

## Fungi isolated from selected birds potentially pathogenic to humans

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The mycology of swabs collected from the beak and the cloaca in 4 species of mud and water waders, migrating through various climatic zones, was analysed. Diagnostic standards were used. 54 isolates of fungi belonging to 5 genera: *Aspergillus* – 53.9%, *Rhodotorula* – 19.6%, *Candida* – 14.8%, *Saccharomyces* – 6.9%, and *Torulopsis* – 4.8%, were obtained. A total number of 15 species of which *Aspergillus fumigatus* and *A. flavus* as well as *Candida albicans*, *C. tropicalis* and *C. glabrata* are of the greatest significance in epidemiology of mycoses, was recorded. Numerous fungi were isolated from the cloaca, which indicates that the birds examined are carriers and suggests that they may take an active part in the transmission of fungi that are dangerous for humans and that colonise water reservoirs.

**Key words:** *Aspergillus* sp., *Candida* sp., epidemiological circulation, waders

### INTRODUCTION

Potentially pathogenic fungi are defined as fungi capable of growth and development at the temperature of the human body and which can survive when the oxidative-reductive potential is reduced. According to Richardson and Warnock (1995), ca. 200 species of free-living fungi, belonging to various systematic and ecological groups, exhibit properties pathogenic to humans. Their great majority are opportunistic, cosmopolitan and euryecological forms, resistant to fluctuations of basic parameters characterising their habitats which may comprise all ecosystems in the biosphere (Dynowska 1995). Aquatic ecosystems, particularly those polluted, characterised by high trophic level, subjected to strong anthropopressure, are some of the greatest natural reservoirs of potentially pathogenic fungi (Dynowska, Biedunkiewicz, Ejdyś 2001). They may constitute a source of dangerous mycoinfections, while animals associated with these habitats often participate in their transmission (Dynowska and Dynowski 1996). Migratory birds and birds feeding in waters rich in organic matter with which fungi are often associated cosmopolitically deserve special attention (Dynowska and Kisicka 2005). Birds' body

temperature (40°C – 44°C) which inhibits the development of most fungi potentially pathogenic to humans is a barrier against the manifestation of pathogenic properties of fungi in their bodies (Baran 1998). Thus, birds in a good physiological form, although colonised by fungi, will not be ill but will be their vectors.

Therefore, mycological examinations of selected species of water and mud waders for which Poland is situated in the main migratory corridor of birds of the European Lowland were launched (Tomiałojć and Stawarczyk 2003).

## MATERIAL AND METHODS

Selected waders, belonging to 4 species – 74 individuals (Tab. 1) from which sterile swabs were taken from the beak (faucial cavity) and the cloaca during spring and autumnal migration through Poland were examined in mycological studies (2001 – 2003). In the case of *Calidris temminckii*, material was collected only from the beak due to the small body size.

Observations were conducted on an inland basin and pools of the Sejna river in the Warmińsko-Mazurskie Voivodeship as part of ornithological studies of the waterbird Research Group 'KULING'.

From the biological material collected, macrocultures on solid and liquid Sabouraud's medium were established and incubated at 37°C for 3 weeks. Yeast-like fungi and yeasts developed after 48 hours while moulds developed after a week. After the colonies from yeast-like fungi were described macroscopically, microcultures were established on Nickerson agar and incubated at 37°C for 48 – 72 hours. They were transferred to room temperature for 48 hours after which their microscopic features, required for the diagnosis of this group of fungi (Dynowska 1995, Kurnatowska 1995), were determined. Fungi were also assessed biochemically using API tests (API 20°C and API 20°C AUX; Biomerieux).

Mould inocula were transferred onto Czapek – Doxa medium. They were incubated at room temperature and at 40°C, required for the growth of *A. fumigatus*. After 3-10 days and after the development of structures characteristic of these fungi, preparations were made using transparent adhesive tape. The mycelia fixed to the tape were transferred onto microscopic slides, stained with cotton blue for about 5 seconds, and covered with cover slips in a drop of lactophenol.

