



Fluorescence Microscopy Detection of *Cryptococcus Neoformans* Isolated From Chicken and Pet Birds Dropping Samples in Mosul Province



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CRYPTOCOCCOSIS is infection with the fungi *Cryptococcus neoformans*, which may occur in the feces of many types of birds, and soil. The mean of study isolation of *Cryptococcus neoformans* from chicken and pet bird dropping sample in Mosul province. One hundred and twenty five samples were collected from dropping belong was of chicken (75) and pet bird (50). Cultured on Sabouraud Dextrose Agar (SDA) and Cryptococcus differential agar, isolates of *Cryptococcus neoformans* were diagnosed micromorphology by fluorescence microscope. The results revealed that the percentage of *Cryptococcus neoformans* isolation in chicken was 10/75 (13.3%), while in Pet bird was 15/50 (30%). All *Cryptococcus neoformans* strains grew on Cryptococcus differential agar at 37°C for 2-5 days, cultural characteristic of positive isolates were observe as light blue. The *Cryptococcus neoformans* appear a positive acridine orange in the image its more sensitive to detection by being yellow against a dark background. The current study proves that the presence of *C. neoformans* in abundance as an environmental contaminant considered a potential source of infection for humans and animals in the Mosul province.

Key words: Pet birds, Chicken, *Cryptococcus neoformans*, Fluorescence Microscopy, Vitek 2 system.

Introduction

Cryptococcus neoformans is an encapsulated yeast. It has been isolated from pigeon droppings, where it is found in the soil rich in organic material [1].

Cryptococcus neoformans has been detected in the environment, primarily in connection with bird droppings, though it has also been isolated in non-avian origins. *C. neoformans* var. *gattii* was discovered in nature with Eucalyptus trees [2]. This type primarily causes contaminations in tropical and subtropical areas because clinically apparent instances of Cryptococcosis in healthy hosts are rare, it is assumed that most persons can establish adequate host defenses against this pathogen [3]. Research has noted variable

proportions of contamination, as it revealed that at least 5%-10% of patients with are infected with cryptococcosis [4; 5]. Definitely, the presence of natural substrate is a fertile surrounding for the growth of the fungus *Cryptococcus neoformans* where bacteria are present with a small quantity, which decreases the antagonism amongst them. This leads to human exposure to this pathogen, which is present as a dust pollutant [6].

The fungus is an enclosed basidiomycete that is globose or sphere-shaped in a diameter of roughly 2–8 μm. The fungus reproduces by budding, and it grows at a temperature of 37°C [7]. It has an antigenic mucopolysaccharid capsule, which is responsible for defense against phagocytic cells [8]. The yeast which extent in

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pigeon feces may struggle deficiency for two years [9]. The high corporeal temperature of pigeons (42°C), attendant with increased macrophage activity, limits cryptococcal growth in these birds. Cryptococcosis can be identified in the research laboratory of is commonly founded on numerous ways straight microscopy, culture, serology, histopathologic investigation and molecular methods [10].

Negative staining of Indian ink has been utilized to make this discovery, despite the fact that it yields positive results in around 60% of infections that are confirmed by culture [11]. The latex agglutination test, which determines the presence of the capsular polysaccharide antigen through the agglutination of antibody-coated latex particles, is an additional method that is now largely available. The idea that fluorochromes like acridine orange may be used to stain the capsular material of *C. neoformans* and produce a bright yellow color when identified by fluorescence microscopy led to the recommendation of another technique [11]. Fluorescence microscopy has microscopic diagnosis of both infectious and non-communicable disorders. With regard to food safety and research, it has permeated many other sectors, particularly those in the health sciences. The knowledge is now accessible for tiny laboratories and public health places in underdeveloped countries thanks to the latest fluorescence microscopes, which are far less expensive and easier to transport than the older types [12]. Acridine orange is a fluorescence dye used to stain DNA, RNA, and acidic vacuoles in living cells. It has been employed to stain samples for epifluorescence microscopy and practical staining, and it is particularly suitable for quick visualization of sterile organic samples from clinical and non-clinical substances, when it attaches to RNA, it emits yellow fluorescence, and when it binds to DNA, it emits green fluorescence [13].

