

Fungal flora in the trachea of birds from a wildlife rehabilitation centre in Spain

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ABSTRACT: In the present work we study the prevalence of fungal flora in the tracheal epithelium of wild birds in a rehabilitation centre. Two hundred and sixteen birds representing 26 species from seven orders were sampled. Yeasts and moulds were isolated from 92 of the birds sampled (42.5%); in 24.5% only yeasts, in 12.5% only moulds and in 5.5% both moulds and yeasts together. The cattle egret was where the greatest number of animals with positive isolation was detected. The prevalence of yeasts shows significant differences between raptors and Ciconiiformes. Within the yeast flora, basically there were strains belonging to the genus *Candida*, mainly *C. albicans*. In the moulds, almost a half of them belonged to the genus *Aspergillus*.

Keywords: birds; yeasts; moulds; fungi; wildlife; *Candida*; *Aspergillus*

Mycoses are among the most frequent and most serious systemic diseases in birds. Most of them are caused by ubiquitous microorganisms to which birds, just like humans and other animals, are continually exposed (Shin et al., 2004). In these processes, members of the genera *Aspergillus* and *Candida* are the most frequently isolated pathogens (Bauk, 1994; Cork et al., 1999; Hubalek, 2004).

The stress appears to be an additional factor in the development of fungal infections (Balseiro et al., 2005). This stress can be associated with captivity, inadequate management, prolonged treatment with antimicrobials, or other debilitating conditions. It can also be associated with a certain form of physiological stress which may be greater at certain times of the year, such as during the breeding season or in winter when environmental conditions are more severe (Redig et al., 1980; Bauk, 1994; Cork et al., 1999). There is evidence that the occurrence of this stress needs to be concurrent with the inhalation of spores to develop a fungal disease (Dixon et al., 1989; Redig, 1993; Bauk, 1994). Anatomical characteristics that might predispose birds to this disease include the absence of the epiglottis for preventing particulate matter from entering the lower respiratory tract, absence of the diaphragm resulting in

the inability to produce a strong cough reflex, and a limited distribution of pseudo-stratified ciliated columnar cells through the respiratory tract (Tell, 2005).

Studies were undertaken in order to collect baseline data on fungal flora and establish the prevalence in tracheal epithelium of wild birds in a Rehabilitation Centre of Wild Animals in Spain. As far as we know, this is the first study of the isolation of fungal flora in the trachea of different birds in rehabilitation centres.

MATERIAL AND METHODS

Animals

Two hundred and sixteen birds representing 26 species from seven orders were sampled, with the highest numbers from the order Falconiformes ($n = 146$): black kite (*Milvus migrans*) ($n = 12$), red kite (*Milvus milvus*) ($n = 1$), griffon vulture (*Gyps fulvus*) ($n = 18$), black vulture (*Aegypius monachus*) ($n = 3$), short-toed eagle (*Circaetus gallicus*) ($n = 5$), goshawk (*Accipiter gentilis*) ($n = 8$), sparrow hawk (*Accipiter nisus*) ($n = 7$), buzzard (*Buteo buteo*)

($n = 21$), golden eagle (*Aquila chrysaetos*) ($n = 5$), booted eagle (*Hieraaetus pennatus*) ($n = 2$), Bonelli's eagle (*Hieraaetus fasciatus*) ($n = 3$), lesser kestrel (*Falco naumanni*) ($n = 42$), kestrel (*Falco tinnunculus*) ($n = 14$), merlin (*Falco columbarius*) ($n = 1$), peregrine (*Falco peregrinus*) ($n = 4$). Samples were also obtained from the order Strigiformes ($n = 14$) or nocturnal raptors: barn owl (*Tyto alba*) ($n = 1$), scops owl (*Otus scops*) ($n = 3$), little owl (*Athene noctua*) ($n = 9$), tawny owl (*Strix aluco*) ($n = 1$). Other birds belonged to the order Ciconiiformes ($n = 49$): cattle egret (*Bubulcus ibis*) ($n = 39$), white stork (*Ciconia ciconia*) ($n = 10$). Other miscellaneous birds sampled were ($n = 7$): mallard (*Anas platyrhynchos*) ($n = 2$), red-legged partridge (*Alectoris rufa*) ($n = 1$), lapwing (*Vanellus Vanellus*) ($n = 1$), yellow-legged gull (*Larus cachinnans*) ($n = 1$), jay (*Garrulus glandarius*) ($n = 1$), raven (*Corvus corax*) ($n = 1$).

