

Isolation of *Histoplasma Capsulatum* from Soil in Mississippi County, Arkansas

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Histoplasma capsulatum is believed to infect 50,000 people a year in the United States. The source of infections appears to be soil or areas related to bird roosts (Furcolow, 1960).

According to epidemiological studies, the endemic area for histoplasmosis is throughout the Mississippi-Missouri and Ohio river valleys. Mississippi County was chosen for a soil survey because of its proximity to the Mississippi River and the center of the endemic area. Also, according to C. E. Palmer (personal communication), 89% of the Navy recruits from this county show positive histoplasmin reactions. This survey was primarily to determine if *H. capsulatum* could be found in the soil of this county.

Mississippi County is located at the extreme northeastern edge of Arkansas and is bounded on the north by Missouri and Tennessee on the east. The county has an area of 932 sq miles and a population of 70,174. Because of the alluvial sediment deposited by the Mississippi River, Mississippi County has some of the richest and deepest top soil in the world. This fact accounts for the predominance of agriculture in the county.

There are several soil types in Mississippi County. Sand, loam and "gumbo" (gray clay) or mixtures of these can usually be found in each sq mile.

The weather in this area of Arkansas is hot and humid in the summer; the average temperature is 27.2 C in summer and 5.5 C in the winter. The annual average temperature is 16 C. The minimum winter low expected is from -18 to -12 C and maximum summer high expected is 32-37.8 C. Rainfall from January first to collection date, 1964, was 29.51 inches. The mean temperature for days on which soil was collected was 19 C, with daytime average of 25.5 C and low of 12.2 C.

One hundred and twenty-two soil samples were collected in late May and early June from 39 sites throughout Mississippi County. Thirty-one samples were taken from areas behind the levee which has an annual overflow from the Mississippi River. Such areas as goat pens, pigeon roosts, cypress swamps, open fields, open virgin land, pig pens, old barnyards, old chicken houses and bayou areas were selected for sampling sites.

Nine samples were taken from a crane roosting area of about 10 acres which was damp, clay base soil covered with crane excrement and protected by trees.

Six samples were taken from two residential sites where one occupant had died of histoplasmosis and the other was a known positive reactor to histoplasmin.

Samples were taken at no greater than 4-in depth. They were placed in paper bags and then in plastic bags which were tied to prevent loss of moisture. All bags were identified and dated. The spade used for collection was carefully cleaned following each sampling. Samples were immediately boxed for shipment to the laboratory. All soils were stored at 25 C until processing could be continued.

The soils were passed through $\frac{1}{2}$ -in mesh screen wire to remove all large extraneous material. Each sample was then mixed thoroughly by hand. Ten-gram samples of each soil were removed and placed into 100-ml sterile physiological saline. This suspension was mixed for 15 min on a magnetic stirring device, then allowed to stand for 1 hr. One milliliter of each supernatant solution was injected intraperitoneally into each of 10 white female Swiss mice weighing 20-25 g. Two weeks later, the mice were sacrificed. The liver and spleen of each mouse were homogenized and 0.5 ml of the homogenate plated onto each of 4 Sabouraud's dextrose agar plates. All plates were incubated at 27 C for 4 weeks before final readings for growth of *H. capsulatum* were made.

Soil pH values were determined by mixing 5.0 ml deaminized water with 5.0 g soil, allowing the sludge to stand in covered beakers overnight and reading using a Beckman Zeromatic pH meter.

In this study, 1200 mice were inoculated; 1142 went to autopsy. *Histoplasma capsulatum* was isolated from 6 of the 120 soil samples tested, or 5% recovery. The organism was found in 36% of the soils collected from chicken houses; 33% of barn soils collected and 33% of cultivated areas of old barn sites. No isolations were made from 31 soil samples collected from the overflow area behind the levee. Two isolations of *H. capsulatum* were made from the site where the occupant died of histoplasmosis and one recovery from the site occupied by the histoplasmin reactor.

The pH of collected soil ranged from 4.3-8.2. Five soils containing *H. capsulatum* were found in the soils with pH 5.0-5.9 and one isolation made from soil in the 7.1-7.9 pH range. Table I shows a breakdown of pH ranges and the number of isolations of *H. capsulatum*. It is estimated that 24% of the soil in Mississippi County has a pH of 5.0-5.9.

Table II shows soil pH, collection site and soil type for those samples from which *H. capsulatum* was isolated. Note that all positive isolations were from barns or chicken houses; however, not all barns and chicken houses checked produced positive isolations and other samples taken in close proximity to the positive sites gave negative findings. All 6 positive soils were of the Sharkey-Clay or Sharkey-Clay associated soil type. A quote from the description of this type soil by the U.S. Department of Agriculture, Soil Conservation Service follows: "The Sharkey series comprises dark, poorly drained, slightly acid to alkaline alluvial soils of the Mississippi River flood plain. These soils are derived from fine textured slack water deposits. These soils are sometimes overlain by sandy loams and loamy sands ranging in depth from 6-24 inches. The Sharkey soils are of moderate extent, are widely distributed and are of considerable agricultural importance in this county."

TABLE I. DISTRIBUTION OF SOILS POSITIVE FOR *Histoplasma capsulatum* AND SOIL PH

pH	No. of Samples	No. Positive
4.3-4.9	3	0
5.0-5.9	29	5
6.0-6.9	53	0
7.0	7	0
7.1-7.9	25	1
8.0-8.2	5	0

TABLE II. COLLECTION SITES ASSOCIATED WITH POSITIVE ISOLATIONS OF *Histoplasma capsulatum*.

Sample No.	Collection Site	pH	Soil Type	No. Mice Autopsied	No. Mice Positive
3	Old barn area, cultivated	7.2	Mhoon-Sharkey	10	1
20	Chicken house	5.4	Sharkey-Tunica-Mhoon Association	9	4
51	Chicken house	5.9	Sharkey-Crevasse	8	8
101	Chicken house	5.8	Mhoon-Sharkey	9	1
102	Chicken house	5.5	Mhoon-Sharkey	9	2
106	Inside barn	5.6	Mhoon-Sharkey	9	3

Table III shows the number of samples made from 4 general soil groups in shaded and open areas and the number of isolations from each group. Four of the 6 positive isolations came from shaded sites and all positive isolations came from loamy and loam-humus type soil.

A total of 122 soil samples were collected of which 6 were found to contain *H. capsulatum* when injected intraperitoneally into white Swiss mice. Five of 6 positive samples showed pH values of 5.0-5.9. All positive soils came from one general soil type, clay subsoil with sandy and silt loam top soil.

These data do not indicate widespread soil infestation in Mississippi County by *H. capsulatum* but perhaps point sources of fungal growth. Definite conclusions concerning soil types of pH range in relation to the

TABLE III. GENERAL TYPE SOILS ASSOCIATED WITH THE ISOLATION OF *Histoplasma capsulatum*

Soil Type	Number of Samples Collected			
	Shaded Sites		Open Sites	
	Total	No. Positive	Total	No. Positive
Loam	38	1	34	2
Sand	5	0	13	0
Clay	10	0	11	0
Humus	8	3	3	0

recovery of *H. capsulatum* cannot be drawn; however, the sandy and silt loams of the Sharkey soil type appear to be most suspect.

Further isolation and comparative studies in this county are indicated.

LITERATURE CITED

- Furcolow, M. L. 1960. *Epidemiology of histoplasmosis*. In *Histoplasmosis*, edited by H. C. Sweany. Charles C. Thomas, Springfield, Ill. p. 113-148.