The objective of this study is to isolation and identification of *Cryptococcus neoformans* in the dropping sample of chicken and pet birds in Mosul Province by using conventional methods and fluorescence microscopy.

Material and Methods

Test sample

A total of 125 samples (N=75 chicken and N=50 pet birds droppings) in Mosul Province,

collected in sterilized saline and transported to the laboratory in sterile condition during the period (October 2022- March 2023). All samples were incubated at 37°C for 24 hours then inoculated into SDA plates with chloramphenicol and incubated at 37°C for 2-5 days.

Conventional Identification

The samples were cultured on Sabouraud's Dextrose Agar (SDA), isolates were subjected to various mycological conventional identification techniques, including morphological according to colonies shape, using two methods: the first involved bright field microscopic examination with Indian ink, and the second involved fluorescence microscopic examination (Optika 40X) with a suspension of 50 µl of a physical solution of acridine orange stain to simulate the micromorphology of *C. neoformans* isolates. Additionally, 04.44 gm of the medium was suspended in 1000 ml of distilled water, then mixed with high-quality and melted via a heating system with time-honored agitation to create the pure colony subculture on specific Cryptococcus difference agar, as per the original company's instructions. The medium did not autoclaved, this medium was used for distinguished between cultural characteristic Cryptococcus species (*Cryptococcus neoformans* appear light blue, dry colony, *Cryptococcus laurentii* appear brown dry colony and *Cryptococcus gattii* appear brown mucoid colony. In addition, Vitek 2 system (BioMerieux, France) as a completely automated device for the identification of used also to isolate [2].

Results

The results isolation of supposed *Cryptococcus neoformans* from Chicken and pet bird's droppings as it showed in Table (1), everywhere the number of positive *Cryptococcus neoformans* isolates were reported as 10/75 (13.3%) from Chicken droppings sample and 15/50 (30%) from pet birds droppings sample.

Macroscopic Identification

Totally, 125 chicken and pet bird droppings samples plated onto Sabouraud's Dextrose Agar, only 25 isolates presented cryptococcus features: creamy to tan color, smooth, mucoid characteristic, and raised surface. All *Cryptococcus neoformans* isolates cultivated on cryptococcus differential agar at 37°C for 2-5 days, cultural typical of positive isolates detected bright blue, dry

colony that distinguished from other species as *Cryptococcus laurentii* and *Cryptococcus gattii* as, shown in figure (1). *Cryptococcus neoformans* species were confirmed with Vitek 2 system (BioMerieux, France).

Microscopic characterization

Observation of India ink preparation appearance for oval or round cells, approximately viewing budding, unequal in size, and surrounded by a large unstained as capsule a zone of clearance or "halo" around the cells. While the image of a positive acridine orange cells as *Cryptococcus neoformans* fluorescing yellow against a dark background, using a blue filter on a fluorescent microscope examination, as shown in the Fig. (2)

Discussion

The environment contains *Cryptococcus neoformans*, which is primarily found in bird secretions like pigeons or dust that has been exposed to bird faces. These conditions are favourable for the growth of pathogenic yeasts like *Cryptococcus* species [14]. Such excreta or contaminated dusts therefore become likely reservoirs and causes the spread of infections.

