CATRINA (2007), 2 (1): 91 -96
© 2007 BY THE EGYPTIAN SOCIETY FOR ENVIRONMENTAL SCIENCES
PROCEEDINGS OF THE SECOND INTERNATIONAL CONFERENCE ON THE ROLE OF GENETICS AND BIOTECHNOLOGY IN CONSERVATION OF NATURAL RESOURCES, ISMAILIA, EGYPT, JULY 9-10, 2007

Domestic Birds as Carriers of *Cryptococcus neoformans*, the Main Cause of the Life Threatening Human Systemic Cryptococcosis

M. Abdel-Razik
Botany Department, Faculty of Science, Suez Canal University, 41522 Ismailia, Egypt

ABSTRACT

Samples of bird excreta including domestic pigeons were collected from farmer houses and aviary shops in rural and urban areas of Ismailia. The collected samples were mycologically processed for the occurrence of the pathogenic yeast, *Cryptococcus neoformans*. It was isolated mainly from pigeon excreta and from only one sample of colored birds excreta collected from decorated colored bird's shops. Pigeon excreta positive for *Cryptococcus neoformans* were suspended in sterilized phosphate buffered saline then three different doses of the supernatant were interaperitoneally injected into groups of experimental albino mice to assess its dissemination and the various pathological effects resulting from systemic infection. Mainly, pulmonary and occasional meningeal lesions were evident in the infected mice.

Keywords: *Cryptococcus neoformans*, pigeon excreta, pulmonary, meningeal infection.

INTRODUCTION

Cryptococcus neoformans, a heterobasidiomycetous yeast like fungus, is the etiologic agent of systemic cryptococcosis. (Kwon-Chung and Bennet, 1992; Casadevall and Perfect, 1998). It is reported to have an affinity to the central nervous system of humans. It mainly attacks the immunocompromised patients and those suffering from other underlying disease (Nildete *et al.,* 1997), and in rare cases it may infect immunocompetent persons (Nunez *et al.,* 2000). Also, diseases of the respiratory tract caused by *C. neoformans* have been detected (Yamaoka *et al.,* 1996; Goldman *et al.,* 1998).

Cryptococcosis is the most frequent systemic mycosis in immunocompromised patients. In HIV patients with advanced infection the prevalence of cryptococcosis ranges from 6-8% and the risk for cryptococcal infection in renal transplant recipients approaches 10%. The clinical presentation of cryptococcosis vary depending on the host and site of infection (Kwon-Chung and Bennet, 1992).

The present investigation was conducted to study the incidence of the yeast fungus *Cryptococcus neoformans* in excreta of housed pigeons and other domestic birds which are fairly common in farmer's houses in the rural areas and some houses of the urban areas, besides the surrounding aviary shops. Also, to clarify its important role in eliciting respiratory and meningeal infections using white mice as experimental animal model.

MATERIALS AND METHODS

Sample collection

Samples of undessicated excreta of domestic pigeons and other birds (chicken, geese, duck) were separately collected from houses and domestic aviary shops in the rural and urban areas of Ismailia town, besides colored birds excreta from Pet bird's shops. Excreta were collected in separate sterilized plastic bags from the pigeon shelters hanged on walls inside farmer houses, and from the floors of bird enclosures mixed with soil.

Isolation of *Cryptococcus neoformans* from birds excreta

Samples of pigeons and other birds excreta were cleansed from straw, grinded in a porcelain mortar and subjected to mycological examination. Ten grams of each sample was suspended in bottles containing 90 ml of sterilized phosphate buffered saline with bacterial antibiotic. Bottles were vigorously shaken for 10 minutes, then allowed to settle for 5 minutes. One ml of the supernatant was inoculated onto each of 3 plates of Wicker ham's malt agar and 3 plates of *Guizotia abyssinica* creatinine agar (GACA) with the addition of 0.1% biphenyl. GACA was prepared according to *Staib et al.,* (1987). All plates were incubated at room temperature (28°C) for one week then examined. *C. neoformans* colonies were enumerated, considering the brown color effect typical to *C. neoformans* which is due to the phenol oxidase production. India ink preparation was made to examine the capsule formation by the isolated suspected *C. neoformans* colonies.

