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Prevalence of *Cryptococcus neoformans* in domestic birds referred to veterinary clinics in Tehran

Seyed Jamal Hashemi[1], Amir Ghaffar Jabbari[1*], Mansour Bayat[1] and Siamak Mashhadi Rafiei[2]

[1]*Department of Mycology, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Department of Small Animal Internal Medicine, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran*

ABSTRACT

Prevalence of Cryptococcus neoformans in domestic birds referred to veterinary clinics in Tehran. Cryptococcus is a sub acute or chronic disease. Cryptococcus neoformans is a factor of this infection. This yeast is found in bird feces and can be spread by dust in the respiratory system. Individuals who are immunocompromised are at risk of fungal infection. In this study, 262 fecal samples from birds such as lovebirds, mynah, parrot, Kasku, finches, bridal Dutch and canaries were gathered. The samples were prepared from suspension cultures in S, Sc and BHI and at 25 and 37 ° C were incubated. After 48 hours of staining in cotton blue and Chinese ink we put them under consideration. Then, using sugar fermentation tests, urea hydrolysis and germ tube were identified. The results included a variety of filamentous fungi such as Aspergilus, Alternaria, Escopolariosis, Mucor, Rhizopus and yeasts like Rodoturela, Candidia albicans, Candidia Sp and Pichia. Cryptococcus Neoformans was not isolated. This disability in isolation was due to the birds' hygiene care, keeping them inside the cage and preventing of direct contact with wild birds.

Key words: Cryptococcus neoformans, Pet birds, Prevalence

INTRODUCTION

Due to the ever increasing importance of fungal disease in the current era and their diagnosis and also the role of micro-organisms in surface and visceral level diseases has caused in most developed countries, lots of studies and research of the unknown issues in micro-organisms should be done. Of course, in our country, depending on climatic conditions and ecology as well as the development of social, economic, industrial and agricultural situations, fungal factors and their relationship with the mentioned conditions of hygiene and preventive medicine, and differentiation of pathogenic, are very important. In this context, it is important to understand these factors.

Cryptococcus is a chronic, sub acute or acute lung, visceral infection. This infection is caused by the yeast fungus called Cryptococcus neoformans. The major importance of this yeast is its opportunistic nature. This organism is the most important life-threatening fungal infections in patients with AIDS. Its major environmental sources are soil contaminated with pigeon droppings or the eucalyptus trees. Cryptococcus has got about 37 species, among which Cryptococcus neoformans is the only specie that is pathogenic. It has four types of A to D. This fungus yeast has

three varieties of Cryptococcus neoformans (C. neoformans), Cryptococcus Gattii (C. gattii), Cryptococcus Grubii (C. grubii). 52 animals, including cats, dogs, foxes, horses, sheep, monkeys, goats, etc. can acquire Cryptococcus. This yeast fungus can be isolated from pigeon droppings. Probably due to the pigeon's body temperature which is about 42 ° C and intestine bacterial flora, it does not cause diseases in birds. Cause of Cryptococcus neoformans in pigeon droppings is the power of Creatinine as a nitrogen source [1-12]. Cryptococcus neoformans is not pathogenic or of a little pathogenic for birds. The fungus can survive in nutrients as saprophytes, and are often seen in manure or birds' manure. This fungus is not invasive in organs and tissues.

This is related to the birds' high body temperature. On the other hand, the aviary and birds' cages, dove nests and poultry units, are the primary means of Cryptococcus infection in mammals and humans. Yeast cells survive for long periods in feces. The fungus can enter the respiratory tract through inhalation of dust and may cause infection. One of the main characteristics of these fungi is the capsule production [3], which is an important factor in Pathogenesis of the yeast. Virulence dependents on the thickness of the capsule. It is lost in soil quickly because it is a good food for all kinds of ameba. In the heat of the direct sun light, contaminated soil is quickly turned to Cryptococcus. In terms of epidemiology, factors such as age and race have little impact, but in terms of job conditions, pigeon breeders' antibodies are higher than normal people, although the infection rate is not more than anyone. About sex, men involve more than 2 to 1 ratio to women. This difference is due to the hormonal differences between contact and employment issues in genders. In general we can say that in prevalence of this infection, these factors have little effect. Cryptococcus infection represents in different clinical forms that pulmonary Cryptococcus is the most important one. In addition to the above conditions mentioned we can refer to other forms like Cryptococcus in nerve centers, skin, systemic and skeletal infection. This fungus due to its opportunistic nature of the organism is as a major cause of infection in patients who are immunocompromised [2,3]. The aim of this study was to investigate the prevalence of yeasts in the feces of pet birds kept indoors. In the case of a fungal infection in birds, it is possible that if the keeper is immunocompromised, he might be infected.

