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Original Research Article

Prevalence of fungal pathogens in broiler chickens and their environment

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ABSTRACT

Fungal diseases of poultry have become problematic as bacterial and viral diseases. This study was designed to investigate the prevalence of fungal agents in broiler chickens and their environment. The prevalence of fungal isolation from broiler chickens was 21.6% including 12.8% moulds and 8.8% yeast while the prevalence of fungal isolation from the environment was 46.8% including 25.5% moulds and 21.3% yeast. Aspergillus species was the most prevalent moulds while *C. albicans* was the most prevalent yeast recovered from broiler chickens and their environment.

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1. Introduction

Fungal pathogens pose serious problems worldwide for both human and animal health, especially in the subtropical and tropical regions. Fungi, bacteria and their toxins are natural contaminants of environment particularly foods even when the best condition of culture, harvest, storage and handling were used. Microorganisms, such as bacteria, moulds, yeasts and viruses, in the living environment are often pathogenic and cause severe infections in both human beings and animals **(Reddy, 2007).**

Fungal infections are frequently associated with morbidity and mortality in birds **(Radwan et al., 2016).** Among the fungi, Penicillium and Aspergillus species are dominantly present **(Plewa-Tutaj and Lonc, 2014).** Species of these genera are commonly found in soil, decaying organic materials, animal feed, stored grains and other materials **(Leite et al., 2012).** Moreover, some of these species can be responsible for spoilage of food and beverages, bio-deterioration of materials and are able to produce dangerous mycotoxins.

Few fungal species are common pathogens in avian species especially Aspergillus spp.; the cause of aspergillosis **(Rippon, 1982).** Manifestations of aspergillosis depend on the organs affected and whether infection is localized or disseminated. Aspergillosis appears to be more significant in confinement situations where stress factors may be involved or where moldy litter or grain is present **(Radwan et al., 2016).** Contaminated poultry litter is often the source of Aspergillus conidia **(Dyar et al., 1984).** A possible effect of aspergillosis is the possible transmission of fungal mycotoxin residues to meat and eggs from infected chickens, which is potentially hazardous to public health (**Anath and Faryd, 2000).**

Aspergillusfumigatus is considered as a major pathogen in birds **(Radwan et al., 2016).** Other species like *A. flavus*, *A. niger*, *A. nidulans*, and *A. terreus* may also be isolated from avian cases of aspergillosis (sometimes in mixed infections) but much less frequently than A. fumigatus **(Kunkle et al., 2003 and Martin et al., 2007).** Active fungal proliferation and sporulation of A. fumigatus on organic material produce large amounts of airborne small-sized conidia that are easily dispersed in air, then potentially inhaled and deposited deep in the respiratory tract of broilers and develops as a bronchopneumonia **(Milos et al., 2011).**

Moreover, Candida species are widely spread throughout the poultry producing areas of the world **(Radwan et al., 2016).** In the past, *C. albicans* was assumed to be the only pathogenic yeast genus Candida. However, it is now known that of the more than 100 species of Candida, seven are of medical significance **(Hopfer, 1985).**

In recent years, the growing economic value of poultry has led to the increase of research of poultry diseases. The fungal diseases of poultry have become problematic as bacterial and viral diseases **(Radwan et al., 2016).** The purpose of this study was to investigate the prevalence fungal pathogens in broiler chickens and their environment

2. Materials and methods

2.1.Samples.

A total of 274 samples were collected frombroiler chickens (*n=227*) and broiler chickens environment (*n=47*; including broilers feed, water and bedding) from different areas in El-Fayoum Governorate during the period from February 2015 to May 2016. The manifestations of studied chickens showed dyspnea, gasping, accelerated breathing, depression, emaciation, ruffled feathers, profuse watery diarrhea, blindness, torticollis, lack of equilibrium, and stunting growth. Postmortem lesions of diseased and freshly dead chickens showed congestion of the lungs, airsacculitis, mucous enteritis with sloughing of the intestinal mucosa and some of them showed greenish gray lesions and caseated nodules of 1-2 mm thickness distributed in lungs, livers, proventriculus, gizzard, intestine and abdomen.

The chickens' samples were collected mainly from crop (*n=20*), air sacs (*n=80*), pericardium

(*n=80*), proventriculus (*n=27*) and liver (*n=20*). All samples were transferred directly to the laboratory of Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suef University for mycological examinations.

2.2.Fungal isolation.

