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PRESENCE OF CRYPTOCOCCUS SPECIES IN DOMESTIC CHICKEN (*GALLUS GALLUS*) DROPPINGS AND THE POSSIBLE RISK IT POSED TO HUMANS IN KABIGERIET VILLAGE, NAKURU COUNTY, KENYA
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ABSTRACT

***Objective*: To isolate and identify Cryptococcus from domestic Chicken dropping.**
***Design*: cross sectional study.**
***Setting*: Kabigeriet village, Olenguorone Division, Nakuru county, approximately 282 km from Nairobi, Kenya.**
***Subjects*: Sixty four domestic chicken droppings were sampled in thirty two homesteads after obtaining the farmers consent.**
***Results*: Two species of Cryptococcus were isolated.**
***Conclusion*: Domestic chicken (*Gallus gallus*) harbor Pathogenic Cryptococcus in their dropping and their close proximity to human habitation poses a risk of AIDS to immunocompromised persons.**

INTRODUCTION

The Chicken (*Gallus gallus*) is a domesticated fowl, which is a subspecies of the Red Jungle fowl. It is one of the most widespread and commonly domesticated birds. In 2003 the total population of birds was more than 24 billion worldwide (Perrins and Christopher, 2003) and out of this population, Chickens were the majority species of birds. Human beings can acquire diseases from domestic Chickens for example avian flu and salmonelosis.

Cryptococcosis is an opportunistic disease mainly caused by an encapsulated fungus; *Cryptococcus neoformans* (Burker, 2001). *Cryptococcus neoformans* is a basidiomycete. In sub-Saharan Africa, adult meningitis is the most common complication of cryptococcosis, mainly caused by *Cryptococcus neoformans* due to high prevalence of HIV. *Cryptococcus neoformans* has been reported to cause infections in persons infected with human immunodeficiency virus worldwide, while *Cryptococcus gattii* causes infection primarily in HIV-uninfected persons both in tropical and subtropical countries (1, 2).

Biochemical and genetic studies have established the existence of the two main varieties of *Cryptococcus neoformans*. *Cryptococcus neoformans var. neoformans* includes serotypes A and D, while *Cryptococcus neoformans var. gatti* includes serotypes B and C (3) and the new species *Cryptococcus bacillispora* var. gatti (4). Due to AIDS pandemic Cryptococcosis has emerged as the major cause of death in HIV/AIDS infected individual (5). *Cryptococcus neoformans* causes cryptococcal meningitis in patients with AIDS which is lethal. In Thailand the incidence of cryptococcal meningitis has been reported to be at 18.5% in individuals suffering from HIV AIDS (6), while in USA the annual incidence is 2-4 cases per 1,000 persons (http://.rightdiagnosis.com/artic/cryptococcossis dbmd.htm). *Cryptococcus neoformans var. gattii* was first isolated from a *Guettarda aereana* tree in the Brazilian Amazon rainforest (7).

MATERIALS AND METHODS

Study area: The study was carried out in Kabigeriet Village, Olenguruone in Nakuru County. Olenguruone is Approximately 282 KM from Nairobi, Kenya. The area lies at about 35° 41'E and 0.1° 35'S. The climate is sub-humid consisting of one rainy season (April to December) and dry season (January – March). The average annual rainfall is 1200 mm and the average temperature is 28ºC with small variations (±5ºC) throughout the year (7). The study area was chosen since it is a typical rural setting where domestic chicken are reared in close proximity with humans. Unlike in other Divisions of Olenguruone, in Kabigeriet village most farmers rear only domestic chicken in a free range system.

Design: The study was a cross sectional laboratory

based study carried out for a period of five months, (April 2010 to August 2010). Sixty four Chicken droppings were sampled in thirty two homesteads after obtaining the farmers consent.

Sample collection: Environmental collection of domestic Chicken droppings was done by scooping fresh droppings from Chicken houses, grass, soil and trees using sterile plastic spoons. Each spoon was used once and discarded into sterile ziplock bags. Droppings which could not be collected using a spoon were swabbed by passing a sterile swab over each sample until it turned "dirty". Figure 1 and Figure 2 show some of the collection sites.

Figure 1
Chicken droppings on top of timber inside an abandoned house

Figure 2
Chicken droppings a human house

Specimen preparation: One Gram of domestic Chicken dropping was weighed on sterile film paper using a scale balance and then transferred to a 10 ml sterile round bottomed tube containing 5 mls distilled water then incubated for one hour with agitation (mix every 15 min) (9). Two 1.7 ml appendoff tubes were filled with 900 µl distilled water and 100 µl of the original sample added to the first tube, (dilution factor 1:10) then 100 µl of the 1:10 dilution added to the last tube containing 900 µl distilled water, giving a dilution factor of 1:100 in the second dilution. Approximately 100 µl of the second dilution were inoculated onto Niger seed agar plate.

