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Mallard duck (*Anas platyrhynchos*) as a potential link in the epidemiological chain mycoses originating from water reservoirs

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Abstract

Swabs sampled from beaks and cloaca of 123 live Mallard ducks (also referred to as wild ducks) were subjected to routine mycological diagnostics. Out of 246 samples analysed, positive results were noted in 210 (85.4%) cases. The taxonomic analysis revealed 30 species of yeast and yeast-like fungi and 24 species of mould fungi, included 35 classified to BSL categories. The most frequently isolated species were: *Candida albicans, C. guilliermondii, C. krusei, Cryptococcus neoformans, Cr. laurentii, Rhodotorula rubra, Saccharomyces cerevisiae, Aspergillus fumigatus, A. flavus, A. niger,* and *Fusarium solani.* The results demonstrated that wild duck breeding in the proximate vicinity of man constitutes a rich reservoir of potentially-pathogenic fungi and a significant link in epidemiology of many mycotic diseases.

Key words: *Anas platyrhynchos,* opportunistic fungi, carrier, epidemiology.

Introduction

A source of mycoinfections posing a risk to man and other vertebrates may be polluted, strongly eutrophicated water ecosystems, whereas vectors of their transmission to man are animals inhabiting this environment (10, 11). Birds feeding in water with substances being rich in organic matter and often colonised by fungi with a various trophic and taxonomic status are worth noticing (12, 13).

As vectors of transmission and dissemination of microbes, birds have been the focus of interest particularly in the case of pathogenic bacteria (16, 29) and viruses, mainly avian influenza viruses (15, 35). Mycological data are significantly scantier and refer mainly to infected or dead birds originating from widely-understood cultures, from which single fungal species had been isolated (2, 10, 33). In turn, information on fungi isolated from wild fowl pertains mainly to microfungi colonising skin, feathers, and nails (22), as well as nests (17) and faeces (4, 13, 21). Mycological analyses of biological specimens collected from organ ontocenoses of live wild birds are sparse (11, 13). Determination of the role of birds living in close proximity of man (23) in the mechanisms of potentially-pathogenic fungi circulation in the biosphere is of a key significance in the context of an extending spectrum of environmental factors predisposing to mycotic infections, as well as in evaluation of the sanitary status of the environment, and assessment of epidemiological risk. Bearing these factors in mind, an attempt was undertaken to evaluate species diversity and prevalence of fungi colonising selected sections of the respiratory system and gastrointestinal tract of Mallard duck (*Anas platyrhynchos*), which is one of the most abundant representatives of the waterfowl inhabiting the northern hemisphere (37), accompanying man and prone to synurbisation (23).

Material and Methods

The experimental material comprised swabs from oral cavity and cloaca of 123 wild ducks, collected from live birds captured by banding (Gdańsk and Olsztyn) and hunted birds (Barczewo/ Olsztyn, Kartuzy,

Kwidzyń). Samples were collected with the use of a sterile cotton swab immersed in a solution of physiological salt before use.

The swabs with the collected material were fixed in a liquid Sabouraud medium with addition of gentamycin and chloramphenicol (0.1%), and transported to the laboratory. The samples were subjected to routine mycological diagnostics (11, 30). Preliminary macrocultures were established with Sabouraud, Czapek-Dox, and glucose-potato (PDA) agars with the addition of antibiotics, and incubated at 25, 37, and 40°C (required for the growth of termophilic species of mould fungi) for 48 h up to 7 d. After 48 h, yeast and yeast-like fungi appeared, whereas after 72 h the first mould fungi showing especially significant growth on the glucose-potato agar. After macroscopic characteristics of the grown colonies, the fungi were passaged onto the same media to proliferate the material for microcultures and for biochemical analyses. The microcultures were run on Nickerson agar enriched with broth and serum (1:1), at 37°C for 48-72 h. Biochemical analyses were conducted with the use of chromogenic media (CHROM-agar Candida) and API-tests by Biomerieux (API 20°C, API 20°C ALX). In the case of mould fungi, imprint specimens were prepared on an adhesive tape that were stained with methylene blue and fixed in lactophenol.

Taxonomic identification was carried out with the use of keys by de Hoog *et al.* (7), Kreger-van Rij (19), Kurtzmann *et al.* (20), Raper *et al.* (25), and Raper and Fennel (26).

During the identification of species, also biosafety classification – BSL was put under consideration (6).

Results

The mycological analyses of 246 samples collected from beak and cloaca of the birds demonstrated positive results in 210 (85.4%) cases. Such a high percentage of positive results was extrapolated to taxonomic diversity of fungi and species abundance of particular isolates in both ontocenoses.

In 126 (60%) cases, the samples contained more than one species. Furthermore, there were 89 two-species samples, 27 three-species samples, six four-species samples, two six-species samples, as well as one five-species and one seven-species sample. The other 84 positive samples were mono-species.

