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Cryptococcus neoformans Isolated from Pigeon Dropping (Preliminary Report)

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Abstract: ***Cryptococcus neoformans*** *species complex is an important agent of opportunistic infection in HIV infected patients whereas* ***Cryptococcus gattii*** *could affect immunocompetent individual and cause invasive infection. Pigeon dropping is known as the natural habitat of* ***Cr. neoformans*** *and plays an important role in the transmisson of the disease. A preliminary study to isolate the fungus from pigeon excreta was conducted in Pondok Gede area near East Jakarta by investigating 30 samples inside and outside the cage and the soil adjacent to it. The study was done by inoculating the samples on the Sabouraud dextrose agar and niger seed agar at room temperature and at 37°C. Two samples from inside the cage were positive and able to grow at both room temperature and 37°C. The environment in that area might be important in the transmission of the disease.*

Key words: *pigeon dropping, bird cage, thermotolerans*

Laporan Pendahuluan: *Cryptococcus neoformans* yang Diisolasi dari Tinja Burung Merpati

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Abstrak: *Kompleks spesies* ***Cryptococcus neoformans*** *adalah penyebab penting infeksi oportunis yang kerap kali fatal pada penderita infeksi HIV/AIDS, sedangkan* ***Cryptococcus gattii*** *dilaporkan menyebabkan infeksi pada individu dengan imunitas normal. Kotoran burung merpati diketahui sebagai habitat alamiah jamur* ***Cr. neoformans*** *dan penting dalam transmisi penyakit. Penelitian pendahuluan untuk menemukan jamur tersebut pada kotoran burung merpati di Pondok Gede dekat Jakarta Timur dilakukan dengan meneliti 90 sampel yang berasal dari 30 kandang burung merpati. Sebanyak 60 sampel berasal dari dalam dan luar kandang dan sisanya 30 sampel berasal dari tanah sekitar kandang yang diduga tercemar kotoran merpati. Pemeriksaan dilakukan dengan menanam sampel pada agar Sabouraud dekstrosa dan agar* ***niger seed*** *pada suhu kamar dan 37°C. Dua sampel yang berasal dari dalam kandang ditemukan mengandung jamur* ***Cr. neoformans*** *yang dapat tumbuh pada suhu kamar dan 37°C. Penemuan ini menunjukkan bahwa lingkungan di daerah penelitian mungkin berperan penting dalam transmisi kriptokokosis.*
Kata kunci: *kotoran merpati, kandang burung, termo tolerans*

Introduction

Cryptococcus neoformans is an encapsulated yeast that may cause cryptococcosis in human. The *Cryptococcus neoformans* species complex comprises of *Cr. neoformans* var *grubii* and *Cr. neoformans* var *neoformans*. The var. *grubii* is opportunistics fungus that can be isolated world wide and mainly affecting HIV/AIDS infected patients induce fatal central nervous system infection, whereas the var *neoformans* is restricted in northern Europe.[1] Another species that consider important and able cause fatal infection in 'immunocompetent' individual is *Cryptococcus gatti* [2]

These two *Cryptococcus* species have close association with the environment which act as source of infection. In nature *Cr. neoformans* is normally lives in the avian excreta and tree hollow[3,4], whereas decaying wood is the natural habitat for *Cryptococcus gattii.*[5] Moreover, animals such as dogs and cats were also reported harbouring the fungus in their nasal cavity.[6] Emmons in 1955[3] reported that the virulent strain of *C. neoformans* was abundant in the pigeon (*Columba livia*) dropping.

In Indonesia, despite the increasing cases of cryptococcal meningitis in HIV infected patient (Wahyuningsih, manuscript), the reports on the natural habitat of this fungus as the source of infection is quite rare. A study was conducted to search for the possible environment of *Cr. neoformans* in pigeon dropping. This preliminary report is a part of an ongoing research on the epidemiology of *Cr. neoformans.*

Materials and Methods

Materials

The material used in this study was pigeon dropping which was collected from 30 bird cages in the Pondok Gede, Bekasi area close to East Jakarta. The total samples tested were 90. It was collected from inside and outside the bird cage, and from the soil adjacent to it which presumed contaminated by the bird excreta. From each location 30 samples were collected. The sample was collected using dispossible plastic spoon then placed in the sealed plastic bag. Each bag containing sample was labeled accordingly to the date, place, and position of the collected sample.