Enzyme activity

a) Evaluation of phospholipase production

The isolated *Cryptococcus neoformans* was inoculated on plates containing egg yolk agar medium prepared according to Price *et al*. (1982)**,** incubated at 37^{o} C for 5 days for the development of preciptation zone of phospholipase activity around colonies (Pz value).

b) Evaluation of urease production

The isolated *C. neoformans* was inoculated on slants of Christensen's agar medium, incubated at 28^{o}C for 5 days for the development of deep pink color (Seeliger, 1956).

* *e-Mail: m.abderazikl@yahoo.com*

Other confirmatory test

Cycloheximide sensitivity test, assimilation and fermentation tests were performed for the confirmation of *C. neoformans* (Lodder, 1971).

***Cryptococcus neoformans* experimental infection**

(A) The samples of pigeon excreta highly positive for *C. neoformans* were used. Twenty five grams of the sample was added to 250ml of sterilized buffered saline, vigorously shaked for 10 minutes, then allowed to settle for 10 minutes, filtered using bacterial filters under aseptic condition, and kept in clean sterilized vials for experimental infection.

(B) Four groups of white allino mice 20-25gm (7 mice each) were kept in separate cages. The first 3 groups were intraperitoneally injected with 0.1ml, 0.3ml and 0.5ml of the filterate respectively. The fourth group was intraperitoneally injected with 0.5ml of sterilized phosphate buffered saline as control. The infected mice were kept in their cages with supply of food and water for 6 weeks under observation for the development of clinical symptoms and recording the mortality rates. Mice showing sever clinical symptoms and dying were dissected, their lungs and brains were excised, fixed in 10% formaline and routinely processed for histopathological examination after staining with Hematoxyline & Eosin and Mucicarmine stains.

RESULTS

Cryptococcus neoformans was isolated from biles of humid pigeon excreta collected from pigeon shelters in farmers houses and domestic aviary shops. These excreta were neither mixed with soil nor subjected to sunlight. Also, only one sample of decorated colored birds excreta collected from colored bird's shops gave a positive result for *C. neoformans*. All other collected domestic birds excreta were negative for the fungus. (Table 1).

The isolated colonies of *Cryptococcus neoformans* were positive for brown color effect on *Guizotia abyssinica*, biphenyl agar, polysaccharide capsule formation in India ink preparation, phospholipase and urease production, with negative growth on cycloheximide and finally confirmed with assimilation and fermentation tests (Fig. 1).

Mortality rate of mice infected with *Cryptococcus neoformans*

All groups of mice inraperitoneally infected with supernatant of pigeon excreta suspension positive for *C. neoformans*, showed a varied range of mortalities. All mice infected with the highest dose (0.5ml) were dead

Table (1): Frequency of *Cryptococcus neoformans* in all collected samples.

Collected samples	*No.*	*+ ve*	*%*
Accumulated humid pigeon excreta collected from shaded areas, not mixed with soil.	19	5	26.3
Dessicated pigeon excreta occasionly subjected to sunlight.	11	0	0
Pigeon excreta mixed with soils from houses floor.	23	0	0
Other birds (Chicken, duck, geese) excreta mixed and without soil from birds enclosures floor.	23	0	0
Pigeon excreta collected from domestic aviary shops.	21	3	14.2
Duck, geese, chicken excreta collected from domestic aviary shops.	21	0	0
Colored birds excreta collected from decorated colored bird shops.	13	1	6.7