The keys Kreger-van Rij (1984); Barnett, Payne, Yarrow (1990); Kurtzman and Fell (2000) and de Hoog et al. (2000) as well as studies by Kurnatowska (1995) and Baran (1998) were used to identify the fungi.

Table 1.

Bird species from which biological material was collected in individual study seasons

N°	Species	2001	2002	2003	TOTAL
I.	<i>Calidris temminckii</i> (Leisl., 1812)	-	-	9	9
II.	<i>Tringa ochropus</i> L., 1758	1	(1)	(7)	9 (8)
III.	<i>Philomachus pugnax</i> (L., 1758)	-	14 (11)	26 (25)	40 (36)
IV.	<i>Actitis hypoleucos</i> (L., 1758)	7	(3)	6 (4)	16 (7)
	Total	8	18 (15)	48 (36)	74 (51)

( ) – figures in parentheses show the number of birds from which swabs were taken in the beak and in the cloaca.

Table 2  
Fungi isolated from individual bird species (I – *C. temminckii*, II – *T. ochropus*, III – *Ph. pugnax*, IV – *A. hypoleucos*)

N°	Fungal species	Beak				Cloaca			
		I	II	III	IV	I	II	III	IV
1	<i>Aspergillus candidus</i> Link	-	+	-	-	-	-	+	-
2	<i>Aspergillus clavatus</i> Desm	+	+	-	+	-	-	+	+
3	<i>Aspergillus flavus</i> Link	-	+	+	-	-	-	+	-
4	<i>Aspergillus fumigatus</i> Fresenius	-	-	+	-	-	-	+	-
5	<i>Aspergillus niger</i> Van Tieghem	-	+	+	-	-	+	+	-
6	<i>Aspergillus terreus</i> Thom	-	-	+	-	-	-	+	-
7	<i>Candida albicans</i> (Robin) Berkhout	-	-	+	+	-	-	+	-
8	<i>Candida glabrata</i> (Anderson) Meyer et Yarrow	-	+	-	-	-	-	-	-
9	<i>Candida krusei</i> (Castellani) Berhout	-	+	-	-	-	-	-	-
10	<i>Candida tropicalis</i> (Castellani) Berhout	-	-	+	-	-	-	+	-
11	<i>Rhodotorula glutinis</i> (Fresenius) Harrison	-	-	+	-	-	-	-	-
12	<i>Rhodotorula mucilaginosa</i> (Jørgensen) Harrison	-	-	+	+	-	-	-	-
13	<i>Rhodotorula rubra</i> (Demme) Lodder	-	-	+	-	-	-	-	-
14	<i>Saccharomyces cerevisiae</i> Hansen	+	-	-	-	-	+	-	-
15	<i>Torulopsis stellata</i> Lodder	-	+	-	-	-	+	-	-

## RESULTS

Fungi were identified in 46 (62.2%) of the 74 birds examined (Tab. 1). A total number of 54 isolates of fungi belonging to 5 genera: *Aspergillus* – 53.9%, *Candida* – 14.8%, *Rhodotorula* – 19.6%, *Saccharomyces* – 6.9% and *Torulopsis* – 4.8%, were obtained. The genera *Aspergillus*, *Candida* and *Rhodotorula* were represented by a few species (Tab. 2), while *Saccharomyces* and *Torulopsis* were represented by *Saccharomyces cerevisiae* and *Torulopsis stellata*, respectively.

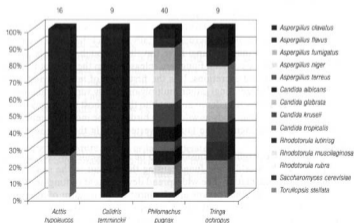


Fig. 1. Percentage participation of fungal species potentially pathogenic to humans isolated from selected waders (figures given above the vertical bars show the abundance of the birds examined).

