The Isolation of Cryptococcus neoformans in Oklahoma City[1]

ROBERT DUKE LONIAN, Douglass High School, Oklahoma City

In many of the nation's larger cities, there is great concern about the effect of large pigeon populations upon health conditions. This concern has greatly increased because of scientific research reports dealing with the relationship between certain disease-causing organisms and the pigeon or the pigeon habitat. One of the most common and dangerous organisms which falls into this category is *Cryptococcus neoformans.*

In view of the absence of literature on the isolation of *C. neoformans* in Oklahoma City, this report is believed to bear significance in pointing to the need for a pigeon control program as a public health measure.

INTRODUCTION

Cryptococcosis, also known as *torulosis* and *European blastomycosis,* is a mycosis of man in which the etiologic agent is *Cryptococcus neoformans.* Approximately 10% of the 300 to 400 fatal mycoses reported each year in the United States are caused by *C. neoformans,* the most frequent etiologic agent of mycotic meningitis in man.

Once considered a rare disease, cryptococcosis is being recognized and reported with greater frequency. There is good reason to believe that many cases of cryptococcosis go unrecognized and unreported, because there is a great deal of discrepancy between its clinical and pathological diagnosis. Cryptococcosis has been reported from the Middle East, the Belgian Congo, Central Europe, Great Britain, Scandinavia, South Africa, India, the Philippines, Australia, and the United States. The greatest number of cases occur in the last two countries mentioned.

CRYPTOCOCCUS NEOFORMANS

The genus *Cryptococcus* is limited to organisms which are nonfermenters and reduce starch under suitable conditions. Each of these organisms is encapsulated, has no true mycelium, and produces no ascospores. The fact that they do not produce ascospores causes them to be classified as *Fungi imperfecti.* Reproduction is by budding.

[1]Appreciation is expressed to Mrs. G. Nix, Dr. F. Felton, and Mr. W. Cook of the Oklahoma City Veterans' Administration Hospital for their invaluable assistance during this investigation.

C. neoformans is a spherical to oval cell which varies from 2.5 to 8.0 microns in diameter. The organism possesses a gelatinous capsule, varying in thickness from very thin or undetectable to 7 microns. In a culture, the cell develops thick walls and contains few to several vacuoles. Parent cells with single buds are always seen. Macroscopically, *C. neoformans* forms a more or less dry cream to orange-tan, opaque, yeastlike, irregularly circular colony within three to five days.

C. neoformans is differentiated from other non-myceliated yeasts by its ability (1) to grow at 37 C, (2) to hydrolize urea, (3) to form capsules on *Cryptococcus* capsule agar, (4) to form wide capsules in mouse brain after intracerebral injection, and (5) by its virulence for white Swiss mice. It is differentiated from other species of *Cryptococcus* (1) by its ability to grow at 37 C; (2) by its ability to assimilate glucose, mannose, trehalose, xylose, galactose, maltose, and sucrose, all provided as separate carbon sources in synthetic media; (3) by its failure to assimilate lactose and potassium nitrate; and (4) by its virulence for mice.

Prevalence in Pigeon Habitats. In 1954, Emmons and his co-workers collected a total of 202 specimens. These consisted of weathered pigeon manure from old nests and under roosting sites and relatively fresh droppings and soil from a city park where visitors fed the pigeons in or near Washington, D. C., and from Loudon County, Virginia. *C. neoformans* was isolated from 126 of the 202 specimens collected. This led Emmons to believe that the pigeon plays a role in the natural occurrence of the organism.

On February 14, 1959, the first of three patients, each from Kingfisher, Oklahoma, reported to a local hospital with *cryptococcosis*. The first patient had spent several years working in a flour mill in Kingfisher. The second patient began to have trouble after he tore the ventilation cupola, which was the roosting site for several pigeons, from his barn. The third patient was troubled after unloading some dusty, moldy hay into a barn near Kingfisher. The first and second soon died at the hospital. Specimens collected from the environments of the patients yielded *C. neoformans* associated with pigeon droppings.