Mycological analysis

Tracheal samples were obtained by introducing a sterile swab (Labcenter, Madrid, Spain) into the trachea as deeply as the animal allows and touching the syrinx if possible, twirled around, and withdrawn, without contacting other mouth parts (contaminated swabs were discarded). Swabs were then placed in transport media, and taken to the laboratory as soon as possible for mycological analysis.

The swabs were cultivated simultaneously in Sabouraud agar (Biocheck, Madrid, Spain) and Sabouraud agar with added gentamycin and chloramphenicol (Biocheck, Madrid, Spain). The plates

were incubated at 37°C, with readings taken after 24 hours and for a maximum of 15 days. Yeast identification was made by macroscopic observation, microscopic morphology, and by the rapid identification method API 20C AUX (Biomerieux, Madrid, Spain). The identification of the moulds was based on macroscopic and microscopic observation and by applying standard taxonomic criteria (de Hoog et al., 2000).

Statistical analysis

Groups of birds with fungal isolates and without fungal isolates were compared using Fisher's exact test (Cochran, 1952; Altman, 1991).

RESULTS

Yeasts and moulds were isolated from 92 out of the two hundred and sixteen birds sampled in the rehabilitation centre, as shown in Table 1. As a result, 42.59% of the animals analysed presented positive fungal isolations, distributed as follows: in 24.53% only yeasts were isolated; in 12.5% only moulds and in 5.55% both moulds and yeasts together. The specific isolation of yeasts and moulds is shown in Table 2 and 3, where it should also be taken into account that at times more than one type of fungus was isolated in a single animal. In all the species analysed, the cattle egret (*Bubulcus ibis*) was where the greatest number of animals with positive isolation was detected, with fungal flora isolated in 87.2% of the animals. In the

Table 1. Groups of birds with the positive isolation of moulds and yeasts sampled in a wildlife rehabilitation centre

Order of birds	Number of sampled birds	Number of birds with isolation of			Total of positive sample birds
		moulds	yeasts	moulds + yeasts	
Falconiformes	146	24 (16.43%)	24 (16.43%)	3 (2.05%)	51 (34.93%)
Strigiformes	14	0 (0%)	2 (14.28%)	1 (7.14%)	3 (21.42%)
Ciconiiformes	49	3 (6.12%)	27 (55.10%)	7 (14.28%)	37 (75.51%)
Others	7	0 (0%)	0 (0%)	1 (14.28%)	1 (14.28%)
Total	216	27 (12.5%)	53 (24.53%)	12 (5.55%)	92 (42.59%)

Table 2. Yeasts isolated from birds sampled in a wildlife rehabilitation centre

Species with positive isolation	Yeasts															
	<i>Candida</i>									<i>Rhodotorula</i>		<i>Saccharo- myces</i>	<i>Trichosporon</i>		<i>Geotrichum</i>	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>Gyps fulvus</i> (n = 18)	2			1								2				
<i>Accipiter nisus</i> (n = 7)	3										1					
<i>Accipiter gentilis</i> (n = 8)	1	1														
<i>Buteo buteo</i> (n = 21)		2														
<i>Hieraaetus pennatus</i> (n = 2)												1				
<i>Circaetus gallicus</i> (n = 5)											2					
<i>Falco tinnunculus</i> (n = 14)	1	1										2				
<i>Falco naumanni</i> (n = 42)	5	2		1							1					
<i>Falco peregrinus</i> (n = 4)	1															
<i>Falco columbarius</i> (n = 1)			1								1					
<i>Strix aluco</i> (n = 1)		1														
<i>Otus scops</i> (n = 3)											1					
<i>Athene noctua</i> (n = 9)	1															
<i>Bubulcus ibis</i> (n = 39)	16	7	3	1	2	1	1	1	1	1	10	6	2	1	1	1
<i>Ciconia ciconia</i> (n = 10)						1										
<i>Larus cachinnans</i> (n = 1)	1															
Total (n = 216)	31	14	4	3	2	2	1	1	1	1	16	11	2	1	1	1