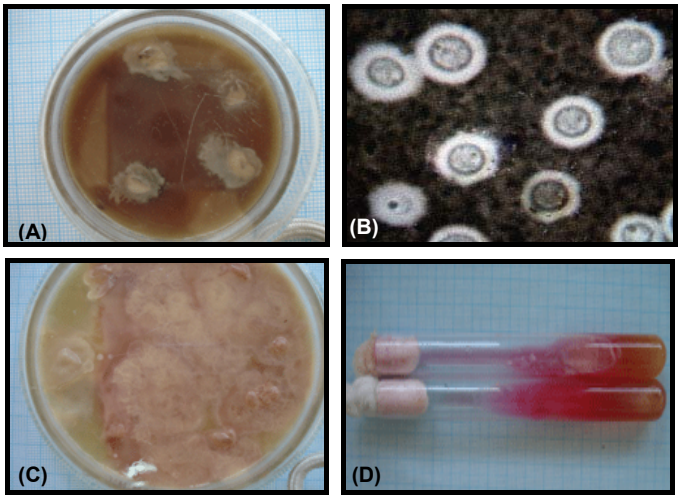

Figure (1): (A) Positive brown color effect on *Guizotia abyssinica*, (B) Polysaccharide capsule formation in India ink preparation, (C) Phospholipase production, and (D) Urease production.

with symptoms of respiratory distress and severe convulsion within 21 days. Mice infected with 0.3 ml had died within a period of 43 days showing symptoms of respiratory distress. While for mice infected with the lowest dose 0.1ml only 2 mice died at the days of 29 and 43 days after loosing their appetite and being sluggish in their cages (Fig. 2).

Detection of *Cryptococcus neoformans* in tissues and histopathological changes

The pulmonary lesions showing fungal granulona with the presence of encapsulated *C. neoformans* in lung tissue was apparent in both groups of mice infected with 0.3 and 0.5ml of the filtrate. The brain infection with the presence of encapsulated yeast in brain tissue was evident only in 2 mice infected with the 0.5ml dose. While those mice infected with (0.1ml) had no yeast cells neither in their lungs nor brain tissue. (Fig. 3).

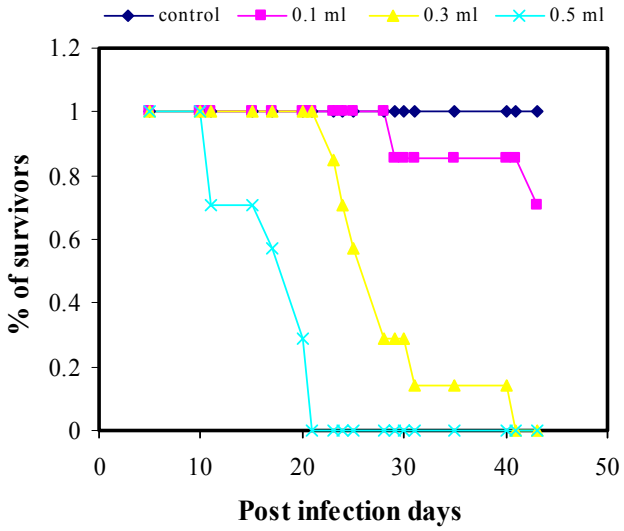

Figure (2): Mortality of mice intraperatoneally infected with various doses.

DISCUSSION

In the present trial the basiodiomycetous yeast fungus *Cryptococcus neoformans* was proven to be found mainly in Egyptian pigeon excreta, accumulated in farmer houses and domestic aviary shops under humid conditions and absence of direct sunlight. Also, in colored birds excreta collected from small colored birds shops. Untill recently, old excreta of pigeon and other bird species including captive parrots and canaries was known to constitue an important reservoir of the yeast fungus *C. neoformans* var. *neoformans* (Kwon Chung and Bennet, 1992; Criseo *et al.*, 1995). *C. neoformans* var. *neoformans* has been isolated regularly throughout the world from accumulated pigeon excreta collected from shelters with appropriate conditions of darkness, humidity and suitable temperature in which *C. neoformans* could survive and flourish (Jen, 1992; Irokanulo *et al.,* 1997; Khosravi, 1997; Caicedo *et al.,* 1999; Kielstein *et al.,* 2000; Abou-Gabal and Atia 1978; Refai *et al.,*1983). As *C. neoformans* is able to grow on such substrates (feces) due to the abundance of nitrogenous compounds as creatinine, xanthine, urea, uric acid (Staib, 1994).

