Association of Bats with Histoplasmosis

CHESTER W. EMMONS, Ph.D.

TWO YEARS ago, a family moved into an old frame dwelling in a rural neighborhood near Clarksburg, Md. Shortly thereafter several members of the family became ill with histoplasmosis. The number of bats living in the attic of the house, the quantity of bat guano in the attic and on the ground adjacent to the foundation walls, and the isolation of *Histoplasma* repeatedly from all four sides of the house out to a distance of 5 feet suggested the source from which one fatal and several clinical illnesses in the family were acquired. A clinical report of this outbreak will be made separately.

The environmental conditions associated with this family epidemic may be significant in explaining the epidemiology of certain cases of histoplasmosis occurring in rural families. They may be especially significant in explaining the puzzling distribution of histoplasmin sensitivity and cases of histoplasmosis in certain towns and cities where there is no known association with chickens and where saprophytic sources of *Histoplasma* have not yet been detected.

The saprophytic growth of *Histoplasma* in soil is so frequently associated with chicken excreta that it receives the immediate attention of the epidemiologist confronted by a case of histoplasmosis. The patient is questioned carefully concerning the presence of chickens in his environment and the extent of his exposure to them. Any episode involving removal of litter and excreta from a chicken house, or its use as a fertilizer on garden or lawn, is at once assumed to have provided the effective exposure. Laboratory isolation of *Histoplasma* from the material under suspicion often strengthens this assumption. However, a preoccupation with the patient's exposure to chicken excreta may lead to oversight of other associations which can be equally important but which have not received general recognition.

Zeidberg and associates (*1*) first called attention to the association between chickens and the saprophytic growth of *Histoplasma* in soil fertilized by their excreta. The first isolation of *Histoplasma* from soil was from a specimen collected under a chickenhouse, although the building was not identified in the report (*2*). This association has been widely recognized and confirmed by many investigators. Many isolations of *Histoplasma* from sites not associated with chickens have been reported, however. These include the earthen floor of a meathouse, miscellaneous farm buildings and a dog pen (*3, 4*), an old unused silo (*5, 6*), river water (*7*), a storm cellar, an old water tower, an old chalk mine, soil under a residence, low moist woodland, an abandoned schoolhouse (*8, 9*), and a hollow tree trunk (*8–10*).

One recognized association has been with caves known to be sources of infection for speleologists, casual visitors, or persons working in caves in the mining of guano or collection of other materials. In 1948, Washburn, Tuohy, and Davis (*11*) reported pneumonitis occurring in persons entering a cave. It is not reported whether bats were present. They called this disease "cave sickness" and suggested that it was a new disease entity. The patients did not re-

Dr. Emmons is with the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Public Health Service. *W. R. Piggott, W. Mitchell, and W. B. Hill, Jr., also with this laboratory, provided technical assistance.*

act to histoplasmin or coccidioidin down to dilutions of 1:100 and 1:10, and no complement-fixing antibodies to these antigens could be shown in any of the 21 persons who became ill. On the basis of X-ray appearance, symptomatology, and one isolation of *Histoplasma* from the mouth of the cave, Grayston and Furcolow accepted this epidemic as histoplasmosis (*8*, *9*).

An epidemic of proved histoplasmosis occurred in persons entering a cave in Venezuela (*12*, *13*). Conversion to histoplasmin sensitivity and significant complement fixation titers were demonstrated, and *Histoplasma* was isolated from 4 of 5 soil samples taken. The cave sheltered a large colony of bats.

Cases of histoplasmosis occurred in 46 persons entering caves sheltering bats in the Transvaal in South Africa (*14*). Animals placed in one cave were also infected, and *Histoplasma* was isolated from the tissues of these animals. Speleologists from the Cape of Good Hope who have not entered caves in the Transvaal have remained histoplasmin-negative.

