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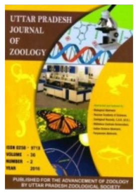

DO PET ANIMALS POSE A RISK OF FUNGAL INFECTIONS TO THEIR OWNERS?

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author ID designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BDK managed the analyses of the study. Author MB managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

ABSTRACT

Introduction: Among various microorganisms, that colonize skin of the pets, fungi, not only acts as opportunistic, but also potentially pathogenic for both humans and animals. It is expected that the closest environment of animals, their dens, collars or brushes could be the potential source of zoonotic pathogens. The aim of the study was to determine the frequency of fungi isolation from the hair coat and skin surface of healthy companion animals, their dens and from the skin of their owners' hands.
Materials and Methods: The samples were obtained in August from the healthy skin of 25 animals' necks by brushing and 25 dens of the same animals` by swabbing and the next 25 specimens were collected from the surface of their owners hands, also with swabbing technique. All samples were cultivated and fungi were identified with routine methods.
Results: A total number of 114 fungal strains, mainly mycelial fungi (93.86%) were isolated. Among them 33 isolates came from animal's skin surface, 39 originated from the skin of owners hands. The next 42 isolates were obtained from the animals' dens. Yeasts or yeast-like fungi were found in 6.14% of positive results of fungal growth. Dermatophytes were not isolated. The most prevalent organisms isolated from investigated samples were different species of *Alternaria* sp.
Conclusion: The skin of healthy companion animals (pets) maybe the source of different, mainly mycelial fungi, which are opportunistic in nature. Asymptomatic carriers of dermatophytes or other pathogenic fungi were not detected among tested animals.

Keywords: Pathogenic fungi; animals; mycoses; yeasts; mycelial fungi.

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1. INTRODUCTION

Medically important fungi can be recognized as opportunistic pathogens or as basic, primary pathogens, like dermatophytes or dimorphic fungi. Opportunistic fungal infections are the majority among described mycoses [1], mainly in immunocompromised hosts [2], such as AIDS patients, transplant receivers and oncological or diabetic patients. Among typical fungal pathogens are dimorphic fungi, eg. *Blastomyces dermatitidis, Coccidioides immitis/posadasii, Histoplasma capsulatum* and *Paracoccidioides brasiliensis* causing endemic mycoses. It is typical for both of the American continents, but usually not found in Poland [2], but the appearence of imported dimorphic infection in Poland cannot be excluded. Typical dermatophytes which are pathogenic for animals belong to the *Microsporum* or *Trichophyton* genus and may be an etiological agent of human dermatophytoses - ringworm (zoonoses), too [3,4]. Dermatophytoses may be easily transmitted from animal to animal or from animal to human. Pets, especially cats, and other companion animals such as rabbits, guinea pigs or dogs are known to be the carriers of dermatophytes [5]. Moreover, the animal's grooming equipment like brushes or dens of dogs and cats, can be the source of infection, because the spores of dermatophytes are highly spreading and resistant to environmental pressures, like drying etc. and may stay viable during very long period [3,5]. In many human clinical cases, dermatophytosis as a zoonosis have been described in literature [5,6,7]. Fungi are well known to be a strong allergens and high concentration of fungal spores in air, might be a serious problem for people suffering for asthma [7,8,9,10] or other hypersensitivities. Therefore, pet owners create a higher risk to contribute a zoonotic diseases, also mycoses.

The aim of the study was to determine the isolation frequency of different fungi from skin of healthy, companion animals (dogs and cats), their dens and the skin of their owners' hands.

