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Fungal Pathogens: Emerging Threats to Birds and Human Health, Assessment the Relative Frequency of Pathogenic Fungi in Ornamental Bird Feces

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A B S T R A C T

Due to the high concentrations of nitrogen compounds and nutrients present in bird droppings, bird droppings provide fertile media for the growth and spread of various disease-causing microorganisms. Understanding the prevalence of these significant pathogenic fungi in pet birds' feces is crucial for effective disease prevention and management. Culture and molecular tests are the most common tests for fungal infections. In the present study, 126 fecal samples were taken from different species of birds' Psittacine, Passeriformes, and Columbiformes. The total fungal infection frequency was 83 (65.87%). Among the positive samples, 83 (71.55%) were filamentous fungi, and 33 (28.44%) were yeasts. *Aspergillus flavus*, *Candida albicans*, and *Cryptococcus neoformans* are the most important pathogens that were found in this study, with frequency of 34 (29.31%), 12 (10.34%), and 2 (1.72%), respectively. Since it is possible to isolate and identify potentially pathogenic fungi and molds for humans, this research and investigation is even more valuable. Therefore, examining and finding hygienic solutions for this issue will be particularly important.

Keywords: *Cryptococcus neoformans, Filamentous Fungi, Candida albicans, Ornamental Birds, Aspergillus flavus.*

1 Introduction

Fungal diseases are not as common as viral and bacterial diseases. This issue is back to their pathophysiology (1). If the immune system is healthy and active, it will be able to neutralize the potential dangers associated with fungi. There are, however, instances where serious diseases can occur in animals and humans (typically as a result of pathological, physiological, immunological, and mechanical disturbances) (2). Public health is affected by the presence

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of these fungi in the environment, even though they may not be pathogenic to birds (3). In research studies, approximately 50% of birds become reservoirs and carriers of fungi that may be pathogenic to humans and themselves (4). On the other hand, antifungal drug resistance is growing up in recent years, so the study of fungal infections is more important (5).

There is a growing popularity of keeping wild birds as pets, but their droppings can be contaminated by a variety of diseases such as *Cryptococcus neoformans*, *Aspergillus spp*., *Candida spp.*, *Mucor spp*., *Rhodotorula*, and others, so it is imperative to understand the factors that influence disease epidemiology (6). The *Cryptococcus neoformans*, for example, does not cause disease in birds but is known to cause pulmonary infections, meningoencephalitis, cutaneous and mucocutaneous infections, and systemic infections in humans (7).

The presence of nutrients and high concentrations of nitrogen compounds in bird droppings, many of which can be transmitted through the air, provides an ideal environment for the growth and spread of a wide variety of pathogenic microorganisms (8). Among the significant diseases, fungi are particularly dangerous due to their ease of transmission through the air, and organisms can become infected by being near them (9, 10).

Invasive fungi are on the rise worldwide, while immunocompromised patients are on the rise as well (11). Several pathogenic fungi can be found in bird droppings, including *Cryptococcus neoformans*, *Aspergillus*, *Candida*, *Rhodotorula*, *Mucor*, and *Penicillium*. It is estimated that *Cryptococcus*, as a global organism, causes over one million new cases of infectious diseases, of which 625,000 result in death (12).

This study has been designed to survey the fungal infection in different species of birds to evaluate the presence and frequency of the most prominent avian fungal infections.

2 Materials and Methods

2.1 Sample Collection:

At the Veterinary Clinic of the Faculty of Veterinary Medicine, University of Tehran, 126 fecal samples were collected from three family of ornamental birds (Psittacine, Passeriformes, and Columbiformes) between November-December 2020 and January-February 2021. In total, ten different species of birds were sampled, including Lovebirds, cockatoos, Dutch canaries, cockatiels, green-cheeked conures, Indian ringnecks, lovebirds, mynah, canaries, and White King pigeons (Table 1). The sample population included both sick and healthy birds. A sterile swab was used to collect fresh bird feces from the surface inside the cages or perches, yielding 0.1 to 1 g of feces. For further processing, the samples were transferred to sterile 1.5-milliliter microtubes, labelled, and stored at approximately four degrees Celsius in a laboratory refrigerator.

In the current study, 126 fecal samples were collected from 10 bird species. In terms of abundance, the Cockatiel constituted 46.8% of the sample, followed by Lovebirds at 15.87%, African grey parrot at 9.52%, Mynahs at 5.5%, and other species at less than 5% each (Table 1).

Table 1. Species Distribution and Percentages

Bird Species	Scientific Name	Number of Samples	Bird Species	Scientific Name	Number of Samples
Cockatiel	*Nymphicus hollandicus*	59	Alexandrine parakeet	*Psittacula eupatria*	8
Lovebird	*Psittacus swindernianus*	20	Rose-ringed parakeet	*Psittacula krameri*	8
African Grey Parrot	*Psittacus erithacus*	12	Domestic Canary	*Serinus canaria domestica*	3
Mynah	*Acridotheres tristis*	7	Sulphur-Crested Cockatoo	*Cacatua sulphurea*	3
Green-cheecked parakeet	*Pyrrhura molinae*	3	Domestic Pigeon	*Columba livia domestica*	3

2.2 Sample Preparation and Culturing:

Upon collection of all 126 samples, 0.5 grams from each sample were transferred to normal saline 0.9% and chloramphenicol in a 1:10 ratio. The mixture was shaken for 20 minutes and allowed to settle for 30 minutes. Subsequently, 100 microliters of the supernatant mixture were cultured on one side of Sabouraud Dextrose Agar 2% plates, while 100 microliters of the sediment were cultured on the other side. The plates were then incubated at 37 degrees Celsius for 7 to 10 days, with daily observations made to monitor fungal growth. Direct microscopic

examination was performed on the grown colonies to aid in identification.