A cave in Peru has been described as the source of histoplasmosis, formerly known locally as "fiebre de Tingo Maria" (*15*). This cave is inhabited by a nocturnal fruit-eating bird (*Steatornis caripensis*), sometimes called the "oil bird" because of the oil obtained from its fat, which is highly prized by residents of the area. Many susceptible persons entering the cave to collect these birds have developed a pneumonitis which is now recognized as histoplasmosis. *Histoplasma* was isolated from soil in the cave, and its presence there was attributed to enrichment of the soil by droppings of *S. caripensis*. However, it is known that bats also inhabit the cave. Grayston and Furcolow, in reviewing epidemics of histoplasmosis (*8*), mention a personal communication from Dr. E. Englert, Jr., who observed pneumonitis in persons who scraped quantities of bat dung from the timbers of a bridge in Maryland.

Methodology

The methods used in the present study for isolating *Histoplasma* from soil were those described in 1954 (*3*), and were only slight modifications of the methods used in the original isolation of *Histoplasma* from soil in 1949 (*2*). Soil specimens were scooped up directly into sterile 25 by 50 mm. pyrex test tubes and the cotton stoppers were replaced to permit drying of the specimens. If the specimen is sealed, as by a screwcap top, the humidity of even a moderately dry soil encourages germination of fungus spores, germination of weed seeds, and activity of microfauna with a resultant rapid change in the microflora.

Immediately upon return to the laboratory, 10 to 15 ml. of the specimen was removed to another 25 by 150 mm. tube, 0.8 percent sodium chloride solution was added nearly filling the tube, a sterile rubber stopper was inserted, and the suspension was shaken vigorously for a half minute. The suspension was allowed to sediment for 10 to 15 minutes and 5 to 10 ml. of the supernatant was removed by pipette to a conical glass or small beaker. For each ml. of the supernatant, 0.25 ml. of an antibiotic solution (2 mg. streptomycin and 5 mg. penicillin per ml. water) was added and 1 ml. of this inoculum was injected intraperitoneally into each of 5 or 10 Swiss, white, general-purpose mice. The mice were killed after 4 weeks and cultures were made from liver and spleen. In the specimens with a high fecal content, it was sometimes necessary to increase the amount of antibiotic or to treat the inoculated mouse with antibiotics the next day. The remainder of the original soil suspension was held at room temperature for reexamination if necessary.

The House and Its Environs

The repeated isolation of *Histoplasma* from soil adjacent to the foundation of a bat-infested house near Clarksburg, Md., where several cases of histoplasmosis occurred, points to a relationship between this disease and the presence of the house bat.

My attention was brought to the Clarksburg episode when Dr. Donald Pohl, of the Children's Hospital in Washington, D. C., referred an infant with severe histoplasmosis to the Clinical Center of the National Institutes of Health in Bethesda, Md. The child had acquired a fatal infection shortly after its family had moved into the old house. Several of its siblings were also ill.

I first visited the premises with Dr. Pohl on

October 10, 1956. The only large building on the premises, where presumably the children were infected, was an old, 2-story frame residence (see illustration). The only other structures were a small shed 40 feet east of the house, which had been used many years before for chickens but was now a doghouse and storage shed, and a privy 150 feet southwest of the house.

The front dooryard consisted of bare, firm, clay soil, with a patchy lawn of bluegrass and other grass beginning about 15 feet from the house. The lawn east of the house sloped to the south and consisted of grass and weeds intersected by a bare soil pathway, deepened by erosion, extending nearly parallel to the east wall and 3–15 feet distant from it. The back dooryard to the south, except for small patches of grass and weeds, was bare for several feet adjacent to the house. Beyond an apple tree, which supported a child's swing, was a scattered accumulation of refuse where trash had been burned and ashes had been dumped. West of the house was a weedy grass lawn, sloping to the south.

