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Isolation and identification of filamentous fungi and yeasts with zoonotic potential obtained from cattle egret (*Bubulcus ibis*) droppings

Fabian-Ricardo Gómez-De-Anda[1], Nancy Guadalupe Flores-Jiménez[2], Jorge Luis de-la-Rosa-Arana[3], Andrea-Paloma Zepeda-Velázquez[1]*

[1] Laboratory of Physiology and Clinical Pathology, Academic Area of Veterinary and Zootechnical Medicine, Institute of Agricultural Sciences, Autonomous University of the State of Hidalgo, Tulancingo, Mexico; [2] Department of Veterinary Medicine and Zootechnics, Faculty of Higher Studies Cuautitlán, National Autonomous University of Mexico, Cuautitlán Izcalli, Mexico; [3] Department of Microbiology in Human Health, Faculty of Higher Studies Cuautitlán, National Autonomous University of Mexico, Cuautitlán Izcalli, Mexico.

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Abstract

The different microorganisms that make up the normal microbiota of birds can be present in different substrates such as the soil and other elements that make up the habitat. In the case of the cattle egret, the intestinal microbiota can change due to partially migratory habits. Thus, this study aimed to isolate and identified fungi and yeasts with zoonotic potential obtained from cattle egret (*Bubulcus ibis*) droppings settler in Tulancingo, Hidalgo, at Eastern economic zone of Mexico. Cattle egret droppings were collected for analysis, a total of 240 pool samples, which were spread on Sabouraud agar and incubated at 25.00 - 37.00 ˚C for 2 to 3 days. Filamentous fungi and yeast were identified by morphology and Lactophenol Blue staining or Chinese Ink stains. Filamentous fungi genera *Mucor* spp. (42.35%), *Rhizopus* spp. (26.71%); *Penicillium* spp. (13.35%); *Paecilomyces* spp. (11.40%); *Scedosporium* spp. (1.95%); and, from yeasts such as *Cryptococcus* spp. (2.29%); *Rhodotorula* spp. (1.95%) were identified. In this work, the presence of filamentous fungi genera and yeasts with zoonotic potential were isolated from droppings of cattle egret. The clinical presentation of fungal infections in humans can occur when immunosuppression is present or different predisposition factors are conjugated. The presence of birds and their dropping in anthropogenic activities is not a predisposing factor for the presentation of the disease in immunologically competent humans.

Introduction

Birds play an important role in the dissemination of microorganisms in the environment associated with indiscriminate dejection of the excrement in different places not only of those birds that are endemic, semi-endemic and exotic in a certain area, but the great variety of species that are present in different geographical areas in different times of the year due to migration.[1] The *Bubulcus ibis* is a semi-migratory bird that belongs to the Ardeidae family and is distributed worldwide except for Antarctica. The distribution in the American continent is closely linked to the expansion of livestock and in north America it is frequently observed in most of Mexican territory.[2] The microbiota of cattle egret is composed of different percentages of multiple microorganisms due to the different eating habits in different geographical locations and different consumable items.[3] The variation of the microorganisms that make up the intestinal microbiota of this type of birds can cause dissemination and in some cases these microorganisms can participate as opportunistic pathogens.[4]

The isolation of filamentous fungi and yeasts from excreta samples, cloaca and from a lower percentage of crop, cecum and proventriculus has been previously carried out on northern bald ibis (*Geronticus eremita*).[5] Some genera and species of filamentous fungi and yeasts which have been isolated from different species of birds with different zootechnical functions include *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Candida kefyr*, *Candida krusei*, *Candida famata*, *Fusarium* spp.,[6] *Rhodotorula* spp., *Trichosporon* spp., *Cryptococcus albidus*,

***Correspondence:**

Andrea Paloma Zepeda Velazquez. PhD
Laboratory of Physiology and Clinical Pathology, Academic Area of Veterinary and Zootechnical Medicine, Institute of Agricultural Sciences, Autonomous University of the State of Hidalgo, Tulancingo, Mexico
E-mail: andrea_zepeda@uaeh.edu.mx

Cryptococcus laurentii and *Microsporum* spp., among others.[7-9] Some fungi that have been isolated from birds are important zoonotic agents and can produce public health problems in compromised immune system people. Infection with this type of fungus can result from improper handling of birds or their feces.[4] Genera of zoonotic fungi that have been reported include *Cryptococcus* spp., *Candida* spp., *Aspergillus* spp., *Geotrichum* spp., *Penicillium* spp., and *Alternaria* spp.[1,9] However, the pathogenic potential of the microbiota compound of filamentous fungi and yeasts in birds depends on the species and genera that are identified as in the case of wild pigeon feces where *Candida albicans* and *A. fumigatus* were isolated[10] or from direct contact of a skin lesion with *Lophophyton* from a fighting cock.[11-13] The purpose of this research work was to isolate and identify zoonotic fungi and yeasts obtained from excreta samples from colonies of cattle egret in the region of Tulancingo, Hidalgo, Mexico.