All samples were taken immediately and transferred directly into pre-enrichment broth Malt extract broth, (Oxoid) and incubated at ^{o}37C for 24-48 h, then cultured on Sabouraud dextrose agar medium (Oxoid) and incubated at ^{o}37C for 24-48 h.

2.3.Identification of fungal isolates.

The recovered fungi were identified morphologically according to Rippon (1988**)**. Mycelial fungi were identified by examination of mycelial morphology, the reverse colour as well as examination of colonial smears using lactophenol cotton blue stain. Yeast like fungi were identified by colonial morphology and examination of Gram`s stained culture.

2.4.Biochemical identification of yeast isolates by using API kit. The appropriate API kit (API 20 C AUX, Oxoid) was used according to the manufacturer's instruction.

3. Results

3.1.Prevalence of fungal pathogens recovered from broiler chickens.

Out of 227 samples collected from different lesions of broiler chickens, 49 fungal isolates were recovered; with a prevalence rate of 21.6%, including 29 (12.8%) mould isolates and 20 (8.8%) yeast isolates. Out of 20 crop, 7 isolates (35%) were from of which 2 (10%) moulds and 5 (25%) yeasts while from 80 air sacs, 17 isolates (21.3%) were recovered of which 12 (15%) moulds and 5 (6.3%) yeast. Also, 10 isolates (12.5%) were recovered from pericardium (*n=80*); 7 (8.8%) moulds and 3 (3.8%) yeast. From 20 livers, 5 isolates (25%) were recovered; 3 (15%) moulds and 2 (10%) yeast; while 10 isolates (37%) were recovered from proventriculus (*n=27*); 5
as follow:

Table (1): Prevalence of fungal pathogens recovered from broiler chickens.

Source	No. of samples	Recovered Fungi					
		Mycelial Fungi		Yeasts		Total	
		No.	%	No.	%	No.	%
Crop	20	2	10	5	25	7	35
Air sac	80	12	15	5	6.25	17	21.25
Pericardium	80	7	8.75	3	3.75	10	12.5
Liver	20	3	15	2	10	5	25
Proventriculus	27	5	18.5	5	18.5	10	37
Total	227	29	12.8	20	8.8	49	21.6

%: Percentage was calculated according to the corresponding No. of chicken samples.

3.2.Prevalence of fungal pathogens recovered from broiler chickenenvironment.

Out of 47 samples collected from broiler environment, 22 fungal isolates were recovered; with prevalence rate of 46.8%, including 12 (25.5%) mould isolates and 10 (21.3%) yeast isolates(Table 2).

Table (2): Prevalence of fungal pathogens recovered from broiler chicken environment.

No. of samples	Recovered Fungi					
	Mycelial Fungi		Yeasts		Total	
	No.	%	No.	%	No.	%
47	12	25.5	10	21.3	22	46.8

%: Percentage was calculated according to the corresponding No. of environmental samples.

3.3. Identification of the recovered fungi.

3.3.1. Identification of mould isolates.

Mould isolates recovered from broiler chickens (*n=29*) were recognized as 5 *A. fumigatus*(2.2%), 6 *A. flavus* (2.6%), 10 *A. niger* (4.4%), 1 *A. nidulans* (0.4%), 1 *Cladosporium* (0.4%) and 4 *Penicillium*species (1.8%) (Table, 3).

Table (3): Prevalence of mould isolates recovered from broiler chickens.

Total No. sample	*A. fumigatus*		*A. flavus*		*A. niger*		*A. nidulans*		*Cladosporium* spp.		*Penicillium* Spp.		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
227	5	2.2	6	2.6	10	4.4	1	0.4	1	0.4	4	1.8	29	12.8

%: Percentage calculated according to Total No. of chickens samples.

Mould isolates recovered from broiler chickens environment (*n=12*) were recognized as 3 *A. fumigatus*(6.4%), 4 *A. flavus* (33.3%), 1 *A. niger* (2.1%), 1 *A. nidulans* (2.1%), 1*A. terrus* (2.1%) and 2 Zygomycetes(2.3%)(Table, 4).

Table (4): Prevalence of mould isolates recovered from broiler chickens environment.

Total No. sample	*A. fumigatus*		*A. flavus*		*A. niger*		*A. nidulans*		*A. terrus*		Zygomycetes		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
47	3	6.4	4	33.3	1	2.1	1	2.1	1	2.1	2	2.3	12	25.5

%: Percentage calculated according to Total No. of samples.

