DE GRUYTER OPEN

Bull Vet Inst Pulawy 59, 65-69, 2015
DOI:10.1515/bvip-2015-0010

Epidemiological importance of yeasts isolated from the beak and cloaca of healthy Charadriiformes

Maria Dynowska, Anna Biedunkiewicz, Iwona Kisicka, Elżbieta Ejdys, Dariusz Kubiak, Ewa Sucharzewska

Department of Mycology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, 10-917 Olsztyn, Poland
dynow@uwm.edu.pl

Received: June 25, 2014 *Accepted: February 20, 2015*

Abstract

The paper presents mycological studies conducted jointly with ornithologists on the epidemiology of mycoses and the taxonomic diversity and prevalence of fungi that colonise the selected onthocenoses in healthy, wild migratory birds. Aquatic ecosystem populations of healthy birds include a percentage of carriers of potential zoo- and anthropopathogens, and this study's purpose was to determine the percentage. The studies were performed on swabs sampled in vivo (during spring and autumn migrations) from the beak and cloaca of nine species of Charadriiformes in two age categories. Macro- and microcultures of fungi were prepared according to the standards for diagnostic mycological laboratories. From the 450 birds examined, fungi were isolated from 130 (26.5%) individuals. The sampling yielded 272 yeast isolates: 170 (62.5%) from the beak and 102 (37.5%) from the cloaca. The isolates represented 23 species, among which *C. albicans, C. neoformans*, and *R. rubra* were predominant. In both onthocenoses in young and adult birds, more fungi were recorded in autumn than in spring. As many as 15 species are included in the biosafety level classification, of which seven are categorised as category 2 and one as category 3.

Keywords: Charadriiformes, yeasts, epidemiology, migrations.

Introduction

Yeasts are a heterogeneous group of microfungi with the encoded pathogenic potential towards the vast majority of vertebrates (21). This pathogenicity may manifest itself when the biological balance is disturbed and the immune mechanisms are impaired (13). These fungi are found in all parts of the biosphere as cosmopolitan saprophytes, which prefer micro-aerophilic conditions and environments rich in organic compounds of a different origin. Most yeasts are opportunistic forms that are unable to invade a healthy organism yet they may colonise it commensally (3, 12). The macroorganism then becomes a carrier, a natural reservoir, and a vector of the fungi (16).

Birds are particularly susceptible to fungal colonisation due to the specific anatomy of the respiratory and tegument systems and very poor or nonexistent antifungal activity of their serum (11). Carrier birds do not usually fall ill as their high body temperature is a physiological barrier, which inhibits the growth and multiplication of most potentially pathogenic fungi. However, the same species become dangerous at the body temperatures of humans or other mammals with impaired immunity (13, 16). Interestingly, mycoses are sporadic in wild birds that are not fed by humans (23), while carrier-state, especially in water fowl, is reported more frequently than in other vertebrates (8, 25). Fungi can be transmitted by birds in a passive manner (on the body surface or in feathering), by biological vectors (ectoparasites), or in the internal organs, mainly in the respiratory and digestive tracts, as evidenced by the close correspondence of the obtained results with the observations of other authors (17, 18, 25).

Due to their particular feeding and migratory routes *via* climatically-diversified habitats, Charadriiformes are exposed to contact with different, and often trophically rich types of water and, consequently, with different types of fungi that are typical of the hydrosphere (9, 19). Therefore, these birds seem to be a good research object, especially in Poland, as the country is situated on their main migratory route (16). Initial studies suggested that fungi

© 2015 M. Dynowska et al. This is an open access article distributed under the Creative Commons Atribution-NonCommercial-NoDerivs license (http://creativecommons.org/licenses/by-nc-nd/3.0/)

associated with the onthosphere of Charadriiformes are much more numerous than previously thought (13, 16). Considering that the interest in birds as vectors of microorganisms is mainly directed to bacteria and viruses, it was decided to conduct mycological analyses of biological materials collected in vivo from the onthocenosis of beak and cloaca of healthy birds of selected Charadriiformes species, taking into account the age and physiological status of these birds and the migration period.

Material and Methods

The studies were performed using swabs collected in vivo (with a swab immersed in saline before sampling) from the beak and cloaca of nine Charadiiformes species in two age categories. The samples were collected during the spring and autumn migration seasons for two years at selected ornithological stations of the "KULING" Water Fowl Research Group on an estuary of the Reda River at Puck Bay, the Pleśno water reservoirs, and the pools of the Sejna River, near Sątopy-Samulewo (in the province of Warmia and Mazury).

