

Detection of *Histoplasma Capsulatum* in Domestic Chicken Droppings in Olenguruone, Nakuru County

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Abstract

Histoplasmosis is a serious community acquired fungal infection among immunocompetent and immunocompromised individuals. Chicken droppings were evaluated for the presence of *Histoplasma capsulatum*, by culturing in Blood agar, SABHI and SDA agar at 25°C and 37°C. Exoantigen were used for confirmation. Two *Histoplasma capsulatum* were isolated in this study. The study found that Chicken droppings harbor *Histoplasma* species.

Keywords: *Histoplasma Capsulatum*, Chicken droppings, dimorphic fungus

Introduction

Histoplasma capsulatum is a level-three pathogenic fungi causing histoplasmosis (Wheat, 1991). The genus, *Histoplasma* consists of three variants namely; *Histoplasma capsulatum* var. *duboisii*, *Histoplasma capsulatum* var. *capsulatum* and *Histoplasma capsulatum* var. *farciminosum*. Variants *duboisii* and *capsulatum* infect mammals (Human beings included) and variant *farciminosum* cause infection in mice and horses (WHO, 2009). *Histoplasma capsulatum* causes a serious community acquired mycosis in immunosuppressed individuals in endemic regions in some countries (Wheat, 1991). Isolation of *Histoplasma* species from the environment has been done mainly in soil enriched with birds and bats guano (Ruchel, 2008). Farmers in

endemic regions are unaware of the health implications associated with coming into contact with bird or bat manure in either active or inactive poultry houses, (CDC, 1997, USAEHA, 1992, CDC, 1977). The risk of Histoplasmosis infections in the environment is difficult to assess since there are no specific, fast or cheap analytical methods for the detection of *Histoplasma capsulatum*. The main aim of this study was to assess the presence of *Histoplasma capsulatum* in chicken droppings.

Sample collections

From January 2012 to April 2012, a total of 84 Chicken droppings were collected from Poultry houses and roosting sites. Approximately 10 to 100g of bat droppings was collected using a sterile plastic spoon, in a plastic bag and transported to the laboratory on the same day using cool boxes.

Materials and methods

Approximately a gram of the Chicken droppings were suspended and vortexed thoroughly in 5 ml (0.5% w/v) saponin. The debris was allowed to settle and the supernatant transferred to a fresh tube. The resulting suspension were centrifuged, and the pellet suspended in a final volume of 500 µl sterile NaCl. One hundred microlitres (100 µl) of the suspension were plated in duplicates on Sabouraud agar BHI and SABHI agar in 25°C for mycelia form and 37°C for yeast form. Microscopic morphology of mycelium was determined by use of Lactophenol cotton blue stain, micro cultures in the slides and tube culture in Blood agar, Sabouraud and SABHI agar with Chloramphenical, Getamycin and cycloheximid (De Hoog and Guarro, 1996, Ajello *et al.*, 1993). The conventional Exoantigen method for detectetion of M and H protein were used for immunoidentification of the *Histoplasma capsulatum* mycelia form and discriminating from other fungi. Exoantigens were obtained by duplicates of 5 to 7 days *H. Capsulatum* cultures grown in BHI broth. After thimerosal sterilization for 24hrs, Exoantigen were obtained from supernatant after concentration and probed by immunoblot against homologous rabbit anti-*Histoplasma capsulatum*.

Results

Two *Histoplasma capsulatum* were isolated and identified in this study. Characterizations of isolates were observed in mycelia phase by development of buff brown to white colonies in culture media incubated at 37°C. Typical *Histoplasma capsulatum* microscopic morphology which show presence of pyriform microconidia, spherical tuberculate macroconidia (thick walled) with finger like projections and thin septate hyphae were observed in all isolates. Production of Exoantigen confirmed Histoplasma

capsulatum, by presence of precipitin lines in double immunodiffusion assay by using human histoplasmosis immune serum.

