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CRYPTOCOCCOSIS IN COLUMBIFORMES AT THE SAN DIEGO ZOO

LYNN A. GRINER[□] and HENRY A. WALCH[□]

Abstract: Two cases of cryptococcosis in columbiformes exhibited at the San Diego Zoo are described. The organism isolated from the first case had morphological, chemical and temperature growth characteristics of *C. neoformans*. The culture from case 2 died before it could be examined biochemically or by mouse inoculation.

INTRODUCTION

For many years *Cryptococcus neoformans* has been associated with avian habitats, especially pigeons. The prevalence of this fungus in pigeon excreta and nests, has been documented by investigators in several countries.^{1,3,4,5,9,11} In one study the fungus was isolated from 14 of 75 pigeon fecal samples, but no isolations were made from 277 chicken droppings.¹² Samples from the habitats of 15 different avian species were cultured but *C. neoformans* was recovered only from pigeon areas.⁵ Reports of isolations of the fungus from the lower digestive tract of birds have been infrequent. Cultures of the digestive tract of 271 pigeons resulted in the recovery of the fungus from 4 birds, and these four came from a pigeon flock whose droppings had been shown to contain *C. neoformans*.¹³ Investigations into the survival of the fungus in the gastrointestinal tract of pigeons demonstrated that organisms given orally could be recovered from the intestines and droppings. However, none of the birds showed evidence of infection.^{7,8} In a recently reported experiment in which *C. neoformans* was given *per os* to pigeons, the fungus initially was recovered from the droppings and crops of 9 of 10 birds on the first day, and from the crops of 2 of these birds at the

termination of the study 86 days post inoculation.¹⁰

Although there have been numerous reports of associations of *C. neoformans* with avian sources, particularly pigeons, actual disease in birds is rare. One such case is cited by Chute,² of a 1938 report by Bisbocci, which described the isolation of a *Cryptococcus sp.* from a pheasant with enterohepatitis. It was reported that the isolated organism produced granulomatous and necrotic lesions in the lung, liver, spleen and intestines of chickens. Since this original publication could not be obtained for review, no comment is possible.

In view of the apparently rare occurrence of cryptococcosis in birds, the present report describing 2 cases of cryptococcosis in columbiformes at the San Diego Zoo, has been prepared.

CASE HISTORIES

Case 1: A female Bartlett's bleeding-heart pigeon (*Gallicolumba c. criniger*) had been exhibited in an aviary along with several species of seed-eating birds, such as finches, weavers and small doves. On 4 January 1974, the bird was observed to be weak and diarrheic. It was moved to the hospital, and a fecal examination revealed trichomonads. It was treated for this protozoan, but died

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on 6 January. At necropsy the bird was in a poor state of nutrition. A tan, nodular lesion, was observed in the superficial pectoral muscles near the thoracic inlet, and additional soft, tan, encapsulated areas were seen in the adjacent fascia. When incised, these lesions had a mucoid or myxomatous texture. India ink preparations of impression smears of the lesion revealed fungi surrounded by a clear capsular zone that varied from 2 to 4 times the diameter of the cell body. Measurements of the fungi and capsule were not made. The brain was removed, and yeast cells, consistent for *Cryptococcus*, were demonstrated in impression smears of the meninges. All other organ systems were grossly normal. Pectoral muscle, heart, arteries, lungs, trachea, esophagus, liver, ventriculus, intestines, kidney and brain, were collected and fixed in buffered 10% formalin, embedded in paraffin and cut

at 3 to 5 μ m. The sections were routinely stained by hematoxylin and eosin and also by the PAS method, and examined under light microscopy. A few individual yeast cells were seen in the lumen of blood vessels in the heart but there was no invasion into the myocardium. An occasional yeast cell was observed on the epicardium, although it is possible that these were merely contaminants acquired in the process of tissue preparation. Many yeast cells were present in the adventitial tissues of the brachycephalic and carotid arteries and in fascial tissues surrounding the esophagus and trachea at the thoracic inlet (Figs. 1 and 2). Accumulations of cryptococci-like yeast were also observed in respiratory bronchioles and alveoli of the lung. Sections prepared by the PAS method revealed that the yeast cells were surrounded by a PAS positive capsule. Yeast cells were present in the meninges and in