The house was in poor repair, with loose siding and cornices containing apertures sufficiently large for the entrance of bats. One conspicuous irregular hole in the siding halfway up the east wall of the house was approximately 4 by 10 inches in size and showed evidence of frequent use. Bat dung was apparent on the ground adjacent to the solid stone and cement plastered foundation on all four sides of the house. It was especially abundant on the west side of the house where large quantities had fallen from the roosting sites of the bats in the attic and wall, and had accumulated between the exterior siding and the inner wall of the house. From this voluminous deposit, it had sifted out between the loose siding boards to the ground.

The family was aware that the house sheltered a colony of bats, and inspection of the attic revealed a large colony of the common brown bat or house bat, *Eptesicus fuscus*, and several bushels of bat guano. Sixteen bats were captured and brought to the laboratory where they were killed, measured, identified by the writer, and spleen, liver, and lung were cultured for fungi. In order to utilize more fully the

Histoplasma was isolated from soil adjacent to the stone and cement plastered basement wall on all four sides of this house. The hole in the east wall of the house and holes in the cornices were used by bats to gain entrance to the attic.

material, the brains were removed, pooled, and injected into mice in a test for rabies. No pathogenic fungus was isolated, and no Negri bodies were found by A. C. Faber who examined the mouse brains.

Isolations From Soil

When the premises were first visited in October, 28 soil samples were collected. Ten of these were adjacent to the doghouse and 18 were taken adjacent to the foundation of the house, the outside entrance to the basement, and inside the basement. As these latter samples were collected, we observed many bat droppings which had sifted out from accumulations under the loose siding or had fallen to the ground as bats entered the attic. Three of the ten samples taken adjacent to the doghouse were positive. *Histoplasma capsulatum* was isolated from 1 of 5 samples taken inside the cellar and from 11 of 13 samples taken adjacent to the foundation. It is noteworthy that positive samples were obtained from all four sides of the house.

On a second visit to the home on March 11, 1957, two samples were collected from soil adjacent to the foundation on the north side of the house. Both were positive.

On June 26, 1957, 24 hours after a 1-inch rain, 21 collections were made. The soil was

moist but well drained. Ten specimens were taken at approximately 1-foot intervals in a northerly direction from the north side of the house, across the front dooryard which was composed largely of bare, hard-packed earth, extending to the bluegrass sod lawn beneath an oak tree 25 feet from the house. *Histoplasma* was isolated from only one specimen collected adjacent to the foundation.

Cryptococcus neoformans was isolated adjacent to the foundation and from specimens taken 3 and 5 feet from the foundation. When this series of specimens was collected, expectorated sputum was observed on the moist soil at several points. The patches of sputum were avoided in collecting soil samples, but it was assumed that many other sputums, no longer visible, had been spit upon the yard adjacent to the front porch and elsewhere on this side of the house. The time interval since recovery from histoplasmosis of all surviving members of the household would preclude relating these sputums to pulmonary histoplasmosis, but they might relate to the isolation of *Cryptococcus* from these sites, where *Histoplasma* was not found.

Three specimens were taken adjacent to the foundation and at 1-foot intervals eastward from the northeast corner of the house; all were positive for *Histoplasma*. All five specimens taken at the foundation and at 1-foot intervals eastward from the southeast corner were positive. Of the three specimens taken near the foundation on the west side of the house *Histoplasma* was isolated from two, and all mice receiving the third specimen died within 24 hours.

Collections were made again on July 11, 1957, at a time when the soil was quite dry. Specimens were taken adjacent to the foundation and at approximately 1-foot intervals along three parallel lines running eastward. Among 10 specimens collected along the first line, only one adjacent to the foundation and one 1 foot from it were positive. Of 10 specimens along the second line, 4 from the sites marked at 0, 1, 2, and 3 feet and a fifth 6 feet from the foundation, were positive. Along the third line, 5 of 6 specimens taken adjacent to the foundation and out to a distance of 5 feet were positive.