Methods

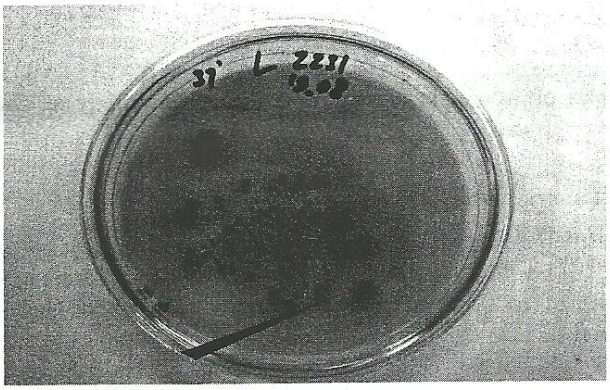
The samples was treated by the method modified from Lazera *et al.*[4] In brief, as much as 5 – 10 g of sample was placed to a 15 ml conical tube and 10 ml of sterile distilled water was added into the tube. Furthermore, the tubes containing sample was shaken gently, then let to stand at room temperature for 15 - 30 minutes. Half mililitre of supernatant was inoculated onto two plates of *niger seed agar* (NSA) according to Staib *et al,*[7] incubated at room temperature (25-29°C) and 37 °C. To the NSA medium 0.05% chloramphenicol

was added to inhibit bacterial growth. The culture were investigated everyday for the maximum seven days. The colony of *Cr. neoformans* will grow as dark brown yeast colony. For purification, the dark brown colony was inoculated onto Sabouraud dextrose agar and fresh NSA, both are incubated at room temperature. For the thermo tolerance test the culture was incubated at 37°C. Further microscopic investigation using India ink was done.

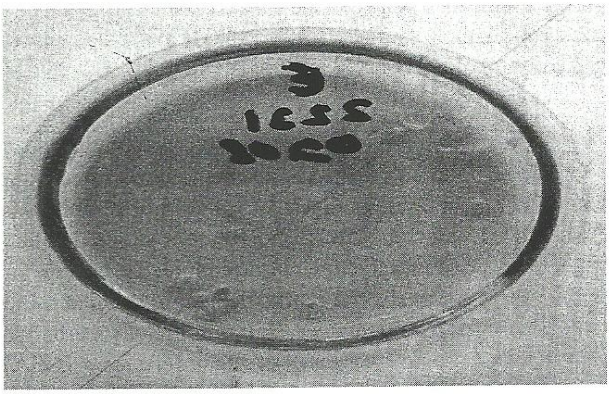
Results

Out of 90 samples investigated, two samples (2.22%) were positive for *C. neoformans,* which grow as dark brown colonies on NSA medium. Microscopically the fungus looks as round yeast cells bearing thin capsule. None of the samples collected out side the birdcage and from the soil adjacent to the cage gave positive result. The positive samples are only from the inner side of the bird cage, thus the positive value is 6.67% out of 30 bird cages investigated.

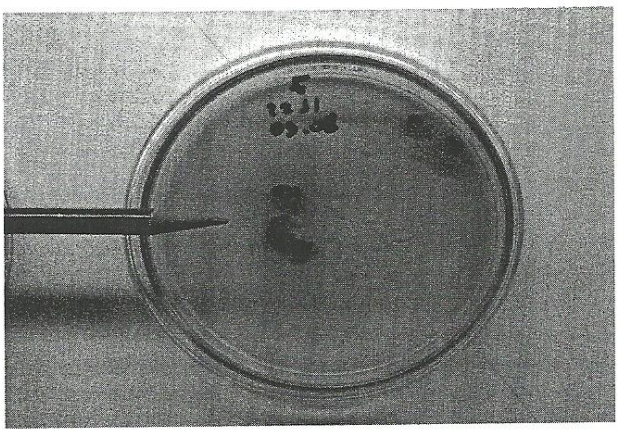
At the thermo tolerance test conducted by growing the yeast at 37°C, all of the suspected colonies grow prosperously as yeast colonies and in India ink test, it appears as a round encapsulated yeast bearing thin capsule.



