Isolation of *Histoplasma capsulatum* from Soil*

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FOR 40 years after its discovery by Darling,[1] histoplasmosis was a rare medical curiosity and thought to be of too little public health significance to excite the interest of the epidemiologist. It was only after Christie and Peterson[2] had demonstrated the common occurrence of human infection with *Histoplasma capsulatum* that attention began to be centered on the source of the agent in nature. The fungus has been recovered from human beings, animals,[3] soil,[4-6] and river water.[7] Man, although apparently one of the most frequently infected hosts, does not appear to transmit the organism to other human or animal contacts. At least there is no conclusive proof of such transmission. Although animals have been found to harbor the specific fungus, there is no evidence to indicate that they are the reservoirs, rather than the victims, of the organism. The almost universal distribution of many of the animal species known to be susceptible hosts, in contrast to the sporadic geographic occurrence of histoplasmosis, makes it highly improbable that animals are the natural sources of *H. capsulatum*. The presence of the fungus spores in river water probably results from the washing of soil into the river during heavy rains. Soil appears, therefore, to be the most likely source of the fungus in nature. It is in the soil that one day may be found the solution to the mystery of the peculiar geographic distribution of histoplasmin sensitivity.

SOIL STUDIES

The Williamson County Tuberculosis Study undertook the epidemiologic investigation of histoplasmosis in 1945 because of the high prevalence of pulmonary calcification in the presence of negative tuberculin reactions among residents of the County[8] and because of the many similarities between tuberculosis and histoplasmosis. An extensive program of histoplasmin skin testing was initiated at first, in order to obtain information regarding the distribution of sensitivity in the population. Later, other epidemiologic factors came under investigation. The results of some of these studies have been reported previously.[9] A program for the mycological study of soil in the County was begun in July, 1950.

Plan—The homes of individuals who had been skin tested with histoplasmin were visited, and usually five samples of surface soil were obtained about the

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premises. In many instances house dust also was collected for study. Soil was taken from places where the residents showed a high prevalence of sensitivity to histoplasmin, as well as from places where few or none reacted to the antigen. Further selection was made to assure the collection of soil samples from every part of the County. Since there was no precedent to indicate the most likely sources of *H. capsulatum* in soil, other than around rat burrows,[4] samples were taken usually near the dwelling and in and around places where animals were concentrated, such as chicken houses, barnyards, and barns. Previous studies had indicated that a damp environment might be a factor in the prevalence of histoplasmin sensitivity,[9] so soil samples were collected near bodies of water whenever they were located reasonably near the dwelling.

At the time of the visit an epidemiologic investigation was made and data were recorded concerning the nature of the terrain around the residence (such as elevation and slope, etc.); evidence of dampness inside and outside the house; the presence or absence of shade trees; the character of the soil; the presence of bodies of water and their proximity to the house; and the kind and number of rodents and domestic animals about the place. The source of each soil sample was also recorded, and an identification number was assigned to each.

The soil specimens were shipped to the Mycology Laboratory of the Communicable Disease Center for study. The mycologist had no knowledge of the source of the soils. Results were reported by the identification number.

Isolation Method—Soil samples were collected by scooping soil into 4-ounce glass jars which, after sealing with plastic screw-caps and labeling properly, were mailed to the mycology laboratory. The samples were examined for the presence of *H. capsulatum* and other pathogenic fungi by using the method developed by Stewart and Meyer.[10] This procedure was later modified by Emmons and successfully used for the isolation of *Histoplasma* from soil.[4] After thorough mixing, a heaping teaspoonful of soil was placed in a 25 x 150 mm. test tube containing 30 ml. of physiological saline and was stirred vigorously with a glass rod. This suspension was allowed to stand for 1 hour, then 5 ml. of the supernatant were pipetted off, and 1 ml. aliquots were injected intraperitoneally into each of 4 mice. At the end of 2 weeks the mice were sacrificed, and 2 tubes of a neutral dextrose-peptone agar * were inoculated with portions of the liver and 2 tubes with portions of the spleen. The agar tubes were incubated at 25° C. and were examined at intervals during a period of 6 weeks.

