A comparative study of digestive tract mycoflora of broilers with layers

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Abstract

Fungal agents, especially yeasts, are resident in digestive system as microflora and they can invade the mucosal tract in animals with predisposing factors. The prevalence of the resident fungal flora in the digestive tract of healthy broilers and layers during April to September 2008 was investigated. The samples were taken from the preventriculus, jejunum and cecum contents of 120 birds and cultured on Sabouraud glucose agar. Both filamentous fungus and yeasts were isolated and a predominance of the *Candida* species was found, followed by *Trichosporon beigelii, Geotrichum candidum*, *Rhodotorula rubra* and *Saccharomyces cerevisiae species*, which were found in 84.6%, 5.5%, 4.6%, 3.3% and 0.5% of cultures, respectively. Among the yeast isolates, *Candida albicans* (45.8%) was the most prevalent fungal agent, representing a significant proportion when compared to the other fungal isolates ($P<0.05$). The frequency of the yeast in the layers' digestive tract was significantly higher than that found in the broilers ($P<0.05$). Mycological examination revealed the highest numbers of yeasts in the cecum and lower numbers in the jejunum and preventriculus in two breeds.

Introduction

The composition of the microbial populations of the digestive tract varies from animal to animal, even within a species (Gabriel *et al*., 2005). Recently, the occurrence of bacteria as natural occupants of the gut flora of the poultry has been well documented. This flora has been found in the highest numbers in the crop and cecum and in lowest numbers in the small intestine (Gabriel *et al.,* 2006).

Another part of gut microflora is fungi, in particular yeasts. Although some yeasts, such as *S. cerevisiae* and *S. boulardii*, are used as probiotics in animals and poultry, the *Candida* species, which are residents of the digestive tract in a broad spectrum of animal species, can act as pathogens in some conditions (Mellado *et al*., 2000). Stress appears to be an important factor in the development of microbial infections, in particular fungal infections. It can be associated with captivity, inadequate management, prolonged treatment with antimicrobials and other debilitating conditions (Balseiro *et al*., 2005). Several reports demonstrated fungal infections of the digestive tract in domestic and wild animals (Hörmansdorfer and Bauer, 2000; Wiesner *et al*., 2001; Ryan and Ray, 2004). To date, the resident fungal flora of digestive tract is still unknown in healthy broilers and layers and this knowledge would be very useful in assessing the accuracy of treatments. This study was undertaken in order to collect baseline data on fungal flora and their frequencies in the digestive tract of broilers and layers collected from different regions of Tehran, Iran.

Materials and Methods

Birds and sampling procedure

One hundred and twenty healthy birds of both sexes, from two breeds, broiler (50 days old) and layer (80 weeks old) were selected from different poultry houses. Information such as sex, age, breed and vaccination history was recorded. In slaughterhouses, the carcasses were immediately opened and sections from the preventriculus to the anus were taken, ligated with a nylon suture and transferred under cool condition to the Mycology Research Center in the Faculty of Veterinary Medicine at the University of Tehran, Iran. After a few hours the preventriculus, jejunum, and cecum were separated and prepared for laboratory tests.

Fungal examination

Approximately 1 g of the contents of the different parts of the digestive tract (the preventriculus, jejunum and cecum) was mixed with 9 ml of sterile distilled water and homogenized for 3 min. From the initial 10^{-1} dilution, 10-fold serial dilutions were subsequently made in sterile

distilled water. The samples were diluted to 10^{-2}, 10^{-3} and 10^{-4}, respectively. For inhibiting the bacterial overgrowth, chloramphenicol (50 µl/ml) and doxycycline (100 mg/50 ml, Daru Pakhsh Co., Tehran, Iran) were added to each tube. Tubes were kept in room temperature for 1 hour before culturing. For each dilution, 0.1 ml was inoculated into Sabouraud glucose agar (Merck Co., Darmstadt, Germany) containing chloramphenicol (50 µl/ml, Daru Pakhsh Co., Tehran, Iran). All the inoculated plates were incubated at 28°C and examined daily for two weeks. The total number of fungal colonies was counted and the number of colonies forming unit per gram (CFU/gr) was determined. In order to identify the yeast isolates, germ tube test, chromagar culture, RapID™ yeast identification test (Remel Inc., USA) and colony morphology and microscopic characteristics of mould fungi were carried out. The results were expressed as the mean CFU/gr. Student's t-test was used to compare the differences between groups using SPSS software (Version 15). A *P*-value of less than 0.05 was considered to be statistically significant.