2. MATERIALS AND METHODS

The samples were collected in August from the hair-coated skin surface of 25 healthy animals (5 cats and 20 dogs) using brushing technique and by swabbing their dens. Also, the swabs from the skin of their owners hands were obtained. A total number of 75 specimens were collected and cultivated on Sabouraud Dextrose Agar (SDA, Becton Dickinson) in two variants: in 30°C supplemented with actidion (for dermatophytes) or without actidion in 37°C, and incubated for 4 weeks in standard conditions. All obtained colonies were passaged and pure cultures were investigated macroscopically by analyzing the rate of growth, texture and color of the colony. Also, microscopic slides were prepared to observe the type of hyphae and to evaluate spores formation, their arrangements and shape. Collected data were applied to identification on routine way; mycelial fungi were identified according to their morphological properties [11]. Classification of yeasts and yeast-like fungi was done on the basis of cells' and colonies' morphology and their biochemical properties, evaluated with API Candida microtests (bioMérieux).

Obtained results were subjected to statistical analysis. The dominance of individual fungal species was calculated according to the method described by Czachorowski [12] using the formula:

$$D_i = n/N * 100\%$$

were:

Di - is the dominance,
n - is number of the i^{th} species,
N - the total number of all species.

On the basis of calculated dominance, isolates were divided into classes, according to those proposed by Biesiadka and Kowalik [13]:

- Eudominants (the species of dominance more than 10%)
- Dominants (5,1-10%)
- Subdominants (2,1-5%)
- Reducers (below 2%)

Also, the Margalef index was used to evaluate the species diversity of all examined sources [14], using the formula:

$$d = S-1/lnN$$

were:

d - is the Margalef index,
S - is number of species,
N - is the total number of isolates.

According to Sienkiewicz [14] the higher Margalef index (d) is equivalent to the greater biodiversity.

3. RESULTS

From all 75 collected samples, a total of 114 fungal isolates were cultured; among them 33 isolates were originated from animals skin, 39 were obtained from the owners skin followed by 42 strains from animals dens. The majority of isolates were mycelial fungi

(93.86%), while the yeasts were identified only in 6.14% of all cultures. Dermatophytes were not found. In 4 investigated samples (2 samples from animals, 1 sample from owner and 1 sample from den) no growth was reported.

Among a total of 114 obtained isolates, 18 different fungal species were identified. The higher species biodiversity according with Margalef index d=2,94 (12 different fungal species), were observed among the isolates from the dens of animals, followed by 11 various species cultivated from their owners hands, what gives biodiversity with d=2,73. Also, 8 different species were obtained from animals skin surface and Margalef index (d) reaching 2.00 was calculated for that source. The most prevalent species was *Alternaria* fungi (47.37%). The fungi belonging to *Aspergillus* genus were isolated (18.42%), which was found less frequently. The scarce growth of *Scopulariopsis* genus (10.53%) and *Penicillium* genus (8.77%) was also observed (Fig. 1.)

The distribution of isolated species in all tested sources is demonstrated Fig. 2.