The greatest number of fungi was isolated from *Philomachus pugnax* (12 species) and *Tringa ochropus* (7 species). The co-occurrence of a number of fungal species in one ontocoenosis was also noticed in these two birds (in *T. ochropus*: *C. glabrata* together with *T. stellata* in the beak and *Rh. rubra* together with *T. stellata* in the cloaca; in *Ph. pugnax*: *A. terreus* together with *Cryptococcus* sp. and *Penicillium* sp. only in the cloaca). Fungal diversity did not exceed 3 species in the other birds (Fig. 1). Fungi were significantly more frequently isolated from the cloaca than from the beak. Fungi of the genera *Aspergillus* and *Rhodotorula*, followed by *Candida*, *Saccharomyces* and *Torulopsis*, certainly dominated in both ontocoenoses.

## DISCUSSION

The results obtained, part of continuing studies, clearly show that the birds examined may take an active part in transferring and transmitting fungi potentially pathogenic to humans throughout the biosphere. Thus, they constitute an important link in the epidemiological chain of mycoses possibly originating in aquatic reservoirs of various climatic zones through which they migrate or directly in infected birds.

It seems disturbing that as many as 6 mould species of the genus *Aspergillus* causing mycoses, mycotoxicoses and mycoallergoses throughout the world were isolated. *A. fumigatus* and *A. flavus* whose carcinogenic mycotoxins cause diseases of parenchymal organs, mostly the liver and the lungs, belong to the most dangerous and most frequently listed species (Orkeley and Rose 1976; Rhodes, Jensen and Nillius 1992; Baran 1998). *A. terreus* and *A. niger* are also frequently recorded in tropical and subtropical countries (Richardson and Warnock 1995). This explains the presence of the species given on migratory birds which may transfer fungi in the digestive tract, lungs and air sacs where the conditions for the development of various fungi are optimal (Dynowska and Dynowski 1996; Shin et al. 1996; Young, Cornish, Little 1998). Birds also transfer fungi on the skin and between feathers (Hubálek, Juricova and Halouzka 1995). While fungi isolated from the beak may occur there accidentally, the fact that fungi are isolated from the cloaca suggests that the fungi have travelled through the birds' digestive tract and that the birds are carriers. Fungi are released from the body of the carrier bird with its secretions and excretions. It is the first stage of interindividual transmission. In the second stage, fungi either multiply or merely survive in the external environment, and then they transfer to and get into the new host which may be the body of another bird or the human body as wild-living birds are the most frequent and the most easily migrating element of the fauna of vertebrates in the environment surrounding humans.

Various forms of aspergillosis, considered to be an exogenic mycosis, are widespread throughout the world while fungi causing them occur in the soil, dust and on decomposing organic matter. The widespread development of fungi in the lungs and a frequent spread to other organs are the most frequent form of aspergillosis (Rhodes et al. 1992). The majority of infections in humans take place via the inhalation route. Fungal spores get into the lungs and paranasal sinuses, the sites most susceptible to infection, together with the air. Infection as a result of the penetration of spores through damaged surfaces occurs most frequently in the case of karatomy-

cosis, the mycosis of the cornea, recorded mostly in warm climatic zones, especially in farmers (Richardson and Warnock 1995).

Apart from dermatophytes, yeast-like fungi of the genus *Candida* which are causative agents of the majority of fungal disorders in Poland are of the greatest epidemiological importance (Dynowska 1993, 1995; Baran 1998). *Candida albicans*, responsible for organ candidioses and generalised candidioses in the majority of vertebrates, are considered to be the most frequent aetiological factor (Ghannaum and Abu-Elteen 1990; Odds 1994; Baran 1998). It occurred with moderate frequency together with other species of this genus: *C. glabrata*, *C. krusei* and *C. tropicalis*, in the birds examined. Invasion by these fungi may occur in all tissues, organs and systems in humans and animals in various developmental periods.

Infection by fungi of the genus *Candida* are considered to be endogenic mycoses as they may be present in the human body in physiological conditions and colonise commensally the oral cavity, digestive tract, vagina, or less frequently the skin. When the biological balance between the fungus and the host is upset and the immunological balance is disturbed, fungi adhere to epithelial cells, hydrolytic enzymes are triggered off by the fungi and the parasitic contact which initiates infection is established (Macura 1993).