For swabs, 100 µl of distilled water was added to a round bottomed tube and the tip of each swab containing sample dipped into distilled water with agitation to make a suspension. Similar amounts for swabs suspension were inoculated onto Niger seed agar plate, pH 6.8±0.2 (BBL, MD). All inoculated plates were incubated at 30°C for 72 hours checking daily for growth. Yeast-like colonies showing a cream to brown pigmentation and mucoid characteristic were isolated in pure culture in Sabouraud dextrose agar pH 5.6±0.2 (Difco, Detroit, MI, USA) (10). Suspected Cryptococcus colonies were tested for urease activity and hyphae production by sub-culturing further onto Christensen's urea agar and corn meal agar respectively (10). Cryptococcus species were confirmed using API 20C AUX (Bio Merieux SA).

RESULTS

Profiles

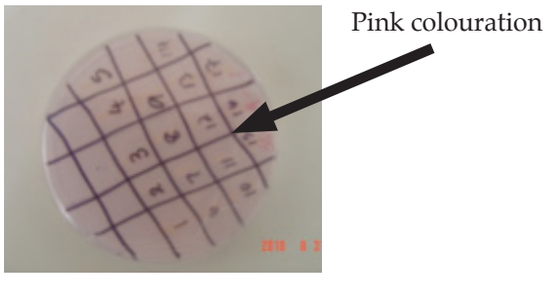
i. Niger seed agar

Of all the 64 samples plated onto Niger seed agar however, only eight isolates showed *Cryptococcus* characteristics; brownish-black colouration due to uptake of diphenolic substrates in presence of phenoloxidase. Phenoloxidase enzyme produced by *Cryptococcus* oxidizes diphenolic via labile dopachrome intermediates, to melanin polymers.

ii. Urease test

All the eight isolates tested were positive for urease (Figure 3). Hydrolysis of urea in Christensen's urea agar by *Cryptococcus* species was indicated by change of colour of the indicator from yellow-orange to pinkish red.

Figure 3
Urease positive yeasts in Christensen's urea media, read after 24 hours at 30°C

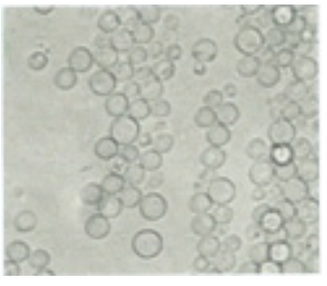


iii. Cornmeal agar

All the *Cryptococcus* species that were urease positive in Christensen's urea media were plated on cornmeal agar to distinguish it from other yeast such as *Candida albicans, C. tropicalis, C. parapsilosis* and *C. krusei*

through the formation of pseudohyphae, true hyphae, arthrospores and chlamydospores. *Cryptococcus* spp. does not form any hyphae or pseudohyphae on corn meal agar.

Figure 4
Urease positive yeasts in Christensen's urea media, read after 24 hours at 30°C. 4: Cryptococcus neoformans in Corn meal agar showing, Round, thick-walled with spaced between cells, indicative of capsules read after 48h at 30°C



iv. Confirmation of Cryptococcus using API 20 C AUX
Out of the eight urease positive isolates which were subjected to API 20 C AUX, only two were confirmed as *Cryptococcus* species, which were isolates from Chicken droppings. This might being due the fact that *Cryptococcus* inoculated onto API 20 AUX were not pure isolates.

Table 1
Cryptococcus species identified using API 20 C AUX

Types of *Cryptococcus*	n(+ve)	%(+ve)
Cryptococcus laurentii	1	12.5%
Cryptococcus neoformans	1	12.5%
None *Cryptococcus species*	6	75%

DISCUSSION

The aim of this study was to investigate the possible presence of *Cryptococcus* in domestic chicken droppings and soil enriched with droppings as a possible source of infections to humans, particularly immunosupressed individuals. *Cryptococcus neoformans* is prevalent in the environment especially in birds excreta such as pigeon or soils contaminated with birds' faeces which provide good nutrients for growth of pathogenic yeasts like *Cryptococcus* species (12). Such excreta or contaminated soils therefore become possible reservoir and sources of infections.

Cryptococcosis is a deep-seated mycosis that can be classified into three forms of diseases; disseminated cryptococcosis, pulmonary cryptococcosis and cryptococcal meningitis (9). High prevalence of *Cryptococcus neoformans* in natural environment especially in birds' droppings and soil enriched with bird droppings has been documented by several investigators in different parts of the world (9, 12, 13). Thus, pigeons and other birds' droppings are vehicles for human pathogenic *Cryptococcus neoformans* (14). There are only few reports of the isolation of this organism from domestic chicken in Kenya, and isolation from such environment is quite low. Kielstein (15) and Walter and Yee (16) attributed this to high pH and presence of high molecular growth inhibitory substances in Chicken droppings. However, infections caused by *Cryptococcus* species have been reported in severe immunosupression cases (17).