All collected samples of pigeon and other bird's excreta, mixed with soil or collected from sites subjected to sunlight, did not reveal any colonies for the

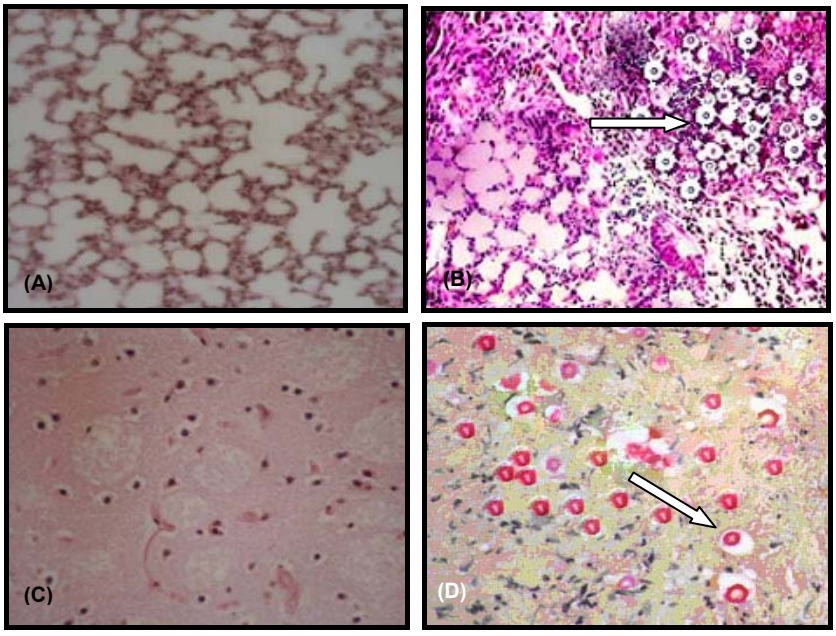

Figure (3): (A) Normal lung tissue, **(B)** Infected lung tissue with encapsulated yeast cell, **(C)** Normal brain tissue, and **(D)** Infected brain tissue showing encapsulated yeast.

fungus. Microbial competition by bacteria, the presence of soil protozoa (*Acanthoamoeba*), with its engulfing and killing effect, in addition to other effects of UV light and soil heavy metals, are the main factors controling *C. neoformans* growth in avian dropping. (Criseo *et al*., 1999; Steenbergen *et al*., 2001).

The distinguishable brown color pigmented colonies of *C. neoformans* on *Guizotia abyssinica* creatinine agar due to its phenoloxidese enzyme which converts a variety of substrates into a dark pigment, melanin, still remain the most important method of laboratory diagnosis. (Hajjeh *et al.,* 1995; Williamson *et al.,* 1998). These melanin pigment plays an important role in environment survival and virulence. It is formed in *C. neoformans* cell wall during tissue invasion (Nosanchuk *et al.,* 1999), and it was isolated from human brain tissue infected with *C. neoformans,* but not from the non infected tissues (Nosanchuk *et al*., 2000). Also, administration of melanization inhibiting compounds (glyophosate) to lethally infected mice was associated with prolonged survival of these mice (Nosanchuk *et al*., 2001).

The diagnostic polysaccharide capsule of *C. neoformans* consists of 3 compounds (manoprotein, galactoxylomanan and a predominant glucoronoxylomanan) forming an intertwining network of strands extending from its cell wall depending on environmental conditions (Casadevall and Perfect, 1998). It can be visualized by light microscope using India ink stain in vitro, as it exclude the ink particles forming a characteristic hallow and constitute a major diagnostic feature of cryptococcosis, because its compounds can be detected in blood stream. Capsule compounds constitute a virulence factor as it inhibit the production of proinflammatory immune response and reduce the leucocytes migration to the site of infection (Buchanan and Murphy, 1998), Also, inhibits the phagocytosis of the fungus by macrophages and neutrophils and inhibit killing of the fungus once being engulfed by the alveolar macrophages. (Dong and Murphy, 1997; Del Poeta, 2004).

