# Notes

## A COMBINED FIELD AND LABORATORY EPIDEMIC OF HISTOPLASMOSIS

### Isolation from Bat Feces in West Virginia[1]

Attempts to isolate *Histoplasma capsulatum* from soil in West Virginia were conducted in the late winter of 1968 when a patient was seen at West Virginia University Medical Center with acute histoplasmosis of the epidemic type. Previous studies had shown the prevalence rate of histoplasmin reactors to vary from 0 to 40 per cent by county[2] with an approximate over-all average of 35 per cent.[3-5] Although sporadic cases of histoplasmosis had been seen throughout the state, *H. capsulatum* had not been isolated from soil in West Virginia.

The present report presents the results of the epidemiologic investigation related to the patient. The investigation resulted in 5 additional infections and in the first isolation of *H. capsulatum* from bat feces in West Virginia.

*Case 1*: A 34-year-old, white man was admitted to West Virginia University Medical Center on March 28, 1968, because of an abnormal chest roentgenogram. The patient was the foreman of a natural gas line maintenance crew, but because the winter had been unusually severe, his outdoor exposure during the preceding months had been limited. Three weeks earlier, he had experienced a flulike illness characterized by malaise, myalgia, fatigue, sweating, and increase of temperature to 103° F. He was admitted to a local hospital where a chest roentgenogram showed a diffuse, bilateral, miliary pattern of nodular infiltration. Therapy was started with a broad spectrum antimicrobial drug, and the fever remitted within 48 hours. The infiltrates, however, did not resolve after one week of therapy, and the patient was referred to West Virginia University Medical Center.

There were no abnormal physical findings on admission, and the patient felt nearly well except for fatigue. A chest roentgenogram revealed the previously described miliary infiltrations. Skin tests with intermediate strength PPD and histoplasmin antigens showed induration 25 mm in diameter at the site of injection of the histoplasmin after 48 hours. No reaction to PPD was present at 48 or 72 hours. Serum complement fixation titers using both yeast and mycelial phases of *H. capsulatum* antigen were increased to greater than 1:128.

*The epidemiologic investigation*: On April 9, 1968, 2 investigators met the patient in Jane Lew, West Virginia. The patient directed the investigators to each of seven sites at which his maintenance crew had dug to repair gas lines during the six weeks preceding the onset of his illness. Soil samples were taken at the sites of digging and then randomly in the vicinity of each digging. All sites were in or near open fields that were used either for pasture or for hay. Samples were also taken from an unused chicken house of a relative of the patient where he had kept some tools.

Approximately two weeks before the onset of his illness, the patient had helped a friend to renovate an unused building. The building was of concrete block construction and had not been in use for some 15 years. During this period, bats had roosted in the eaves of one wall. The patient indicated that in running electric wiring in the wall of the building, they had found the wall to be filled with bat manure.

The building was located on a side street in Jane Lew directly behind a grouping of stores on the main street and removed from farmland by approximately one block. At the time of the

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2 Andrews, C. E., Lawless, J. J., and Ruggers, J.: Amer. Rev. Resp. Dis., 1964, *89*, 409.

3 Jarvis, M. A., Chick, E. W., and Schwerba, J. J.: Arch. Environ. Health (Chicago), 1967, *14*, 668.

4 Stewart, W. C., Gustke, S., Carder, H., Stout, B. M., Chick, E. W., and Andrews, C. E.: W. Virginia Med. J., 1965, *61*, 189.

5 Bender, T. R., Chick, E. W., and Bauman, D. S.: W. Virginia Med. J., 1970, *66*, 191.

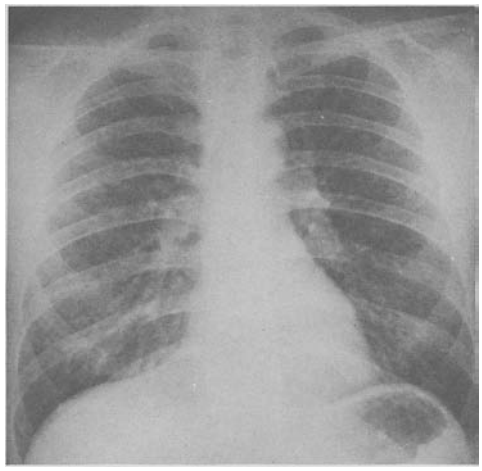

Fig. 1. Case 2. Chest film taken five days after onset of symptoms shows bilateral diffuse lesions in both lung fields.

investigation, the building was undergoing thorough cleaning, painting, and wiring; however, the opening in the wall through which the electric wiring was run, as indicated by the patient, was still open to inspection via a trap door in the floor. The opening in the wall for the wiring was approximately six inches in diameter and considerable amounts of bat manure could be observed within the wall. A number of samples of the bat manure were taken by the investigator. During the collection of the soil samples the owner of the building, in an effort to be helpful, ran a stick into the opening and raked forward the manure, raising an obvious and considerable cloud of dust before he could be stopped by the investigator. In addition, soil samples were taken from a garden where some of the bat manure had been placed several weeks before.

