Isolation of Histoplasma capsulatum From Soil in Washington, D.C.

CHESTER W. EMMONS, Ph.D.

IN THE FALL of 1960, *Histoplasma capsulatum* was isolated from 10 of 10 specimens of soil collected in a small park in downtown Washington, D.C. In order to clarify and emphasize the significance of the demonstration that this pathogenic fungus grows freely in urban as well as rural habitats on the Atlantic seaboard, it is necessary to review some of the history of *Histoplasma* and histoplasmosis.

H. capsulatum was isolated from a saprophytic source for the first time in 1948 (*1*). Both the local site and the geographic area from which the first positive soil specimen came are historically and scientifically significant. This specimen was collected near the entrance to a rat burrow under the edge of a chickenhouse in Loudoun County, Va. Later isolations of *H. capsulatum* from soil taken from many farm premises in both northern Virginia and eastern Maryland (*2*), the report of four fatal human cases of histoplasmosis in northern Virginia in 1946 (*3*), the discovery that in this area seven species of animals are natural hosts of *H. capsulatum* (*4*), and the observation that as many as 83 percent of persons in one intensively studied community are hypersensitive to histoplasmin (*5*) conclusively demonstrate that *H. capsulatum* is a frequent contaminant of man's environment and that histoplasmosis is a common disease and an important medical problem on the Atlantic seaboard. Some workers, in discussing the epidemiology of histoplasmosis, have persistently ignored these facts.

Dr. Emmons is head of the Medical Mycology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Public Health Service. (Paper received Feb. 6, 1961.)

Epidemiologic studies of many cases of histoplasmosis in residents of the District of Columbia have included searches for *H. capsulatum* in the urban environments of the patients (*2*). Up to the time of this study no isolations of this fungus had been reported from soil collected within the District of Columbia. We had isolated the fungus many times from soil (usually around chickenhouses) to which District of Columbia resident patients had been exposed during visits or temporary residence in nearby Maryland or Virginia.

Because of persistent confused or erroneous concepts expressed in the literature of the mycoses, the epidemiology of histoplasmosis cannot be discussed without reference to the epidemiology of cryptococcosis. The present study, although it resulted in isolations of *Histoplasma*, was directed toward a search for *Cryptococcus neoformans*. The first reported isolation of *C. neoformans* since 1896 from a source unrelated to human or animal infection was also from the Washington, D.C., area (*6*). This report was followed by the demonstration that *C. neoformans* is a frequent inhabitant of accumulations of pigeon droppings in old nests or beneath roosting sites under conditions in which this material has not become composted with soil (*7*). This purely saprophytic association between virulent strains of *C. neoformans* and pigeon droppings has been confirmed in several parts of the world and in Washington, D.C., by repeated examinations of such material collected from the attics of old school houses, cupolas on school houses and other public buildings, the ledges outside windows of office buildings, and many similar locations (*8*). Both *H. capsulatum* and *C. neo-*

formans, therefore, are commonly present in man's environment in the Washington, D.C., Maryland, and Virginia areas.

I have continued to look for *C. neoformans* in Washington and particularly in the soil of city parks. It is assumed that *C. neoformans* probably is present in the soil of city parks frequented by pigeons, and although examinations of specimens so far have failed to demonstrate it, the search has been continued. Collected specimens have always been examined by methods known to be adequate to demonstrate the presence of *H. capsulatum*, should it also be present.

Materials and Methods

Specimens were collected as described in previous papers (*9*) by scooping up the superficial layers of soil directly into large test tubes (22 by 150 mm). Portions of each specimen were removed in the laboratory to similar sterile tubes. Physiological sodium chloride solution was added in the proportion of about 5:1. Each tube was stoppered with a sterile rubber stopper and shaken vigorously. An 8-ml. portion of fluid was taken from the upper part of the liquid column after sedimentation of solid material; 2 ml. of an antibiotic solution (2 mg. streptomycin and 5 mg. penicillin per milliliter of water) were added to the 8 ml. of soil suspension, and 1 ml. of this mixture was injected intraperitoneally into each of five mice. Mice were killed 1 month later, and portions of spleen, omentum, liver, and any abscesses or lesions which were observed were placed on modified Sabouraud's agar by mincing a tissue on the agar slant with the scissors by which it was taken from the organ. The tissues were spread with a stiff loop and the exertion of pressure, and the cultures were incubated at 30° C.

Results

Without reviewing the specimens of park soil collected during previous years and from which no significant fungus isolations were made, this report is concerned with collections made during the fall of 1960. Ten specimens were taken from a park about 2 acres in size. This park is traversed by numerous paths near which benches are located, and it usually contains many pigeons. No significant isolations were made in this area.