Results

The fungal isolates from the digestive tract are illustrated in Table 1. From some of the samples cultured, only a single fungus was isolated. Within the yeast flora, there were strains belonging to the genus *Candida* (84.6%), with the majority of isolates of *C. albicans* (45.8%). The other predominant *Candida* isolates included: *Candida spp*. (9.6%), *C. tropicalis* (8.8%), *C. kefyr* (5.4%), *C. parapsilosis* (4.2%), *C. krusei* (3.3%), *C. glabrata* (3.3%), *C. guilliermondii* (2.1%) and *C. lusitaniae* (2.1%). Of the moulds identified, almost all the isolates belonged to the species *G. candidum* (4.6%).

Table 1: Frequency of fungal isolates from the digestive tract of broilers and layers.

Breed/Site	Broiler				Layer				
Species	Preventriculus	Jejunum	Cecum	Total	Preventriculus	Jejunum	Cecum	Total	Total
Candida albicans	2	5	7	14	22	25	49	96	110
C. tropicalis	1	2	5	8	5	2	6	13	21
C. krusei	1	-	2	3	3	2	-	5	8
C. glabrata	-	2	2	4	2	1	1	4	8
C. kefyr	3	6	3	12	-	-	1	1	13
C. guilliermondii	-	1	-	1	1	-	3	4	5
C. parapsilosis	2	1	3	6	-	1	3	4	10
C. lusitaniae	2	1	2	5	-	-	-	-	5
Saccharomyces cerevisiae	-	-	-	-	1	-	-	1	1
Geotrichum candidum	-	-	3	3	1	-	7	8	11
Trichosporon beigelii	-	1	5	6	4	-	3	7	13
Rhodotorula rubra	-	1	-	1	4	2	1	7	8
Candida spp.	4	7	2	13	2	5	3	10	23
yeast	1	1	-	2	1	-	-	1	3
Total	15	25	34	79	46	38	77	161	240

In terms of the location of the isolates in the digestive tract, the yeast isolates were predominantly found on the cecum ($P<0.05$). Also, the frequency of the yeast found in the layers' digestive tract was significantly higher than the frequency found in the broilers ($P<0.05$).

Fungal counts were made in Sabouraud glucose agar for the three digestive organs from chickens, in which 10^{-4} was countable and considered for colony forming unit (CFU) in this study. The total counts from the preventriculus and jejunum were relatively stable and similar, while those of the cecum contents fluctuated to a greater degree, as shown in Table 2. The most yeast was found on the layers. Regarding the CFU/gr, there was only a significant difference found in the number of yeast colonies in the cecum between broilers and layers ($P<0.05$). Also, significant difference was found in total colonies counted from digestive tract between two breeds ($P<0.05$).

Table 2: Total fungal counts associated with the digestive tract of broilers and layers at 10^{-4} dilution

Site / Breed	Cecum	Jejunum	Preventriculus	Total
Broiler	$10^{3}\times 1.6$	$10^{3}\times 3.9$	$10^{3}\times 2.2$	$10^{3}\times 7.7$
Layer	$10^{4}\times 2.34$	$10^{3}\times 3.5$	$10^{3}\times 1.4$	$10^{4}\times 2.83$
Total	$10^{4}\times 2.5$	$10^{3}\times 7.4$	$10^{3}\times 3.6$	$10^{4}\times 3.6$
P-value	S*	NS**	NS	S