The swabs were placed in liquid Sabouraud medium with gentamycin and chloramphenicol (0.1%) and transported to a laboratory. Laboratory tests were performed in accordance with routine mycological diagnostics procedure (13, 24). Macrocultures were prepared with Sabouraud agar and the above-mentioned antibiotics and incubated at 25, 37, and as thermophilic species require, 40°C for the period between 48 h and 7 d. The first signs of growth were recorded after 48 h and fungi only sporadically appeared on d 7 of incubation. Having established the gross characteristics, microcultures were set on Nickerson agar. Chromogenic media (CHROMagar Candida, CHROMagar, France) and API-tests (API 20°C, and API 20°C AUX, BioMérieux, France) were used for biochemical analyses (14).

The following keys were used for taxonomic identification: Kreger-van Rij (19), Kurtzman, Fell and Bekhout (21), De Hoog *et al*. (6), and the methods published by Richardson and Warnock (24).

BSL classification was applied to characterise the isolated fungi (5).

Results

Of the 490 birds examined, fungi were isolated from 130 (26.5%) individuals. In total, 272 yeast isolates were obtained: 170 (62.5%) from the beak and 102 (37.5%) from the cloaca; the isolates represented 23 species (Table 1). In onthocenoses in both young and adult birds, more fungi were recorded in autumn than in spring. This applied to the number of isolates and taxonomic diversity of fungi (Fig. 1). In the latter case, the differences were smaller or did not exist. The examples include: the identical number of fungal species in spring and autumn in the beaks of young birds (15), and in the cloaca of adult individuals (10). Interestingly, the diversity in the number and taxonomy of fungi in the beak of all birds was higher in autumn, and the number of species in the cloaca of young birds doubled, *i.e.* 5 species in spring and 10 species in autumn (Fig. 1).

The highest taxonomic diversity of fungi was recorded in *G. gallinago* – 16 species (14 in the beak and 11 in the cloaca), *C. alpina* – 14 species (14 in the beak and 7 in the cloaca), *T. glareola* – 13 (13 in the beak and 8 in the cloaca), and *Ph. pugnax* – 11 (10 in the beak and 8 in the cloaca). The smallest number (2) of species, identified as *A. hypoleucos*, was found in the beak (Table 1).

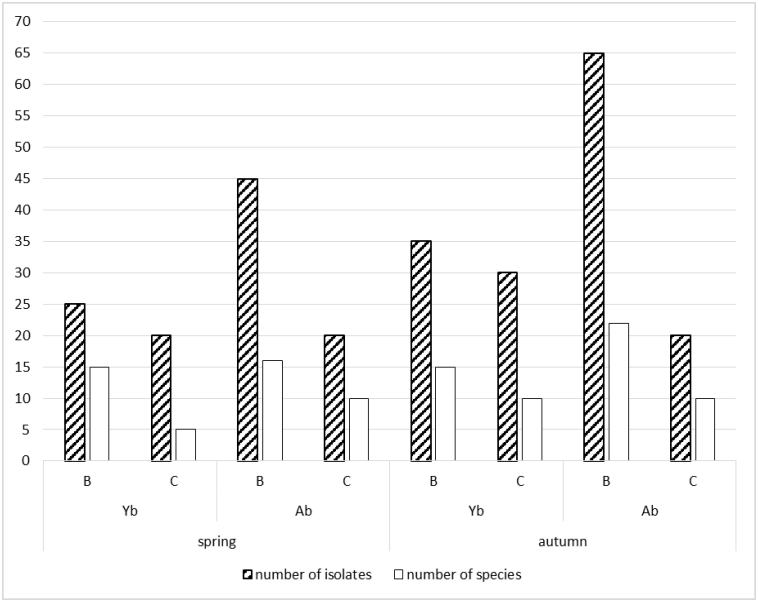


Fig. 1. Number of isolates/species of fungi obtained from the beak (B) and cloaca (C) of young birds (Yb) and adult (Ab) in spring and autumn

