ISOLATION OF *CRYPTOCOCCUS NEOFORMANS* SPECIES FROM HONEYBEE COLONIES IN MAU FOREST, KENYA

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Abstract

Cryptococcus neoformans is an opportunistic pathogen causing infections mainly in immunosupressed individuals. The objective of the study was to evaluate the presence of *Cryptococcus* species in Mau forest. A total of forty five samples were collected by removing the upper lids of the hive and rubbing the inner surfaces of the beehives with a swab soaked in 0.9% NaCl. The samples were cultured in the Niger seed agar containing 0.1% biphenyl media and incubated at 30°C then observed daily for 15 days. Yeasts producing brownish colouration were further tested for urease activity, cycloheximide resistance and carbohydrate assimilation. Yeasts identifications were done by Canavanine-glycine-bromothymol and API 32C. Serotypes confirmations were done using Crypto check system. A total for two *Cryptococcus neoformans* species were isolated: *Cryptococcus neoformans* var. *grubii* and *Cryptococcus neoformans* var. *gattii*. The study found that bee's act has porters and an indicator, of the presence of *Cryptococcus* plant flora in the vicinity.

Keywords: *Cryptococcus*, HIV/AIDS, yeasts, bees

Introduction

Cryptococcosis is an opportunistic infection mainly caused by an encapsulated fungus; *Cryptococcus neoformans* (Burker, 2001). Three *Cryptococcus* varieties have been indentified: *Cryptococcus neoformans* var. *gattii* (serotypes B and C) *Cryptococcus neoformans* var. *grubii* (Serotype A) and *Cryptococcus neoformans* var. *neoformans* (serotype D), each species present fundamental epidemiological and ecological differences. *C. neoformans* has been reported to cause infections in persons infected with human immunodeficiency virus worldwide, while *C. gattii* causes infection primarily in HIV-uninfected persons both in tropical and subtropical countries, its habitat is restricted to subtropical and tropical regions (Dixit *et al*., 2009, Lazera *et al*., 1993, Sorrel and Ellis, 1997, Correa *et al*., 1999). The *Cryptococcus neoformans* ecological studies have left many questions unanswered, how it spreads in nature and characteristics of *Cryptococcus* niche which are unknown. The repeated isolation of *Cryptococcus* from the same niche may be an explanation for reservoir and porter of the plant. However, some yeast infections have been reported in regions where plant ecology is absent. Hence this could explain the possibility of a different mechanism.

Beekeeping is a common agricultural activity in communities living in Mau forest, Kenya. In these regions there is large plantation of cypress; eucalyptus, pawpaw trees among others, Honeybee colonies in these regions are kept from April to December and the bees collect pollen and nectar from wild flowers (Yulmaz *et al*., 1987). *Cryptococcus neoformans* have been isolated from different sources: Pigeon excreta and chicken excreta (Kemoi *et al*., 2013, Filiu *et al*., 2002). The fungus it has also been isolated from droppings of budgerigars, Canaries and parrots. The objective of the study was to investigate the relationship between honey bee colonies which can act as a porters and the spread of *Cryptococcus neoformans*.

Materials and methods

Collection of samples

A total of forty five samples were collected by removing the upper lids of the hive and rubbing the inner surface of the beehives with a swab soaked in 0.9% NaCl (Ergin *et al*., 2004).

Identification of *Cryptococcus neoformans* isolates

The samples were cultured in the niger seed agar containing 0.1% biphenyl media (Teknova, France) (Mseddi *et al*., 2010) and incubated at 30°C and observed daily for 15 days. Yeasts producing brownish colouration were further tested for urease activity, cycloheximide resistance and carbohydrate assimilation (Lazera *et al*., 1996). Yeasts identifications was

done by Canavanine-glycine-bromothymol and API 32C (bio-merieux, France) (Ergin *et al*., 2004). Serotypes confirmations were done using Crypto check system (Iatron, Tokyo, Japan).

Results

A total of two *Cryptococcus neoformans* species were isolated: *Cryptococcus neoformans* var. *grubii* and *Cryptococcus neoformans* var. *gattii*. Fast growth of saprophytic fungus such as Zygomycetes in may hamper cultivation of yeasts. However, Zygomycetes and other saprophytic fungi were not observed in this study. These results facilitate the detection of *Cryptococcus neoformans* in niger seed agar as melanin producing colonies.

Discussion

Cryptococcus neoformans causes life threatening infections namely cryptococcal meningitis, pulmonary cryptococcosis and disseminated cryptococcosis, especially in patients living with AIDS and other conditions associated with cellular deficiency (Esaki *et al*., 2006, Rinaldi *et al*., 1986, Shimizu *et al*., 1986). In the current study, two species of *Cryptococcus neoformans* i.e. *Cryptococcus neoformans* var. *grubii* and *Cryptococcus neoformans* var. *gattii* were isolated from the honey beehives in Mau forest. These findings are consistent with studies where *Cryptococcus* species have been isolated from various environmental and non environmental sources: hollow of living trees, soil, oral cavity, brain, lungs, liver and intestinal canal of striped grass mouse and its association with avian guano has been reported (Montenegro and Paula, 2000, Filiu *et al*., 2002, Bauwens *et al*., 2004, Kartrin and Heidemarie, 2005, Reimao *et al*., 2007, Kidd *et al*., 2007). In other studies, high numbers of yeasts have been isolated from pigeon weathered droppings and the environment also favors the growth of most bacteria and fungi (micro-organism). There has been isolation of *Cryptococcus* species in pigeon excreta in Western countries where pigeon and pet/captive birds are kept in homesteads (Kielstein *et al*., 2000, Ferreira-Paim *et al*., 2010, Casadevall and Perfect, 1998). However, few authors have reported the isolation of *Cryptococcus* species from honey bee colonies and the isolation of the yeast from such habitat is low. Ecological niche for *Cryptococcus neoformans* is still not clear. It is not known why in some regions where there is no specific plant flora; *Cryptococcus neoformans* infection is still being reported.

It has been reported by some authors that plants act as an important ecological reservoir for *Cryptococcus neoformans*. Soil enriched with bird's droppings has also been reported to be another habitat for the yeasts (Kemoi *et al*., 2013). The finding of this study is that bees act as a porter and an indicator, that there is presence of *Cryptococcus* plant flora in the vicinity.

It has been reported that some animals like koalas that feeds on *Eucalyptus tereticornis* and *Eucalyptus camaldulensis* leaves also act as carriers of *Cryptococcus* (Krockenberger *et al.*, 2002, Connolly *et al*., 1999). This is only true for *Cryptococcus neoformans* var. *gattii*. The transportation of *Cryptococcus* yeast via different vectors has been proved by *Cryptococcus* infections have in reported in different countries with different plant flora and different climatic conditions.

Conflict of interest

The authors declare that there is no conflict of interest among them, and no financial relationship with theirs affiliated institutions.

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