Collections were made on August 22, 1957, when the soil was dry, and specimens were taken adjacent to the foundation and at approximately 15-inch intervals running eastward from the house foundation, most of these specimens being from underneath bluegrass sod. Of 10 specimens, only the 4 adjacent to the foundation and extending out to 3½ feet were positive.

Eighteen soil samples were collected on September 30, 1957, again adjacent to the northeast corner of the house and running eastward at intervals of 1 to 3 feet. The first 5 specimens, extending to 3 feet from the foundation, were positive for *Histoplasma*. *C. neoformans* was isolated from the sixth specimen, which was in a footpath 5 feet from the house. *Histoplasma* was isolated from one specimen 9 feet from the house.

It seems apparent from the sampling already done that *Histoplasma* can be isolated consistently from soil adjacent to the foundation of this house and that its ability to grow here is related to the presence of bat droppings. It is further remarkable that there is no apparent association with chickens or other birds. The precise interval since chickens had been kept on the premises was not known by the residents except that it had been many years before. It is further remarkable that, although most of the studies of the spatial relationship were made on the north and east sides of the house, *Histoplasma* was isolated on all four sides, including the south side which was somewhat shaded by an elevated porch and a tree and the west side which had no protection from the sun.

Since the study was begun, we have been comparing selected soil samples taken near the foundation and positive for *Histoplasma* with samples taken at a distance of several feet and from which *Histoplasma* was not isolated. The soil is acid (pH 5.3–6.7) without consistent or significant differences in pH reactions between positive and negative soils. Unweathered bat dung has a pH reaction of 6.2 to 7.1. To date, although several hundred fungi have been isolated, we have not recognized significant differences in the microflora of positive and negative specimens. Sampling will be continued throughout 1958 in an investigation of the extent to which distribution of *Histoplasma* is limited on these premises and in a further search for seasonal differences, for histoplasmosis in

bats, and especially for micro-organisms which may either stimulate or inhibit *Histoplasma* in the highly competitive environment of soil.

Conclusion

The special significance of this study relates to a possible explanation of the prevalence of histoplasmin sensitivity and the occurrence of clinical cases of histoplasmosis in certain urban areas where the sources of infection are not yet recognized. It is a well-known habit of the brown bat (*E. fuscus*), sometimes called the "house bat," to live in crevices and attics of old houses, and "it is often seen about street lights in large cities where it finds congenial habitation in dark nooks in the roofs or in accessible crannies in the buildings" (*16*). Solitary individuals or pairs may roost throughout the active season behind the shutters of residences and seek shelter for hibernation in more protected spaces in the same or neighboring buildings or in caves in the winter. Fairly large colonies may inhabit the false fronts of shops or store buildings of an architectural style popular 50 years ago. The house bat is urban as well as rural in its choice of residence.

In view of the findings reported here, *Histoplasma* should be sought in soil samples adjacent to any buildings infested or colonized by bats in towns where histoplasmin sensitivity is observed under circumstances not readily explained by known exposures to the litter of chicken houses or other commonly recognized sources. It should be sought in towns where there is a differential histoplasmin-reactor rate, especially if the higher incidence of reactors is in an older part of town. Large old houses, even if in good repair, offer more crevices and shelters for bats than the small compact houses of new real estate developments.

This remarkable association of bats with a severe family epidemic of histoplasmosis and with the presence of *Histoplasma* adjacent to the foundation of the house may not be unique. Enrichment by bat feces of soil near a building infested by the house bat appears to be as effective in supporting the saprophytic growth of *Histoplasma* as bat guano is generally supposed to be in those caves associated with cases of histoplasmosis. The wide distribution of the house bat and its intimate association with man and human dwellings may well support a widespread reservoir of *Histoplasma* in soil to which man is frequently exposed.

Summary

Histoplasma capsulatum was isolated from 50 of 105 soil samples from 6 collections made between October 1956 and September 1957 on the premises where a family epidemic of histoplasmosis had occurred. Chickens, often associated with the saprophytic growth of this fungus, had not been kept on the premises for many years.