1 – *C. albicans*, 2 – *C. parapsilosis*, 3 – *C. zeylanoides*, 4 – *C. ciferrii*, 5 – *C. famata*, 6 – *C. colliculosa*, 7 – *C. lipolytica*; 8 – *C. guillermondi*, 9 – *C. inconspicua*, 10 – *C. lambica*, 11 – *R. mucilaginis*, 12 – *R. glutinis*, 13 – *S. cerevisiae*, 14 – *T. capitatum*; 15 – *T. cutaneum*, 16 – *G. penicillatum*

Table 3. Moulds isolated from birds sampled in a wildlife rehabilitation centre

Species with positive isolation	Mould							
	<i>Aspergillus</i>	<i>Penicillium</i>	<i>Alternaria</i>		<i>Mucor</i>	<i>Nigrospora</i>	<i>Gliocladium</i>	<i>Chrysosporium</i>
<i>Milvus migrans</i> (n = 12)	1		3				1	1
<i>Gyps fulvus</i> (n = 18)	3		1					
<i>Accipiter gentilis</i> (n = 8)			1		1			
<i>Buteo buteo</i> (n = 21)	3		3		1	1		
<i>Circaetus gallicus</i> (n = 5)			1					
<i>Falco tinnunculus</i> (n = 14)	1							
<i>Falco naumanni</i> (n = 42)	5		2		1			
<i>Strix aluco</i> (n = 1)						1		
<i>Bubulcus ibis</i> (n = 39)	6		1		1		1	
<i>Ciconia ciconia</i> (n = 10)	2							
<i>Larus cachinnans</i> (n = 1)			1					
Total (n = 216)	21		13		4	2	1	1

Table 4. Univariate comparisons between the values of four independent variables recorded as isolate and unisolate in birds of a wildlife rehabilitation centre

	Falconiformes (n = 146)		Strigiformes (n = 14)		Fisher exact test P-value	Falconiformes and Strigiformes (n = 160)		Ciconiiformes (n = 49)		Fisher exact test P-value
	yes	no	yes	no		yes	no	yes	no	
Prevalence moulds and yeasts	51	95	3	11	P = 0.3863 n.s.	54	106	37	12	P < 0.0001 ***
Prevalence yeasts	27	119	3	11	P = 0.73 n.s.	28	132	34	15	P < 0.0001 ***
Prevalence moulds	27	19	1	13	P = 0.47 n.s.	28	132	10	39	P = 0.67 n.s.

n.s. = non-significant

Strigiformes, all the positive isolations belonged to yeasts or to yeasts and moulds, but not to moulds only. In contrast, no isolates were found in *Milvus milvus*, *Aegyptius monachus*, *Aquila chrysaetos*, *Hieraaetus fasciatus*, *Tyto alba*, *Vanellus vanellus*,

Anas platyrhynchos, *Garrulus glandarius*, *Corvux corax* and *Alectoris rufa*.

Fungal isolates, both moulds and yeasts, among Falconiformes and Strigiformes found by the Fisher exact test indicate that there were no significant differences between both groups (Fisher exact test P = 0.3863) (Table 4).

Mould isolates between raptors and Ciconiiformes found by the Fisher exact test indicate that there were no significant differences (Fisher exact test P = 0.67), although the prevalence of yeasts was significant (Fisher exact test P < 0.0001) (Table 4).