MATERIALS AND METHODS

254 out of 262 cases were selected as the samples of the current study. Birds, including parrots, kaskv, canaries, finches, bridal Dutch, mynah and love birds were referred to veterinary clinics to visit and diagnose diseases. Number of selected veterinary clinics in Tehran was 7. In this study, about 20-10 grams of birds' droppings were collected and all information about the birds and their keepers was written in a questionnaire. At first two slides were prepared of the birds' droppings. One of them was stained with cotton blue and the other one with Chinese ink to see fungi objects and Cryptococcus neoformans capsule. In the next step dropping samples were placed in a mortar and then 5 g of the sample was poured in a sterile flask with distilled water of a volume of 250 cubic centimeters, 100 cubic centimeters of solution 1/0% of glycine as a solution to reduce osmotic pressure and is added to suspend the fungal spores in suspension and is shaken well for 10-15 minutes and then is set to rest for 30 minutes to suspended solids to be settled. The supernatant suspension of a cubic centimeter is cultivated to each agar medium Sabrodkestroz, Sabrodkestroz Chloramphenicol agar in a linear culture and Hartaynfyuzhen. Culture plates were incubated at 25 ° C and 37 ° C. Initially the cultures after 48 hours and then every day for one week, were seen due to the growth of yeast organisms such as control of colonies grown in number and shape of the media. Suspected yeast colonies were stained with Chinese ink to survey capsule and then in niger culture, Cryptococcus neoformans was cultured. If not approved, the yeasts have been measured due to sugar and the germ tube test for diagnosis. The study of filamentous fungi in culture slides were also used.

RESULTS

In 262 specimens of birds, Cryptococcus neoformans was isolated. Birds, including love birds, parrots, kaskv, finches, canaries, bridal Dutch and myna. Their number and frequencies are presented in Table 1. In our study, we were disable to isolate a variety of microbial organisms according to Table 2 containing a variety of saprophytic filamentous, yeast fungi and bacteria were found. 201 out of 262 samples of saprophytic fungi were able to grow. Yeasts and bacteria were grown in 211 and 152 samples respectively. Saprophytic filamentous fungi based on culture slides testing and diagnosis of species included Aspergilus alternaria, mucor, Rhizopus and Askopolarysis among which different kinds of Aspergilus were the most frequent. According to Table 3, the frequency of other filamentous fungi were studied. Yeasts isolated from the feces of birds included Serozyeh Saccharomyces, various species of Candidia, Candida albicans, Candidia tropicalis, Candidia torolopsys, Candidia agzyloaskoy, Candidi ankospy siva and other yeasts like Rodoturela, Pichia and Aysateknyka. Among them, 62 cases of Candida albicans

yeast was more frequent (Table 4). Based on the yeast sugar fermentation tests using sugars such as glucose, lactose, terhalose, maltose, and sucrose, as well as test of germ tube and urea hydrolysis we were able to identify yeasts (Table 5).

TABLE 1- Birds

Kind	Number	Number in %
lovebirds	52	19.8%
Parrot	40	15.2%
Kasku	62	23.7%
Finch	25	9.5%
Canary	10	3.8%
bridal Dutch	28	10.7%
Mynah	45	17.1%
Total	262	100%

TABLE 2- Growth Rate of Organic Factors

Kind of Organism Work	Growth No.	Non-Growth No.	Growth No. in %	Non-Growth No. in %
saprophytic fungi	201	61	76.71%	23.29%
Yeast	211	51	80.53%	19.46%
Bacteria	152	111	58.01%	41.99%

TABLE 3-Amount of saprophytic fungi

Kind of fungi	Amount	Amount in %
Aspergilus	92	26.8%
Alternaria	86	25.1%
Mucor	50	14.6%
Escopolariosis	65	19%
Rhizopus	50	14.6%
Total	343	100%

TABLE 4-Amount of Yeast Fungi

Kind of Fungi	Amount	Amount in %
Serozyeh Saccharomyces	45	10.1%
Candidia Sp	55	12.3%
Candida albicans	62	13.9%
Candidia torolopsys	40	9%
Candidi trodifaz	57	12.8%
Candidia agzyloaskoy	20	4.5%
Candidi ankospy siva	25	5.6%
Rodoturela	40	9%
Pichiasis bensis	60	13.5%
Aysateknyka orintalis	42	9.4%
Total	343	100%

TABLE 5-Yeast Identification

	Born pipe	Glucose	Maltose	Sucrose	Lactose	Terhalose	Urea Hydrolysis
Candida albicans	+	+	+	+	-	+	-
Candidia tropicalis	-	+	+	+	-	+	-
Rodoturela	-	+	+	+	-	-	+
Saccharomyces	-	+	+	v	v	-	+