2.3 Culturing on Chromogenic Media:

To further aid in identification, chromogenic media were utilized. Fresh yeast colonies (24-hour culture) were streaked linearly on Chrom Agar medium and incubated at 28 degrees Celsius for 48 hours. After incubation, the colonies were observed for color changes. *Candida albicans* colonies appeared green, *Candida tropicalis* appeared blue, and *Candida krusei* appeared pinkish on this medium. Other Candida species displayed purple coloration, while other yeasts appeared white.

3 Results

Among 126 samples included in this study, 83 (65.87%) were positive for fungal infection. There were 116 different colonies were grew that 83 (71.55%) of them were identified as filamentous fungi and 33 (28.44%) were yeasts. Twelve samples have concurrent infection with both filamentous fungi and yeasts. Further details of fungal species were shown in Table 2.

Table 2. Infection frequency of Fungal species

Fungi Species	Fungal Type	Frequency of Infection
Aspergillus flavus	Filamentous Fungi	34 (29.31%)
Mucor	Filamentous Fungi	22 (18.97%)
Aspergillus niger	Filamentous Fungi	13 (11.21%)
Penicillium spp.	Filamentous Fungi	7 (6.03%)
Aspergillus terreus	Filamentous Fungi	5 (4.31%)
Aspergillus fumigatus	Filamentous Fungi	1 (0.86%)
Aspergillus ochraceus	Filamentous Fungi	1 (0.86%)
Candida albicans	Yeast	12 (10.34%)
Candida tropicalis	Yeast	8 (6.90%)
Cryptococcus neoformans	Yeast	2 (1.72%)
Rhodotorula	Yeast	2 (1.72%)
Candida glabrata	Yeast	1 (0.86%)
Candida krusei	Yeast	1 (0.86%)
Undetected by culture method	-	7 (6.03%)

4 Discussion and Conclusion

Various fungal infections and diseases were well documented (7, 13, 14). Otherwise, the epidemiological aspect of these bird's pathogens didn't discuss in Iran. The present study highlights the infectious rate of the mycotic pathogens that presented in the avian feces. The most prevalent fungi, were belonged to *Aspergillus spp.* and *Candida spp.*

Culture is considered the gold standard for diagnosing fungal infections as it allows for species-level identification and antifungal susceptibility testing (15). However, culture methods have limitations such as low sensitivity and long growth time (16). To overcome these limitations, new methodologies that are independent of pathogen isolation have been developed (17). These culture-independent methods, such as PCR-based tests, have shown promise in improving the accuracy and speed of diagnosis (18, 19).

Among the fungal avian diseases, Aspergillosis, candidiasis and cryptococcosis are very important (7, 13, 14). The most abundant filamentous fungus that reported from psittacine dropping are *Aspergillus* spp.(20). In 2019 a study in Brazil (21) has been surveyed 149 dropping and found the *Aspergillus niger* was the most frequent filamentous fungus in the parrots dropping whereas the present study showed that the most frequent is *Aspergillus flavus*.

Aspergillus fumigatus, the most important fungal respiratory pathogen, is found in 1 sample. In a recent study about Aspergillosis in birds in 2021, *Aspergillus fumigatus* reported as most frequent fungus that were found in both wild and domesticated birds. Whereas *Aspergillus flavus* was less common (22).

On the other hand, *Cryptococcus spp*. are one of the most dangerous fungal systemic infections and can be lethal (21). Cryptococcosis is the most important yeast-related fungal disease with global prevalence, affecting humans, especially those with immunodeficiencies, and some mammals. In humans, the disease is often associated with immune suppression or high levels of yeast exposure (23). In Iran, numerous studies have been conducted on animal samples, showing the presence of different *Cryptococcus* species in

bird droppings (24). In the present study, two fecal samples out of 126 samples were identified as positive for *Cryptococcus* contamination, accounting for 1.72% of the samples. Both samples were from pigeons. No *Cryptococcus* strains were isolated from parrots or canaries.

Some other fungal infections are important in another viewpoint. They are mostly come after antibiotic therapy and/or immunosuppression (25). The conditions that other microorganisms can't grow very well. This issue is more important in pet birds due to the various stress that they are faced; illegal trading, wild catching, breeding in high population and etc.

In conclusion, this study reveals the prevalence of *Aspergillus* spp. and *Candida* spp. as the most common fungal pathogens in avian feces. While culture remains the gold standard for diagnosis, alternative methods such as PCR-based tests show promise in improving accuracy and speed. Aspergillosis, candidiasis, and cryptococcosis are significant avian fungal diseases, with *Aspergillus flavus* being the most frequent filamentous fungus observed. The presence of *Cryptococcus* spp. highlights the potential health risks associated with these pathogens, particularly in immunocompromised individuals. Further research is needed to understand epidemiology and develop effective prevention and treatment strategies to safeguard avian and human health.

Conflict of Interest

No conflicts of interest were reported between the authors.

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Author Contributions

ZM and AKH contributed to the original idea and study design. The research supervised by AKH.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

Ethical Considerations

The Ethics Committee of University of Tehran, Iran, approved the study.

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