3.3.2. Identification of yeast isolates.

Out of 274 samples, 30 yeast isolates (10.9%) were recovered and arranged as 20 (8.8%) from 227 chickens samples and 10 (21.3%) from 47 environmental samples. All yeast isolates were recognized as *Candida* species. *C. albicans* was the most prevalent with a total of 15 isolates (5.5%); 11 isolates (4.8%) from chickens and 4 (8.5%) from the environment. Then, both of *C. tropicalis* and *C. krusei*; with a total of 6 isolates for each (2.2%) of which 3 isolates (1.3%) and 4 isolates (1.8%) from chickens, respectively, as well as 3 isolates (6.4%) and 2 isolates (4.3%) from environment, respectively. Finally, *C. glabrata* with a total of 3 isolates (1.1%); 2 isolates (0.9%) from chickens and 1 (2.1%) from the environment(Table, 5).

Table (5): Prevalence of yeast isolates recovered from broiler chickens and their environment.

Source	Total No. sample	*C. albicans*		*C. tropicalis*		*C. krusei*		*C. glabrata*		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%
Broiler chicken	227	11	4.8	3	1.3	4	1.8	2	0.9	20	8.8
Environment	47	4	8.5	3	6.4	2	4.3	1	2.1	10	21.3
Total	274	15	5.5	6	2.2	6	2.2	3	1.1	30	10.9

%: Percentage was calculated according to the corresponding Total No. of samples in chikens and their environment.

4. Discussion

Fungi are found on a wide variety of substances such as soil, plants, water and exudates of animals **(Radwan et al., 2014).** Among the infectious diseases, the fungal diseases have their own importance and seem to be one of the great obstacles for the poultry farmers.

Species of the genus Aspergillus are important fungal infection, which affects the respiratory tract of the birds causing high morbidity, mortality and production losses **(Richard et al., 1991).** Aspergillosis appears to be more significant in confinement situations where stress factors may be involved or where moldy litter or grain is present. Contaminated poultry litter is often the source of Aspergillus conidia **(Dyar et al., 1984).** Moreover, mycological examination to investigate the mycotic flora of chicken population revealed isolation of fungal isolates such as *A. niger, A. fumigatus* and *A. flavus* indicating the ubiquitous nature of these fungi **(El -Badry and Sokkar, 1988).** Moreover, Candida species are widely spread throughout the poultry producing areas of the world **(Radwan et al., 2016).** Poultry of all ages are susceptible to the effects of such organism.

Recently, the growing economic value of poultry has led to the increase of research of poultry diseases. The fungal diseases of poultry have become problematic as bacterial and viral diseases **(Darwish, 1989).** Therefore, the current study was designed to investigate the prevalence of fungal agents in broiler chickens and their environment.

In the present study, the prevalence of fungal isolation from broiler chickens was 21.6% including 12.8% mycelial fungi and 8.8% yeast isolates (Table 1). The present prevalence was lower than that of **Radwan et al. (2014)** who reported that the prevalence rate of fungal isolation from broiler chickens was 39% of which 3% were mycelial fungi and 36% were yeast and these results were opposite to those obtained in the present study. Also, Radwan et al. (2016) recorded a prevalence rate 53.1% (42% mycelial fungi and 11.1% yeast). These results were nearly similar to those of **Pennycott et al. (2003)** who isolated yeast from chicken samples in a percentage of (12.15 %).

Results illustrated in table (2) showed that the prevalence of fungal isolation from broiler chickens environmental samples (feed, water and bedding) was 46.8% of which 25.5% were mould isolates and 21.3% were yeast isolates. These results supported that obtained by **Lovett et al. (1971)** who recorded 12 fungal genera in feed with total densities varying from 7×10^2 to 3.2×10^5 CFU/g. Also, **Bacon and Burdick (1977)** isolated 18 fungal species were from poultry litter and air.

Presence of fungi in the environment of poultry implies bad managemental techniques which represents the main source of infection of broilers directly by fungi or indirectly by their metabolites (mycotoxins). Humidity and temperature conditions encountered in poultry farms promote the rapid growth of hyphae and efficient asexual multiplication resulting in a

copious production of easily airborne hydrophobic conidia, which are subsequently dispersed and inhaled by the birds **Pinello (1977).** Initial contamination of poultry farms may occur through use of a mouldy litter or introduction of one-day old birds that has retained conidia in hatchery facilities. Further contamination may involve inappropriate bedding management **(Dyar et al., 1984).** A short-time exposure to heavily contaminated wood shavings induced an experimental pulmonary aspergillosis in chickens **(Julian and Goryo, 1990).** The negative correlation between relative humidity and the number of Aspergillus conidia in air may indicate that xerophilic Aspergillus conidia more readily discharge in dry conditions than in humid atmosphere. Interestingly, high counts of A. fumigatus conidia in air coincided with high levels of respirable dust particles of poultry houses suggesting a possible physical association or a similar response to indoor conditions **(Debey et al., 1995).**

