

# Fungal flora of the combs and wattles of Iranian native chickens

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#### ABSTRACT

**Background and Objectives:** Poultry are more susceptible to receiving and spreading of fungal infections in exact conditions. The goal of this study was to identify the normal fungal flora and dermatophytes agent of the combs and wattles of adult native chickens in Tehran, Iran.

**Materials and Methods:** A total of 150 combs and wattles samples were collected by skin scraping or brushing of the margin of the suspected lesion and skin of organ. The mycological analyses were performed by direct microscopy and culture media. **Results:** One hundred and ninety fungi were isolated from the combs of 150 native chickens' samples that including

non-dermatophytes isolates 165 (86.8%), dermatophytes 6 (3.2%) and yeast 19 (10%). Among different fungal isolates, *Aspergillus* was the predominant species.

**Conclusion:** Our results showed that human in contact with poultry, both at the household and the industrial level, have a clear risk factor for exposure to fungal pathogens, especially dermatophytes.

Keywords: Native chicken, Zoonotic, Dermatophytes, Microsporum

### INTRODUCTION

Traditionally livestock and poultry production systems are important national resources in Iran (1). Poultry are more susceptible to receiving and spreading of fungal infections in exact conditions (2, 3). Although these diseases occur mainly sporadically, but at times they may take the form of outbreaks. Dermatophytes are among the most frequent causes of dermatological problems in poultry birds (4-6).

Disease caused by *Aspergillus flavus* in chickens is economically more important than those caused principally by *Microsporum gallinae* and sometime by *Trichophyton simii*. The disease is found sporadically worldwide where it appears most commonly in backyard flocks and those poultry which kept under

\* Corresponding author: Prof. Ali Reza Khosravi Address: Mycology Research Center, Faculty of Veterinary Medicine, Azadi Street, Tehran, Iran. Tel: +98-21-61117151 poor husbandry and management conditions (6, 7). It is well known that there is a bilateral relationship between the presence of pathogenic fungi at indoor poultry house and especially in apparently healthy poul-try flora, with animal health and productivity (3, 8).

Currently, rooster is the most important domestic avian species for the industrial production. Additionally, under natural conditions male compared to female chickens have more ability to transit disease to others especially in fungal disease (9, 10). On the other hand, livestock and poultry birds are major causes of zoonotic diseases in transmission circle. Animals serve as reservoirs of the zoophilic dermatophytes, and may act as a direct source of human and animal infection for subjects in contact with them, or as an indirect source of infection by contaminating working areas and dwelling places (11-14).

Besides dermatophytes, saprophytic fungi (predominantly *Aspergillus*, *Alternaria*, *Mucor*, *Malassezia* and *Candida* species) have been the most important agents in veterinary medicine and public health. In this regard, several researcher groups have focused

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#### FUNGAL FLORA OF COMBS AND WATTLES

Isolated fungi	Positive samples (N)	Percentage (%)
Non-dermatophytes	(165)	(86.8)
Aspergillus spp.	103	54.2
Mucor spp.	16	8.4
Penicillium spp.	10	5.3
Alternaria alternata	10	5.3
Fusarium spp.	4	2.1
Paecilomyces spp.	4	2.1
Chaetmium spp.	4	2.1
Scopulariopsis spp.	3	1.6
Sterile hyphae	11	5.8
Yeast	(19)	(10)
Candida albicans	19	10
Dermatophytes	(6)	(3.2)
Microsporum gallinae	6	3.2
Total	190	100

Table 1. Fungi isolated and identified from combs of Iranian native chickens under study (September to November 2011).

on the study of normal flora of different animals and birds (6, 15).

Up to now there is a few data on the poultry dermatophytosis in Iran; therefore, our results can provide knowledge to prevent some disorders in humans which working in poultry house and slaughterhouse. The goal of this study was to identify the normal flora and dermatophytes agent of combs and wattles of adult rooster in Tehran, Iran.

