

PATHOGENIC FUNGI ASSOCIATED WITH TAIPEI PIGEONS⁽³⁾

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Abstract: Fungal isolations from the pigeon population in the metropolitan area of Taipei were investigated. Five potential pathogenic species found included *Aspergillus fumigatus*, *A. niger*, *Cryptococcus neoformans*, *Geotrichum candidum*, and *Histoplasma capsulatum*. Twenty four other species of taxonomic interest were also observed.

INTRODUCTION

Pigeons kept as pets and for the sport of racing is a very common practice throughout the Republic of China. Each day toward evening the birds are allow to exercise and are released from roof top cages for limited flight around the community. Some individuals has a few as one or two birds while other owners have flocks numbering well over one hundred. The birds remain within a flock per individual owner, however, their exercising flights serve as a method of dispersing microorganisms that might be carried by the birds. Some fungi have the ability to grow within the intestinal tract of birds while other fungal species find suitable nutritional requirements in birds. Pigeon owners and handlers as well as other residents of the community have varying degrees of exposure to the fungi possibly carried by the birds. It was learned in a study by Emmons (1960) that wild pigeons in Washington D.C. were heavily infected with a potential human fungal pathogen and all office workers in that city were exposed to the mycosis by high fungal cell counts present in pigeon droppings found on office buildings. The pigeons found numerous window sills and building ornamentation quite suitable as bird roosts.

Geographic areas have been identified as endemic to certain mycoses, and individuals living within those areas generally produce positive skin reactions indicating some involvement with the fungal pathogens (Conant *et al.*, 1971; Emmons *et al.*, 1970; Rippon, 1974). It is the purpose of this study to examine the pigeon population of the Taipei metropolitan area for fungal species potentially pathogenic to man.

MATERIALS AND METHODS

A total of 185 collections of bird droppings and dust were made during the month of June from pigeon roosts and cages equally distributed throughout Taipei city and suburbs. Sterile cotton swabs were used in making the collections. Swabs were placed in sterile test tubes and transported to the laboratory where the material was streaked on petri plates containing Sabouraud's maltose agar for incubation at 25 C. For each swab three plates were streaked. Plates were considered positive for each potential pathogen when species were identified and pure cultures were made. Plates were held up to two weeks for culture identification, however, most colonies were found with 48 hours growth.

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- (3) Supported by National Science Council Grant NSC-64-0501-0202(10) to Dr. Zuei-Ching Chen, Mycology Laboratory, Department of Botany, National Taiwan University.

Blood agar base medium containing heart infusion was used for growth of selected isolates at 37 C for study of the yeast phase. Malt agar was also employed for yeast colony study. In addition, all colonies under investigation were grown at 37 C since avirulent species and strains grow poorly if at all at 37 C (Hazen *et al.*, 1970). Species of the representative genera under study were identified according to the literature by colony morphology, colony color, limited nutritional requirements, and the size and shape of various cell types obtained from pure culture isolates (Conant *et al.*, 1971; Lodder, 1971; Rippon, 1974). Slide mounts for microscopic observations were made with lactophenol cotton blue. Nonpathogenic fungal species of taxonomic interest isolated from pigeon droppings were noted according to descriptions in the literature (Agnihotrudu and Barua, 1957; Barnett and Hunter, 1972; Barron, 1968; Booth, 1971; Cooney and Emerson, 1964; Ellis, 1971; Gilman, 1966; Lodder, 1971; Morton and Smith, 1963; Raper and Fennell, 1965; Raper and Thom, 1968; Rebell and Taplin, 1964; Tubaki, 1954).

RESULTS

Fungal species identified as causal agents of human mycoses and other fungi were studied for frequency of distribution in pigeon populations throughout Taipei. *Cryptococcus neoformans* (Sanfelice) Vuillemin was isolated from 62% of the sample collections. The yeast was equally distributed throughout the metropolitan area. Colonies have a mucoid texture, smooth margin, cream color, and a moist appearance. The capsule of the yeast cells is quite evident when examined microscopically. Cells are spherical and at times somewhat elongated, 3.5 to 7.0×3.7 to 8 microns in size. Single buds are most frequent, occasionally two or more can be found. Individual cells are easily separated from the colony and they are found singly or in groups of two or more (Fig. 1A).

A profusely sporulating filamentous fungus was frequently found in the pigeon roost collections. *Histoplasma capsulatum* Darling appeared in material from 59% of all sampling locations. The numerous spores are the most distinguishing structures of this organism. Both smooth walled and tuberculate macroaleuriospores are found in the same colony (Fig. 1B). Spores are 8 to 14 microns in diameter, round to pyriform in shape. Smooth walled spores are devoid of the tubercles or finger like projections. Some cells have very pronounced tubercles giving the spores a warty appearance. Young colonies are white but soon they become light brown with age. Heavily sporulating colonies were more pigmented. At 37 C incubation, oval budding yeast cells were produced, however, hyphal filaments found in colonies grown at 25 C were also present at the higher temperature.