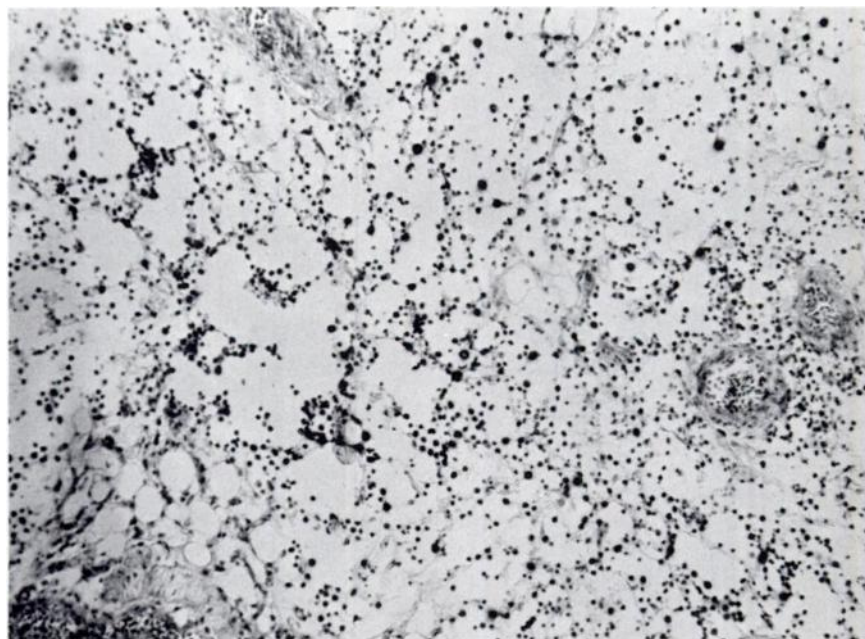


FIGURE 1. *Cryptococcus* in fascia at thoracic inlet of Bartlett's bleeding-heart pigeon. PAS stain \times 100.

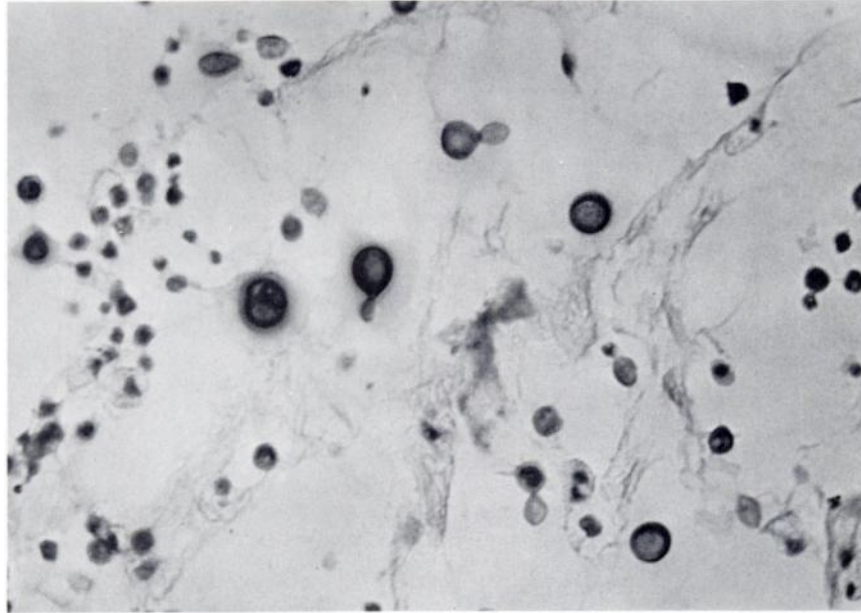


FIGURE 2. *Cryptococcus neoformans* showing budding and capsule in fascia of Bartlett's bleeding-heart pigeon. PAS stain $\times 400$.

paravascular spaces adjacent to the meninges. Pseudovacuated spaces containing yeast were observed in the parenchyma of the cerebrum and cerebellum. These organisms had not elicited an inflammatory response of significance in any of the tissues examined. Cultures from the meninges and the muscle lesions were made on Phytone media.⁵ The isolated yeast was consistent in colonial and microscopic characteristics for *C. neoformans*, and it grew well at 37 C. Four mice, inoculated intracerebrally, died within a week. Many heavily capsulated yeast cells were demonstrated in the mice brains. This culture was held in a stock culture collection and recently was retested by mouse inoculations and for temperature growth characteristics, biochemical activity and assimilation pattern. Six mice,

inoculated intracerebrally, were dead within 8 days and all had many capsulated yeast cells in the brain. A growth temperature profile was done at temperatures ranging from 37 to 42 C, and no growth was obtained beyond 40 C. Control cultures of a human and cat isolate showed an identical temperature growth response and therefore indicated nothing unusual about the pigeon isolate that might enhance its invasive capability at the higher avian body temperature. Biochemical activity and assimilation pattern were determined to be consistent for *C. neoformans*, using the system of Huppert *et al.*⁶

Case 2: A male Beccari's crowned pigeon, (*Goura victoria beccari*) was hospitalized on 6 February 1977, for an enlarged right infraorbital sinus. A biopsy taken on 7 February, was diagnosed

⁵ Baltimore Biological Laboratory, Cockeysville, Maryland.

