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Mycoflora of Ostrich (*Struthio camelus*) gastrointestinal tract as a human hazard

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Abstract:

Ostriches are susceptible to bacterial, fungal and parasitic diseases. One of the most common strategies to reduce microbial contamination in animal production systems is to identify microbe sources. In this regard, a first critical component for comprehensive farm-to-fork strategies to reduce the burden of foodborne illness is the identification of the pathogenic fungi in foodstuffs with animal sources, and the reduction of human pathogen contamination in food production. This study was carried out to identify to mycoflora in the ostriches' (*Struthio camelus*) gastrointestinal tract (GIT), in the northwest of Iran. The samples were taken from different parts of the gut tract, including crop, gizzard, intestine and caecum of 50 ostriches. A total of 396 fungal colonies were obtained from GIT. These isolates belonged to 17 genera, and *Candida* (18.7 %), *Aspergillus* (16.7 %), *Monascus* (10.6 %), *Trichosporon* (6.6 %) and *Fusarium* (6 %) were predominant isolates. Among the *Candida* isolates, *C. tropicalis* was the most predominant isolates following by *C. albicans*, *C. glabrata* and *C. krusei*. *Aspergillus* spp. and *Monascus ruber* were predominant isolates among the mould fungi.

Keywords: mycoflora, foodborne, *Monascus*, Ostriches.

Introduction

The ostrich has a long history dating back to ancient Africa and Egypt, but today they are also bred in Iran (Cooper, 2000). Commercial ostrich farming initially began for the feathers only; much later, they were bred for leather as well, and only relatively recently for meat. In recent years, increasing attention has been paid to ostrich breeding in Iran with interest focusing on using the ostrich as a meat producer with a major role in agriculture, the economy and the meat production system (Cooper, 2000b).

Even though the nutritional value of ostrich meat is well documented, very little information is available worldwide on the microbiological aspects and quality retention of this foodstuff, especially regarding the source of food-poisoning microbes which most frequently occur in slaughterhouses. One of the most common strategies to reduce microbial contamination in animal production systems is to identify microbe sources and farm management practices that lead to animals' exposure to the pathogen on the farm (Cooper, 2004; Ruma et al., 2008; Maciorowski, 2007).

The lower GIT of most poultry species including the ostrich is normally populated by a large number of microorganisms. Historically, the microbial composition of the GIT of avian species, especially the ostrich, has not been extensively defined compared to that of ruminants (Gabriel et al., 20006; Brisbin et al., 2008; Shokri, et al., 2011).

According to other studies, ostriches are more susceptible to fungal disease, especially aspergillosis. 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, contamination of feed with microbes and other debilitating conditions (Cooper, 2005; Sajid, et al., 2006; Khosravi, et al., 2008).

A first critical component in comprehensive farm-to-fork strategies to reduce the burden of foodborne illness is the identification of the pathogenic fungi in foodstuff with animal sources, and the reduction of human pathogen contamination in the food production (Doyle and Erickson, 2012; Daniels, 2003).

However, there is a lack of published research characterizing the mycoflora of GIT of domesticated ostriches. The purpose of this study was to provide current data on mycoflora in the ostrich (*Struthio camelus*) GIT in northwest of Iran.

Materials and Methods

Ostriches and sampling procedure: Samples were collected from GIT of 50 healthy mature ostriches and information such as sex, age, breed and vaccination history were recorded from April 2012 to January 2013. In slaughterhouses, the carcasses were immediately opened and sections from the proventriculus to the anus were obtained and transported in cool conditions to the Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Iran.

Isolation and identification: Samples were cultured onto Sabouraud glucose agar (SGA) (Merck Co., Darmstadt, Germany) containing chloramphenicol (0.005%) and incubated at 30^{o}C for 2-5 weeks. In order to identify the fungal colonies, visual examinations of the fungal colonies were made and their characteristics, including texture, pigment and rate of growth on the medium, were recorded. The identification of yeasts was confirmed by germ tube test, CHROM agar, urease test, sugar fermentation and assimilation tests by RAP ID yeast plus system (Remel Inc., USA). Additional tests were done for exact identification of *Aspergillus* spp. in species levels.