In total, 386 isolates of fungi were obtained, *i.e.:* 260 (67.3%) isolates of yeast and yeast-like fungi classified to 30 species belonging to 15 genera, and 125 (32.7%) isolates of mould fungi (covering 24 species of nine genera (Table 1). Amongst the first, the highest number of species belonged to the genus *Candida* – six, *Rhodotorula* – four, and *Cryptococcus, Saccharomyces*, and *Trichosporon* – each with three species. The remaining genera, except for *Rhodosporidium* – two species, were represented by single taxa: *Aureobasidium, Dipodascus, Geotrichum, Hansenula, Kloeckera, Lipomyces, Monoascus, Saccharomycopsis,* and *Trichosporonoides* (Table 1). The predominating genera of mould fungi were *Penicillium* – seven species and *Aspergillus* – six species. The genus *Mucor* was represented by four species, *Fusarium* by two species, and *Absidia, Acremonium, Monilia, Syncephalastrum,* and *Taeniolella* by single taxa (Table 1).

The prevailing part of species was potential zoo- and anthropopathogens. As many as 35 species were found in biosafety classification, including ten species with category 2 and one species with category 3 (Table 3).

Analysis of the number of isolates of particular species demonstrated clearly that the most frequently detected fungi included: *Candida albicans* – 66 isolates, *Cryptococcus neoformans* – 31, and *Aspergillus fumigatus* – 30 isolates. Species: *C. krusei* – 20 isolates, *C. guilliermondii* – 16, *Cryptococcus laurentii* – 14, *Aspergillus niger* – 17, *A. flavus* – 13, *Rhodotorula rubra* – 15, and *Saccharomyces cerevisiae* – 16, and *Fusarium solani* – 11, were less frequently noted.

Irrespective of the number of isolates, due to sanitary and epidemiological reasons of a great attention are: *Absidia corymbifera* – two isolates, *Candida tropicalis* – five, *Fusarium oxysporum* – two, *Syncephalastrum racemosum* – one, and *Trichosporon cutaneum* – nine.

Nevertheless, generally more fungi species were noted in the beak than in the cloaca, the prevalence of particular species in both analysed ontocenoses was comparable. It especially refers to those species that were noted most frequently and had a high pathogenic potential. Furthermore, eight species were isolated only from beak and four species only from cloaca (Table 1). The latter included *Fusarium oxysporum* and *Syncephalastrum racemosum* with BSL 2 category (Table 1).

Discussion

The study has proven that wild ducks constitute a very rich reservoir of potentially-pathogenic fungi with great significance in aetiology of mycotic diseases of man and animals. Birds are especially susceptible to colonisation by fungi due to favourable conditions for their growth in poorly vascularised air sacs, very poor antimycotic activity in serum or its lack, and exceptionally thin skin devoid of sweat and sebaceous glands (5, 10, 28). In the case of wild birds not fed by man, mycoses are sporadic (5), whereas the carrier state – especially in waterfowl – is noted more frequently than in other vertebrates (2, 11-13). A physiological barrier that inhibits the growth and proliferation of most of potentially-pathogenic fungi in tissues of birds is high temperature of their body (40-42°C). In addition, an important role in protecting birds against fungal infection is ascribed to the secretion of the uropygial gland, exhibiting fungicidal and fungistatic effects (13).

Table 1. Fungi isolated from Mallard duck (*Anas platyrhynchos*) with consideration of BSL classification, number of isolates, and ontocenosis