DISCUSSION

Cryptococcus is a sub acute or chronic fungus disease. The disease invades mainly up to the brain, meninges, lungs and skin in humans and domestic animals such as dogs, cats, cows and horses. The organism, Cryptococcus neoformans, is a foreign saprophytic presented on wood, plants and fruits. However, it is rather grows on bird droppings. The yeast cells inter humans and animals bodies via dust inhalation and through the skin. Cryptococcus is more common in the tropics and is only to be seen individually. Immune deficiency and impaired immune systems are predisposing factors. The prevalence of Cryptococcus has increased sharply in recent years and is one

of the major causes of the common infections in AIDS patients [1]. People tend to keep animals such as birds and pets and feel close to them and also children have an interest in holding and playing with birds, which cause the risk of infection. The presence of this organism in the dropping and lack of the above mentioned disease has been confirmed in birds. As a result of an immune defect in the keeper, it leads to the risk of infection [2]. In this study, we did not succeed in the isolation of Cryptococcus neoformans from birds' droppings and domestics. It can be observed in maintaining the good health of the birds, including the cleaning of birds' cages, using clean, fresh food, seed and bird control in terms of diseases. In a research run by Shadzi et al. in the city of Isfahan, they were able to isolate a sample of Cryptococcus neoformans from lovebirds [8]. In another study by Emami et al. entitled presence of Cryptococcus neoformans in birds' droppings took place in Qom, they could isolate 10 varieties of Cryptococcus from birds' feces [4]. Cryptococcus neoformans has also been included. Another study with Moghadami et al. in surveying the ratio of infection in pigeons in Mashhad and the suburb to Cryptococcus neoformans fungus about 11 cases equal to 37/8% were identified [6].

In another study conducted by Michaeli, the prevalence of yeasts was reported 0.8% [7]. 136 samples of pigeon droppings in a similar study on Pigeon houses in Isfahan has been reported 1.8% of infection [8] and in another study of 470 pigeon crop in Tehran, there has been a 2.1% infection [10]. In a study on soil of the Caspian Sea, run by Forouzesh et al. Cryptococcus yeast was isolated from 203 samples [9]. In another study by Khosravi et al. on the prevalence of Cryptococcus neoformans fungi in the pigeons of northern province, 175 samples were successfully isolated [5]. Ben Field in the United States isolated this fungus from pigeons [11]. In our study we were able to isolate filamentous fungi such as Aspergilus, alternaria, Mucor, Rhizopus, and Escopolariosis. The amount of contamination of birds' feces with fungi, were 76.71% and in Ms. Irani Khah's study this infection rate was 78.50% [4]. Yeast infection rate in this study was 80.52% while the rate of 70.59% was reported in Ms. Irani Khah's study. In the case of bacterial infection in our study, 58.01% and 17.24% in another study were reported. The high prevalence of Candidia albicans in birds could be due to living of this yeast in digestive system of healthy and unhealthy birds. The global spread of the fungus Aspergilus is one of the reasons. One important factor in the case of not being able to separate this yeast can be the isolation of these birds from wild birds or even pet pigeons.

CONCLUSION

In conclusion, the results of the present study indicate high levels of hygiene and monthly visits by veterinary specialists can prevent the risk of disease in the birds' keepers.

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REFERENCES

[1] A Khosravi, H Shokri, R Yaharaeat. Veterinary Mycology, Tehran, Jahad Daneshgahi. **2005**.
[2] EG. Evans, MD. Richardson. Medical Mycology A Practical Approach. Tehran, Jahad Daneshgahi **2003**.
[3] AR. Khosravi. Fungal Diseases and Immune Responses. First Edition. Tehran University, Tehran. **2008**.
[4] M. Emami, MR. Shidfar, YS. Khorasani, M. Irani Khah. Master thesis. Azad University of Qom. **2003**.
[5] A.R. Khosravi. Isolation of Cryptococcus neoformans from Pigeon (columba Livia) dropping in Northern Iran. Mycopathologia, 139: 93-95.
[6] A. Ebrahimzadeh, M. Moghadami. Master thesis Modares university. **1985**.
[7] A. Mikaeli. In search of Cryptococcus neoformans in birds' excrements of Kermanshah city. **2000**.
[8] Sh. Shadzi, M. Chadganipoor, B. Naser, N. Elchi. Isolation and distinction Cryptococcus neoformans of pigeon excrements in pigeon house "Esfahan". 3rd Parasitological congress. Sari **2000**.
[9] M. Forozesh. PhD thesis. Tehran University **1979**.
[10] V. Gedli. PhD thesis. Tehran University **1979**.
[11] RW. Benham. *J. Infect. Dis* **1935,** 57:325.
[12] E. Drouhet. *Imycology* **1997**, 7:10-27.
[13] C. Kao, Schwarts. *Am. J. Clin.Path*. **1957**. 22, 625-663.
[14] JW. Rippon. Medical Mycology Saunders Company. **1988**.
[15] KJ. Kown Chung, JE. Bennette, Medical Mycology, Lea/Febiger. **1992**.
[16] JR. Perfect, GM. Cox. *Drug*. **1999**. 2: 259-269.

[17] J. Bava, A. Troncoso. *J. Infect*. **2009**. 3 (7) 572-574.
[18] S. Chuck, M. Sande. *N. Engl. J. Med*. **1989**. 321: 794-799.
[19] AR. Khosravi, H. Shokri. Fungal Disease Immunocompromised Patients. Tehran. Jahad Daneshgahi **2008**.