Prevalence of Cryptococcus neoformans in Pigeon Habitats

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THE frequent occurrence of virulent strains of *Cryptococcus neoformans* in pigeon droppings under roosting sites and in old pigeon nests was first reported in 1955 (*1*). Prompt confirmation of this observation from Japan (*2*), Cincinnati, Ohio (*3*), and New York City (*4*) showed that this is not an unusual or geographically restricted association. The pigeon is not a host to *C. neoformans* but this pathogenic fungus grows as a saprophyte in pigeon manure and can be isolated from the majority of specimens collected in either rural or urban areas.

I have continued investigations of the prevalence of *C. neoformans* in the environment in order to estimate the frequency of man's possible exposure to the fungus and to evaluate in retrospect the accuracy of diagnoses in certain epidemics or focal outbreaks of pneumonitis which have been accepted as histoplasmosis.

Materials and Methods

Most of the specimens studied have been collected in or near Washington, D.C. The specimens, consisting of dried and weathered pigeon manure from old nests or under roosting sites and relatively fresh droppings and soil from a city park where pigeons are fed by visitors, were scooped up into large glass tubes closed with cotton plugs.

Direct cultures were made by transferring material from the specimen to agar slants. The slants were incubated at 37° C. in order to inhibit growth of many fungi. Part of each specimen was suspended in salt solution and injected intraperitoneally in mice in order to isolate *Histoplasma capsulatum* if it were present. Mice were killed after 1 month and cultures were made from spleen and liver in the manner previously described (*1*).

Results

In the series reported here, virulent strains of *C. neoformans* which grew at 37° C. were isolated from 63 of 91 specimens. *H. capsulatum* was not isolated from any of the samples, although the methods used have been shown to be adequate for isolation of *H. capsulatum* from hundreds of other soil specimens. The following table shows the types of buildings or environments from which specimens were taken.

Sources of collections	*Total number specimens collected*	*Number specimens positive*
Warehouse, former barn	15	14
Old school building, now offices	10	7
Grain-milling establishment	5	3
Cupola on high school building	7	7
Window ledges, Federal and municipal office buildings	18	17
Public park	7	0
Railroad station	4	1
Barns (Virginia and Maryland)	25	14
Total	91	63

The warehouse was formerly a dairy barn with two attached silos. For several years

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prior to this study it had been a government warehouse, and at the time the specimens were taken it had been emptied in preparation for demolition. It stood on the grounds of Junior Village in Washington, D.C., and I am indebted to officials of that institution for permission to collect specimens there.

The former public school building in the District of Columbia, now occupied by offices, is an old brick building with a large attic. The floor of the attic was covered with pigeon dung, nests, broken eggs, and carcasses of pigeons. It apparently fits very closely the description of a similarly infested old school building at Plattsburg, N.Y. (*5*). During the demolition of that building 23 men became ill with a pneumonitis diagnosed in retrospect and on inadequate evidence as histoplasmosis.

The grain-milling establishment attracted large numbers of pigeons and a few birds nested in some of the open buildings.

The cupola of a high school building and the window ledges of Federal and municipal buildings in Washington, D.C., presented the familiar types of shelters used by urban pigeons for nesting and roosting. I am greatly indebted for assistance in collection of specimens from these buildings to Wallace Coleman and Baker Wingfield, General Services Administration, and Clarence Travis, District of Columbia Health Department.

The one positive specimen from a railroad station was on a concrete floor beneath a ledge where pigeons nested.

Collections from barns were from nests on sills and other protected supports and from floors of haymows, as well as on hay piled deeply above mow floors under roosting stations.

Failure to isolate *C. neoformans* from seven soil specimens in a city park, is not conclusive evidence that the fungus does not grow in such areas. Additional samples will be taken from this and other parks.