Statistical analysis: The chi-square (X2) test was used to assess statistical differences between the groups. A P-value less than 0.05 was considered statistically significant.

Results

Fungal agents were isolated from different parts of the ostriches' GIT in this study. The fungi were isolated and the mean numbers of fungal species are summarized in Table 1 and Table 2. A total of 396 fungal isolates were obtained from the samples, which were related to 17 predominant genera: *Candida* (18.7%), *Aspergillus* (16.7%), *Monascus* (10.6%), *Trichosporon* (6.6%), *Fusarium* (6%), *Mucor* (4.5%), *Acremonium* (4.5%), *Geotrichum* (4.5%) and *Cladosporium* (4.5%). From 396 fungal isolates, 140 (35.3%) and 256 (64.7%) were yeasts and moulds, respectively. Among the *Candida* isolates, *C. tropicalis* was the most predominant isolate, followed by *C. albicans*, *C. glabrata* and *C. krusei* (Table 1). *Aspergillus* and *Monascus ruber* were also isolated more than the other mycelial fungi. Among *Aspergillus* isolates, *A. niger* was the most widely distributed in the ostriches examined. *M. ruber* was one of the most common species with a percentage prevalence of 10.6 % of all obtained fungi in the GIT of ostriches (Table 2).

Discussion

The ostrich is an important animal in the commercial farming sector. Ostrich chicks are particularly susceptible to fungal disease, especially aspergillosis, the most susceptible individuals being young birds kept in enclosed facilities and exposed to dust or hay which is either wet or dry (Yegani and Korver, 2008.)

The lower GIT of most animal species, including poultry, is normally populated by large numbers of microorganisms (Savage et al., 1968). Historically, the microbial composition of the GIT of ostriches has not been extensively defined compared to GIT microorganisms in other poultries. On the other hand, the presence of microbiota has several impacts on the digestive system of the host. However, it is obvious that the

Table 1. Frequency of yeast species isolated from different parts of gut tract of apparently healthy ostriches (April 2012 to January 2013).

Yeasts	Crop No. (%)	Gizzard No. (%)	Small intestine No. (%)	Large intestine No. (%)	Cecum No. (%)	Total No. (%)
Candida species						74(52.9) a
Candida albicans	0	2(1.4)	8(5.7)	5(3.6)	5(3.6)	20(14.3)
Candida tropicalis	1(.7)	0	10(7.1)	6(4.3)	7(5)	24(17.1)
Candida krusei	0	0	5(3.6)	3(2.1)	2(1.4)	10(7.1)
Candida famata	0	0	4(2.9)	3(2.1)	1(.7)	8(5.7)
Candida glabrata	0	2(1.4)	4(2.9)	4(2.9)	2(1.4)	12(8.6)
Non-*Candida* species						66(47.1)
Geotrichum spp	3(2.1)	4(2.9)	4(2.9)	4(2.9)	3(2.1)	18(12.9)
Rhodotorula spp	6(4.3)	4(2.9)	4(2.9)	1(.7)	1(.7)	16(11.4)
Kluyveromyces	2(1.4)	2(1.4)	1(.7)	1(.7)	0	6(4.3)
Trichosporon spp	7(5)	4(2.9)	6(4.3)	4(2.9)	5(3.6)	26(18.5)
No. (%)	19(13.5)	18(12.9)	46(32.9) b	31(22.1)	26(18.5)	140(100)

Table 2. Frequency of mold fungi isolated from different parts of gut tract of apparently healthy ostriches (April 2012 to January 2013).