Cryptococcus neoformans have been isolated in immunocompromised patients particularly those with acquired immune deficiency syndrome (AIDS) (18). Its caused cryptococcal meningitis which is a life-threatening infections in patients living with AIDS with prevalence of 46% in Africa (1,19-21) with mortality rate of 13 - 44% (22,23). The present study has found out that Domestic Chickens are reservoirs of human pathogenic *Cryptococcus neoformans*. Thus, these might been another source of infections to people living with acquired immunodeficiency syndrome. There were minimal cases of *Cryptococcosis* before the HIV / AIDS pandemic (17). Due to HIV / AIDS pandemic the disease has emerged as a major cause of infirmity and death in infected HIV / AIDS victims.

In Kabigeriet village, Olenguruone, most people reared chickens in their yards and out of 32 homesteads sampled during the study; 17 (53.1%) homesteads kept their chicken inside their houses. In this study we succeeded in isolating two *Cryptococcus* species (Table 1) from Kabigeriet chicken droppings, with isolation rate of 3.1%. Isolation of *Cryptococcus* from chicken droppings is difficult since it can be affected by some biotic factors such as soil bacteria when grown on media by inhibiting or killing the yeasts (9,24).Once *Cryptococcus* species is detected from one house, it is likely that there is *Cryptococcus* species in droppings from other homesteads in the same location. There was higher isolation rates of yeasts in chicken droppings (100%) compared to soils (0%). This could be attributed to soil exposure to harsh environmental condition like; less availability of nutrient in the soil, dilution of soil especially during rainy seasons and sunlight that could not support yeast survival for a long time.

The weather in Kabigeriet village, Olenguruone is considered appropriate for the growth of *Cryptococcus*; with a mean temperature of 28°C with small variations (±5°C) throughout the year and mean annual rainfall of 1200 mm. It has been reported that cryptococcosis in AIDS patients is predominant during the rainy season (25), although our data showed a different pattern. It is believed during dry season patients inhaled a lot of the spores and the infection could

have gone into latency period of six months before its manifestation. Therefore, it is conceivable that the seasonal predominance of *Cryptococcus neoformans* is an important factor in cryptococcal infection (9). This seasonal difference in the environmental isolation does necessarily account for the latent period of the infection. The immune status of the host is a very important factor that affects cryptococcal sideration, considering a years-long latency report. Also reactivation of *cryptococcosis* is possible (26).

The mechanism by which the birds' excreta get infected with pathogenic yeasts is still not known (27). The screening of new target such as the domestic chickens in the environment for Cryptococcus species ecology niche can be an important step in explaining the spread mechanism of the yeast.

Further research work should done to identifying simple methods to prevent cryptococcosis, such as regular cleaning of Chicken roasting sites to avoid human exposure.