The most frequently isolated organism was *Geotrichum candidum* Link with 81% of all collections positive for this species. The organism dominated the streak plates with numerous colonies. The white to cream colored colonies soon appeared on the agar surface. At 25C the growth was more rapid than at 37C, however, good colony development appeared at both temperatures. Colony growth is somewhat raised and the texture appears smooth and dry. The filamentous colony perimeter produces an indefinite margin. Hyphae are a distinguishing characteristic of *G. candidum*. Soon after growth, hyphal filaments increase in septations and cells fragment into chains of arthrospores of varying size. Arthrospores appear rectangular to oval in shape and frequently germinating arthrospores are seen giving an appearance of a budding yeast cell (Fig. 1C).

Aspergillus fumigatus Fresenius was isolated with a 51% frequency while *Aspergillus niger* Van Tieghem was found in 4% of all collections. Both species are capable of producing human mycoses, however, *A. fumigatus* is more pathogenic when considering primary agents of disease. *Aspergillus fumigatus* colonies are grey-green in color with a velvet to deep felt texture,

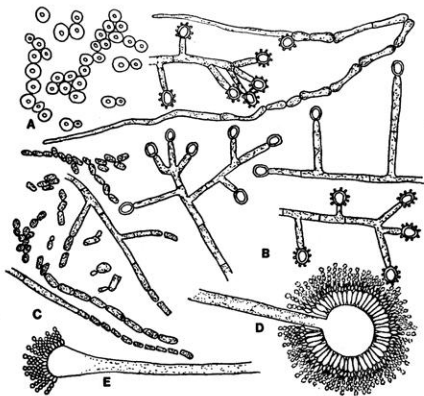


Fig. 1. A. *Cryptococcus neoformans* encapsulated yeast cells and three budding cells. B. *Histoplasma capsulatum* smooth walled and tuberculate macroaleuriospores with variations in hyphal growth patterns. C. *Geotrichum candidum* hyphae and arthrospore production. D. *Aspergillus niger* conidiophore with a round vesicle, 2 rows of sterigmata, and smooth walled conidia. E. *Aspergillus fumigatus* club-shaped vesicle, one row of sterigmata, and echinulate conidia.

floccose, and slightly folded. The reverse colony surface is white to cream. The short conidiophores are columnar, 5 to 8 microns in diameter with a gradual broadening apex adjacent to the vesicle. Conidiophore length can reach 300 microns. A green to brown color can be seen in the wall of the expanded area. The vesicle is flask-shaped, 20 to 30 microns in diameter with a single row of sterigma on the upper portion only (Fig. 1E). Sterigmata are 6 to 8 microns long. Globose to subglobose conidia are echinulate, 2 to 3 microns in diameter. No sclerotia are found.

Aspergillus niger colonies are black due to the abundant large conidiophores produced on the colony surface. Conidiophores are 5 to 6 microns in diameter, 100 to 250 microns in length. Vesicles are round, 10 to 16 microns in diameter. Two rows of sterigmata variable in length are found. Conidia are smooth walled, 2 to 2.5 microns in diameter. Aleuriospores are frequently found in the vegetative mycelium.

Other fungi were isolated from the pigeon coprophilic material, however, they were recovered with a less than 1% frequency. None of the species cause severe mycoses but their presence could establish themselves as opportunists. The species included *Arthroderma multifidum* Dawson, *Aspergillus candidus* Link, *A. clavatus* Desmazieres, *A. flavus* Link, *A. ochraceus* Wilhelm, *A. ornatus* Raper, Fennell and Tresner, *Cephalophora tropica* Taxter, *Chrysosporium tropicum* Carmichael, *Epicoecum nigrum* Link, *Pusarium tricinctum* (Corda) Saccardo, *Humicola*

lanuginosa (Griffon and Maublanc) Bunce, *Mucor fragilis* Bainier, *M. racemosus* Fresenius, *Oedocephalum glomerulosum* (Buller) Saccardo, *Penicillium capsulatum* Raper and Fennell, *P. digitatum* Saccardo, *P. janthinellum* Biourge, *P. rugulosum* Thom, *Rhizotorula rubra* (Dnmm) Lodder, *Scoyulariopsis brevicaulis* Bainier, *Torula herbarum* f. *quaternella* Saccardo, *Trichoderma lignorum* (Tode) Harz, and single isolates of *Neurospora* sp. Shear and Dodge, and *Silbum* sp. Tode ex Fries.

DISCUSSION

Numerous references can be found in the literature on the species under study concerning their association with pigeons and other birds such as starlings or domestic fowl of economic importance including chickens. Previous studies give particular attention to a case history or epidemic and the source of infection which frequently involved pigeons. The purpose of this study is to investigate pet pigeons of a densely populated urban community in order to isolate and identify all potential pathogenic fungal species and to evaluate their frequency of isolation. Pigeons in most cities are considered a hardy pest of urbanization, birds that enjoy public buildings and statuary, and this fondness is demonstrated with visible disrespect. However, avian enthusiasts can be found in any large city who have pigeons as pets or racing purposes. From observations, pet pigeons are more common in Taipei than other cities of equal size.