* S: Significant; ** NS: Non-significant

Discussion

Fungi are ubiquitous in the environment and enter the digestive tract via an oral route, however they are rarely pathogenic in healthy individuals (Shin *et al*., 2004). There are numerous reports of fungal infections in domestic poultry and occasional reports of the disease in wild birds (Moretti, Fioretti and Bonchio, 2000; Hubalek, 2004; Ryan and Ray, 2004). Since fungal flora of the digestive tract have not previously been described in both broilers and layers, this study was undertaken to identify the indigenous gut fungal flora in these birds. In this study, all of the organisms isolated were yeasts, with the exception of *G. candidum*. The most frequently isolated fungi belonged to the *Candida* species (84.6%), followed by *T. beigelii* (5.5%), *G. candidum* (4.6%), *R. rubra* (3.3%), *S. cerevisiae* (0.5%) species, with other yeasts making up the remaining number (1.4%). The yeasts isolated in this study, and their relative frequency, both demonstrate the similarity between healthy digestive tract of these birds and those of other animal species (Tully, 1995; Brown *et al*., 2005). *Candida* species, in particular *C. albicans*, may be a normal inhabitant of the digestive tract in birds. It is also an opportunistic yeast that can cause a variety of problems associated with the avian digestive tract, the magnitude and outcome of which may depend on the age and

immune system status of a bird (Gerlach, 1994). In addition, some *Candida* antigens are known to have immunosuppressive components, so the rapid growth of *C. albicans* in exact conditions, such as antibiotic and corticosteroid therapies, could affect local and even systemic immune functions (Odds, 1988). The prevalence of yeast flora in the digestive tract of layers was also demonstrated. Specifically, strains belonging to the genus *Candida* were identified and, of these, more than a half has been identified as *C. albicans*. This prevalence was significantly different than that found in the broilers. Previous work has indicated that birds such as layers that are subjected to different stresses and/or specific diets have higher levels of corticosteroids (Deem, 2003; Park, 2003; Balseiro *et al*., 2005; Blanco *et al*., 2007). This can affect the immune system and result in the multiplication and invasion of yeast cells into the body (Griffin, 1989). Also, because the layers were kept for a longer period in poultry houses, they were repeatedly exposed to environmental fungi. Under these conditions, fungal elements are transmitted into the birds' digestive and respiratory tracts and sometimes even invade the intestinal mucosal barriers in exact birds. Among the different sites of the digestive tract, the cecum showed significantly the highest total counts (2.5×10^4) and a significant difference was found between the broilers and layers' caeca. The jejunum had the next highest count (7.4×10^3). The preventriculus, as may be expected from its lower pH, contained the fewest organisms (3.6×10^3). The reasons of the highest fungal density in the cecum could be related to the lower digestive, enzymatic and decomposition processes occurring in this site of digestive tract and its higher pH degree in comparison to other sites. The lower total fungal count found in the cecum of broilers, compared to that of layers, may be due to the fact that broilers, who are younger than layers, have a less significant intestinal microbiota that leads to lower total fungal counts. A low percentage of strains of the genus *Trichosporon*, *Geotrichum* and *Rhodotorula* were also isolated; the pathogenic roles of which have been discovered in recent years as those of emerging fungi (Fleming, Walsh and Annaissie, 2002). It seems that the certain factors, combined with a specific generalized debilitation of each individual, predispose individuals to the development of infection by these fungi (Haupt *et al*., 1983; Fleming *et al*., 2002). On the basis of data obtained in this study, it was concluded that *C. albicans* was the most frequently isolated yeast from broilers and layers with healthy digestive tract. In addition, the frequency of the yeast population in the digestive tract of layers, in particular in the cecum, was more than that of broilers.