The present study showed into being positive of *C. neoformans* isolation as was 10/75 (13.3%) from Chicken droppings sample and 15/50 (30%) from pet birds droppings sample. indicating higher isolation rate from chicken dropping samples with the accepted obtainable by some authors in Egypt regarding 1/170 isolates of *C. neoformans* from Chicken Dropping [2]. The researchers looked into the potential for *Cryptococcus* to be present in domestic chicken droppings and dust that which been increased by droppings as a potential source of contamination to humans, mainly immunosuppressed persons [15,16]. Disseminated cryptococcosis, pulmonary cryptococcosis, and cryptococcal meningitis are the three subtypes of deep-rooted mycosis known as cryptococcosis [17]. Numerous investigators in various parts of the world have identified *Cryptococcus neoformans* as being prevalent in the natural environment, particularly in bird droppings and dust contaminated with bird droppings [18,14]. There is a little information on the isolation from such an environment, and this organism originated from domestic chicken in Kenya, this was linked to a high pH and the presence of high molecular development inhibiting elements in chicken droppings

[19,20]. Nevertheless, infections brought on by *Cryptococcus* species have been documented in situations of severe immunosuppression [21].

The results of the current study isolated 15/50 (30%) from pet birds droppings sample which companionable with work in Italy by Abbas *et al.* [22] who show that birds of prey may performance as transporters and spreaders in the surroundings of *C. neoformans* and other possibly zoonotic yeasts. *C. neoformans* and *C. gattii* can by of Cryptococcosis in humans as well as in both wild and domestic animals. Also in the current study the isolated which were higher than study in Baghdad [23], who described the proportion of *C. neoformans* (13%) isolated from bird dropping. Also in Malaysia [24] found (20) isolates of *Cryptococcus neoformans* from feces of zoo birds. The severe health dangers for human originated either from direct interaction or from inhaling of the fungal spores from communicable organisms which cultivate in the nutrient-rich builds of bird droppings and spread of fungi spores by wind extracts, causing diseases in humans [25,26]. The fluorescent microscopy may also more accurate and dependable in mass screening programs for the discovery of Cryptococcal species. This difference in fluorescence of Cryptococcal yeasts caught our attention as we investigated acridine orange as an alternative for India ink. Acridine orange, which is used to stain the capsule with Indian ink, is a non-specific stain, but fluorescence has the ability to quickly cross the threshold of dead or living cells, fix to essential nucleic acids, and increase the sensitivity of determining sterility of cerebrospinal fluid. Other fungal-specific fluorescent dyes have been employed in the past to effectively stain fungi in human and animal tissue [27, 28, 29]. However, in our source-restricted area, these are no longer freely available.

Conclusion

The present study determined that the probability of contamination of the environment by *C. neoformans* in chicken and pet birds dropping and the danger for humans and animals in a contact with those birds. Therefore, further investigations are important employed to study the health status of birds in addition using staining fungal nucleic acids with fluorescent dyes instead of the capsule with India ink since it sensitivity for the diagnosis of Cryptococcales as hypothesized in this study.

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Ethical approval

A procedure authorized by the Ethical Council

for Animal Research was employed to care for the cows used in this study (No=UM.VET.2022.049).

Conflict of interest

The authors declare no conflict of interest

Funding statement

University of Al-Hamdaniya

Authors contributions

Hawraa Faisal: Conception and design of

TABLE 1. Isolation of *Cryptococcus neoformans* from Chicken and pet birds droppings.

Source of samples	Number of samples	Number of isolates <i>Cryptococcus neoformans</i>	%
Chicken droppings	75	10	13.3
Pet birds droppings	50	15	30
Total	125	25	20

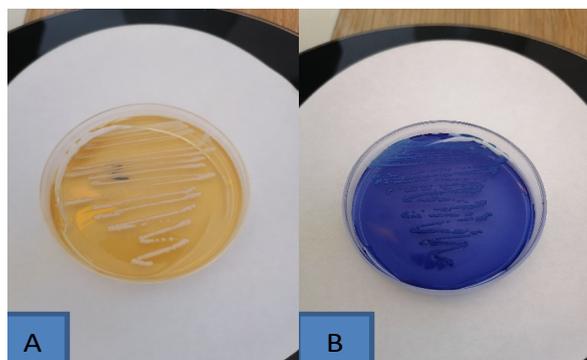


Fig. 1. A- *Cryptococcus neoformans* seem creamy to tan color, smooth, mucoid typical and elevated surface on Sabouraud's Dextrose Agar. B- *Cryptococcus neoformans* bright blue, dry colony on Cryptococcus differential agar.

