic parasites in fecal samples and fur from dogs and cats in the Netherlands. Vet Parasitol **7**: 115-122

Sedlák K, Tomšíčková M 2006: Deset nejvýznamnějších zoonóz v České republice; Dermatofytózy (in Czech, Ten Most Important Zoonoses in the Czech Republic; Dermatophytoses). In: Sedlák K (Ed.): Nebezpečné infekce zvířat a člověka. Scientia, Prague, 167 p.

Sullivan A, Edlund C, Nord CE 2001: Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect Dis **1**: 101-114

Statista, Bedford E, Consumer Goods & FMCG Pets & Animal Supplies: Dog owning households in Europe 2010-2021. Available at: www.statista.com/statistics/515299/households-owning-a-dog-europe/ Accessed September 27, 2022.

Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusbe, W-H, Li D-Z, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF (Eds) 2018: International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books

Westgarth C, Pinchbeck GL, Bradshaw JW, Dawson S, Gaskell RM, Christley RM 2008: Dog-human and dog-dog interactions of 260 dog-owning households in a community in Cheshire. Vet Rec **5**: 436-442

Wipler J, Čermáková Z, Hanzálek T, Horáková H, Buchta V 2018: Pets (dogs/cats) as a possible source of opportunistic pathogenic fungi in humans. Klin Mikrobiol Infekc Lek **24**: 41-49