REFERENCES

24. Akram, M.S.H., Sinasi, T.Y., Ahmet, G., Mehmetm, A.S. Cryptococcus neoformans varieties from material under the canopy of eucalyptus trees and pigeon dropping samples from four major cities in Jordan. *Mycothologia*. 2005; **158**:195-199.
4. Barreto de Oliveira, M.T., Boekhout, T., Theelen, B. Cryptococcus neoformans shoos' remarkable gemotypic diversity in Brazil. *J. Clinical Microbiology.* 2004; **42**:1356-1359.
14. Bauwens L., Swinne D., de Vroy C.H., de Meurichy. Isolation of Cryptococcus neoformans var. neoformans in the aviaries of the Antwerp zoological gardens. *Mykosen*. 1986; **29**:291-294.
25. Bell M., Archibald L.K., Nwanyanwuo O. Seasonal variation in the etiology of bloodstream infection in a febrile inpatient population in a developing country. *International journal of infectious diseases*. 2001; **5**:63-69.
26. Blasi E., Brozzetti A., Fracosci D. Evidence of microevolution in a clinical case of recurrent Cryptococcus neoformans meningoencephalitis. *Eur. J. of microbiology of infectious diseases*. 2001; **20**:535-543.
23. Corbett, EL., Churchyard, G.J., Charalambos, S., Sanb, B., Moloi, V., Clayton, T.O. Morbidity and mortality in South African gold miners, impact of untreated disease due to human immunodeficiency virus. *Clinical Infectious Diseases.* 2002; **34**:1251-1258.
2. Dixit, A., Carroll, S.F., Qureshi, S.T. Cryptococcus gattii: an emerging cause of fungal diseases in North America. Interdisciplinary Perpect infectious Diseases 840452, Dmid, 9503836, published online May 25, 2009.
17. Esaki, M.S., Nagaligeswatan, K., Devaleenol, B., Srinivasan, R. Pneumonia and pleural effusion due to Cryptococcus laurenti in clinical proven case of AIDS. *Clinical Respiratory Journal.* 2006; **13**:275-278.
7. Fortes, S.T., Lazera, M.S., Nishikawa, M.M., Macedo, R.C.L., Wanke, B. First isolation of Cryptococcus neoformans var. gatii from a native jungle tree in Brazilian Amazon forest rainforest. *Mycoses*. 2001; **44**:137-140.
27. Filiu, W.F., Wanke, B., Anguena, S.M., Vilela, V.O., Macedo, R.C., Lazera, M. Avian habitats as a source of Cryptococcus neoformans in the city of Campo Grande, Mato Grussu do Sul, Brazil. Rev. Sol. *Brazil Medicine Tropical*. 2002; **35**:591-595.
22. French, N., Gray, K., Watera, C., Nakiyingi, J., Lugada, E., Moore, M. Cryptococcal infection on cohort of HIV-1 infected Uganda adults. *AIDS*. 2002; **16**:1031-1038.
5. Grusse, P., Tinteinot, K., Sullver, O., and Schmitz, B. Encephalomyelitis due to Cryptococcus neoformans var. gattii presenting as spinal tumor: Case report and review of the literature. Journal of Neurology, Neurosurgery and psychiatry. 2001; 70:113-116.
19. Kaplan, J.E., Hanson, D., Dworkin, M.S., Frederick, T., Bertolli, J., Lindegren, M.L. Epidemiology of human immunodeficiency virus-associated opportunistic infections in the United States in the era of highly active antiretroviral therapy. *Clinical Infectious Diseases*. 2000; **30**:S5-S14.
15. Kielstein P. Studies on the ecological behavior of Cryptococcus neoformans. Mycoses 1996; **39**:113-117.
11. Kwon-Chung K.J., Bernnett, J.E. Medical mycology. Philedelphia.PA; Lea and Febiger, 1992.
1. Lakshimi, V, Sodha, T, Jeja, V.D, Umabala, P. Prevalence of central nervous system cryptococcosis in AIDS patients. *Indian Journal of Medical Microbiology*. 2007; **23**:146-149.
13. Lazera, M.S., Salmito Cavalcanti, M.A., Londero, A.T., Trilles, L., Nishikawa, M.M., Wanke, B. Possible primary ecological niche of Cryptococcus neoformans. Medical Mycology. 2000; **38**: 379-383.
8. Metrological Kenya, (2009), unpublished annual rainfall and temperature data. Ngong road branch. www.meteo.go.ke.
3. Monica Cheesbrough. District laboratory practice in tropical countries, Cambridge university press, Cambridge UK, 2004; 244-245.
9. Misuzu, K., Chesenda, P., Akra, Y., Piphat, C., Toru, C., Petchsri, S., Taiga, M., hiroshi, K., Yasuhito, H., Yushitsuru, M., Yutaka, I., Shungeru, K. Environmental isolation of Cryptococcus neoformans from an endemic region of HIV-associated cryptococcosis in Thailand. *Yeast*.2004; **21**:809-812.
20. Mirzan, S.A., Phelan, M., Rimland, D., Gravis, E., Hamil, R., Brandt, M.E. The changing epidemiology of cryptococcosis: an update from population-based active surveillance in 2 large metropolitan areas, 1999-2000. Clinical Infectious Diseases. 2003; **36**:789-794.
12. Reimao, J.Q., Drummund, E.D., Tercett, M.D.S., Lyon, J.P., Franco, M.C., De Siqueria, A.M. Isolation of Cryptococcus neofmans from hollows of living trees in the city of Alfenas, MG, Brazil. *Mycoses*. 2007; **50**:261-264.

18. Rodero, L., Cordora S., Cahn P., Soria M., Lucarini M. Timed-kill curves for Cryptococcus neoformans isolated from patients with AIDS. *Medical Mycology*.2000; **38**: 201-207.
6. Suwat, C., Thira, S., Orapan, S., Kenrad, E.N. Clinical presentation and risk behaviors of patients with acquired immune-deficiency syndrome in Thailand, 1994-1998: regional variation and temporal trends. *Clinical Infectious Diseases*. 2001; **32**:958-962.
16. Walter J.E., Yee R.B. Factors that determine the growth of Cryptococcus neoformans in avian excreta. *America Journal of Epidemiology*.1968; **88**: 448-450.
21. Wong, M.L., Back, P., Candy, G., Nelson, G., Murray, J. Cryptococcal pneumonia in African miners of autopsy. *Int. J. Tuberculosis and Lung Diseases*. 2007; 11:528-533.
10. Yamamoto, Y., Kahno S., Noda T. Isolation of Cryptococcus neoformans from environment (pigeon excreta) in Nagasaki. Kansenshugaku Zasshi. 1995; 69:642-645.