C. neoformans phospholipase was detected in the sera of patients with meningeal *cryptococcosis*, it was considered as avirulence factor involved in tissue invasion, mainly acting on phospholipids of the outer mamalian plasma membrane and the main constituent of pulmonary surfactant (Chen *et al*., 1997). Also, the correlation of large capsule formation with high phospholipase production and the dramatic decrease in virulence of non phospholipase producers mutants demonstrated in macrophage studies confirm its role as a virulence factor (Steenbergen *et al*., 2001).

The yeast fungus *C. neoformans* has the propensity to cause a life threateming chronic infection in both immunocompromised and immunocompetent hosts, (especially those individuals with impaired immunity), it elicits a wide range of inflammatory response mainly granulaoma formation which represent the main histological finding produced in the lung with its large population of resident macrophages. (Yamaoka *et al*., 1996). On the other hand, in brain, the cellular response is sparcely observed and the lesions are characterized by the growth of encapsulated *Cryptococcus* cells with abundant capsular polysaccharides in the surrounding tissue. (Goldman *et al*., 1998).

The infection usually occur when fungal particles are inhaled and enter the alveolar space. In most immunocompetent individuals, this infection is either cleared or remain dormant until immune imbalance leads to further development (Rodrigues *et al*., 1999). Also, subsequent dissemination via accidental trauma may lead to continous invasion along the organs until fungal growth is established (Nildete *et al*., 1997). Cases of cryptococcal pneumonia with fatal outcome in immunocompetent patients have been reported (Robinson, 1995). The dessimination of infection to the central nervous system in normal hosts also may occur (Diamond, 1995; Nildete *et al*., 1997). Moreover, *Cryptococcus* infection was confirmed in sera of immunocompetent children (2-5 years old), residing at an area characterized by the large numbers of pigeons, and its highly accumulated excreta. The infection signs and symptoms were determined as primary pulmonary cryptococcosis, which may be asymptomatic or produce symptoms confused with viral infection and therefore not correctly diagnosed as fungal infection. (Goldman *et al*., 2001). In conclusion**,** the accumulated excreta of domestic pigeons and some other birds constitute a serious health hazard, as being the main reservoir of the yeast fungus *Cryptococcus neoformans*, the cause of the life threatening pulmonary and meningeal infections, especially between those individuals with certain underlying diseases and also children with their immature immune response. Thus all sanitary percutations should be achieved, including the regular getting rid of accumulated excreta, cleaning and disinfecting their sites, besides the daily exposure to direct sunlight, to control the propagation and spread of this yeast fungus in the domestic environment.

REFERENCES

ABOU-GABAL, M., AND M. ATIA. 1978. Study of the pigeons in the dessimination of *Cryptococcus neoformans* in nature. Sabouraudia **18**: 63-68.

BUCHANAN, K.L., AND J.W. MURPHY. 1998. What makes *Cryptococcus neoformans* a pathogen. Emerging Infectious Diseases **4**: 71-83.

CAICEDO, L.D., M.I. ALVAREZ, M. DELGADO, AND A. CARDENAS. 1999. *Cryptococcus neoformans* in bird excreta in the city zoo of Cali, Columbia. Mycopatholgia **147**: 121-124.

CASADEVALL, A., AND J.R. PERFECT. 1998. *Cryptococcus neoformans*. Washington D.C. ASM Press.

CHEN, S.C.A., M. MULLER, J.Z. ZHOU, L.C. WRIGHT, AND T.C. SORRELL. 1997. Phospholipase activity of *Cryptococcus neoformans*: a new virulence factor.

Journal of Infectious Diseases **175**: 414-420.