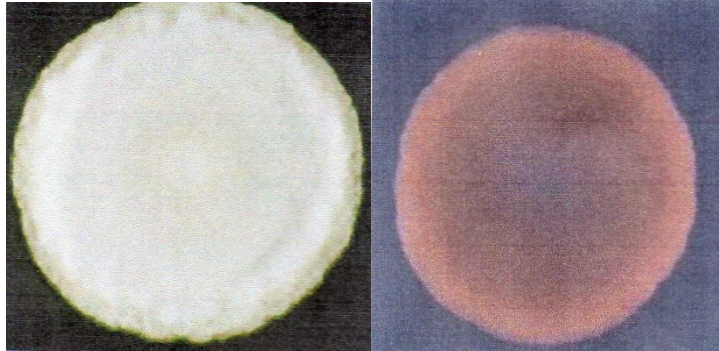


Fig 1: *Histoplasma capsulatum* colonies on SDA incubated at 37°C showing white-brown cottony colony with Brown yellow reverse.

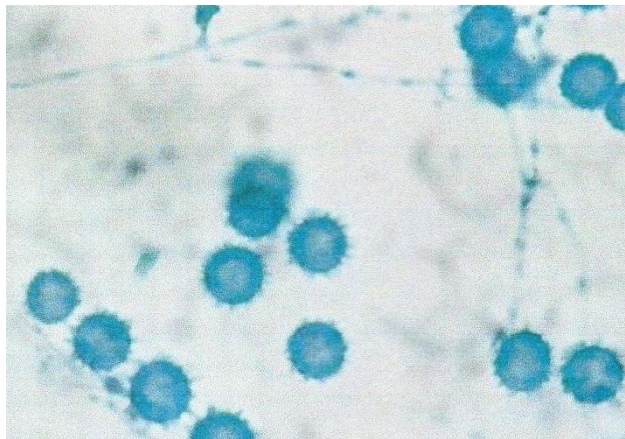


Fig 2: Lactophenol cotton blue mount of *Histoplasma capsulatum* showing numerous tuberculate macroconidia.

Discussion

Histoplasmosis has been reported in different countries from a variety of environments, which could be related to different *Histoplasma* strains isolated from different sources such as Soil, patients' samples and infected animal samples. This study isolated two *Histoplasma capsulatum* from chicken droppings which is normal, since isolation of *Histoplasma capsulatum* in nature is low, as reported by Mc Murray and Russell (1982). Histoplasmosis in Mexico and most of western countries has been associated with enclosed spaces where Bats roost (Taylor *et al.*, 1994, Taylor *et al.*, 1996, Taylor *et al.*, 1997). In Kenya there is insufficient information that has been documented and that can link the disease and *Histoplasma* known habitats. In Africa, infections with *Histoplasma capsulatum* in HIV/AIDS patients has been reported by several authors in different countries (Amayo

et al., 1993, Kakou *et al.*, 1993, Bayes *et al.*, 1996, Schoffer and Baur, 1998, Outarra *et al.*, 1998). In West Africa, disseminated histoplasmosis has been reported in 3% of AIDS patients.

Bats are one type of vertebrates known to be infected with *Histoplasma capsulatum*. Most of Bats infected with this fungi exhibit a variety of diets and colonial habits: pollinivorous, hematophagous, piscivorous, insectivorous, nectarivorous and frugivorous. These suggest that the diet does not have any association with the occurrence of the infections in bat species (Kunz *et al.*, 1988). The circumstances that foster the infections of Bats with *Histoplasma capsulatum* have not been reported. However, findings of this study shows close relationship between bats, characteristics of their roosting sites and parameters of the population that are involved in dispersion of spores and fungal growth hence bats are exposed to infection. In bat captured sites there is high risk of infections due to air currents produced by bats when flapping wings and animal movements, leading to dispersion of fungal spores. This way bats inhale the spores and get infected (Mc Murray and Russell, 1982, Carvajal, 1977). Also the physical conditions of caves, ceiling of the caves and guano deposits can influence the infection risk factor for bats and humans.

The present data from this study establish the close relationship between, Environmental conditions, characteristics of Chicken roosting sites with the risk of *Histoplasma capsulatum* infections. The fungus *H. capsulatum* grow well in soils with high nitrogen content enriched with chicken and bat droppings. The organism might carried on feet, wings and beaks of birds and infect roosting sites. Chicken have not been shown to present health risk for histoplasmosis because birds does not get infected with *H. capsulatum*, unlike Bats which they become infected with *H. capsulatum*. The high frequency of *Histoplasma Capsulatum* infections in different Birds and Bat species hence act as porters for fungal dispersion in the environment due to their migratory habits (Di Salvo *et al.*, 1969). Other studies have reported isolation rate up to 35.2% (Gonzalez *et al.*, 2014).

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