The frequent isolation of pathogenic fungi in Taipei directs attention to the disease symptoms. Most commonly, the diseases are manifested in mild forms. Pulmonary infection is the primary manifestation of *C. neoformans*. Cryptococcosis in the lungs usually is asymptomatic and only detected by x-ray. Infection takes place after yeast cells are inhaled but the disease is usually resolved with minimal symptoms due to the defense mechanisms of a normal patient (Howard and Bolande, 1966). When pulmonary symptoms are present, a low grade fever, cough, pleuritic pain, and some weight loss may be noted (Campbell, 1966). At times a heavy discharge of mucoid sputum containing numerous organisms may occur. Cryptococcosis as a debilitating disease is less common, however, when it does occur it is quite severe. Any internal organ can be infected, frequently the central nervous system is involved. Cutaneous, mucocutaneous, and bone infections occasionally occur, however the subclinical lung infections are most frequent and quite common (Rippon, 1974). It is estimated that at least 15,000 subclinical cryptococcal respiratory infections occur in New York City each year (Ajello, 1969; Littman and Walker, 1968).

Histoplasmosis is similar to cryptococcosis in the fact that lung infection is initiated after spores are inhaled, and most cases are subclinical and inapparent. Approximately 95% of all cases are unnoticed by the patient and disease discovery is made only by x-ray that identifies calcified lung areas and by a positive histoplasmin skin test (Rippon, 1974). Many areas of the world are endemic for the disease and very common positive skin tests in inhabitants of these areas are found (Ajello, 1971). In the United States alone, it is estimated that 40 million people have had the disease (Furcolow, 1962; Ajello, 1970). The common mild symptoms of the disease include a cough, pleuritic pain, some shortness of breath, hoarseness, and a slight fever (Little, 1960). In addition to the common short term infections, other cryptococcal cases include progressive lung involvement, chronic cutaneous lesions, chronic internal diseases, and the acute rapidly fatal systemic infection.

Climatic conditions favoring growth of *H. capsulatum* and disease development have been investigated (Fonseca, 1971; Furcolow, 1958). Average temperatures of 22 to 29 C, an annual precipitation of 52 to 73 cm, and a 67 to 87% relative humidity favor growth of the organism in nature and disease involvement in humans.

Geotrichum candidum is the specific causal organism of geotrichosis, and lung infections are the most frequent form of the disease (Conant *et al.*, 1971). Symptoms simulate tuberculosis including the thick grey mucoid sputum. Rales are heard in breathing and the condition is most often chronic (Rippon, 1974). Other clinical cases less frequently found include bronchopulmonary geotrichosis with severe asthmatic symptoms (Ross and Reid, 1966), bronchial geotrichosis without lung infections with a prominent chronic cough, a gelatinous sputum and coars rales (Morenz, 1971), and rare occurrences of oral, gastrointestinal and cutaneous infection (Goldman and Lipscomq, 1969; Morenz, 1971). Frequently *G. candidum* is isolated as a normal inhabitant of human oral microflora but the same species under various conditions becomes the causal agent of disease.

Numerous species belong to the genus *Aspergillus* (Raper and Fennell, 1965), and many disease forms are included in aspergillosis (Rappon, 1974). A limited number of species are involved in the disease but any species may cause an allergic reaction. Illness can result from toxicity by eating food supporting growth of various species, asthmatic reactions to spore inhalation, localized infection, cutaneous aspergillosis, nasal orbital aspergillosis, necrotic and granulomatous infection of specific organs, central nervous system infection, and widespread systemic involvement which is rare yet fatal. Aspergillosis involving the bronchopulmonary system produces a gelatinous sputum which is sometimes bloody. Additional symptoms include fever, cough, wheezing, chills, malaise, and general aches. *Aspergillus fumigatus* is the causal species for most all forms of the disease (Tan and Sugai, 1966; Welch and Buchness, 1955; Young and Jennings, 1972) while *A. niger* frequently causes otomycosis and cutaneous infections (Cahill and Mofty, 1967).

Aspergillosis (Hinson *et al.*, 1952), histoplasmosis (Lehan and Furcolow, 1957), and cryptococcosis (Fink and Barboriak, 1968; Mira and Anzola, 1968; Staib and Bethauer, 1968) have a common association in the literature with individuals in contact with pigeons while geotrichosis does not have this relationship. Generally the diseases are subclinical and transitory, noticed by the patient only as a minor irritation.

ACKNOWLEDGEMENTS

Research support was provided by the U. S.-R. O. C. Cooperative Science Program, The authors wish to thank the many individuals who assisted in these investigations.

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