Within the yeast flora, basically there were strains belonging to the genus *Candida* (65.21%), with a majority isolation of *C. albicans*. Data to be taken into account is the elevated prevalence of *C. parapsilosis* (Table 5).

In the moulds identified, almost a half (48.9%) belonged to the genus *Aspergillus*, with a high percentage of *Penicillium* also identified (Table 6).

Table 5. Species of yeasts isolated from birds sampled in a wildlife rehabilitation centre

Yeast	Number of isolates	Percentage
<i>Candida</i>	60	65.21
<i>C. albicans</i>	31	33.69
<i>C. parapsilosis</i>	14	15.21
<i>C. zeylanoides</i>	4	4.34
<i>C. ciferrii</i>	3	3.26
<i>C. famata</i>	2	2.17
<i>C. colliculosa</i>	2	2.17
<i>C. lipolytica</i>	1	1.08
<i>C. guillermondi</i>	1	1.08
<i>C. inconspicua</i>	1	1.08
<i>C. lambica</i>	1	1.08
<i>Rhodotorula</i>	27	29.34
<i>R. mucilaginosa</i>	16	17.39
<i>R. glutinis</i>	11	11.95
<i>Sacharomyces cerevisiae</i>	2	2.17
<i>Trichosporon</i>	2	2.17
<i>T. capitatum</i>	1	1.08
<i>T. cutaneum</i>	1	1.08
<i>Geotrichum penicillatum</i>	1	1.08
Total	92	100

Table 6. Genera of moulds isolated from birds sampled in a wildlife rehabilitation centre

Mould genera	Number of isolates	Percentage
<i>Aspergillus</i>	21	48.84
<i>Penicillium</i>	13	30.23
<i>Alternaria</i>	4	9.30
<i>Mucor</i>	2	4.65
<i>Nigrospora</i>	1	2.33
<i>Gliocadium</i>	1	2.33
<i>Chrysosporium</i>	1	2.33
Total	43	100

DISCUSSION

An incubation temperature of 37°C was used to isolate fungi that could be implicated in infectious processes in the birds, not including environmental ones, which could be found in the trachea for merely accidental reasons.

In birds the trachea often bifurcates immediately after entering the thoracic inlet, with the syrinx at this bifurcation. The syrinx is the vocal organ of birds and shows considerable variation between species, in males exhibiting large bullae with unknown functions in some species (Powell, 2000). The syrinx is a common site for fungal lesions in raptors, psittacines, and waterfowl (Tully, 1995). There is also a small area of the stratified squamous epithelium in the syringeal area of some birds that can be modified, allowing colonization by inhaled aspergillosis spores (Bauk, 1994). In our study, samples were obtained from the trachea and from the syrinx when the size of the animal allowed. In the respiratory processes of birds, the usefulness of tracheal cultures has been documented by several authors, with a proper sample collection necessary and appropriate interpretation of culture results (Redig et al., 1980; Redig, 1983).

Fungi are ubiquitous in the environment, and as with saprophytes or commensals, they coexist without effect with the host. Airborne fungal spores, e.g. *Aspergillus*, enter the upper and lower respiratory tract by inhalation, but are rarely pathogenic in healthy individuals (Shin et al., 2004). The ubiquity of the organism suggests that birds may be carriers of the fungi but do not develop overt disease unless stimulated by a decreased resistance of the host elicited by some stress such as an infectious disease, a toxicant, or malnutrition. Experiments indicated that birds subjected to a handling stress had higher levels of corticosteroids (Fowler et al., 1995; Fowler, 1999; Deem, 2003; Park, 2003; Balseiro et al., 2005), which can affect the immune system in many ways (Griffin, 1989).

Raptors are included as a species especially susceptible to aspergillosis (Tell, 2005). In this context, aspergillosis has been described in most diurnal raptors, although some authors consider that nocturnal raptors are rarely affected (Wolf et al., 1992; Redig, 1993). These same authors also suggest the existence of raptor groups with a greater predisposition to the colonization of fungal flora and thus more susceptible to a possible infection of this type. In this sense, Dufty and Belthoff (1997) showed

that the daily pattern of corticosterone secretion is reversed in nocturnal birds and is correlated with the activity period rather than with the light/dark cycle. They also found that captive and free-living owls were not subjected to chronically high levels of stress. In contrast to this, our results indicate that there is no significant difference in the number of fungal isolates between nocturnal and diurnal raptors (Table 3).