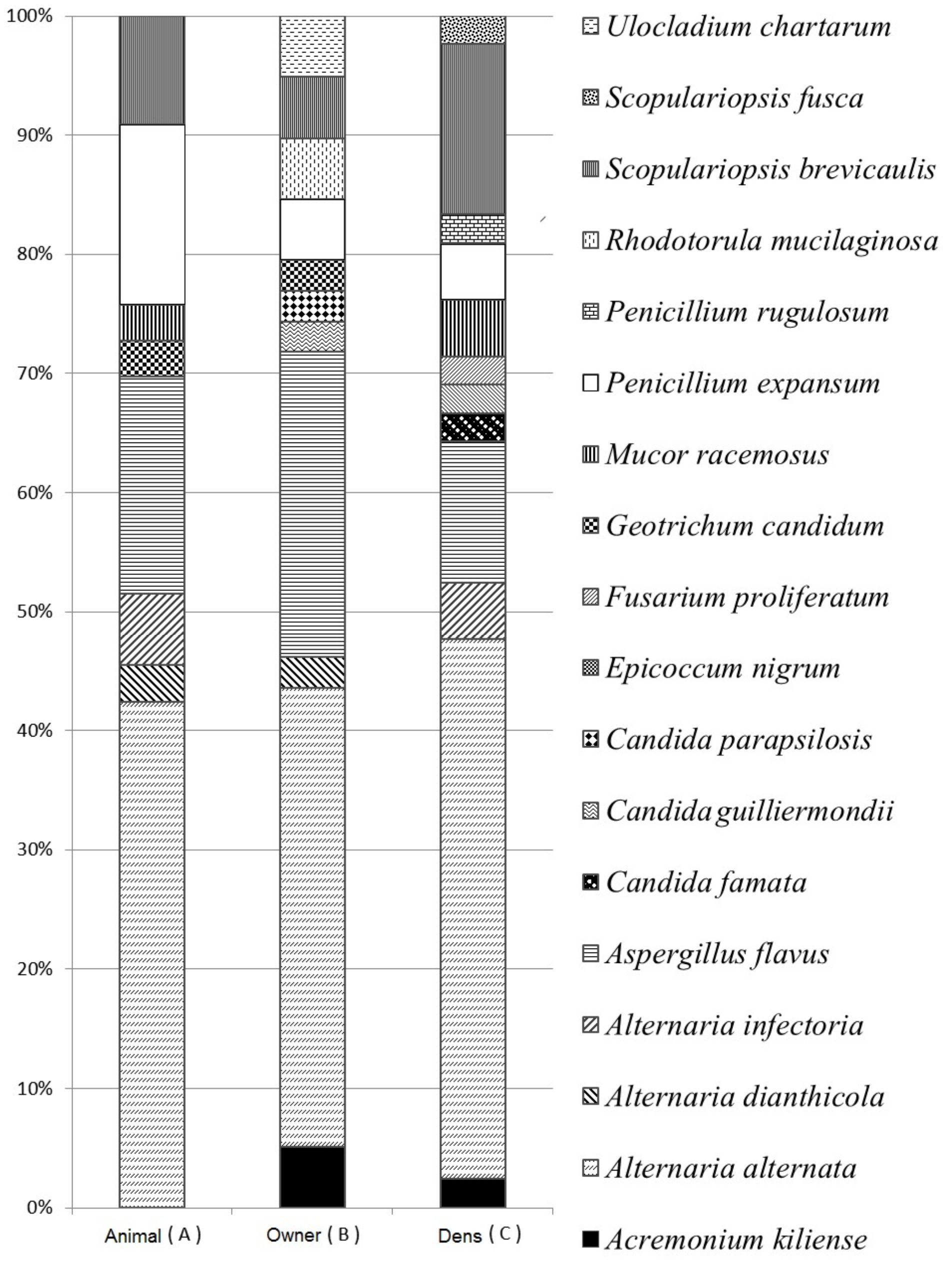

Fig. 1. The contribution of individual fungal species divided into examined sources