Early in the course of the study this procedure was modified on the basis of findings by Strauss and Kligman,[11] who demonstrated that in fungus infections in mice, gastric mucin altered the agent-host relationship in favor of the fungus. Accordingly, 5 ml. of the soil supernatant were mixed with 5 ml. of 5 per cent gastric mucin,† and 1 ml. aliquots were injected into mice as described above. It was soon learned, however, that the use of mucin necessitated treating the mice for the first week with daily intraperitoneal injections of a mixture of streptomycin (1,000 units) and penicillin (12,500 units) to prevent lethal bacterial infections.

This procedure and some preliminary results were described previously by Ajello and Zeidberg in 1951.[5]

Results—By August, 1951, 299 soil, 18 chicken manure, and 35 house dust samples had been obtained from 70

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* Dextrose 1.0 gm.
Neopeptone 1.0 gm. adjusted to *p*H 7.0
Distilled water 100.00 ml.
Agar 2.0 gm.

† Granular mucin, Type 1701-W, The Wilson Laboratories, Chicago, Ill.

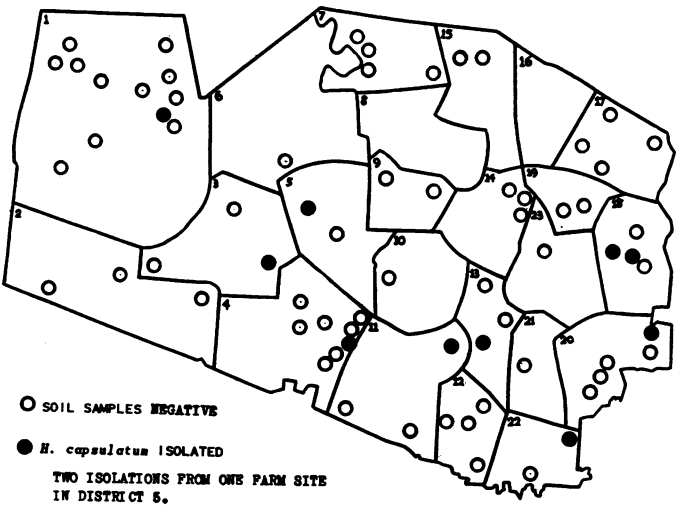

FIGURE 1—Location of 70 Premises from Which Soil Samples Were Obtained and Premises with *Histoplasma capsulatum* Isolated, Williamson County, Tennessee, July, 1950–August, 1951

premises distributed widely over the County (Figure 1). *H. capsulatum* was isolated from 11 samples of soil collected on 10 different places, concentrated in the southern and eastern part of the county (Figure 1). None of the samples of chicken manure or of house dust yielded the specific fungus.

The sources of the samples and the percentage of isolations of *H. capsulatum* from each source are shown in Table 1. Although soil was obtained from 9 different source categories, the fungus was isolated from only 3 of these: under dwellings, inside chicken houses, and from chicken yards.

The highest percentage of isolations was obtained from samples collected in chicken houses and chicken yards.* Of 51 specimens of soil taken from these 2 sources, the organism was found in 7 or 13.7 per cent. Of the remaining 248 samples, only 4 were positive (1.6 per cent). These differences are statistically significant. The possible importance of the association of chickens with the occurrence of *H. capsulatum* in soil will be discussed in the next section.

The influence of shelter came under consideration because it had been observed that most of the isolations were obtained from soil in protected sites. Ninety-two samples of soil were from sheltered places, such as inside chicken houses, underneath dwellings, and inside

TABLE 1

Results of Examinations of Soil Samples Collected from 70 Premises, by Sources of Samples, Williamson County, Tennessee, July, 1950–August, 1951

Source of Sample	*Number of Samples*	*H. capsulatum Isolated*	
		Number	*Per cent*
Total	299	11	3.7
Near house	93	–	–
Under house	51	4	7.8
Inside chicken house	37	5	13.5
Chicken yard, run, etc.	14	2	14.3
Barn yard	25	–	–
Inside barn	4	–	–
Bank of water course	45	–	–
In open	25	–	–
Other	5	–	–

* This association of chickens and *H. capsulatum* in soil was reported in a paper presented at the annual meeting of the Tennessee Veterinary Medical Association in Chattanooga, Tenn., January 15, 1952.

barns. Of these, *H. capsulatum* was isolated from 9 (10 per cent). Only 2 positive soils (1 per cent) were found among the 207 specimens from open places, but these 2 were from chicken yards. The association of chickens may in some way mitigate the effect of exposure.