Table 1. Number of isolates from different species of birds in beak and cloaca

No.	Species of fungi	BSL*	I		II		III		IV		V		VI		VII		VIII		IX		Total	
			B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	C
			2		14		7		6		7		16		11		13		6		82	
1	*Candida albicans*	2	5		5	4	5				1	1	7	4	5	2	5		1	3	34	14
2	*Candida dubliniensis*	2											1								1	
3	*Candida glabrata*	2			4	2			2	2	1	1	4	2	1	1	1	1			13	9
4	*Candida guilliermondii*	1											2				3	1			5	1
5	*Candida inconspicua*	1/ 2											1				1				2	
6	*Candida krusei*	2			2	2							3	2			2		1	1	8	5
7	*Candida parapsilosis*	1			1																1	
8	*Candida stellata*	ns											1			2					1	2
9	*Candida tropicalis*	2													1	1	1	1			2	2
10	*Citeromyces matritensis*	ns			1																1	
11	*Cryptococcus neoformans*	2 / 3			3	3	2	2	1		1		8	8	10	5	2	2	1		28	20
12	*Cryptococcus satoi*	ns			1																1	
13	*Geotrichum candidum*	1									3	3	2	3							5	6
14	*Pichia anomala*	ns			1																1	
15	*Rhodotorula glutinis*	1			1	1	1	1	1				2	2	1	1	2	2	1		9	7
16	*Rhodotorula muscilaginosa*	1			1		1		1				1		2		2				8	
17	*Rhodotorula rubra*	1	1		4	4	2	2	3	3	2	2	3	2	1	1	3	3	1	1	20	18
18	*Saccharomyces cerevisiae*	ns					1	1			4	1	2	1	1	1	2				10	4
19	*Saccharomycopsis capsularis*	ns			1										2						3	
20	*Trichosporon asteroides*	2			1									1							1	1
21	*Trichosporon cutaneum*	2			2	2	1		1		1		5	5	2	1	2	2	1	1	15	11
22	*Trichosporon fermentans*																1	1			1	1
23	*Trichosporon pullulans*													1							0	1

Legend: B – beak; C – cloaca; ns – not specified; I. *Actitis hypoleucos*; II. *Calidris alpina*; III. *Calidris ferruginea*; IV. *Calidris temminckii*; V. *Charadrius hiaticula*; VI. *Gallinago gallinago*; VII. *Philomachus pugnax*; VIII. *Tringa glareola*; IX. *Tringa ochropus*; * - according to de Hoog (3)

Among fungi, *C. albicans* and *C. neoformans* were predominant (48 isolates each) and *R. rubra* was markedly present (38 isolates). *T. cutaneum* (26 isolates) and *C. glabrata* (22 isolates) were also common. *C. krusei, R. glutinis*, and *S. cerevisiae* were reported less frequently. *S. cerevisiae* was reported in all birds examined, while *C. krusei* was not detected only in *C. ferruginea*, and *R. glutinis* was not detected only in *A. hypoleucos*. The other species enumerated in Table 1 were represented by few or single isolates.

The majority of isolated fungi were potential zoopathogens and anthropopathogens. As many as 15 (65%) species have been already included in the biosafety level classification, of which seven were classified as category 2 and one species was classified as category 3.

Discussion

Fungi that colonise macroorganisms have most often previously been isolated from their natural habitat (13). The species identified in Charadriiformes are commonly isolated from different types of water rich in organic compounds and with different degrees of microbiological purity. This applies both to freshwater and seawater, especially coastal zones that are the most common feeding grounds of Charadriiformes. Some of the isolated fungi (*C. albicans, C. guilliemondii, C. tropicalis, T. cutaneum*, and fungi from *Rhodotorula* genus) prevail in fresh and untreated sewage (16, 20). Their association with the hydrosphere is of a cosmopolitan nature and their prevalence is limited more by ecological than by geographical factors. Assuming the specificity and spatial discontinuity of biotopes of these fungi, their cosmopolitism is determined by, among others, transport by overflying water fowl (2, 16, 25).

It is believed that about 50% of wild birds are reservoirs and carriers of fungi which are potentially pathogenic to birds themselves and to humans. The cloaca is considered the most frequently colonised onthocenosis (2), however in the present study, more fungi were identified in the beak than in the cloaca. It seems that it may be related to the anatomical structure of the beak in Charadriiformes and to the way and grounds on which they feed. All the Charadriiformes studied except *C. hiaticula* search for food by exploring the ground with their long beak and by penetrating coastal waters at different depths, which increases the possibility of contact with fungi found in specific parts of the aquatic environment. *C. hiaticula* searches for food mainly on the intertidal region. The sanitary status of the water which the birds use seems to be a determiner for whether fungi originating from different climatic zones can colonise the bird organism (15).

In the Polish climate, the fungi of *Candida* genus are the most important in an epidemiological context, as they are the causative agent of most superficial and systemic mycoses in Poland, with *C. albicans* being the most common aetiological factor (13, 16, 20). The results of long-term studies (12) do not permit such an unambiguous statement. *C. dubliniensis, C. glabrata, C. guilliemondii, C. tropicalis*, or *C. parapsilosis* are isolated from healthy and diseased adults and children equally often, or sometimes even more frequently than *C. albicans*. All these species were identified in Charadriiformes.