In the present study, the mycological identification of the recovered moulds from broiler chickens (n=29) revealed that the isolates were identified as *A. fumigatus* (2.2%), A. flavus (2.6%), *A. niger* (4.4%), *A. nidulans* (0.4%), Cladosporium (0.4%) and Penicillium species (1.8%) (Table, 3). These result run parallel to those obtained by **(Radwan et al., 2016)** who recorded 2.2% *A. fumigatus*, 8.4% for both *A. flavus* and *A. niger*, 1.3% *A.nidulans*, 0.4% Cladosporium spp. and 1.8% Penicillium spp. **Abd El-Aziz (2015)** reported that A. fumigatus represented 28.0%, *A. niger* 18.6%, Cladosporium spp. 11.9% and Penicillium Spp. 8.5%. Similarly, the presence of the aforementioned fungal species was described from poultry farm by (El-Zarka, 1988), who reported the presence of *A. niger, A. fumigatus*, Penicillium spp. and Cladosporium spp. in addition to A. flavus. Comparable results were also stated where fungal isolates represented about 41.7 % of poultry samples and *A. niger, A. fumigatus* represented the majority of these isolates with 12.5% and 10%, respectively (El-Badry and Sokkar, 1988). In another study conducted by **Moustafa (1995)**, 35.2% of recovered isolates were *A. flavus*, 27.5% A. *niger*, 23.1% *A. fumigatus* and 14.3% *A. terreus*. However, neither Cladosporium nor Penicillium spp. were recovered.

Concerning the mycological identification of the recovered moulds from broiler chickens environment (n=12) they were recognized as 6.4% *A. fumigatus*, 33.3% *A. flavus*, 2.1% for each of *A. niger A. nidulans* and *A. terrus* as well as 23% Zygomycetes (Table, 4). The direct application of feed on the litter and an average concentration of 1.3×104 Aspergillus CFU/g of wood shavings were incriminated in an outbreak of aspergillosis affecting a broiler breeder flock **Akan et al. (2002).** A wider variety of fungal genera were isolated from litter than from feed. Up to 12 fungal genera, with dominant Aspergillus, Fusarium, Mucor, and Penicillium, were isolated in feed **(Lovett et al., 1971).** The species *A. fumigatus* yielded at a maximum of 2.3CFU/g in turkey commercial feed **(Lair-Fulleringer et al., 2006).**

Identification of the recovered yeast isolates from broiler chickens and their environment illustrated in table (5) revealed that all isolates (n=30, with a total prevalence of 10.9%) belonged to Candida spp. and arranged as 20 (8.8%) from 227 chickens and 10 (21.3%) from 47 environment. *C. albicans* was the most prevalent with a total prevalence of 5.5% followed by both of *C. tropicalis* and *C. krusei* (2.2% for each) and finally, *C. glabrata* (1.1%). These results were lower than those of **Pennycott et al. (2003)** who isolated *C. albicans* from chicken's samples in a percentage of (12.15 %). On the other hand, these results run hand to hand with those of **Radwan et al. (2014)** who found that *C. albicans* was the most prevalent (19%) followed by *C. pseudotropicalis* (6 %) and *C. krusei* (5%). Also, Radwan et al. (2016) recorded the same where *C. albicans* was the most prevalent (2.7%) followed by C. krusei (2.2%). Moreover,

Wyatt and Hamilton (1975) found that *C. albicans* comprised 95% of the isolates and the mean incidence of Candida in the crops was 32.3%. They studied the crops from four field outbreaks of crop mycosis. Three of the four cases of crop mycosis were characterized by multiple strains of *C. albicans* in the crop. They also found that less than 1% exhibited visible lesions attributable to Candida. *C. albicans* comprised 95% of the isolates. The population of Candida in the crops of birds found positive was of low magnitude in the majority of the chickens examined. Finally, we can conclude that The fungal diseases of poultry have become problematic as bacterial and viral diseases. Presence of fungi in the environment of poultry implies bad managemental techniques which represents the main source of infection of broilers directly or indirectly. The prevalence of fungal pathogens isolation in examined broiler chickens and their environment were 21.6% and 46.8%, respectively. Aspergillus species was the most prevalent moulds while *C. albicans* was the most prevalent yeast recovered from broiler chickens and their environment.

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