A few species of *Rhodotorula* as well as *T. stellata* and *S. cerevisiae* were found among the isolated fungi. They are listed as potential pathogens to humans and animals that may cause diseases identical to those caused by fungi of the genus *Candida* (Dynowska 1995; Baran 1998).

The yeast-like fungi and true yeasts discussed above are typical of polluted waters that have undergone strong eutrophication, rich in plant and animal substrates (Dynowska 1995; Dąbrowski, Bogusławska-Wąs and Daczkowska-Kozon 1998; Dynowska, Biedunkiewicz and Ejdys 2001). These waters are also preferred as feeding grounds by waders whose various ontocoenoses may be colonised by various species of fungi during long journeys from breeding grounds to winter habitats.

It is particularly difficult to record routes and sources of fungal infection as the process of infection transmission is different for various groups of fungi, while individual life habitats have an enormous influence on their morphology and biological features. This is confirmed by the present authors' earlier studies on the enzymatic activity of potentially pathogenic fungi isolated from waters and biological material collected from humans (Dynowska, Sucharzewska and Biedunkiewicz 2001).

The increasing development of infection caused by fungi shows their ecophysiological expansive nature, and is usually associated with growing prevalence of fungi in the human environment. Wild-living birds that constitute a live, natural reservoir of potentially pathogenic fungi constitute its permanent component.

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Grzyby potencjalnie chorobotwórcze dla człowieka  
izolowane od wybranych ptaków

## Streszczenie

Praca jest fragmentem kontynuowanych badań mikologicznych wśród ptaków siewkowych, prowadzących błotno – wodny tryb życia, migrujących przez różne strefy klimatyczne oraz ocena roli tych ptaków w transmisji grzybów potencjalnie chorobotwórczych dla człowieka ze środowisk wodnych.

Badaniami objęto 4 gatunki ptaków (*Actitis hypoleucos*, *Calidris temminckii*, *Philomachus pugnax*, *Tringa ochropus*), od których pobierano jałowo wymazy z dzioba (=jama gardła) i kloaki podczas wiosennej i jesiennej migracji przez tereny Polski

Ogółem uzyskano 54 izolaty grzybów, należących do 5 rodzajów: *Aspergillus* – 53.9%, *Candida* – 14.8%, *Rhodotorula* – 19.6%, *Saccharomyces* – 6.9% i *Torulopsis* – 4.8%. Trzy pierwsze rodzaje reprezentowane były przez kilka gatunków, dwa następne przez *S. cerevisiae* i *T. stellata*.

Najwięcej grzybów wyizolowano od *Philomachus pugnax* – 12 gatunków i *Tringa ochropus* – 7 gatunków. Również u nich stwierdzono współwystępowanie kilku gatunków grzybów w jednej ontocenozie (u *T. ochropus* w dziobie *C. glabrata* wraz z *T. stellata* i w kloace *Rh. rubra* wraz z *T. stellata*; u *Ph. pugnax* tylko w kloace *A. terreus* wraz z *Cryptococcus* sp. i *Penicillium* sp.). U pozostałych ptaków różnorodność grzybów nie przekraczała 3 gatunków. Znacznie częściej grzyby izolowano z kloaki niż z dzioba. W obydwu ontocenozach zdecydowanym dominantem były grzyby z rodzaju *Aspergillus* i *Rhodotorula*, a w następnej kolejności *Candida*, *Saccharomyces* i *Torulopsis*.

Uzyskane wyniki wyraźnie wskazują, że analizowane ptaki mogą brać czynny udział w przenoszeniu i przekazywaniu grzybów potencjalnie chorobotwórczych dla człowieka w całej biosferze. Tym samym stanowią ważne ogniwo w łańcuchu epidemiologicznym grzybic mogących mieć swoje źródło w rezerwuarach wodnych różnych stref klimatycznych, przez które migrują lub bezpośrednio u zainfekowanych ptaków.