STUDIES OF FOWL

The comparatively frequent isolation of *H. capsulatum* from the soil of chicken houses and yards, as noted above, led to a consideration of the role of domestic fowls in the occurrence and distribution of the fungus. Suggestive evidence of this association has appeared in the literature recently. Several instances are reported of the development of acute respiratory disease in individuals who were exposed to high concentrations of pigeon and chicken manure dust.[12-16] Although in none of these reports was *H. capsulatum* conclusively implicated as the etiologic agent, in some, the affected individuals developed relatively high histoplasmosis complement-fixation titers, strong skin test reactions to histoplasmin antigen, and subsequent extensive pulmonary calcification so commonly seen in areas of high prevalence of histoplasmin sensitivity. The occurrence of these cases in close association with the excreta of fowl was quite suggestive, particularly in the light of the findings in the present study.

At least three possibilities concerning the association of chickens and *H. capsulatum* in soil came to mind: either (1) chickens were the natural reservoir and contaminated soil by excretion of the fungus; or (2) chickens, if infected and diseased, were by the nature of their feeding habits victims of their close association with soil that was naturally the habitat of the fungus; or (3) chickens neither were infected by soil nor contaminated it, but provided nutrients for the growth of the fungus. Since chickens are among the commonest of domestic animals in the United States and in many parts of the world, it seemed unlikely that they could be the reservoir of the organism, otherwise the sporadic geographic distribution of infection with *H. capsulatum* would not obtain. The greater probabilities were either that they were infected by soil, or that they were merely contributors to the enrichment of the natural culture medium.

In order to determine whether chickens are susceptible to infection with the specific fungus, 2 hens were purchased from each of 6 places where *H. capsulatum* had been isolated. All 12 were tested with histoplasmin,* 0.1 ml. inoculated intradermally in the wattle. The first 8 were tested with the antigen in a dilution of 1–1,000, but full-strength histoplasmin was used on the remaining 4. All failed to react. The 12 hens were sacrificed, and cultures of organ tissue were made. All cultures were negative for *H. capsulatum.* These numbers are too small to draw any sweeping conclusions. The most that may be said is that thus far, on premises of proved occurrence of *H. capsulatum* in soil, hens that were closely associated with these soils showed no evidence of infection or disease. Eighty-nine chickens from several areas in Williamson County were tested with full-strength histoplasmin,† and only 2 were positive. These 2 were sacrificed and cultures were made of their internal organs. Cultures were negative for *H. capsulatum.*

Menges [17] tested 98 hens in an area of high prevalence of histoplasmin sensitivity and found only 1 reactor to full-strength antigen. Eighteen samples of hen manure obtained in the present study failed to yield *H. capsulatum* on mouse culture. It appears that fowls probably do not harbor the organism

* Lot 1, CDC Antigen.

† CT-189, Histoplasmin, Concentrated, supplied by Eli Lilly & Co., Indianapolis, Ind.