Materials and Methods

Study area. The resting colonies of the cattle egret are found in the geographical location 20°03'47.5"N 98°22'51.8"W in the region of Tulancingo, Hidalgo, Mexico.[3] The temperature in the area ranges from 10.00 ˚C to 24.00 ˚C, the climate is mainly dry and warm with a predominant forest ecosystem.[14] The study site was characterized by presenting long extensions of land that are destined for agriculture with crops of alfalfa, clover, grass in general, prickly pears, mezcal and corn plants.[15]

Samples and their handling. A simple random sample collection was adopted. A total number of 10 droppings were collected per sampling area (40 droppings per day). The collection was carried out from 07:30 to 08:30 hr since Monday to Friday.[16] With a sterile swab a sample was taken from the center of each dropping to obtain one pool sample for 10 collected droppings. The pool samples were spread in Sabouraud agar medium added with 0.10% chloramphenicol (Merck-Sigma-Aldrich, Toluca, Mexico) to prevent bacterial grow and incubated at 25.00 - 28.00 ˚C for 72 hr.[17] Isolations were then observed after 2 - 3 days. No birds were handled or captured to obtain droppings.

Fungi and yeast morphology identification. The colonies were inactivated with 10.00% formaldehyde for 1 hr and samples of fungal hyphae were obtained with adhesive tape which was placed with the adhesive on the surface of the colony and finally on a slide using a drop of Cotton Blue stain. For yeasts, a drop of Cotton Blue or Chinese ink was placed on a clean slide with a bacteriological loop a sample of the colony that was taken and diluted in the stain and finally a coverslip was placed.[18] The samples were observed using a compound microscope (Velab, Puebla, Mexico) at 40×.[19]

Isolation percentages. The positive cultures were recorded by the colony type to obtain the percentage of individual isolation based on the morphological identification and the isolation percentages were established.[20]

Results

Fungi and yeast Identification. Based on the macroscopic morphology of the colony's microscopic identification of fungi structure and the use of Cotton Blue staining, genera filamentous fungus and yeast were confirmed (Fig. 1). White cottony colonies turning to brown and hyaline hyphae without segmentation and oval columellae and absence of rhizoids were observed in *Mucor* spp.; *Rhizopus* spp., presented dense mycelles with the appearance of grayish sheep's wool and globose sporangiospores as well as sporangiophores, stolons and, rhizoids. The colonies of *Penicillium* spp., presented a velvety, filamentous and powdery texture in white color and in the center turning to yellow colors, later to blue-green tones and the septate hyaline hyphae, the coniophores, the filiade as well as the conidiospores, the metula and the phialide were observed. In the case of *Paecilomyces* spp., white velvety colonies were observed turning light green in the center and with a convex appearance the hyphae coming out of the phialides, the conidial were elongated, elliptical or oblong that presented small chains.

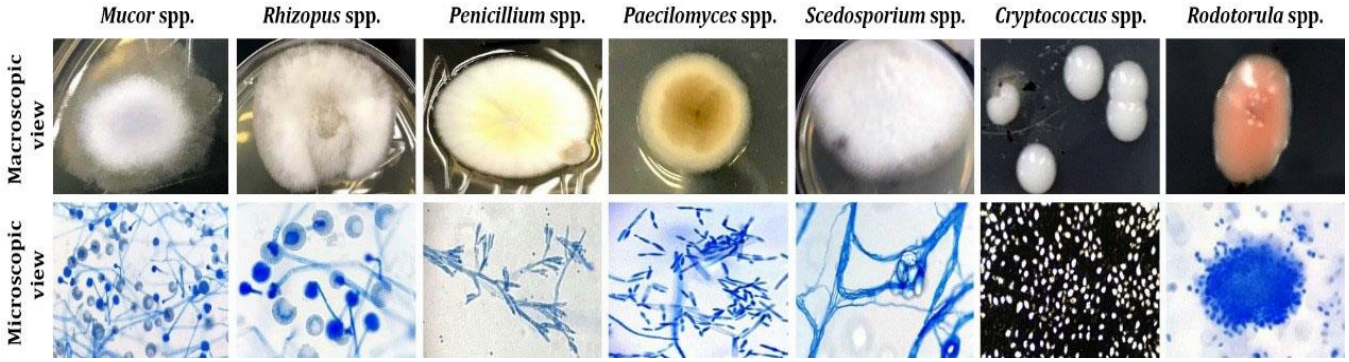

Fig. 1. Fungi and yeasts isolated from dropping of cattle egret. Microscopic views (Lactophenol Cotton Blue stain or negative stain, 40×).