as a granulomatous inflammation containing cryptococci-like yeast (Fig. 3). A culture was made and *C. neoformans* was isolated. Histopathologically, it could not be ascertained that *Cryptococcus* was the primary pathogen, since the tissue contained small, old granulomas, which did not contain the yeast. Bacteria were present in these lesions. A second biopsy, taken on 27 April, was cultured and processed for histopathology. There was an initial growth of a yeast morphologically resembling *C. neoformans*. Later when the culture was removed from refrigeration, preparatory to biochemical testing and mouse inoculation, it was found that the organism was no longer viable. Possibly, residue of the therapeutic agent weakened the viability of the organisms. Yeast cells were abundant in the tissue sections. On 2 May, the pigeon was found moribund and was

ethanized. At necropsy, the lungs grossly appeared to be congested, and a pinkish caseous exudate was found to be present in the right infraorbital sinus. Histopathologically, the lungs contained several granulomatous lesions, but *Cryptococci* were not observed in the pulmonary tissue. Numerous *Cryptococci* were present in the infraorbital sinus tissues and many, but not all, appeared to be degenerated (Fig. 4). Fungal cells were observed within the marrow cavity of the bony wall of the infraorbital sinus. Cultures prepared from the sinus exudate did not produce growth of the yeast organisms.

In both cases there was evidence that *Cryptococci* had invaded tissues and had become pathogenic for the host. We were unable to determine the source of the pathogen or the mode of entry into the host.

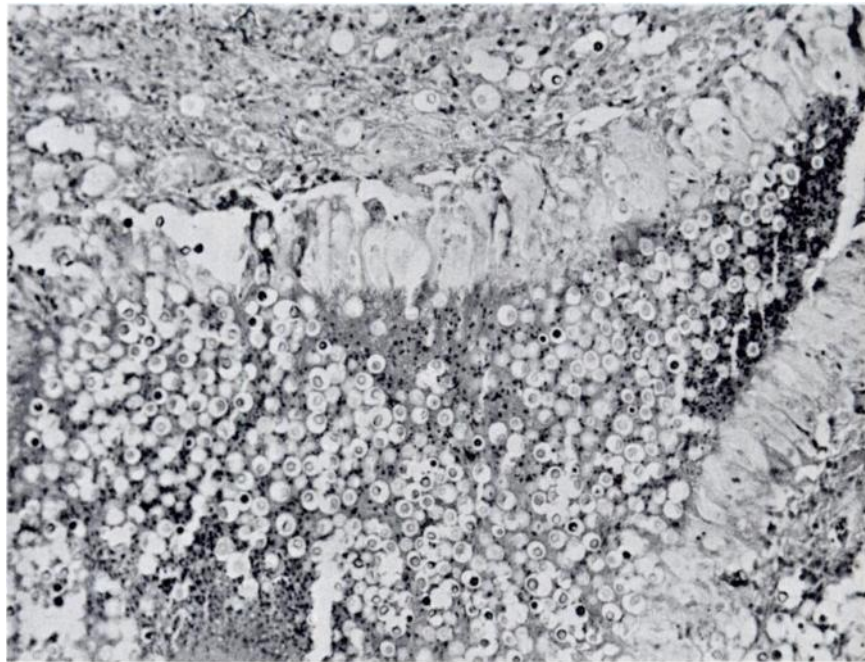


FIGURE 3. Biopsy section of the cryptococcal granuloma in the infraorbital sinus of a Beccari's crowned pigeon. PAS stain \times -100.

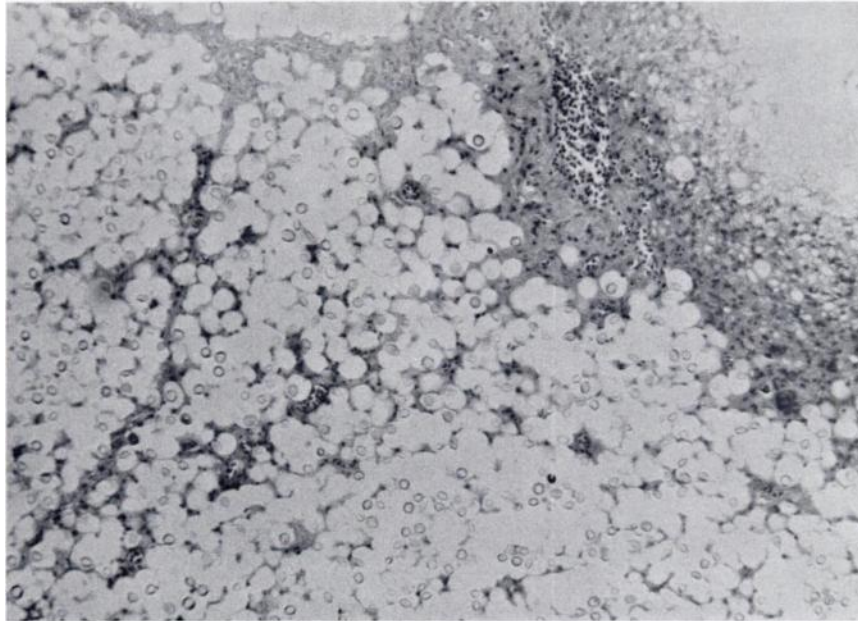


FIGURE 4. Necropsy sections of the infraorbital sinus granuloma following therapy. Most of the fungi were dead or inactive. PAS stain \times 100.

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