Ten additional soil collections were made on the same day from a small park adjacent to Pennsylvania Avenue NW. This park contains a few paths and benches and numerous sycamore trees. The park is well kept, and when these collections were made it had been raked and cleaned recently. It appeared that litter and other material on the surface had been scraped up and removed that morning. There was no conspicuous evidence of bird droppings, although this is known to be an important roosting area for a concentration of starlings (*Sturnus vulgaris*). *H. capsulatum* was isolated from all 10 of the specimens of soil collected from this small park. *C. neoformans*, the fungus actually being sought, was not found.

Five specimens were taken from an area adjacent to the street pavement near the intersection of Massachusetts Avenue and E Street NE., under pin oak trees. This was an area which showed little promise of yielding a pathogenic fungus. The soil contained gravel and cinders, was eroded by rains, and at the time showed little evidence of contamination by bird excreta. However, *H. capsulatum* was isolated from one of the five specimens collected at this site.

Discussion

In general the most productive type of location for isolation of *H. capsulatum*, both before and after Zeidberg and co-workers (*10*) called attention to this association, has been in, under, or near chickenhouses. There have been isolations from other locations, some of them associated with bird habitation and some without any obvious association of this type. These have been reported or reviewed by numerous investigators (*11–14*). Another association, the presence of *H. capsulatum* in decayed bat guano in caves, has been demonstrated by many investigators (*15–20*). A family outbreak of histoplasmosis with one fatal case was reported from Maryland by Emmons (*9*). On the premises occupied by the family, no chickens had been

kept for many years, but *H. capsulatum* was isolated repeatedly at all seasons of the year adjacent to the basement wall of the house where the soil was contaminated by droppings of the house bat (*Eptesicus fuscus*). These habitats from which *H. capsulatum* has been isolated, although varied, were essentially rural or in small villages. A possible role of the house bat in the epidemiology of urban histoplasmosis was suggested, however (*9*).

Ajello's report of a school outbreak of histoplasmosis, in which manure from a grackle (*Quiscalus quiscula*) roost was used to enrich soil for a gardening project, associates this bird with the saprophytic occurrence of *H. capsulatum* (*21*). I have failed in two attempts to isolate *Histoplasma* from a large grackle roost 75 miles from Washington, probably because sampling has been inadequate. It is probable that further studies will show additional associations of the types mentioned above.

The occurrence of urban hypersensitivity to histoplasmin with probable urban exposure to *Histoplasma* has been reported by Aronson and Edwards (*22*). An outbreak mentioned by Larsh (*23*), to be reported by Furcolow and associates, demonstrated an association between an urban starling roost and several severe cases of histoplasmosis (*24*). The source of infection was on an old, neglected, 11-acre estate, overgrown with vines, weeds, and trees in Mexico, Mo. Although urban in location it was actually a densely wooded area. Histoplasmin sensitivity in school children in Milan, Mich., has been associated with the roosting of starlings in trees on a playground (*25*).

In order to place the solved and unsolved epidemiologic problems presented by histoplasmosis in perspective and to evaluate the significance of the observations reported here, it is necessary to examine currently established facts about the distribution of *Histoplasma* and histoplasmosis. Many epidemiological features of histoplasmosis have been elucidated by surveys of hypersensitivity to histoplasmin (*26–28*), by studies of individual cases and of focal outbreaks of histoplasmosis (some of which are cited), and by isolations of *H. capsulatum* from saprophytic sources as reported and reviewed here. It is obvious that new epidemiological relationships have been discovered recently, and it is probable that other significant features remain unknown.

Certain erroneous concepts concerning histoplasmosis have persisted in the face of current evidence.

Despite the many reported isolations of *Histoplasma* (including its first isolation from and visible demonstration in soil) in the Maryland-Virginia area, two of the latest textbooks use a map (*29*) which gives no indication of the known high percentage (up to 83 percent) of histoplasmin reactors in communities of that region (*2, 5, 27*). Commenting on this map, Furcolow (*29*) states, ". . . the fungus does not grow in any great quantity—at least insofar as we know" east of the Appalachian Mountains. He speaks also of ". . . the absence of infection east of the Appalachians. . . ." But he adds, "It is true that in northern Virginia, and possibly in Maryland and Pennsylvania and certain other areas, there are foci of fairly high sensitivity."

This same report implies a relationship between "epidemics of histoplasmosis" and exposure to pigeon manure on upper floors of buildings, although the only reported isolations of *Histoplasma* associated with pigeon excreta have been in instances where soil was mixed with pigeon droppings, as was the case in the outbreak reported by Sabin (*30*). On the other hand, *C. neoformans*, which is known to cross react with *H. capsulatum* (*31*), is very frequently present in accumulations of pigeon manure in old nests and on upper floors of buildings (*6, 7*).