CRISEO, G., M.S. DE BOLIGNANO, F. LEO, AND F. STAIB. 1995. Evidence of Canary droppings as an important reservoir of *Cryptococcus neofarmans*. International Journal of Medical Microbiology Virology, Parasitology and Infectious Diseases **282**: 244-254.

CRISEO, G., M. GALLO, AND A. PERNICE. 1999. Killer activity at different PHs against *Cryptococcus neoformans var neoformans* serotype A by environmental yeast isolates Mycoses **42**: 601-602.

DEL-POETA, M. 2004. Role of phagocytosis in the virulence of *Cryptococcus neoformans*. Eukaryotic cell.

DIAMOND, R.D. 1995. *Cryptococcus neoformans* in. G.L Mandell, J.E. Bennet, and R. Dolin (Eds.), Principles and practice of infectious diseases. 4th Ed. 2331-2340.

DONG, Z.M., AND J.W. MURPHY. 1997. Cryptococcal polysaccharide bind to CD18 on human neutrophils. Infection and Immunity **65**: 557-566.

GOLDMAN, D.L., B.C. FRIES, S.P. FRANZOTS, L. MONTELLA, AND A. CASADEVALL. 1998. Phenotypic switching in the human pathogenic fungus *Cryptococcus neoformans* is associated with changes in virulence and pulmonary inflammatory response in rodents. Proceedings of Natural Academic Science **95**: 14967-14972.

GOLDMAN, D.L., H. KHINE, J. ABODI, D.J. LINDENBERG, L.P. PEROFSKI, R. NIANG, AND A. CASADEVAL. 2001. Serologic evidence for *Cryptococcus neoformans* infection in early childhood. Pediatrics **107**: 66

HAJJEH, R.H., M.E. BRANDT, AND R.W. PINNER. 1995. Emergence of cryptococcal disease: epidemiologic perspectives 100 years after its discovery. Epidemiology Review **17**: 303-320.

IROKANULO, E.I., A.A. MAKINDE, AND C.O. AKUESGI. 1997. *Cryptococcus neoformans var neoformans* isolated from dropping of captive birds in Nigeria. Journal of Wildlife Disease **33**: 343-345.

JEN, T.M. 1992. *Cryptococcus* isolated from pigeon excreta in Taiwan. Change Hua Min Kuo Wei Ching Wuchi Mien I Hsueh Tsa Chin **25**: 270-275.

KHOSRAVI, A.R. 1997. Isolation of *Cryptococcus neoformans* from pigeon (*Columbia Livia*) dropping in northern Iran. Mycopathologia **39**: 93-95.

KIELSTEIN, P., H. HOTZEL, A. SCHMALRECK, D. KHASCHABI, AND W. GLAWIS CHING. 2000. Occurrence of *Cryptococcus* spp. In excreta of pigeon and Pet birds. Mykosis **43**: 7-15.

KWON – CHUNG, K.I., AND J.E. BENNET. 1992. Medical Mycology Philadelphia. Lea and Febiger 397.

LODDER, J. 1971. The yeasts taxonomic study. North Holland pulbshing company Amsterdam.

NILDETE, G.L., M.B. GOMEZ, AND C.C. PRIMO. 1997. The invasive behavior of *Cryptococcus neoformans*: a possibility of direct access to the central nervous system. Mycopathologia **140**: 1-11.

NOSANCHUK, J.D., R. OVALLE, AND A. CASADEVAL. 2001. Glyophosate inhibits melanization of *Cryptococcus neoformans* and prolongs survival of mice after systemic infection. Journal of Infectious Diseases **183**: 1093-1099.

NOSANCHUK, J.D., A.L. ROSAS, S.C. LEE, AND A. CASADEVALL. 2000. Melanization of *Cryptococcus neoformans* in human brain. Lancet **355**: 5049-2050.

NOSANCHUK, J.D., P. VALADON, M. FELDMESSER, AND A. CASADEVALL. 1999. Melanization of *Cryptococcus neoformans* in murine infection. Molecular cell Biology **19**: 745-750.