We estimated the prevalence of fungal isolates between nocturnal and diurnal raptors on the one hand and Ciconiiformes on the other, to establish if we could find any differences between different orders of carnivorous birds. We found that there were no significant differences in the mould flora, while the prevalence of yeasts was very significant (Fisher exact test $P < 0.0001$) (Table 4).

Aspergillus was the most common mould isolated (48.84%). Aspergillosis is one of the most frequent infectious diseases affecting stressed and immunosuppressed animals (Briggs et al., 1996; Carrasco et al., 2001; Friend, 2001). It is known that the disease will result from a natural infection of *Aspergillus* carried in the birds and brought to a clinical climax by the individual or cumulative stresses of capture, transportation, confinement, and nutritional deficiency. The number of colonies for establishing a definitive diagnosis is usually between one and four; the recovery of large numbers carries a severe prognosis. A simple colony is significant and should not be disregarded as an incidental finding (Redig, 1993).

With regard to other kinds of moulds, the order Mucorales includes a number of saprophytic fungi associated with an underlying diseased condition. The most common infection route is by inhalation of the spores (Reavill, 1996). Mucor infections have been reported as possible aetiological agents of meningoencephalitis in birds (Orcutt and Bartick, 1994); they have also been isolated from a military macaw (*Ara militaris*) with severe bronchopneumonia (Dorrestein et al., 1985) and from a penguin with pneumonia and air sacculitis (Pelto, 1988). There are a few documented cases of diseases caused by *Penicillium*, described as the aetiological agent of a localized beak infection in a macaw (*Ara ararauna*) (Bengoa et al., 1994), associated with feather lesions and found in the lungs, air sacs, liver and other tissues of a captive New World toucanet (Reavill, 1996).

Chrysosporium was identified in the plumage of birds as keratinolytic fungi. The ecological relation-

ships between birds and feather-degrading fungi are poorly studied, but they appear to be similar to those for birds and feather-degrading bacilli (Burt and Ichida, 1999).

In our study we describe an important prevalence of yeast flora in the trachea, fundamentally strains belonging to the genus *Candida* (65.21%); and of these, more than a half has been identified as *C. albicans*. The relationship between this high prevalence and the later incidence of clinical candidiasis has not been established yet. *Candida* spp. are usually opportunistic pathogens that infect birds suffering from primary infectious diseases or malnutrition (Tully, 1995). One fact to keep in mind is the high prevalence of isolates of *C. parapsilosis* (15.21%), a species which recently has been increasingly isolated in cases of clinical candidiasis in very different animal species and in humans (Blanco et al., 2000; Garcia and Blanco, 2000). A high percentage (28.3%) of strains of the genus *Rhodotorula* was also isolated, whose pathogenic role has yet to be discovered.

Most of the yeast species isolated in this study were described previously and implicated in different animal pathological processes, and specifically *C. humicola*, *C. inconspicua*, *T. cutaneum* and *Rhodotorula* sp. have been implicated in respiratory disorders in birds (Costa-Durao et al., 1991). In any case, no sufficiently reliable criteria could be established to prove that positive culture results were associated with disease.

It seems clear that the predisposition factors and a specific generalized debilitation of each individual are fundamental for the development of infection by fungal flora, in accordance with what has been noted by different authors, in the sense that all birds are probably prone to infection (Greenacre et al., 1992). For this reason, efforts should be made to reduce a stress in the recovery of birds and strict hygiene should also be encouraged at all times.

Projects to rehabilitate wildlife birds must carefully consider the risks of disease when determining whether to release these animals back into the wild or to incorporate them into captive breeding programs.

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