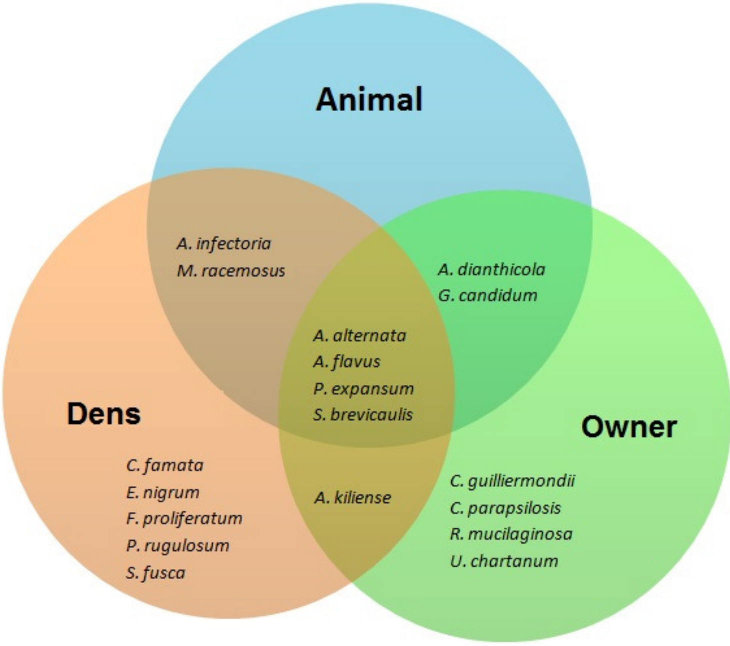

Fig. 2. Distribution of fungal species to all tested sources

The following fungal species: *Alternaria alternata* (Fig. 3), *Aspergillus flavus, Penicillium expansum* (Fig. 4.) and *Scopulariopsis brevicaulis* were commonly isolated from all investigated sources, while *Alternaria diathicola* and *Geotrichum candidum* were “common factors“ cultivated from skin, both pets and their owners.

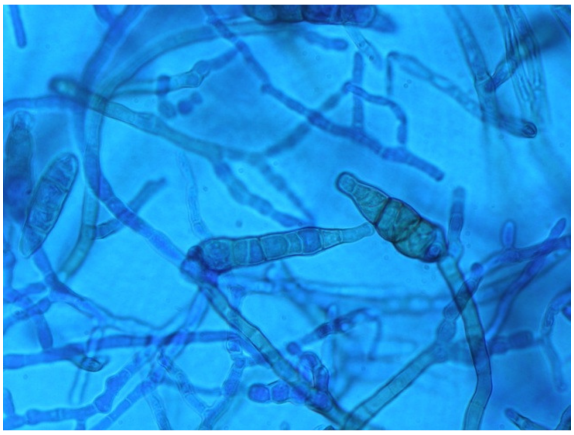

Fig. 3. Example of photomicrograph of Alternaria alternata. (Phot. I. Dąbrowska)

Among all isolated fungi, the eudominant for all tested sources were *Alternaria alternata* (42.42% of the isolates from animals skin, 38.46% of the isolates from the their owners skin and 45.24% of the isolates from their dens). Other significant eudominant was *Aspergillus flavus*; domination factor for this species was calculated for all tested sources as 18.18%, 25.64% and 11.90%, respectively. Furthermore, *Scopulariopsis brevicaulis* was shown to be a strong eudominant among the isolates from the animals dens (14.29%), while in the isolates from the skin of animals and their owners hands, it was observed as dominant species (9.09% and 5.13% respectively). Among the fungi isolated from the skin of humans' hands, *Acremonium kiliense, Peniciullium expansum, Rhodotorula mucilaginosa* and *Ulocladium chartanum* were the dominants.

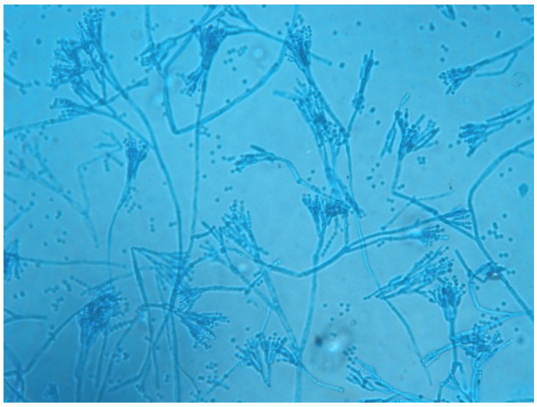

Fig. 4. Example of photomicrograph of Penicillium expansum. (Phot. I. Dąbrowska)

According to Margalef index (Table 1) the greatest species diversity was observed among isolates obtained from the animals dens (d = 2.94). The lowest species heterogeneity was noted among the samples isolated from animals (d=2.00).