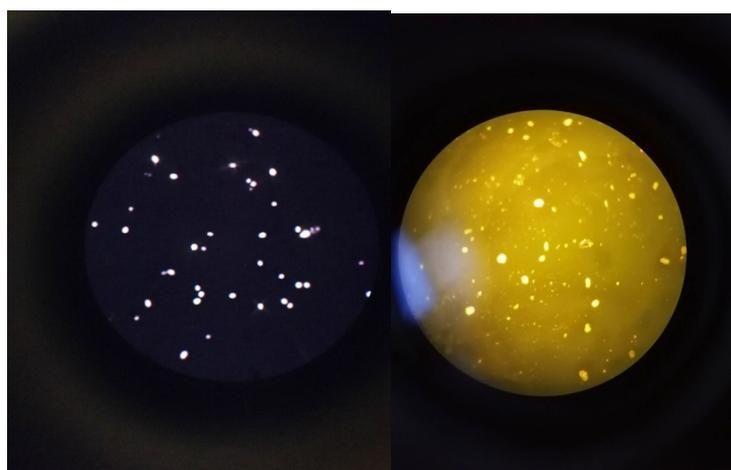


Fig. 2. Acridine orange and Indian ink-stained *Cryptococcus neoformans* cells (A) A bright field light microscope image of a positive Indian ink slide (B) Fluorescence microscope image a blue filter and a positive acridine orange slide showing *Cryptococcus neoformans* fluoresces yellow against a dark background. Each of them examined at 40X.

the study, wrote the first draft of the manuscript and design figures. Mumtaz Mati and Karrar Ali: critically revised the manuscript, funding acquisition. Adil Jabbar Atiyah: Writing one of the topics and critically revising the manuscript.

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الفحص بالمجهر الفلوريستنت عن المستخفيات الحديثة المعزولة من عينات البراز الدجاج والطيور الأليفة في محافظة الموصل

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المكورات المستخفية هي عدوى بالفطريات المستخفية ، والتي قد تتواجد في براز أنواع عديدة من الطيور، وفي التربة. في الدراسة الحالية عزل فطر *Cryptococcus neoformans* من عينات براز الدجاج والطيور الأليفة في محافظة الموصل. تم جمع مائة وخمسة وعشرين عينة من البراز كانت للدجاج (75) وطيور أليفة (50). مزروعة على (Sabouraud Dextrose Agar) وأجار المكورات التفريقي ، تم تشخيص عزلات *Cryptococcus neoformans* بواسطة مجهر الفلورسنس. أوضحت النتائج أن النسبة المئوية لعزل *Cryptococcus neoformans* في الدجاج كانت 10/75 (13.3٪) بينما في الطيور الأليفة كانت 15/50 (30٪). نمت جميع سلالات *Cryptococcus neoformans* على أجار المكورات التفريقي عند 37 درجة مئوية لمدة 2-5 أيام ، لوحظت الخصائص الثقافية للعزلات الإيجابية باللون الأزرق الفاتح. ظهرت عزلات *Cryptococcus neoformans* برتقالي أكردين إيجابي في الصورة وكانت أكثر حساسية للكشف لكونها صفراء على خلفية داكنة. تثبتت الدراسة الحالية أن وجود فطر *C. neoformans* بكثرة كملوث بيئي يعتبر مصدرا محتملا للعدوى للإنسان والحيوان في محافظة الموصل.

الكلمات المفتاحية: الطيور الأليفة، الدجاج، المكورات المستخفية الحديثة، الفحص بالمجهر الفلورسنس، نظام فيتيك².