TABLE 1

RESULTS OF ISOLATION STUDIES FOR *HISTOPLASMA CAPSULATUM* FROM SOIL, A CHICKEN HOUSE, AND A BAT ROOST

| Site | Mouse Inoculation (+/−)* |
|---|---|
| Left side road/McWhortle, old well site | 0/5 |
| Right side road/McWhortle, old well site | 0/5 |
| Ben's Run gas line | 0/5 |
| Ben's Run — under trees near stream | 0/5 |
| Lit Burn No. 1 | 0/5 |
| Lit Burn No. 2 | 0/5 |
| Lit Burn No. 3 | 0/5 |
| Chicken house | 0/5 |
| Wall of renovated building (bat roost) | 2/5 |
| Dirt from wall of renovated building taken to garden | 0/5 |

*+ = *Histoplasma capsulatum* present; − = *H. capsulatum* not present.

*Case 2*: Ten days after the field trip, one of the investigators became acutely ill with chills, fever, and progressive malaise. Pertinent physical findings at the time of hospitalization at West Virginia University Medical Center two days after onset of the illness were temperature of 102° F, warm, flushed skin, and slight enlargement of the spleen. A chest film revealed multiple fluffy infiltrates throughout both lung fields. Previously nonreactive to histoplasmin, he became reactive. The yeast phase complement fixation titer increased to 1: 16. It was assumed that acute histoplasmosis had developed, and he was allowed to return home after 24 hours. He continued on bed rest, aspirin, and Darvon®,[6] The fever, cough, chest discomfort, and malaise continued for approximately one week and then slowly decreased over two and one-half weeks.

*Case 3*: The patient was standing across the room from the site of sampling of the bat manure. Approximately 14 days later, lethargy, malaise, and low grade fever developed. His skin test became reactive, and the yeast phase serum complement fixation titer increased to 1:8. The patient remained at home partially confined to bed for approximately one week and then returned to school on limited activity during the next three weeks.

*Isolation studies*: All soil and bat manure specimens were treated similarly. Five g of each specimen were placed in 50 ml of saline containing 10,000 units of penicillin, 1,000 μg of streptomycin, and 0.05 mg of chloramphenicol per ml of saline. The mixture was thoroughly shaken and allowed to settle. One-milliliter aliquots of the supernatant were injected intraperitoneally into 5 albino mice. After four weeks, the mice were killed, and the livers and spleens were removed and homogenized. The homogenates were planted on Sabouraud 0.5 per cent dextrose agar, using five plates per homogenate.

None of the samples of soil yielded *H. capsulatum*. Samples of the bat manure, however, did yield *H. capsulatum* (table 1). Cultures showed typical morphologic features in both mycelial and yeast phases, except that an un-

[6] Mention of commercial concerns or products does not constitute endorsement by the U.S. Public Health Service.

TABLE 2

RESULTS OF SKIN TESTS, SEROLOGIC TESTS, AND ONSET OF ILLNESS FOR EACH PATIENT

| Patient* | Skin Test | Yeast CF | Mycelial† ID | Onset of Illness |
|---|---|---|---|---|
| 1 | 3/28/68 | 1:128 | (H, M+) | 3/7/68 |
| 2 | Periodic tests over past 15 years: nonreactive | | | |
| | 4/19/68 + | 1:16 | ND | 4/16/68 |
| 3 | 4/22/68 + | 1:8 | ND | 4/21/68 |
| 4 | 3/15/68 – | | | None |
| | 10/22/68 + | 1:8 | 10/22/68 (M+) 3/14/69 | |
| 5 | 3/17/68 – | | | |
| | 10/28/68 + | ND | 10/22/68 (M+) | None |
| 6 | 9/27/68 – | | | |
| | 3/14/69 + | ND | 3/14/69 (H, M+) 5/23/69 | 3/69 |

Definition of symbols and abbreviations: CF = complement fixation; ID = immunodiffusion; ND = not done; + = reactive; – = nonreactive; H = "H" band; M = "M" band.