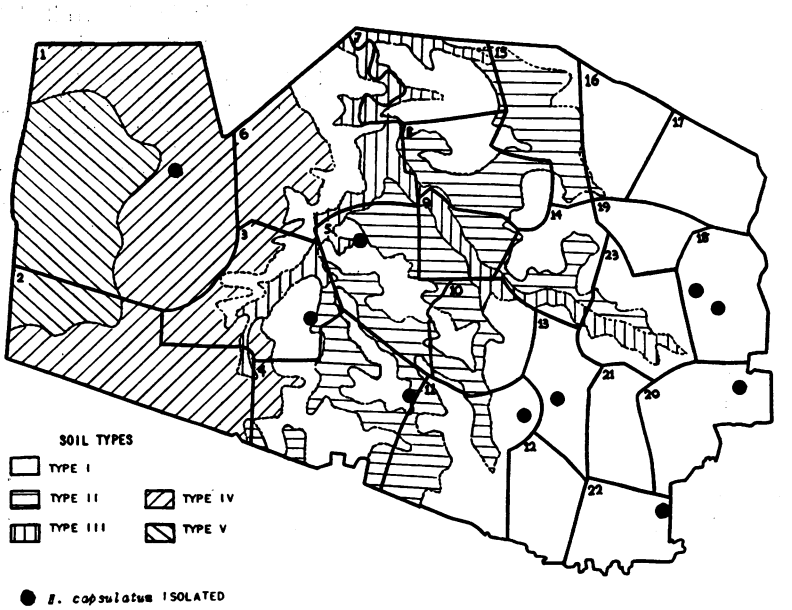

FIGURE 2—Distribution of Soil Types and Location of Premises Where *Histoplasma capsulatum* Was Isolated from Soil, Williamson County, Tennessee, July, 1950–August, 1951

and therefore cannot be directly responsible for its presence in soil.

RELATION OF SOIL TYPE AND THE PRESENCE OF *H. capsulatum* IN SOIL

Williamson County has 5 general soil types which are differentiated by variations in depth, *p*H, permeability, texture, organic and inorganic components, etc. The distribution of these types is shown in Figure 2.* The places from which *H. capsulatum* was isolated in the present study are also spotted on this map. In 10 instances the fungus was recovered from Type I soil, which is described as shallow, acid, of slow permeability, and low organic content. The only other isolation was made from Type IV soil, which differs from Type I chiefly in its high organic content. The other soils in the County are generally deeper, more alkaline, and of faster permeability. Although many samples have been taken from these other soils (see Figure 1), no isolations have been obtained thus far.

No definite conclusions may be drawn from these few observations. They do suggest, however, with what is known of the cultural requirements of fungi, that certain factors may operate to influence the effectiveness of soil as a culture medium for *H. capsulatum*. A shallow soil with slow permeability, particularly if ground slope is minimal, tends to retain moisture over prolonged periods of time in a climate where the rainfall is adequate and the humidity is high.

The acid character of the soil may also enhance fungal activity since it is known that many fungi decompose organic matter most effectively in an acid medium. *H. capsulatum* is known to grow over a wide range of *p*H in artificial culture media, but its preferential *p*H in nature is not known.

The occurrence of the fungus in a soil type with a characteristically low or-

* The original soil maps of the County were prepared by Douglas Moyers and C. H. Jent of the U. S. Dept. of Agriculture, Soil Conservation Service.

ganic content is, on the surface, somewhat contradictory in view of the known nutritional requirements for fungal growth. However, most of the positive soil samples were obtained from sites where chickens were concentrated, and it might be presumed that these soils were rich in organic matter. This presumption was proved correct when chemical analyses by Dr. L. G. Olson of the Georgia Agricultural Experiment Station showed that the positive soil samples had a high organic matter content.

The influence of these various factors on the occurrence of *H. capsulatum* in soil is no more than speculative at present. It is suggested, however, that further investigation is in order, and may eventually uncover the true explanation for the observed geographic differences in the distribution of *H. capsulatum*.

SUMMARY

1. Mycological studies of soil were undertaken as part of the general epidemiologic investigation of histoplasmosis in Williamson County, Tenn.

2. A modification of the soil-flotation, mouse-inoculation method for isolating pathogenic fungi from soil was used.

3. Two hundred and ninety-nine soil, 18 chicken manure, and 35 house dust samples were collected from 70 premises distributed over the County. Isolations of *H. capsulatum* were obtained from 11 of the soil samples.

4. Of 51 specimens taken from inside chicken houses or chicken yards, 7 were positive, a percentage of 13.7 compared to only 1.6 per cent isolations for 248 samples from other sources.

5. Nine of 92 soil samples from sheltered sites were positive, while the fungus was isolated from only 2 of 207 samples from open places, and these 2 were from chicken yards.

6. The association of domestic fowls with *H. capsulatum* in soil was investigated. The prevalence of histoplasmin sensitivity in chickens is apparently extremely low, and no cases of chicken histoplasmosis were found.

7. The soils in which *H. capsulatum* was found were shallow, acid, and of slow permeability. The possible significance of these findings is discussed.

8. Further studies of soil are suggested as a means of discovering the explanation for the observed sporadic geographic distribution of *H. capsulatum*.

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