Fungi	Crop No. (%)	Gizzard No. (%)	Small intestine No. (%)	Large intestine No. (%)	Cecum No. (%)	Total No. (%)
Absidia corymbifera	4(1.6)	1(.4)	0	2(.8)	3(1.2)	10(3.9)
Acremonium spp	3(1.2)	5(2)	1(.4)	4(1.6)	5(2)	18(7)
Alternaria alternate	2(.8)	4(1.6)	2(.8)	2(.8)	2(.8)	12(4.7)
Aspergillus spp	12(4.7)	20(7.8)	10(3.9)	10(3.9)	14(5.5)	66(25.8) [a]
Botrytis spp	2(.8)	0	0	2(.8)	2(.8)	6(2.3)
Chrysosporium spp	3(1.2)	3(1.2)	1(.4)	2(.8)	3(1.2)	12(4.7)
Cladosporium spp	2(.8)	4(1.6)	3(1.2)	3(1.2)	6(2.3)	18(7)
Fusarium spp	6(2.3)	7(2.7)	2(.8)	5(2)	4(1.6)	24(9.4)
Monasacus ruber	9(3.5)	10	6(2.3)	7(2.7)	10(3.9)	42(16.4) [a]
Mucor spp	5(2)	6(2.3)	0	3(1.2)	4(1.6)	18(7)
Penicillium spp	3(1.2)	4(1.6)	1(.4)	2(.8)	4(1.6)	14(5.5)
Scopulariopsis spp	6(2.3)	5(2)	0	2(.8)	3(1.2)	16(6.3)
Total b No. (%)	57(22.2)	69(27)	26(10.2)	44(17.2)	60(23.4)	256(100)

fungal flora of GIT is affected by environmental conditions (Iji et al., 2008).

In this study, a range of fungal flora was isolated from the GIT samples, indicating the presence of these organisms in the healthy ostriches that live in the arid regions of northwest Iran. A total of 396 fungi were identified from the samples. Fungal isolates were predominantly *Candida* spp., *Aspergillus* spp., *Monascus* spp., *Trichosporon* spp. and *Fusarium* spp. The majority of the fungal species isolates were ubiquitous, and most of the genera match with those reported in human GIT and in the GIT of other avian and mammalian species. Also, some isolates are opportunistic agents for animal and humans (Gabriel et al., 2005). Fungal flora in poultry have been previously reported in different geographical areas in the world. Yudiarti et al. reported the *A. niger*, A. fumigatus, Chrysonilia crassa, *Mucor* circinelloides, *Mucor* spp., Rizhopus oryzae and Rizhopus oligosporus were the most frequently obtained fungi from the GIT of chickens (Yudiarti, 2012).

In our study, the total mould isolates were generally higher than the yeasts. Among the filamentous fungi isolates, *Aspergillus* occurred most frequently (16.7%), and *A. niger* (24/6%), *A.fumigatus* (20/5%), *A. nidulans* (12/3%), and *A. flavus* (10/2.5%) were the predominant *Aspergillus* isolates. These findings are in accordance with some reports regarding the bird and broiler GIT (Minami, 2010). Aspergillosis is an

economically important disease in ostriches and other poultries. This disease mainly affects the respiratory system, but sometimes infection may spread to other visceral organs and can also develop in the form of an outbreak (Jalahtii, 2004).

In the present study, the most other predominant filamentous fungi were genera *Monascus* (42/10.6%), *Fusarium* (24/6%), *Mucor* (18/4.5%), *Cladosporium* (18/4.5%), *Acremonium* (18/4.5%), *Scopulariopsis* (16/4%) and *Penicillium* (14/3.5%). Several previous reports have showed *Penicillium*, *Acremonium*, *Cladosporium*, *Scopulariopsis* and *Mucor* species are the most common saprophytes in the GIT of different animal and birds. It is necessary to mention that all our examined ostriches were domestic animals with no clinical signs of GIT disease; most probably, they had no time to suffer immunodepression due to malnutrition-associated stress and, in consequence, exhibited no indicators for spreading any subclinical infection in the studied farm.

There are several factors that influence the microbe population, such as different feed ingredients and sub-therapeutic levels of antibiotics in their diets (Yudiarti et al., 2012).

Gulbahar reported that zygomycosis is a fungal infection of the upper GIT of ratites caused principally by Rhizomucor; incidence of this disease is sporadic and rare (Gulbahar, 2000).