*Animal studies on soil samples began April 11, 1968.

†Immunodiffusion tests were performed using patient's serum undiluted and HKC-43 histoplasmin concentrated 10 times before use.

usually high number of tuberculate macroconidia and microconidia were observed on each colony.

*Cases 4 to 6*: This strain of *H. capsulatum* was studied intensively during the several months after isolation and also was used in some experimental procedures. During this time, 3 persons (Cases 4 to 6) working with this isolate in the laboratory became reactive to histoplasmin (table 2). Two showed one band, and one person showed two bands on immunodiffusion tests. Only one had clinical symptoms (Case 6). He had mild fever, malaise, and cough for approximately three weeks but did not seek medical attention. All had normal chest films.

*Follow-up studies*: Approximately one year later, samples were again obtained from the site of the first isolation. At this time, the building was in constant use. Holes were drilled in the base of the wall, and samples of remaining bat manure were obtained.

Holes were then drilled in each concrete block at the top of the wall. Forty per cent formalin and potassium manganate were introduced through the holes into the wall. The holes were then plugged. Repeat samples were taken from the base of the wall one hour after treatment.

The samples were prepared as before and injected into ten mice. None of the samples yielded *H. capsulatum*.

* * *

The present investigation resulted in the first isolation of *H. capsulatum* from natural sources in West Virginia. It is of interest that the source was the concrete block wall of a previously abandoned building containing abundant bat manure. No cases other than those presented could be associated with this site.

Skin test surveys in West Virginia have revealed an over-all reactor rate of between 35 and 40 per cent. Rather wide variations within this rate, however, have been observed among counties. Further isolation studies are needed to determine the prevalence of *H. capsulatum* under natural conditions in these areas.

The strain of *H. capsulatum* isolated from the source exhibited a high degree of infectivity. The investigator obtaining the samples from the wall obviously breathed in a number of spores from the dust cloud raised by the owner. The second investigator standing across the room had significantly less exposure but obvious clinical symptoms still developed. Three persons working in the laboratory at the time this strain was isolated and studied became reactive

to histoplasmin and serologic responses to histoplasmin developed despite strict precautions.

It is of interest that repeat sampling of the site approximately one year later yielded no *H. capsulatum*. During this time, however, the wall had been sealed and painted, the bats chased away, and the building had been used constantly. Formalin was placed in the wall at the time of this sampling to sterilize the area. Because the initial cultures yielded no fungi, its effectiveness was unknown.

*In summary*: The first isolation of *Histoplasma capsulatum* in West Virginia was obtained from bat manure in an abandoned building. The investigation resulted in demonstrations of three methods of human infection: inhalation of fungus by natural or accidental circumstances (one person), inhalation during collection of soil samples (2 persons), and inhalation while working with cultures in the laboratory (3 persons).

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## ACCELERATED LUNG MATURATION AND INCREASED SURVIVAL IN PREMATURE RABBITS TREATED WITH HYDROCORTISONE[1]

The ability to alter selectively the maturational rate of an organ system within a developing fetus was demonstrated in the classic studies of Moog.[2] Buckingham and associates[3] first suggested that Moog's conclusions might have applicability to the developing lung. Acting on observations of Liggins,[4] deLemos and co-workers[5] demonstrated that treatment of fetal lambs with hydrocortisone accelerated the appearance of pulmonary surfactant. Extending these observations in fetal rabbits, Kotas and Avery[6] and Wang and associates[7] found that over-all lung maturity, as defined by accelerated morphologic development, increased pulmonary surfactant, increased aeration at low deflation pressures (stability), and increased aeration at maximal distending pressure, was increased with steroid treatment.[6,7] Because it has been suggested that surfactant deficiency and pulmonary immaturity might be causally related to the respiratory distress syndrome (hyaline membrane disease) in premature infants,[8,9] these studies hint at a new mode of treatment for this disease.

Motoyama and co-workers[10] confirmed the

[1] This work was supported by the Medical Research Council of Canada and the American Thoracic Society.

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[7] Wang, N., Kotas, R., Avery, M. E., and Thurlbeck, W.: J. Appl. Physiol., 1971, *30*, 362.

[8] Boughton, K., Gandy, G., and Gairdner, D.: Arch. Dis. Child., 1970, *45*, 311.

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[10] Motoyama, E. K., Kikkawa, Y., Orzalesi, M., Kaibara, M., Wu, B., Zigas, C., and Cook, C.: Pediatrics, 1971, 48, 547.