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References

1. Balseiro, A.; Espi, A.; Marquez, I.; Perez, V.; Ferreras, M.C.; Garcia Marin, J.F. and Prieto, J.M. (2005) Pathological features in marine birds affected by the prestige's oil spill in the north of Spain. J. Wildlife Dis. 41: 371-378.
2. Blanco, G.; Lemus, J.A.; Grande, J.; Gangoso, L.; Grande, J.M. and Donázar, J.A. (2007) Geographical variation in cloacal microflora and bacterial antibiotic resistance in a threatened avian scavenger in relation to diet and livestock farming practices. Environ. Microbiol. 9: 1738-1749.
3. Deem, S.L. (2003) Fungal diseases of birds of prey. Vet. Clin. North Am. Exotic Anim. Pract. 6: 363-376.
4. Fleming, R.V.; Walsh, T.J. and Annaissie, E.J. (2002) Emerging and less common fungal pathogens. Infact. Dis. Clin. North Am. 16: 915-933.
5. Gabriel, I.; Mallet, S. and Sibille, P. (2005) Digestive microflora of bird: factors of variation and consequences on bird. INRA Prod. Anim. 18: 309-322.
6. Gabriel, I.; Mallet, S.; Sibille, P. and Guillot, J.F. (2006) Microflora of the digestive tract: critical factors and consequences for poultry. World Poult. Sci. J. 62: 499-511.
7. Gerlach, H. (1994) Bacteria. In: Ritchie, B.W., Harrison, G.J., Harrison, L.R., (1 st ed). Avian medicine: principles and application. Wingers, Lake Worth, FL, pp: 949-983.
8. Griffin, J.F.T. (1989) Stress and immunity: a unifying concept. Vet. Immunol. Immunopatholol. 20: 263-312.
9. Haupt, H.M.; Merz, W.G.; Beschorner, W.E.; Vaughan, W.P. and Saral, R. (1983) Colonization and infection with *Trichosporon* species in the immunosuppressed host. J. Infect. Dis. 147: 199-203.
10. Hörmansdorfer, S.; Bauer, J. (2000) Yeast infection in veterinary medicine. Contrib. Microbiol. 5: 54-78.
11. Hubalek, Z. (2004) An annotated checklist of pathogenic microorganisms associated with migratory birds. J. Wildlife Dis. 40: 639-659.
12. Mellado, E.; Cuenca-Estrella, M.; Regadera, J.; Gonzalez, M.; Diaz-Guerra, T.M. and Rodriguez-Tudela, J.L. (2000) Sustained gastrointestinal colonization and systemic dissemination by *Candida albicans*, *Candida tropicalis* and *Candida parapsilosis* in adult mice. Diagn. Microbiol. Infect. Dis. 38: 21-28.
13. Moretti, A.; Fioretti, D.P. and Bonchio, L. (2000) Isolation of *Candidia* rugosa from Turkey. J. Vet. Med-B. 47: 433-439.
14. Park, F. (2003) Behaviour and behavioural problems of Australian raptors in captivity. Sem. Avian Exotic Pet Med. 12: 232-241.
15. Raquel Brown, M.; Thompson, C.A. and Mohamed, F.M. (2005) Systemic candidiasis in an apparently immunocompetent dog. J. Vet. Diagn. Invest. 17: 272-276.
16. Ryan, K.J.; Ray, C.G. (2004) Sherris Medical Microbiology. 4th edition. McGraw Hill, New York, USA.
17. Shin, S.H.; Ponikau, J.U.; Sherris, D.A.; Congdon, D.;

Frigas, E.; Homburger, H.A.; Swanson, M.C.; Gleich, G.J. and Kita, H. (2004) Chronic rhinosinusitis: An enhanced immune response to ubiquitous airborne fungi. J. Allergy Clin. Immunol. 114: 1369-1375.

18. Tully, T.N. (1995) Avian respiratory diseases: clinical overview. J. Avian Med. Surg. 9: 162-174.
19. Wiesner, S.M.; Jechorek, R.P.; Garni, R.M.; Bendel, C.M. and Wells, C.L. (2001) Gastrointestinal colonization by *Candida albicans* mutant strains in antibiotic-treated mice. Clin. Diagn. Lab. Immunol. 8: 192-195.