No.	Species	BSL	Number of isolates	Ontocenosis
1	*Absidia corymbifera (=Lichthemia corymbifera)*	2	2	B + C
2	*Acremonium alabamense*	1	1	B
3	*Aspergillus aculeatus*	1	1	B
4	*A. flavus*	2	13	B + C
5	*A. fumigatus*	2	29	B + C
6	*A. nidulans*	1	2	B + C
7	*A. niger(=A. brasiliensis)*	1	17	B + C
8	*A. versicolor*	1	4	B + C
9	*A. glaucus*	—	2	B + C
10	*Aureobasidium pullulans*	1	2	C
11	*Candida albicans*	2	66	B + C
12	*C. guilliermondii (=Meyerozyma guilliermondii)*	1	16	B + C
13	*C. krusei (=Issatchenkia orientalis)*	2	20	B + C
14	*C. lipolytica (=Yarrowia lipolytica)*	1	1	B
15	*C. parapsilosis*	1	4	B + C
16	*C. tropicalis*	2	5	B + C
17	*Cryptococcus laurentii*	1	14	B + C
18	*C. laurentii var. magnus (=C. magnus)*	1	2	B + C
19	*C. neoformans*	2/3	31	B + C
20	*Dipodascus albidus*	—	10	B + C
21	*Fusarium oxysporum*	2	1	C
22	*F. solani (= Haematonectria haematococca)*	2	11	B + C
23	*Geotichum candidum*	1	2	B + C
24	*Hansenula silvicola* (*=Wickerhamomyces silvicola)*	—	4	B + C
25	*Kluyveromyces fluoresccens* (lack in IndexFungorum)	—	4	B + C
26	*Lipomyces tetrasporus*	—	2	B + C
27	*Moniliella suaveolens (=Saprochaete suaveolens)*	1	2	C
28	*Monascus ruber*	1	2	B + C
29	*Mucor circinelloides*	1	1	C
30	*M. hiemalis*	1	1	B
31	*M. mucedo*	—	4	B + C
32	*M. racemosus*	1	3	B + C
33	*Penicillium caseicola (=P. camemberti)*	—	2	B + C
34	*P. chrysogenum*	1	11	B + C
35	*P. citrinum*	1	9	B + C
36	*P. expansum (=P. glaucum)*	1	1	B
37	*P. gorgonzola (=P. roqueforti)*	—	3	B + C
38	*P. urticae (=P. griseofulvum)*	—	3	B + C
39	*Rhodosporidium dacryoideum (= Sakaguchia dacryoidea)*	—	2	B + C
40	*R. kratochvilovae*	—	2	B + C
41	*Rhodotorula glutinis*	1	5	B + C
42	*R. minuta*	1	6	B + C
43	*R. muscilaginosa*	1	8	B + C
44	*R. rubra*	1	15	B + C
45	*Saccharomyces cerevisiae*	—	16	B + C
46	*S. roseus*	—	1	B
47	*S. rouxii (=Zygosaccharomyces rouxii)*	—	2	B
48	*Saccharomycopsis capsularis*	—	2	B + C
49	*Syncephalastrum racemosum*	2	1	C
50	*Taeniolella stilbospora*	—	1	B
51	*Trichosporon aquatile*	—	3	B + C
52	*T. cutaneum (=T. beigelii)*	2	9	B + C
53	*T. fermentans (=Geotrichum fermentans)*	—	4	B + C
54	*Trichosporonoides madida*	—	1	C

B- beak, C – cloaca

However, when transferred from bird to the body of humans and other mammals, where temperature is lower, this species becomes hazardous when microbiological homeostasis and immunological mechanisms (immunosuppression) are disturbed in the body (3, 7, 11).

Although diasporas of fungi isolated from wild ducks occur commonly in different parts of the biosphere, including ontosphere of man and animals (7, 11), a very high number of taxa (33% out of noted species) classified to category 2 of biosafety, BSL2 seems to be alarming (6). This category includes fungi with high capability of survival in tissues of different organs of the vertebrates that may induce severe opportunistic infections (7, 11). The remaining taxa are either classified to a specified biosafety category (6) or are enumerated in medical and veterinary mycology as potential anthropopathogens and zoopathogens (7, 11, 18). The fungi species noted in the wild ducks may induce superficial or organ mycoses, mycotoxicoses, and mycoallergoses, and most of them are angio-invasive microfungi that may simultaneously play a role of coloniser, allergen, or invasive pathogen (3, 10, 11). An example in this respect may be *Aspergillus, Fusarium, Mucor*, or *Candida* isolated from the analysed wild ducks.

Waterfowl and the ciconiiformes bred in captivity (34), as well as wild birds (10, 28, 33) are characterised by high incidence of aspergillosis. In veterinary mycology, aspergillosis is an infectious disease lethal to ca. 5% of wild birds (24). It affects malnourished birds with deficiency of vitamin A, as well as wounded and immunosuppressed birds (5). Young birds are most susceptible to infections with *Aspergillus* (33), and the most frequent routes of the infection include inhalation of fungi spores or ingestion of contaminated feed (10). The most frequent causative agents of aspergillosis include: *A. fumigatus, A. flavus, A. niger,* and less frequently – *A. nidulans, A. glaucus,* and *A. versicolor* (28, 31). All these species were detected in the analysed material. Apart from the respiratory system, *Aspergillus* frequently invades the gastrointestinal tract, skin, bones (10), eyes (9), and the central nervous system (10). Spores of these fungi may penetrate through an eggshell to a developing embryo (5, 28). Similar modes of invasion are observed in case of *Penicillium, Mucoraceae* (Absidia), *Mucor,* or *Fusarium* infections. Diseases induced by these fungi in birds were noted very rarely (8, 28), whereas in the mammals – significantly more often (6, 7). The literature on avian diseases provides more data on mucormycosis of meninges and brain, as well as pneumonia and inflammation of air sacs (5, 28). In the case of the analysed wild ducks, apart from four species of the genus *Mucor*, analyses also revealed the presence of *Absidia corymbifera* – a species classified to BSL 2 category, hazardous to man and other mammals (7). This category also includes the aforementioned *A. fumigatus* and *A. flavus,* that produce aflatoxins claimed to be the strongest fungal carcinogens, and showing affinity to hepatocytes (10), as well as *F. solani* and *F. oxysporum* with a very rich composition of mycotoxins displaying carcinogenic, mutagenic, and teratogenic activities (5, 28). Fungi of the genus *Fusarium* deserve special attention owing to their increasing expansiveness in human tissues, especially in keratinised tissues and blood vessels (7, 30). These fungi have been reported to display affinity to bone marrow and to induce symptoms that resemble preliminary stages of leukaemia (7, 30).