NUNEZ, M., J.E. PEACOCK, AND C. ROBERT. 2000. Pulmonary cryptococosis in the immunocopetent host. Chest **118(2):** 527-534.

PRICE, M.F., I.D. WILKINSON, AND L.O. GENTRY. 1982. Plate method for detection of phospholipase activity in *Candida albicans*. Sabouraudia **13**: 110-115.

REFAI, M., S.A. TAHA, F. SELIM, AND H.H. YOUSSEF. 1983. Isolation of *Cryptococcus neoformans Candida albicans* and other yeasts from pigeon dropping in Egypt. Sabouraudia **31**: 163-165.

ROBINSON, C.R. 1995. Severe cryptococcal pneumonia. New England Journal of Medicine **332**: 1752

RODRIGUES, M.L., C.S. ALVIANO, AND L.R. TRAVA. 1999. Pathogenicity of *Cryptococcus neoformans*: virulence factors and immunological mechanism. Microbos infect **1**: 293-301.

SEELIGER, H.P.R. 1956. Use of a urease test for the screening and identification of cryptococci. Journal of Bacteriology **62**: 127-131.

STAIB, F. 1994. *Cryptococcus neoformans* and *Cryptococcus* pecularities and challenge J. Mycol. Med. **4**: 56-60.

STAIB, F., M. SEIBOLD, E. ANTWEILER, B. FROLISH, S. WEBWER, AND A. BLISSE. 1987. The brown color effect (BCE) of *Cryptococcus neoformans* in the diagnosis control and epidemiology of *Cryptococcus neoformans* infections in AIDS patients. Zbl Bokt. Hyg. **266**: 167-177.

STEENBERGEN, J.N., H.A. SHUMAN, AND A. CASADEVALL. 2001. *Cryptococcus neoformans* interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic. strategy in macrophages. PNAS **98(26)**: 15245-152502.

WILLIAMSON, P.R., K. WAKAMATUS, AND S. ITO. 1998. Melanin biosynthesis in *Cryptococcus neoformans*, Journal of Bacteriology **150**: 1570-1572.

YAMAOKA, H., N. SAKAGUCHI, K. SANO, AND M. ITO. 1996. Intra vascular granuloma induced by intravenous inoculation of *Cryptococcus neoformans*. Mycopathologia **133**: 149-158.

Received July 16, 2007
Accepted October 1, 2007

CATRINA (2007), 2 (1): 91 -96
© 2007 BY THE EGYPTIAN SOCIETY FOR ENVIRONMENTAL SCIENCES
PROCEEDINGS OF THE SECOND INTERNATIONAL CONFERENCE ON THE ROLE OF GENETICS AND BIOTECHNOLOGY IN CONSERVATION OF NATURAL RESOURCES, ISMAILIA, EGYPT, JULY 9-10, 2007

الطيور المنزلية كمصدر لفطر كريبتوكوكس نيوفورمانس المسبب الرئيسى للعدوى الجهازيه المهدده لحياة الانسان

محمد عبد الرازق
قسم النبات، كلية العلوم، جامعة قناة السويس، الإسماعيلية، مصر

الملخص العربـــى

تم عزل فطر كريبتوكوكس نيوفورمانس من عينات زبل الحمام التى تم جمعها من داخل منازل الفلاحين وأبراج الحمام ومحلات بيع الطيور بالإضافه إلى روث الطيور الملونه التى تستخدم فى المنازل بغرض الزينه.
وأيضاً دراسة تأثير هذا الفطر على الجهازين التنفسى والعصبى لحيوانات التجارب بحقن تركيزات مختلفه من محلول زبل الحمام الذى أظهر نتائج إيجابية للفطر فى التجويف البريتونى للفئران البيضاء.

وقد أثبت الفطر قدرته على الإنتشار فى داخل الأنسجه الرئويه والعصبيه للمخ مسببا أعراض باثولوجيه وهستوباثولوجيه أدت إلى موت فئران التجارب على فترات متفاوته بما يتناسب مع تركيز الجراثيم فى محلول الحقن.