Table 1. Biodiversity of fungal species

Sources	Number of samples [n]	Number of species [S]	Total number of isolates [N]	The average number of species per samples [α=S/n]	The Margalef index of biodiversity [d]
Animal	25	8	33	0,32	2,00
Owner	25	11	39	0,44	2,73
Dens	25	12	42	0,48	2,94

4. DISCUSSION

Molds are present everywhere – they are an important part of the natural environment and they are able to "clean the world" by decomposing dead organic matter. Fungi, including molds, can also colonize skin and mucous membranes of healthy host, both animals and humans [15,16,17]. In common opinion and according to literature [18,19,20,21,22], humans, working with the animals have higher risk to be infected by fungi. Moreover, the higher air contamination with the fungal spores was reported in the areas where animals were housed [20,22,23]. It is also suggested, that asymptomatic pet's carriers of potentially pathogenic fungi may pose a risk for human health, especially for immunocompromised persons.

Surprisingly, in our investigation, no dermatophyte growth from none of tested sources was found. It may suggest that healthy companion animals, kept in proper conditions are not the carriers of dermatophytes, as frequently as it was thought. Their role and importance as asymptomatic carriers in pathogenesis of human dermatophytosis should be carefully reconsidered. However, while the majority of known cases of asymptomatic carriers of dermatophytes were cats [23-27]. In our study, we have the predominance of dogs among examined animals, which may have influenced the results. But similarly, in the results of our other investigation (data yet not published) we didn't isolate any dermatophyte strain from hair coat of clinically healthy cats from the shelters for homeless animals (catteries).

In the present study, fungi isolated from the surface of animals skin or from pets nearest environment (dens) were identified as typical molds, mainly from *Alternaria*, *Aspergillus* or *Penicillium* species. Molds' spores readily enter indoor environments by circulating through doorways, windows, heating, ventilation and air conditioning systems or may be carried on the surface of people and animals skin or clothing, shoes and bags into indoor environments. According with report of the Committee on Environmental Health [28], the most common prevalent indoor molds are fungi from *Cladosporium, Penicillium, Aspergillus* and *Alternaria* genus. These fungi grow in rich, moisturised environments and may colonize surface of the living host. Some harmful molds may cause an opportunistic infections in the hosts with impaired immunity. Moreover, some airborne fungal spores eg. *Cladosporium, Penicillium, Aspergillus* or *Alternaria* are the common allergens and may develop clinical cases of allergy in both humans and pets, such as upper respiratory tract irritation, cough and eye or skin irritation. In some cases, the clinical signs of hypersensitivity are moderate, but some patients may require immediate and intensive medical assistance, after contact with fungal allergens. Among pet animals a lot of dogs suffer from allergic dermatitis and atopy. An allergy to mold and other environmental antigens maybe a genetic predisposition [29,30] that manifests as a skin irritation, that causes dog to itch. Other symptoms include secondary skin lesions,that are developed from excess scratching. Skin in these areas might be crusted over, raw or oily. When a dog inhales mold spores, his immune system, similarly to humans, start an overproduction of the IgE protein, which attaches to tissue mast cells in the dog's skin, and stimulates them to release the mediators of inflammation, including histamines, which further irritate the skin [30].

In our study the prevalence of molds on pets skin surface and in their dens was comparable to molds prevalence, usually found in indoor air [31,32]. Among 18 different isolates of fungi only 4 were commonly isolated from all investigated possible sources of fungi and only 2 species: *Alternaria diathicola* and *Geotrichum candidum* were isolated from skin surface from both human and animals, which eventually may suggest possibility of strain transmission.

5. CONCLUSIONS

On the basis of these results it can be concluded:

1. The skin of pets and their owners hand may be colonized by molds, but usually the similar prevalence of molds occurs in indoor environment.
2. The most frequently isolated organisms from animals skin, their dens and the hands of their owners, were fungi from *Alternaria* genus,

what may have significance for allergic patients
3. The asymptomatic carriage of dermatophytes on the skin of healthy pets animals was not shown.
4. It is suggested, that healthy owners of the healthy pets usually are not exposed to the higher risk of fungal infection.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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