### MATERIAL AND METHODS

A total of 150 clinically healthy and sick adult roosters (Lari) in poultry farms situated in Tehran were examined during September to December of 2011. Specimens were collected mainly from 7 farms in different geographic location in these areas by skin scraping or brushing of the margin of the suspected lesion and skin of organ with using a sterile scalpel blade in to presterilised polythene bags or petri dishes. Scalpels were changed for each sample to avoid contamination of specimens. Then collected specimens transferred immediately to the laboratory of Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Iran.

After direct microscopic examination with 10% KOH/DMSO solution, specimens were cultured onto

Sabouraud glucose agar (Merck Co., Darmstadt, Germany) containing chloramphenicol (0.005%) and onto SGA plus chloramphenicol (0.005%) and cycloheximide (0.005%) and incubated at 30°C for 2-5 weeks. Visual examinations of the fungal colonies were made and their characteristics including texture, pigment, and rate of growth on medium for identification of different pathogen fungi were recorded. Colonies were examined under a light microscope to determine the morphological structures of the yeasts on slide mounted in lactophenol-cotton blue. 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 to identify Aspergillus spp at the species levels. The species were identified by gross and microscopic morphology and by in vitro tests, if required based on the criteria enumerated by Rebell and Taplin and Frey et al. (16, 17).

The chi-square  $(x^2)$  test was used to assess statistical differences between the groups. A P value less than 0.05 was statistically considered significant.

## RESULTS

Fungal agents were isolated from 140 (93.3%) of



Fig. 1. Percentage of *Aspergillus* species isolated from combs of Iranian native chickens under study (September to December 2011).

the 150 adult Iranian native chickens in this study. The organisms isolated and the mean numbers of fungal species were represented in Table 1. A total of 190 fungal isolates were obtained from the samples. Fungal isolates belonged to 10 genera: Aspergillus (54.2%), Candida (10%), Mucor (8.4%), Penicillium (5.3%), Alternaria (5.3%), Microsporum (3.2%), Fusarium (2.1%), Paecilomyces (2.1%), Chaetmium (2.1%) and Scopulariopsis (1.6%) and sterile hyphae (5.8%). Non-dermatophytes isolates were 165 (86.8%), dermatophytes were 6 (3.2%) and yeast were 19 (10%) of the total isolates (Tables 1 & 2). Aspergillus spp were the first most frequently occurring non-dermatophytes species (54.2%). M. gallinae and A. flavus were the most commonly encountered dermatophytes and non-dermatophytes respectively, while C. albicans was the only yeast recovered (Table 1).

*Aspergillus* species were widely distributed and the *A. flavus* was the most common species with a percentage prevalence of 62.4% of all *Aspergillus* in the poultry investigated (Fig. 1).

#### DISCUSSION

More than 150 species of fungi have been identified as pathogens for human and animals under predisposing conditions (15, 18). Dermatophytosis is an infection of the skin, hair or nails caused by dermatophytes, a group of related filamentous fungi also known as ringworm fungi which may pose public health problems in many parts of the world. There are numerous reports on the incidence in dermatophytosis due to zoophilic dermatophytes in human and animals around the world (13, 19-21).

Occurrences of dermatophytes have been previously reported in domestic animals in Iran by Khosravi *et al.* (13, 22). In the present study, *M. gallinae* was the only dermatophytes isolated from adult roosters, that confirming some reports on isolation of dermatophytes from birds (4, 23, 24). However, the frequency of occurrence in this study was slightly higher. This could be due to suitability and susceptibility of combs tissue for these fungi. Also rooster combs are bigger than other birds and could harbor fungi which can cause opportunistic infections. It is notable to mention that, the infected scale and feathers act as source of infection to humans and animals (7, 24).

However there is a bilateral relationship between incidence of dermatophytosis with climate and natural reservoirs. In this regard, pattern of the species of dermatophytes can be different in both human and animals even in similar geographical conditions (14, 25).

It is necessary to discuss that isolated dermatophyte, *M. gallinae*, is among the important infectious agents of human. In a previous study of human dermatophytosis in Iran, zoophilic dermatophytes were isolated from 37.9% of the patients with different tineae and *Microsporum* spp were the commonest etiologic agent (13, 16, 26). It has been pointed out repeatedly that no person or geographic area is free of these fungi (12, 17, 21).