Histoplasma was isolated from 45 of 66 soil specimens (68 percent) taken adjacent to or within 5 feet of the foundation wall of the house and from only 2 of 29 (6.8 percent) taken 6–18 feet from the house. Three of ten samples taken near a doghouse were also positive.

The house sheltered a colony of the brown, or house, bat (*Eptesicus fuscus*), and bat dung was found adjacent to the foundation. The presence of bats is the apparent factor responsible for the constant saprophytic infestation of soil on these premises with *H. capsulatum*.

The house bat inhabits suitable shelters in towns and cities, as well as rural areas, and may be the ecologic factor responsible for the presence of *Histoplasma*, prevalent histoplasmin sensitivity, and clinical histoplasmosis in observed instances of urban histoplasmosis in towns and cities where reasons for the occurrence of these phenomena have not yet been determined.

REFERENCES

(*1*) 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.

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

(*3*) Emmons, C. W.: The significance of saprophytism in the epidemiology of the mycoses. Tr. New York Acad. Sc., Ser. II, 17: 157–166, December 1954.

(*4*) 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.

(5) 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.

(6) Loosli, C. G., Procknow, J. J., Tanzi, F., Grayston, J. T., and Combs, L. W.: Pulmonary histoplasmosis in a farm family: a three year follow-up. J. Lab. & Clin. Med. 43: 669–695, May 1954.

(7) Gordon, M. A., Ajello, L., and Georg, L. K.: *Microsporum gypseum* and *Histoplasma capsulatum* spores in soil and water. Science 116: 208, Aug. 22, 1952.

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

(9) Furcolow, M. L., and Grayston, J. T.: Occurrence of histoplasmosis in epidemics; etiologic studies. Am. Rev. Tuberc. 68: 307–320, September 1953.

(10) 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.

(11) Washburn, A. M., Tuohy, J. H., and Davis, E. L.: Cave sickness. A new disease entity. Am. J. Pub. Health 38: 1521–1526, November 1948.

(12) 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 Carácas 62: 85–109 (1955).

(13) Campins, H., Zubillaga Z., C., Gómez López, L., and Dorante, M.: An epidemic of histoplasmosis in Venezuela. Am. J. Trop. Med. Hyg. 5: 690–695, July 1956.

(14) Murray, J. F., Lurie, H. I., Kaye, J., Komins, G., Borok, R., and Way, M.: Benign pulmonary histoplasmosis (cave disease) in South Africa. South African Med. J. 31: 245–253, March 1957.

(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) Anthony, H. E.: Field book of North American mammals. New York, N. Y., G. P. Putnam's Sons, 1928.

Cerebrovascular Diseases Classified

All known types of brain "strokes" have been classified and defined for the first time in a 2-year study conducted by a committee appointed by the National Institute of Neurological Diseases and Blindness, Public Health Service. The committee, comprising eight of the Nation's leading physicians, was formed to explore ways of facilitating research in cerebrovascular diseases.

In addition to setting up a common language for the exchange of information of researchers, the classification serves as a useful tool in the treatment of "strokes." Precise diagnosis is imperative in new treatments for several types of brain blood vessel diseases, the study points out.

Nine major categories are set up in the classification, with changes in tissue the determining factor in the groupings. Two categories include the majority of cases: blocks in a blood vessel and rupture or hemorrhage of a blood vessel. Next important is temporary lack of blood without tissue death.

In emphasizing the pioneering character of the study, Dr. Clark H. Millikan, neurologist of the Mayo Clinic, Rochester, Minn., and committee chairman, said that many statements in the study may undergo modification as understanding of the diseases increases.

Copies of the study, A Classification and Outline of Cerebrovascular Diseases, which appeared in the May 1958 issue of *Neurology*, may be obtained from the National Institute of Neurological Diseases and Blindness, Public Health Service, Bethesda 14, Md.