The colonies of *Scedosporium* spp., presented a floppy appearance with white and later grayish colors, with a brown color on the reverse. It presented hyaline, cylindrical and septate hyphae with emerging conidiogenic cells. The globose asci contained eight ascospores and these were unicellular, ovoid, or ellipsoidal, smooth and pale yellow to copper in color. In the case of yeasts, *Cryptococcus* spp., colonies with a mucoid, shiny and cream-colored appearance were observed and ovoid structures with a wide capsule were observed under microscopy when negative staining was performed. *Rhodotorula* spp., showed colonies of coral pink color and soft consistency, smooth, moist with a mucoid appearance to the microscopy, ovoid and/or slightly elongated and monogematic narrow-based blastoconidia were observed.

Isolation percentages. A total number of 307 isolates of filamentous fungi genera with zoonotic potential such as *Mucor* spp., *Rhizopus* spp., *Penicillium* spp., *Paecilomyces* spp. and *Scedosporium* spp., as well as yeasts of the genus *Cryptococcus* spp., and *Rhodotorula* spp., were identified (Table 1).

Table 1. Percentages of isolates of fungi and yeasts with zoonotic potential, isolated from droppings of the cattle egret (*Bubulcus ibis*) in the region of Tulancingo de Bravo, Hidalgo, Mexico.

Fungus/ yeast	No. of isolation	Percentage
***Mucor* spp.**	130	42.35
***Rhizopus* spp.**	82	26.71
***Penicillium* spp.**	41	13.35
***Paecilomyces* spp.**	35	11.40
***Scedosporium* spp.**	6	1.95
***Cryptococcus* spp.**	7	2.29
***Rodotorula* spp.**	6	1.95
Total	307	100

Discussion

The fungal microbiota present in different types of birds is diverse and can present variations according to the diet consumed also with a temperature range of avian species.[21] In wild birds, their habits of residence in different places are an important factor in the health of the host due to the changes in feeding due to migratory or partially migratory behaviors.[9] Other factors such as climate changes and coexistence with other types of animals as in the case of cows,[3] can also cause and facilitate the dissemination of possible microorganisms that can be potentially pathogenic in different hosts including humans. In the different scientific research, the isolation of different genera of filamentous fungi and yeasts, Sabouraud Dextrose Agar was added with chloramphenicol that was the most used culture medium.[22]

In this research, it was identified that the filamentous fungi with the highest percentage of isolation was *Mucor* spp. (42.35%). This fungal genus may play an important role in the clinical presentation of acute fungal infections of the respiratory tract[23] and *Rhizopus* spp. genus was 26.71%, the identification of which could be explained for the contamination appeared in the feces at the time of dejection and falling to the ground, because this fungus could be isolated from the soil, wood and organic remains[24] or based on the type of feeding of the cattle egret that was an omnivorous bird obtaining its food from agriculture and livestock areas.[3] However, the isolation could be related to different eating habits due to the percentage of isolation of 18.42%. This fungus can produce diseases mainly related to the upper respiratory tract.[25]

The results obtained in isolation of *Mucor* spp. genus, in the present work were in agreement with Marinho *et al.*,[22] that identified the fungus of cloaca isopods, in different bird species such as the Golden-billed Saltator (*Saltator aurantiirostris*), the Orange Tanager (*Pipraeidea bonariensis*), the Blue Cardinal (*Stephanophorus diadematus*), and others which were in captivity in a 4.00%. This filamentous fungus is considered a contaminant, so the differences identified in the percentage of isolation are related to the nature of the habitat in captive birds versus wild birds.

Paecilomyses spp is related to skin lesions and develop keratitis lesions,[26,27] because these types of filamentous fungi and other genera such as *Alternaria* spp., and *Thrichophyton* spp., have a keratinophilic activity due to the presence of keratinase enzymes.[28] In poultry farms, the identification of the genus was 4.00% in dust from poultry houses,[29] while this research was isolated in 8.00% from fresh droppings because these birds can eat smaller birds.[3] The presence of this type of fungi in the microbiota fulfills the role of partially disintegrating the feathers of the consumed birds.