The unusual nesting habits of the pigeon, no doubt, play an important part in the relationship between the pigeon and *C. neoformans*. Contrary to the habitats of most birds, the pigeon does not remove fecal material from its nest. As the year progresses and additional broods of young are raised, the simple nest of loose twigs, straw, and weed stems is transformed into a plasterlike mass of dried fecal material.

The actual role of the pigeon in aiding the growth of *C. neoformans* has been studied by Emmons. He collected twenty pigeons (all were young birds and included half-grown squabs) from four farms which had yielded positive specimens. Cultures from the spleen, liver, kidney, crop, gizzard, upper intestine, and lower intestine were all negative, which indicates that the relationship between the pigeon and *C. neoformans* is not of a pathogenic nature.

PROCEDURE

Fifty-one specimens of pigeon droppings, soil, egg shell and other organic debris, and sparrow droppings were collected in various parts of Oklahoma City. Forty-five of these specimens were obtained on three different visits to the same fifteen sites. Collections were made on July 9, August 15, and September 1, 1963.

Each specimen was collected aseptically in a sterile stoppered test tube. A portion of each sample was placed in a capped test tube containing sterile mycological broth. These tubes were shaken in order to suspend as many organisms as possible in the broth.

Culture plates containing Sabouraud's dextrose agar with penicillin and streptomycin or Littman's oxgall agar were inoculated with portions of the mycological broth suspensions. The inoculations were aseptically made, using the pour and streak methods. These plates were incubated at 37 and 21 C.

Organisms from colonies resembling *C. neoformans* were viewed under the microscope. Secondary isolations were made by streaking plates containing Sabouraud's dextrose agar with organisms from colonies which resembled those of *C. neoformans* and whose cells resembled *C. neoformans*. Organisms from these secondary isolations were used to inoculate urease agar tubes, *Cryptococcus* capsule agar tubes, and cornmeal agar plates. All were incubated at room temperature.

Those specimens which hydrolyzed urease, grew no mycelia nor spores on cornmeal agar, and grew capsules on capsule agar were tested for their carbon assimilation. Those organisms which assimilated dextrose, galactose, sucrose, maltose, ammonium sulfate, and pectin yeast nitrogen base, and which failed to assimilate lactose and potassium nitrate when each was presented as separate carbon sources in synthetic media, were tested for their virulence for white Swiss mice. Four mice (females, between the ages of six and eight weeks) were inoculated with each specimen. Two were inoculated intracerebrally, and two were inoculated intraperitoneally. The intracerebral injections consisted of 0.05 cc of a 1×10^6 cells per cc concentration of *C. neoformans* suspended in sterile distilled water. The intraperitoneal injections consisted of 0.15 cc of a 3×10^6 cells per cc concentration of *C. neoformans* suspended in sterile distilled water.

Those mice which died were autopsied, and cultures were made on Sabouraud's dextrose agar, using portions of the brain and spinal fluid as an inoculant. It was assumed that those cultures which grew *C. neoformans* were from mice killed by that organism.

RESULTS

Out of a total of 51 specimens collected on the three trips, four reacted positively. All of these contained pigeon droppings, and all were from the September 1 collection.

DISCUSSION

The isolation of *C. neoformans* from samples collected in Oklahoma City suggests that it exists in several of the city's large buildings, churches, and houses. There are places with attics, roofs, and/or gables from which *C. neoformans* could be blown by the wind and spread to other pigeon roosts and nests or maybe inhaled by humans. The fact that many of these structures are places that large numbers of people frequently visit should encourage a greater interest upon the behalf of the citizens of Oklahoma City for this ever increasing danger.

The local isolation of *C. neoformans* also implies that there might be several, or at least a few, undiagnosed cases of human *cryptococcosis* in Oklahoma City. It also suggests that new infections might be occurring at anytime to anyone, visitor or resident, in Oklahoma City.

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