Fungi from the *Trichosporon* genus show an increasing range of viable host organs in humans with *T. cutaneum* being increasingly frequently isolated from the respiratory tract, digestive tract, and skin, and *T. asteroides* being responsible for superficial infections (10). However, *C. neoformans* is a fungus that is thought to be the most dangerous to humans, especially to children. It is believed to be the most common commensal of birds (13, 17, 18, 22) in which it asymptomatically colonises the skin, subcutaneous tissue, and lungs. This fungus is common in the lithosphere and easily develops in the Polish climate (particularly in autumn) on the faeces of different avian species and in faeces-contaminated soil (13). In present studies *C. neoformans* was isolated from Charadriiformes with identical frequency as *C. albicans*. The former is believed to most often colonise the air sacs where it has the best conditions to grow thanks to the circulation of fresh, heated, and moist air (23). It was demonstrated that this species may also often colonise the cloaca. In humans, *C. neoformans* shows an affinity to the central nervous system, causing meningoencephalitis, which may be fatal in extreme cases (7). The identification of as many as three species of *Rhodotorula* in Charadriiformes, with the predominance of *R. rubra*, indicates that the birds feed on sewage waters (9, 16). These are species that are more commonly reported in hydromycology as the important species in the water self-purification process of self-cleaning of water rather than reported in medical or veterinary mycology as potentially pathogenic factors. However, their presence should not be neglected in individuals with impaired immunity, because similarly to *S. cerevisiae* they may cause local or even generalised mycoses (12, 20).

The isolation of fungi from the beaks of healthy birds may indicate their accidental occurrence and a transient association with this onthocenosis, although it does not exclude their solid relationship based on commensalism. The presence of the same species in the cloaca demonstrates that these fungi passed through the gastrointestinal tract and create a carrier state. Additionally, it was demonstrated that birds in which the fungi were identified in the cloaca have poorer mechanisms protecting against invasion and development of the fungi (3). It is best seen in young birds in which the number of species in the cloaca doubled in autumn. It is emphasised that age is an important physiological factor which determines the

emergence of fungi in the onthosphere of all vertebrates (15, 16). Apart from the gaps in the first and second lines of protection, young Charadriiformes are exposed to numerous stress-inducing factors (*e.g*. predators) during the first independent migration of their lives, which is energy-depleting and weaken their condition that is already much more vulnerable than in adults. However, looking at a significant increase in the number of strains and species in all birds in autumn initially suggests an increase in the number and diversity of these fungi in the environment. Breeding is of vital importance as, thereafter, the physiological condition and immunity of adult birds drop (15). That is favourable for infection with potentially pathogenic microfungi, which are definite opportunists.

Fungi are released from the bird with excreta. This is stage I of inter-individual transmission with a vital role during copulation, when fungi colonise the cloaca, or during feeding the offspring, when fungi colonise the beak. In the latter case, when fungi pass from parents to offspring, vertical transmission occurs. Stage II of transmission involves multiplication of fungi and/or survival in the environment. During stage III, transmission and penetration into a new host take place - this may be another bird or a human (14).

It seems that the obtained results have greatly expanded fragmentary knowledge on the taxonomic diversity and prevalence of fungi associated with the onthocenoses of the respiratory and digestive tracts in wild birds. This new knowledge may be applied to develop epidemiological calendars related to the carrier state of potentially pathogenic fungi in these birds.

Modern medicine – in search for the ecological background of a number of diseases – increasingly often conducts biomonitoring studies (1, 9) to analyse not only the diversity and prevalence of aetiological factors, but also their reservoirs and vectors (4, 13, 14). The latter are the basis for evaluating epidemiological hazards, which are particularly important in the case of mycoses, belonging as they do to the group of environmentally-mediated diseases (13, 14, 25).