In most instances urban isolations of *H. capsulatum* have been in villages or small towns, often with possible association with old chicken-houses, exposure to the former site of a chicken-house, or exposure to chicken manure used to fertilize gardens or flower beds (*32*). One urban outbreak (*23, 24*) involved severe exposure to bird excreta on a large, neglected, wooded estate which was being cleared for a city park. The Washington, D.C., site described here is quite different from the Mexico, Mo., site (*23, 24*), being without rural characteristics, even in miniature.

The specimens of soil reported in the present paper were taken from a well-kept small park adjacent to a busy street in a densely populated

downtown business section of Washington, D.C. The park had been periodically and recently cleaned, and there was no obvious contamination with bird excreta, although it is known that the soil under the sycamore trees where the collections were made is regularly contaminated with such material. It is obvious that roosting starlings can create a soil environment suitable for the growth of *H. capsulatum* even though bird excreta does not accumulate and remain on the surface of the soil. Studies will be continued by the collection of soil contaminated by starling excreta in this and similar urban areas.

Isolations of *H. capsulatum* from this typical urban environment demonstrate and define an urban type of human exposure to *Histoplasma* already suggested by others (*21–24*), and they reemphasize the well-known and adequately documented importance of histoplasmosis as a frequent disease and an important medical problem in Washington, D.C., and surrounding areas. The role of house bats (*9*) in producing urban habitats suitable for growth of *H. capsulatum* remains as a possibility not yet evaluated.

Summary

The recent isolation of *Histoplasma capsulatum* from soil in downtown Washington, D.C., justifies and requires a review of the frequency and importance of histoplasmosis in the Maryland-Virginia area. The importance of the Atlantic seaboard of the United States in the worldwide distribution of histoplasmosis has been ignored by some workers.

H. capsulatum was isolated from 10 of 10 soil specimens collected from a small, clean park adjacent to Pennsylvania Avenue NW., Washington, D.C., and from 1 of 5 specimens collected adjacent to another downtown Washington street. The first 10 isolations were from soil not obviously contaminated by bird droppings, but the soil specimens were taken under sycamore trees which are used as roosting places by a large flock of starlings (*Sturnus vulgaris*).

These isolations confirm, in a dramatic manner, the opinion expressed by others that roosting birds may play important roles in the epidemiology of urban histoplasmosis. They do not support the contention that pigeons are important in the maintenance of *H. capsulatum* in soil, although it has been well known since 1955 that virulent strains of *Cryptococcus neoformans* are commonly present in accumulations of pigeon droppings in both urban and rural areas.

REFERENCES

(*1*) Emmons, C. W.: Isolation of *Histoplasma capsulatum* from soil. Pub. Health Rep. 64: 892–896, July 15, 1949.

(*2*) Emmons, C. W., and Campbell, C. C.: Histoplasmosis in the District of Columbia, Maryland and Virginia. Clin. Proc. Children's Hosp. 13: 225–235, November 1957.

(*3*) McLeod, J. H., Emmons, C. W., Ross, S., and Burke, F. G.: Histoplasmosis. J. Pediat. 28: 275–295, March 1946.

(*4*) Emmons, C. W., et al.: Histoplasmosis: proved occurrence of inapparent infection in dogs, cats and other animals. Am. J. Hyg. 61: 40–44, January 1955.

(*5*) Olson, B. J., Bell, J. A., and Emmons, C. W.: Studies on histoplasmosis in a rural community. Am. J. Pub. Health 37: 441–449, April 1947.

(*6*) Emmons, C. W.: Isolation of *Cryptococcus neoformans* from soil. J. Bact. 62: 685–690, December 1951.

(*7*) Emmons, C. W.: Saprophytic sources of *Cryptococcus neoformans* associated with the pigeon (*Columba livia*). Am. J. Hyg. 62: 227–232, November 1955.

(*8*) Emmons, C. W.: Prevalence of *Cryptococcus neoformans* in pigeon habitats. Pub. Health Rep. 75: 362–364, April 1960.

(*9*) Emmons, C. W.: Association of bats with histoplasmosis. Pub. Health Rep. 73: 590–595, July 1958.

(*10*) Zeidberg, L. D., Ajello, L., Dillon, A., and Runyon, L. C.: Isolation of *Histoplasma capsulatum* from soil. Am. J. Pub. Health 42: 930–935, August 1952.

(*11*) Grayston, J. T., Loosli, C. G., and Alexander, E. R.: The isolation of *Histoplasma capsulatum* from soil in an unused silo. Science 114: 323–324, Sept. 28, 1951.

(*12*) Grayston, J. T., and Furcolow, M. L.: The occurrence of histoplasmosis in epidemics—epidemiological studies. Am. J. Pub. Health 43: 665–676, June 1953.