At least dermatophytic fungi and poultry dermatophytosis can be transferred to farmers; poultry slaughter house employees and veterinarians by direct contact with lesions on animals or other humans, contact with contaminated skin flakes (dander), or indirectly through spores in the environment (4, 7, 24, 25). In this study, the total mold isolates were generally higher than the yeasts. However, *C. albicans*, an opportunistic pathogen for human and animals, was also isolated (19 / 10%). In this field our results are in agreement with Mbata and similar to Gründer, results (7, 24).

In this work, all of the yeasts isolates from poultry combs were *C. albicans*, it is valuable for determination of poultry combs fungal flora. *C. albicans* is an important zoonotic fungus which can cause widely range of mucosal, cutaneous, subcutaneous and systematic mycoses. Systemic candidiasis in poultry is rare but has been reported in humans and different animals (11, 15). In contrast, Gründer *et al.* isolated other yeast genera such as *Trichosporon, Kloeckera* 

and *Malassezia* from chicken combs in Germany (23).

Among the mold fungi isolated during the survey, A. flavus occurred most frequently (33.8%), whereas other Aspergillus species recovered were A. nidulans, A. carbonicus, A. fumigatus, and A. tereus. These findings are in accordance with some report about bird and broiler toe web and feathers (2, 5, 7, 23). These species are also reported as potential pathogens for humans and animals (15). Aspergillosis is an economically important disease in poultry industry that mainly affects the respiratory system, but sometimes infection may spread to other visceral organs, also aspergillosis can emerge in the form of an outbreak (8, 27). On the other hand, aspergillus spp especially A. fumigatus which isolated from chickens combs were the commonest allergenic funguses that play a significant role in the occurrence of allergic bronchopulmonary mycoses (ABPM) (27). Therefore, our results provide information on chickens fungal agents and prevention the respiratory allergic disorders in poultry workers (24, 27).

In this study, the most other predominant isolated saprophytic fungi from the poultry combs belonged to these genera: *Mucor* (8.4%), *Alternaria* (5.3%), and *Penicillium* (5.3%). Several previous reports have showed *Penicillium*, *Aspergillus*, *Alternaria* and *Mucor* species are as most common saprophytes on the skin of different animal and birds (7, 28- 30).

Other keratinophilic fungi obtained from specimens in decreasing order of occurrence were *Fusarium* spp (2.1%), *Paecilomyces* spp (2.1%), *Chaetmium* spp (2.1%) and *Scopulariopsis* spp (1.6%). Among these *Fusarium* spp and *Scopulariopsis* spp are reported by other investigators (7, 23, 24). As far as we know there is no previous report of isolating *Chaetmium* spp from poultry skins and combs. The significance of the *Chaetmium* spp is even more difficult to estimate.

*Scopulariopsis* spp has also been isolated from roosters combs and can often cause inflammatory lesions on the skin and it may have aggravated the skin infections in exact conditions (22- 24). Moreover *Scopulariopsis* spp has been described as a human nail pathogen and a possible pathogen to other animals (15, 24).

It is necessary to mentioned that household, workers, veterinarian and persons with specific medical conditions such as a chronic illness, immunodeficiency and pregnancy may be at higher risk of developing disease or complications from a zoonotic fungal disease by contact with poultry at the household and the industrial level or pet birds (12, 14, 15, 27). Recently, in Iran, more people have begun to keep birds and roosters in their homes as pets and they have close contact with them, and dermatophytes could be transferred to humans by direct contact (12, 18).

It is possible that since poultry spend most of the time on the ground of poultry house and contact of other poultry body and faceses, they may be acquiring continually keratinophilic fungi from the soil, dust and other source (4, 8, 23). Due to the lack of comprehensive studies on poultry dermatophytosis in Iran, there are no reliable data about exact economical losses from disease annually.

In conclusion, our results showed that Iranian native chicken are carriers to saprophytic and pathogenic fungi in their comb and wattles. Also human contact with poultry, both at the household and the industrial levels, has a clear risk factor for exposure to avian fungal pathogens especially dermatophytes. Improvements in public health care play an important role in monitoring and controlling zoonotic disease.

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