Discussion

The close association between man and pigeons in cities and in barns in the country, and the growth of *C. neoformans* in pigeon manure in old nests and under roosting sites present theoretical and practical problems of considerable public health importance. The strains of *C. neoformans* isolated from these sources exhibit the same patterns of variability with respect to degree of virulence for mice, thickness of capsule, and acid production in various carbohydrates as strains of the fungus isolated from fatal cases of cryptococcal meningitis in man. It seems certain that man must be frequently exposed to this pathogenic fungus by inhalation of windblown cells from saprophytic environmental sources.

Knowing that virulent strains of *C. neoformans* are present in man's environment and assuming that cells of the fungus must be inhaled frequently, one can only speculate on the reasons for the infrequent recognition of pulmonary cryptococcosis in man. The most probable explanation is that primary pulmonary lesions occur as they do in histoplasmosis, that these lesions heal spontaneously because of innate resistance of the individual or because of immunity acquired during the slow evolution of the pulmonary lesion, and that the fungus reaches the central nervous system in only a small percentage of cases. Pulmonary lesions are indeed found in some cases of cryptococcal meningitis, and *C. neoformans* has been demonstrated in healed "coin lesions" in persons without active cryptococcosis (*6*).

Pulmonary cryptococcosis, limited in extent and duration, may be much more prevalent than is currently recognized. Presumptive evidence of extensive but benign pulmonary infection, without dissemination to the central nervous system, can be deduced from a study of certain epidemics of pneumonitis in which the type of exposure almost certainly precludes the presently accepted diagnosis of histoplasmosis, but makes the presumptive diagnosis of pulmonary cryptococcosis very probable.

One of the most interesting of these epidemics was the one at Plattsburg, N.Y., previously mentioned (*5*). In 1938, 23 men who tore down a school building became ill. The tower of the building "was filled with dead pigeons of various sizes and with pigeon droppings to a height of about 4 feet. Between the roof of the building and the ceiling of the gymnasium was another deposit of manure and dead pigeons about

2 feet in depth." The diagnosis of ornithosis was apparently ruled out. In retrospect, 15 years later, a diagnosis of histoplasmosis was made. The evidence accepted for the diagnosis in this and similar epidemics was hypersensitivity to histoplasmin, positive serologic tests, development of miliary calcification, and isolation of *H. capsulatum* from soil at the point source of the epidemic (*7*). Serologic cross reactions between cryptococcosis and histoplasmosis occur so that this evidence is equivocal.

In the case of the Plattsburg epidemic, the isolation of *H. capsulatum* from 1 of 24 soil specimens was reported. The soil sample was taken from the dirt floor of a church two doors from the site of the demolished school house some 15 years after the epidemic. The probability of contaminating a soil sample from the air of a laboratory has been discussed in a previous paper (*1*). The most convincing retrospective evidence against a diagnosis of histoplasmosis in the Plattsburg epidemic is that *H. capsulatum* has never been isolated from accumulations of pigeon manure in the upper floors of a building, but virulent strains of *C. neoformans* are regularly present in such material. Similar evidence against a diagnosis of histoplasmosis and for a diagnosis of pulmonary cryptococcosis can be presented for epidemics of pneumonitis at Warrenton, N.C. (*7*), and Mandan, N. Dak. (*8*).

Summary

Virulent strains of *Cryptococcus neoformans* are generally present in pigeon manure in old pigeon nests and under roosting sites. Recent additional isolations of this pathogenic fungus from varied types of rural and urban environments are reported. The frequent occurrence of *C. neoformans* in man's environment presents a hazard not recognized prior to 1955.

Histoplasma capsulatum has not been isolated from pigeon manure in upper floors of buildings. *C. neoformans*, however, is very frequently present in pigeon manure from haymows in barns and from upper floors of buildings which have sheltered pigeons. In view of these facts, the circumstantial evidence for a diagnosis of a previously unrecognized pulmonary form of cryptococcosis is as strong as the circumstantial evidence for a diagnosis of histoplasmosis in certain past epidemics of pneumonitis in men who were exposed to pigeon manure while demolishing old buildings which had sheltered pigeons. Until such epidemics can be studied systematically at the time they occur, instead of in retrospect, and until cultures and adequate serologic studies are made, the etiology of the type of pneumonitis reported will remain equivocal.

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