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

References

1. Brilhante R.S., Castello Branco D.S., Duarte G.P., Paiva M.A., Teixeira C.E., Zeferino J.P., Monteiro A.J., Cordeiro R.A., Sidrim J.J., Rocha M.F.: Yeast microbiota of raptors: a possible tool for environmental monitoring. Environ Microbiol Rep 2012, 4, 189–193.
2. Cafaria C., Camarda A., Romito D., Campolo M., Quaglia N.C., Tullio D., Otranto D.: Occurrence of yeasts in cloacae of migratory birds. Mycopathologia 2006, 161, 229–234.
3. Casadevall J., Clayton Y.M.: Bacterial and fungal flora of seagull droppings in Jersey. J Clin Pathol 1971, 24, 317–319.
4. Chryssanthou E., Wennerg H., Bonnedahl J., Olsen B.: Occurrence of yeasts in faecal samples from Antarctic and South American seabirds. Mycoses 2011, 54, 811–815.
5. De Hoog G.A.: Risk assessment of fungi from humans and animals. Mycoses 1996, 39, 407–417.
6. De Hoog G.S., Guarro J., Gene J., Figueras M.S.: Atlas of clinical fungi. Universitat Rovira and Virginii, Reus, 2000, p. 1126.
7. Decostere A., Hermans K., De Baere T., Pasmas F., Haesebrouck F.: First report on *Cryptococcus laurentii* associated with with feather loss in a glossy starling (*Lamprotornis chalybaeus*). Avian Pathol 2003, 32, 309–311.
8. Dupont C., Carrier M., Higgins R.: Bacterial and fungal flora in healthy eyes of birds of prey. Can Vet J 1994, 35, 699–701.
9. Dynowska M.: Yeast-like fungi possessing bio-indicator properties isolated from the Łyna river. Acta Mycol 1997, 32, 279–286.
10. Dynowska M., Biedunkiewicz A., Ejdys E., Sucharzewska E., Rosłan M.: Fungi of the genus *Trichosporon* isolated from the skin in hospitalized patients. Acta Mycol 2013, 48, 145–151.
11. Dynowska M., Dynowski J.: A case of aspergillosis and candidosis of black stork - *Ciconia nigra* (Linnaeus 1758). Med Weter 1996, 52, 127–128.
12. Dynowska M., Góralska K., Troska P., Barańska G., Biedunkiewicz A., Ejdys E., Sucharzewska E.: Results of long-standing mycological analyses of biological materials originating from selected organ onthocenoses yeast and yeast-like fungi. Wiad Parazytol 2011, 57, 97–102.
13. Dynowska M., Kisicka I.: Fungi isolated from selected birds potentially pathogenic to humans. Acta Mycol 2005a, 40, 141–147.
14. Dynowska M., Meissner W., Pacyńska J.: Mallard duck (*Anas plathyrhynchos*) as a potential link in the epidemiological chain mycoses originating from water reservoirs. Bull Vet Inst Pulawy 2013, 57, 323–328.
15. Dynowska M., Wojczulanis-Jakubas K., Pacyńska J., Jakubas D., Ejdys E.: Potentially pathogenic yeast isolated from the throat and cloaca of an Arctic colonial seabird: the little auk *(Alle alle*). Polar Biol 2012, 36, 343–348.
16. Dynowska M., Kisicka I.: Participation of birds in the circulation of pathogenic fungi descend from water environments: case study of two species of Charadriiformes birds. Ecohydrol Hydrobiol 2005, 5, 173–178.
17. Foti M., Rinaldo D., Guercio A., Giacopello C., Aleo A., De Leo F., Fisichella V., Mammina C.: Pathogenic microorganisms carried by migratory birds passing through the territory of the island of Ustica, Sicily (Italy). Avian Pathol 2011, 40, 405–409.
18. Hubálek Z.: An annotated checklist of pathogenic microorganisms associated with migratory birds. J Wild Dis 2004, 40, 639–659.
19. Kreger-Van Rij N.J.W.: The yeasts. A taxonomic study. Elsevier, Amsterdam, 1984, p. 1082.
20. Kurnatowski P., Rózga A., Wójcik A.: Potentially pathogenic yeast-like fungi in lowland water reservoir. Mycoses 2001, 55, 40–41.
21. Kurtzman C.P., Fell J.N., Bekhout T.: The yeasts. A taxonomic study. Elsevier, Tokyo, 2011, p. 2080.
22. Raso T.F., Werther K., Miranda E.R.T., Mendes-Giannini M.J.: Cryptococcosis outbreak in psittacine birds in Brazil. Med Mycol 2007, 42, 355–362.
23. Reding P.: Fungal diseases. In: *Avian medicine*, edited by J. Samour, Elsevier Science, Edinburgh, 2003, pp. 273–291.
24. Richardson M.D., Warnock D.W.: Fungal infection. Diagnosis and management. Blackwell Scientific Publication, Oxford, 1993, p. 352.
25. Tsiodras S., Kelesidis T., Kelesidis L., Bauchinger U., Falagas M.E.: Human infections associated with wild birds. J Infect 2008, 56, 83–98.