Yeast and yeast-like fungi strongly linked with various ontocenoses of man, particularly with the digestive, respiratory, and urogenital systems and with skin are also of a great attention (7, 11, 30). The prevailing majority of these fungi are capable of colonising mucous membranes of the vertebrates in a commensal relationship (3). According to some authors (36), even 100% of wild fowl are carriers of different fungi, mainly *Candida* and *Cryptococcus*, therefore, candidosis and cryptococcosis are considered as endogenous mycoses (10, 11). Apart from individual predispositions and density of population of wild birds, a significant effect on infections in inter-individual transmission is ascribed to external factors such as temperature, humidity of environment, pollution, and degradation of habitat (12), as well as stress and poor diet (2).

Fungi of the *Candida* and *Cryptococcus* genera that were prevailing in the analysed material, as opportunistic forms, do not invade organisms with maintained homeostasis (10). They activate their pathogenic potential in case of a decreased immunity of the organism they are linked to (3). In the temperate climate, apart from dermatophytes (17), the greatest significance in aetiology and epidemiology of mycoses is ascribed to fungi of the genus *Candida* (1, 11, 12). The most frequently mentioned aetiological factor includes *C. albicans* (10), which is claimed to be responsible for most of superficial, organ, and whole-body invasions. This species is, however, increasingly displaced in the body of vertebrates by *C. krusei, C. parapsilosis, C. tropicalis*, or *C. guilliermondii* (11, 12). Avian candidoses are most frequently observed in the gastrointestinal and respiratory systems (1, 2, 10), skin and its products, as well as in the nervous system (10). In birds, *Candida* sp. organisms were detected in blood, bone marrow, and parenchymatous organs (5, 28). In humans and animals, candidosis usually locates in the same regions of the body (11).

One of the most severe pathogens to man and other mammals is *Cryptococcus neoformans*, which is simultaneously a frequent commensal of birds, especially of pigeons (32) and galliforms (8, 27, 28). In the material originating from the analysed wild ducks, its presence was significant. Its biosafety category is still under discussion (BSL 2/3). *Cr. neoformans* is a typical fungus of lithosphere that grows easily on bird faeces, especially in the autumn, and in soil

contaminated with these faeces (11). Cryptococcosis of birds is one of the severe zoonoses (5, 8). In humans, infection may develop by direct contact with a sick bird, healthy carrier, or with faeces of birds regarded to be the greatest reservoir of *Cryptococcus*, which shows an exceptional affinity to the central nervous system (7, 11, 14). These fungi also invade skin and subcutaneous tissue, as well as the respiratory system. Often, they are a causative agent of fatal fungemia (30). Additionally, *Cr. neoformans* has been implicated in some cases of chronic inflammation of brain meninges revealing itself after 15 and even 20 years since infection (30). The isolation of the same species from the ontosphere of man (6, 7) and wild fowl is the best proof for the circulation of potentially-pathogenic fungi in the entire biosphere (12).

While analysing the results obtained in the study, a high number of single strains of fungi detected in the experimental material is of a great attention. Their appearance may be accidental due to their presence in water (12, 18), bioaerosol of air or feed (10), but it may also indicate the onset of fungi invasion inside the ontosphere of birds. Significantly increasing expansiveness to tissues of man and other vertebrates is observed in case of *Saccharomyces* and *Trichosporon,* especially *T. cutaneum* – with BSL 2 category (11, 20).

An international epidemiological survey (36) conducted under auspices of the World Health Organization points explicitly to the role of wild birds in dissemination of aetiological factors of diseases noted contemporarily in humans. The authors of this survey (36) enumerate more than ten taxa of terrestrial and water fowl that take part in the transmission of bacteria, viruses, and fungi posing threat to man, to significant distances all over the globe. The "bird-carriers" also included Mallard ducks that prefer coastal waters of urbanised areas, rich in organic matter, and subjected to strong anthropopression. Simultaneously, these are typical habitats of potentially-pathogenic fungi (11, 12, 18).

Concentrations of wild ducks in urban areas, especially in winter, may exceed even 10 000 birds (23). Populations of birds fed additionally by men are dense, which tightens links of transmissions of potentially-pathogenic fungi not only between different populations of the wild ducks but also between birds and humans.

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