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## ***Cryptococcus neoformans* ISOLATION FROM SWALLOW (*Hirundo rustica*) EXCRETA IN IRAN**

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### SUMMARY

*Cryptococcus neoformans* is an encapsulated yeast that can cause cryptococcosis, a life-threatening infection that mainly occurs in immunocompromised patients. The major environmental sources of *C. neoformans* have been shown to be soil contaminated with avian droppings. In the present study, we evaluated the isolation of *C. neoformans* from swallow (*Hirundo rustica*) excreta in two northern cities of Iran. Ninety-seven swallow droppings were evaluated and 498 yeast-like colonies were isolated and identified as *Rhodotorula* spp. (62.8%), *Candida* spp. (28.5%) and *C. neoformans* (8.7%). *Cryptococcus neoformans* was isolated from 5/97 (5.2%) of collected samples. Min-Max colony forming units (CFU) per one gram for the positive samples were 3-10 *C. neoformans* colonies. The total mean CFU per one gram for the positive samples was 4.8. The results of this study demonstrate that excreta of swallow may harbor different species of potentially pathogenic yeasts, mainly *C. neoformans*, and may be capable of disseminating these fungi in the environment.

**KEYWORDS:** Swallow; *Hirundo rustica*; Excreta; *Cryptococcus neoformans*.

### INTRODUCTION

*Cryptococcus neoformans* is an encapsulated yeast that causes cryptococcosis, a life-threatening infection that is usually manifested as meningoencephalitis mainly in immunocompromised patients<sup>3,8,9,16</sup>. The genus *Cryptococcus* includes nearly 90 species but the taxonomy of the genus is currently being re-evaluated. Among these, *C. neoformans* and *C. gattii* are pathogenic. Following its first identification in nature from peach juice samples<sup>24</sup>, the major environmental sources of *C. neoformans* have been shown to be either soil contaminated with avian droppings (*C. neoformans*) or eucalyptus trees and decaying wood forming hollows in living trees (*C. gattii*)<sup>4,11,12,26</sup>.

*Cryptococcus neoformans* can be divided in four serotypes (A to D). Until recently, serotypes A and D were included in var. *neoformans*, while serotypes B and C were included in *C. gattii*. However, in addition to the previously observed phenotypic differences, recent molecular studies have detected significant genetic variations between the two serotypes. Thus, it was proposed that a new variety, var. *grubii*, be created to contain serotype A<sup>5</sup>. This leaves serotype D as the sole serotype in var. *neoformans*.

Swallows are passerine birds in the family *Hirundinidae* which are characterized by their adaptation to aerial feeding. The family contains around 83 species in 19 genera. *Hirundo rustica* (barn swallow) is the most widespread species of swallow in the world. A distinctive passerine

bird with blue upperparts, a long, deeply forked tail and curved, pointed wings, it is found in Europe, Asia, Africa and the Americas. There are six subspecies of *Hirundo rustica*, which breed across the Northern Hemisphere. Four are strongly migratory. *Hirundo rustica* is the type of swallow which migrates to northern Iran in spring season. Swallows have readily adapted to nesting in and around human habitation. They often return to the same breeding area each year, and may select the same nest site if they were previously successful in that location<sup>8</sup>. On the other hand, they are considered as a holy bird in the north and some other regions of Iran. Because of the high prevalence of swallows around human habitation, and the seriousness of cryptococcosis in humans, there is an interest in the relationship between the birds, their excreta, and human cryptococcosis. In addition, some investigators from Iran have suggested that the number of cryptococcosis cases is increasing<sup>1,18,23,27</sup>. Therefore, in the present study we evaluated the isolation of *C. neoformans* from swallow excreta in Sari and Qaemshahr, two northern cities of Iran.

### MATERIAL AND METHODS

**Study area.** Sari, the capital of Mazandaran province is located in the north of Iran, between the northern slopes of the Alborz Mountains and southern coast of the Caspian (Mazandaran) Sea. Its population is estimated to be 250,000. It has a land area of 3,923 square kilometers. Sari is one of the entertainment and sports centers of Iran. Qaemshahr, another city of Mazandaran province is situated 23 kilometers southwest of Sari. Its population is estimated to be 180,000.

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During spring 2010, ninety-seven swallow desiccated droppings samples were collected from the two cities including two general hospitals and human dwellings.

**Isolation and identification of *Cryptococcus* spp.** The collected droppings were processed in a biohazard safety hood (Class II), each sample was ground into powder by a mortar with a porcelain pestle and then 1 g was suspended in 50 mL of sterile physiological saline (0.9% aqueous NaCl) containing 0.4 g/L chloramphenicol, vigorously vortexed for 1-3 min and let stand for 15-20 min for decantation. Supernatants were removed by micropipettes and 10, 100, 200, and 500 µL aliquots were seeded on Sabouraud dextrose agar plates with chloramphenicol (0.05 mg/mL), incubated for two to 15 d at 30 °C, and assessed daily. The grown yeast-like colonies were sub-cultured onto corn meal agar + tween 80 and CHROMagar media (HiMedia-India) and they also assessed for phenoloxidase activity on Niger seed agar (*Guizotia abyssinica* (niger seed) 50 g, dextrose 1 g, KH<sub>2</sub>PO<sub>4</sub> (potassium dihydrogen orthophosphate) 1 g, creatinine 1 g, agar 15 g, Chloramphenicol 1 g, Distilled water 1000 mL) for 72 h at 30 °C and subjected to complementary tests as urease production on Christensen Urea Agar and ability to grow at 37 °C. *Cryptococcus neoformans* variety was determined by growth and change of color in the canavanine-glycine-bromothymol blue medium.