(*13*) Hazen, E. L., Little, G. N., and Mordaunt, V.: Isolation of *Histoplasma capsulatum* from two natural sources in the Mohawk Valley; one the probable point source of two cases of histoplasmosis. Am. J. Pub. Health 46: 880–885, July 1956.

(*14*) Hosty, T. S., et al.: A small outbreak of histoplasmosis. Am. Rev. Tuberc. 78: 576–582, October 1958.

(*15*) Lazarus, A. S., y Ajello, L.: Aislamiento de *Histoplasma capsulatum* del suelo de una cueva en el Peru. Rev. med. exper. Lima 9: 5–15 (1955).

(*16*) Campins, H., Zubillaga Z., C., Gómez López, L., y Dorante, M.: Estudio de una epidemia de histoplasmosis en el Estado Lara, Venezuela. Gac. méd. de Caracas 62: 85–109 (1955).

(*17*) Alarcón, D. G.: Histoplasmosis pulmonar epidemica. Gac. méd. Mexico 87: 745–750, Octubre 1957.

(*18*) Murray, J. F., et al.: Benign pulmonary histoplasmosis (cave disease) in South Africa. South African M. J. 31: 245–253, March 1957.

(*19*) Montemayor, L., Heredia Osio, B., y Bellard Pietri, E. P.: Aislamiento del *Histoplasma capsulatum* en el suelo de dos cavernas en Venezuela. Rev. san. y asis. social. 23: 39–54, Abril 1958.

(*20*) Gonzalez Ochoa, A.: Histoplasmosis primaria pulmonar aguda en la republica Mexicana. Rev. d. Inst. salub. y. enferm. trop. (Méx.) 19: 341–350, Diciembre 1959.

(*21*) Ajello, L: *Histoplasma capsulatum* soil studies. Mykosen. 3: 43–48 (1960).

(*22*) Aronson, D. L., and Edwards, P. Q.: An urban focus of histoplasmin sensitivity. Am. Rev. Tuberc. 79: 83–86, January 1959.

(*23*) Larsh, H. W.: Natural and experimental epidemiology of histoplasmosis. Ann. New York Acad. Sc. 89: 78–90, Aug. 27, 1960.

(*24*) The APHA conference report. Histoplasmosis epidemic attributed to starlings. Pub. Health Rep. 76: 150, February 1961.

(*25*) Whitehouse, W. M., Davey, W. N., Engelke, O. K., and Holt, J. F.: Roentgen findings in histoplasmin-positive school children. J. Michigan M. Soc. 58: 1266–1269 (1959).

(*26*) Christy, A., and Peterson, A. J.: Pulmonary calcification in negative reactors to tuberculin. Am. J. Pub. Health 35: 1131–1147 (1945).

(*27*) Palmer, C. E.: Geographic differences in sensitivity to histoplasmin among student nurses. Pub. Health Rep. 61: 475–487, Apr. 5, 1946.

(*28*) Edwards, L. B., Peeples, W. J., and Berger, A. G. Prevalence of sensitivity to tuberculin and histoplasmin among high school students in Montgomery County, Maryland. Pediatrics 21: 389–396, March 1958.

(*29*) Furcolow, M. L.: Recent studies on the epidemiology of histoplasmosis. Ann. New York Acad. Sc. 72: 127–164, Apr. 10, 1958.

(*30*) Sabin, A. B.: An epidemic of miliary granulomatous pneumonitis caused by *Histoplasma*. *In* Proceedings of the Conference on Histoplasmosis, 1952. PHS Publication No. 465 (Public Health Monogr. No. 39). Washington, D.C., U.S. Government Printing Office, 1956, pp. 20–23.

(*31*) Heller, S., McLean, R. A., Campbell, C. C., and Jones, I. H.: A case of coexistent nonmeningitic cryptococcosis and Boeck's sarcoid. Am. J. Med. 22: 986–994 (1957).

(*32*) Kier, J. H., Campbell, C. C., Ajello, L., and Sutliff, W. D.: Acute bronchopneumonic histoplasmosis following exposure to infected garden soil. J.A.M.A. 155: 1230–1232, July 31, 1954.

Directory of Parent Education Specialists

A directory of at least 500 persons professionally identified with the field of parent education through their work, position, title, special training, or publications is being compiled by Dr. Armin Grams of the Institute of Child Development of the University of Minnesota and Muriel Brown of the Children's Bureau. The Bureau expects to publish this "Directory of Specialists in Parent Education" in the fall of 1961.

An extensive list of names has been acquired, but the editors hope, if possible, to include all those eligible. Persons who wish to know more about the project are invited to write Dr. Armin Grams, Institute of Child Development, University of Minnesota, or Miss Muriel Brown, Division of Research, Children's Bureau, Department of Health, Education, and Welfare.