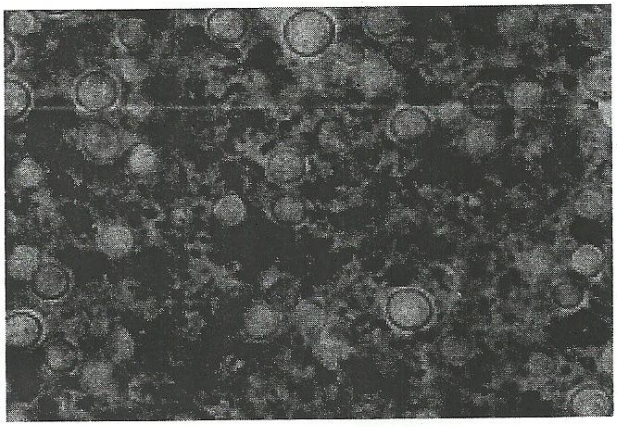
Picture 1. Isolation of *C. neoformans* from Pigeon Dropping on Niger Seed Agar (NSA), Incubated at 37° C. The Colony Appears as Black Brown Yeast Colony



Picture 2. Purification of Suspected Colony on Sabouraud Dextrose Agar



Picture 3. Purification of Suspected Colony on NSA Incubated at 37° C



Picture 4. Cryptococcus Neoformans in India Ink

Discussion

Cr. neoformans is a fungus, known as an infecting agent of cryptococcosis in human. In the host with compromised immunity such as HIV/AIDS or even in immunocompetent individual, the fungus may disseminate and can cause fatal cryptococcal meningitis. In nature this fungus could be found in bird excreta which act as its natural habitat and responsible for the transmission to human via inhalation. In this study, *Cr. neoformans* was isolated in the two out-of 30 samples collected from the inner part of the birdcage. Our result is slightly different with the work of Susilo, who in 1968[8] isolated *Cr. neoformans* by inoculation of the contaminated soil to the mice. He found out that 9% of 77 bird droppings tested were positive for *Cr. neoformans*. This difference may lies on the methods of isolation. Our study use special medium NSA for the isolation of the fungus and the previous study use mice as the tool of isolation, which may be more sensitive in isolating the fungus. On the other hand, NSA medium in which content extract of *Guizotia abyssinica* accommodates *Cr. neoformans* to produce melanin and therefore it grows as dark brown yeast colony that easily recognized.

All the strains isolated in this study were growing well at 37°C. It proved that the fungus isolated are virulence organism because one of the virulence factors that permit the fungus eliciting a disease in human is its ability to overcome temperature barrier make them able to grow within the host. *Cr. neoformans* is one of the genus *Cryptococcus* that could grow at 37°C and able to cause disease in human. The ability of the fungus isolated to grow at 37°C, confirmed that it is pathogen and may cause disease.[9,10] The fungus isolated presumed as *Cr. neoformans*, although speciation is not yet done, but it was isolated from pigeon dropping, which known harboring that fungus in nature.[3]

The positive samples was collected from the bird cage, and none of the samples collected from outside and from the soil adjacent of the cage were positive. Since the place outside is exposing to the sunlight, the samples originated from that place were negatives. Exposure to the sunlight will rapidly sterilize the place from *Cr. neoformans.* Sun light killed this fungus.[9] In addition, there is a possibility that the yeast of *Cr. neoformans* is ingested by free-living amoeba (*Acanthamoeba palestinensis)* or sterilized by bacteria *Pseudomonas aeruginosa* and *Bacillus subtilis*, which often isolated from the pigeon dropping, so it cannot be found in the sample tested.[10]

Cr. neoformans is available in our environment. The environment as the natural habitat of that fungus may act as the source of infection. In the last view years, in Indonesia particularly Jakarta, we saw the sharp increase of the incidence of HIV infected patients,[8] a special group with deteriorated immunity. Due to their abnormal immunity, they prone to opportunistic infection cause by many organisms including *Cr. neoformans.* That fungus is able in inducing fatal brain infection.[9] Its existence in our environment and the fact that *Cr. neoformans* is able to induce fatal cerebral infection in immunocompromised group warns us that the danger of this infection in HIV infected patients is coming close.

Conclusion

The existence of presume virulent *Cr. neoformans* in the environment might be important in the transmission to human and cause fatal invasive infection.

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