## RESULTS

Out of 97 collected samples, 498 yeast-like colonies were isolated. The isolated yeast-like colonies were identified as *Rhodotorula* (62.8%), *Candida* spp. (28.5%) and *C. neoformans* (8.7%). *Cryptococcus neoformans* was isolated from 5/97 (5.2%) of collected samples. Of five positive samples, two samples were collected from around the Qaemshahr General Hospital. None of the collected samples from Sari were positive for *C. neoformans* growth. Table 1 shows the number of isolated *C. neoformans* colonies based on sampling places. Min-Max CFU per one gram for the positive samples was 3-10 *C. neoformans* colonies. The total mean CFU per one gram for the positive samples was 4.8.

**Table 1**

Number of isolated *C. neoformans* colonies based on sampling places

Sample code	Sampling place	Number of isolated colonies
B6	Human dwelling	5
B11	Human dwelling	10
R2	Hospital	3
R3	Hospital	3
H10	Human dwelling	3
Total		24

None of collected samples from Sari were positive for *C. neoformans* growth.

## DISCUSSION

In the present study, 5.2% of collected samples from swallow excreta were positive for *C. neoformans*. Although pigeon droppings have been documented as the major source of *C. neoformans* in nature,

the presence of this yeast in many bird species other than pigeons, i.e., dove, psittacines, budgerigars, canaries, parrots, cockatoos and starlings had also been reported<sup>2,7,13,20,21,25</sup>. In previous studies from Iran, *C. neoformans* was also isolated in 17.8%<sup>10</sup> and 34%<sup>28</sup> of pigeon droppings. However, there are two reports of the isolation of *Cryptococcus* from captive passerine excreta<sup>6,17</sup> but the current report appears to be the first describing the isolation of *C. neoformans* from *Hirundo rustica* excreta.

In this present study, all identified strains were found to be *C. neoformans*, no *C. gattii* was found in this study. Our observation is consistent with the findings of previous investigations that reported the predominance of *C. neoformans* in avian excreta<sup>14,15,19,22</sup>.

## CONCLUSION

The results of this study demonstrate that excreta of swallows may harbor different species of potentially pathogenic yeasts (mainly *C. neoformans* and *Candida* spp.) and may be capable of disseminating these fungi in the environment. Furthermore, presence of the swallow, which houses in close proximity to human dwellings as well as around the special places such as hospitals, makes these birds a focus of interest for possible carriers and spreaders of pathogenic fungi. Swallows dwelling around hospitals is particularly interesting because of immunocompromised patients that are considered high risk for contracting opportunistic diseases. Therefore, our findings emphasize the potential importance of swallows in the contamination of the public and domestic environments and reinforce that bird excreta is a dangerous reservoir and potential source of inhaled *C. neoformans*.

Considering the incidence of human cryptococcosis in Iran and the fact that swallows are common in this country and in peaceful coexistence with humans in Iran, our results also emphasize the need for further study on the distribution of *C. neoformans* in swallow excreta from other regions of Iran as well as throughout the world.

## RESUMO

### **Isolamento de *Cryptococcus neoformans* de excrementos de andorinhas (*Hirundo rustica*) do Irã**

*Cryptococcus neoformans* é levedura encapsulada que pode causar criptococose, infecção potencialmente mortal que ocorre principalmente em pacientes imunocomprometidos. As principais fontes ambientais de *C. neoformans* são o solo contaminado com fezes de aves. No presente estudo, avaliamos o isolamento de *C. neoformans* de excreta de andorinhas (*Hirundo rustica*) em duas cidades do norte do Irã. Noventa e sete amostras de fezes de andorinhas foram avaliadas e 498 colonias semelhantes à levedura foram isoladas e identificadas como *Rhodotorula* spp. (62,8%), *Candida* spp. (28,5%), *C. neoformans* (8,7%). *Cryptococcus neoformans* foi isolado a partir de 5/97 (5,2%) das amostras coletadas. Unidades Min-Max formadoras de colonias (CFU) por 1 grama das amostras positivas foram 3-10 colonias de *C. neoformans*. A média total de CFU por 1 grama das amostras positivas foi de 4,8. Os resultados deste estudo demonstram que excrementos de andorinhas podem abrigar diferentes espécies de leveduras potencialmente patógenas, principalmente *C. neoformans*, e podem ser capazes de disseminar estes fungos no meio ambiente.

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