Ostriches as meat production animals which play an important role in agriculture and economy. In the last few years, predictive microbiology has been focused on foodborne pathogens, whereas predictive modelling of filamentous fungi has not received the same level of attention. *M. ruber* is a widespread ascomycetous fungus in Europe, as it is common in silage and deteriorating grain (Gulbahar, 2000).

In the present study, among different obtained isolates, *M. ruber* was one of the most frequent species isolated from different parts of ostriches' GIT, in particular the intestine. The isolation of *M. ruber* has not been recorded before, although its involvement in foodborne pathogens and GIT infection in humans is well documented (Minami et al., 2010). The reasons for the repeated isolation of *M. ruber* in our study are not fully understood, but may be associated with contamination of the ostriches' feed (Doyle, 2012). In an unpublished paper, the ostriches' feed was shown to be contaminated with mycelial fungi such as *M. ruber*, *A. flavus*, *A. niger* and *Fusarium* spp. Toxigenic species such as *A. flavus*, *Fusarium* spp. and *Penicillium* spp. were also identified .

The yeasts isolated in this study, and their relative frequency, demonstrate the similarity between the healthy digestive tracts of these birds and those of other animal species (Jalahtii et al., 2004).

Yeast isolates were predominantly *Candida*, *Trichosporon*, *Rhodotorula* and *Geotrichum* species. In the present study, *Candida* spp. were the most frequent yeast isolates, including *C. tropicalis* (24/6%), *C. albicans* (20/5%), *C. glabrata* (12/3%), *C. krusei* (10/2.5%) and *C. famata* (8/2%).

Our results show a high prevalence of *Candida* species (18.7%) in the GIT of examined ostriches. The predominance of the yeasts was expected, since their role as members of GIT flora has been reported as a natural condition of humans and animals, such as other poultry and birds. *Candida* is widely distributed in the environment, and frequently colonizes the skin and mucous membranes such as in the oral cavity and GIT of avian species. There is evidence that animal meat production can be the source of pathogenic fungal infections in humans. Based on these reports, it would appear that yeasts are substantially represented in the total microbial ecology of spoiled poultry carcasses.

Moreover, the total number of saprophytic fungal isolated in this work was higher than the number published by others. The presence of saprophytic species has been considered as an indicator of transient GIT contamination from soil or the environment (Yang et al., 2012).

Different populations of moulds may be found in growing versus stored grain, and can be divided into two large groups: field fungi and storage fungi. When pathogenic fungi contaminate animals or

human food, it becomes a potential route of transmission of disease to both populations, and consequently of great concern to producers and consumers. Some moulds, such as *A. flavus*, are known plant pathogens, causing kernel rot in maize and a storage fungus. The growth of mycoflora on crops is highly dependent on climatic conditions, e.g., rainfall and temperature (Khosravi et al., 2007).

Regarding the abovementioned points, intestinal microbiota are referred to as commensal as they coexist without initiating inflammatory or infectious responses. It is becoming clear that these same bacteria and fungi provide at least three key functions in the poultry intestine, including epithelial cell health, nutrient metabolism and breakdown, and indirect mucosal defence against pathogenic bacterial strains (Gabriel et al,. 2003).

There are several studies of mycobiota carried out with samples from domestic animals, like ruminants and poultry; in all these studies, the most frequently isolated fungal genera were classified as saprophytes, mainly *Aspergillus*, *Alternaria*, *Penicillium* and *Cladosporium* spp., which is in agreement with our studies (Jalahtii, 2004).

Households, workers, veterinarians and people with specific medical conditions, such as chronic illness, immunodeficiency and pregnancy, may be at a higher risk of developing disease or complications from a zoonotic fungal disease caused by contact with poultry and ostriches at the household and the industrial level (Khosravi et al., 2007).

In conclusion, our study provides the first description of the GIT mycoflora of domestic ostriches in Iran, showing a large number of saprophyte and opportunistic species in different parts of the GIT. With respect to our results, ostriches are in close contact with some important mycelial and yeast fungi. To prove the role of fungal isolates from the GIT of ostriches in microflora and/or their pathogenesis, this study should be continued in the future.

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