Penicillium spp. gender has been isolated from 33.44% to 60.00%;[21] of poultry houses and 42.30%, from dirty litter[30] compared to the present work, which was isolated in 13.35%. The isolation from aerosols inside a poultry house is 0.88%, depending on the conditions found in an open or closed environment and humidity that can be an important factor that favors the fungus isolation from soil substrates.[31] The presentation of *Penicillium* spp., in humans is related to respiratory problems, allergic rhinitis, and asthma.[32]

Scedosporium spp. can be naturally isolated from contaminated soil and water as well as from urbanized environments. The fungus becomes established in immunocompromised human patients causing local and/or systemic infections, where the central nervous system can be infected.[25] The isolation of *Scedosporium* spp., may be due to the contamination of the samples with the soil, with the low percentages of isolations (1.95%). Al-Yasiri *et al*. obtained isolation of *Scedosporium* spp., from fecal samples of yellow-legged gulls (*Larus michahellis*) in

5.20% in a coastal area. The isolation differences may be related to the areas and substrates that make up each locality. [33]

In previous studies, the isolation of yeasts such as *Cryptococcus* spp., was observed to be in percentages ranging from 11.11% to 85.00% prevalence and other authors have obtained percentages of 8.50%.[21] However, in the present research work, a very low percentage of isolation was obtained (2.29%) compared to 39.13% of isolates obtained from lovebirds kept as pets.[34] The isolation of *Cryptococcus* spp. is usually obtained from the ground and bird droppings especially pigeons, however, up to 100 species have been identified in this genus and only *C. neoformans* and *C. gattii* are considered pathogenic species for humans.[35]

The presentation of mycosis in an immuno-compromised person infected with this *Cryptococcus* species can cause headaches and in severe cases of the disease, meningitis will be developed.[18]

In the case of the yeast *Rhodotorula* spp., it is found in the environment and has been isolated from excreta of wild birds (6.66%),[21] in pet lovebirds (*Agapornis* spp.; 13.05%)[25] and Harris's hawk (*Parabuteo unicinctus*).[36] In this work it was isolated in 1.95% of cases. The importance of this yeast lies in being an emerging opportunistic pathogen as well as in the participation of clinical presentations related to meningitis, endocarditis, peritonitis and exophthalmitis.[37]

Although in the cases of *Alternaria* spp., a pathogenic role in humans has been described, the clinical presentation is mainly caused by the production of toxins in foods such as fruits, vegetables and tubers, and not directly from the fungus.[38]

The different percentages obtained from the fungal isolates are mainly related to the differences of each bird species, zootechnical function and area where they will naturally live. Birds with a zootechnical production function remain in poultry houses until they are transferred to the slaughterhouse which increases the number of filamentous fungi and yeasts in each area.[28,30,39] Those birds that are in captivity and belong to zoos or are pets, the cleaning of artificial habitats or areas where birds are kept are cleaned up regularly preventing the accumulation of different microorganisms with a pathogenic potential.[36] Finally, in the case of wild birds whose resting areas are not at ground level, they benefit from the distance between the treetops.

Previous studies have reported the isolation of other fungal genera such as *Trichosporon* spp., (13.90%), *Rhizomucor* spp., (8.30%),[23] *Cladosporum* spp. (20.00%),[30] *Malassezia pachydermatis* (2.00%) and *Aspergillus* spp., (28.20%).[22] Glushakova *et al.*, identified that the incubation temperature of freshly excreta samples can affect the growth of different species of mainly pathogenic and opportunistic yeasts as in the case of *C. albicans*, that can grow between 25.00 ˚C up to 37.00 ˚C, while, at temperatures of 25.00 ˚C yeasts present in plant elements can grow.[1] In this research work, the macroscopical and microscopical identification was used to identify the genera of fungi and yeasts, isolated from cattle heron droppings. However, due to the importance of the different genus identified in this research, it is necessary to carry out molecular identification. Therefore, this work will be the basis for future studies on the microbiota of filamentous fungi and yeasts of cattle egret.

In the present research work, the isolation of eight different genera of fungi and yeasts isolated from cattle egret excreta was reported. The genera *Mucor* spp., *Rhizopus* spp., *Penicillium* spp., *Paecilomyces* spp., *Scedosporium* spp., *Cryptococcus* spp., and *Rhodotorula* spp.; have been previously reported as zoonotic agents mainly in hosts with some type of immunosuppression. However, the presence of these fungi and yeasts in different places where birds live is not a definitive factor for development a disease. People who are in optimal immunological conditions can develop some clinical condition from the participation of different factors related to the environment, the host and the pathogen. Therefore, the presence of avian species in anthropogenic activities does not predispose to the presentation of a filamentous fungi or yeast zoonosis.

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Conflicts of interest

The authors declare no conflict of interest.

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