A SEARCH FOR PATHOGENIC FUNGI IN CONNECTICUT SOILS

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A GRADUAL increase in the incidence of infections due to *Cryptococcus neoformans* and *Microsporum gypseum* observed at the Yale-New Haven Medical Center has led us to determine the frequency of these organisms as well as other pathogenic fungi in certain Connecticut soils.

Although it is recognized that *C. neoformans* and *Histoplasma capsulatum* are most likely to be isolated from soil containing the droppings of pigeons, chickens, starlings, and probably other birds, our samples were taken primarily from soils of gardens, yards, and the countryside since our patients with cryptococcosis have resided in urban or suburban areas and have no history of contact with sites containing guano.

Materials and Methods

Two hundred and four samples of soil were collected from 119 sites throughout Connecticut, as shown in the map. The collections were made between the months of August and November 1959, and from April to July 1960. Seven of the samples were collected in, under, or around chicken houses, two were from a barn floor under a pigeon roost, and two were from the yards of children with *M. gypseum* infections. The remaining samples were collected from sites not contaminated with guano.

All samples were scooped into one pint waxed containers from the top soil to a depth of 1 inch, and were kept at room temperature until processed.

A modification of the technique of Emmons (*1*) was used in examining the samples for *C. neoformans* and *H. capsulatum*. One part of soil was mixed with 10 parts of sterile, physiological saline in an 18 by 150 mm. test tube. A nontoxic rubber stopper was inserted and the specimen shaken vigorously for several minutes to obtain a homogeneous suspension of soil particles; 4 ml. of this suspension was immediately pipetted into a 13 by 100 mm. test tube. One milliliter of an antibiotic solution containing 5,000 units per milliliter of penicillin and 25,000 micrograms per milliliter of streptomycin was mixed with the sample, making the final concentration in the specimen 1,000 units per milliliter of penicillin and 5,000 micrograms per milliliter of streptomycin. After sedimenting at room temperature for 1 hour, 1 ml. of the supernatant was inoculated intraperitoneally into each of four white, male, Swiss mice weighing 15 to 20 grams.

The mice were killed at the end of 4 and 6 weeks. The liver and spleen of each mouse was removed and cut into several fragments. Impression smears were made from the cut surface of one fragment, which was then placed in 10 percent formalin for histological studies should the need arise. The smears were stained with Giemsa and carefully examined for mycotic agents. The remaining tissue was minced with sterile scissors and inoculated to the surface of Sabouraud's glucose agar, Mycosel agar, trypticase soy agar, and brain-heart infusion agar. The latter two media contained 20 units of penicillin per milliliter and 40 micrograms of streptomycin per milliliter. All tubes were sealed with parafilm and incubated at room temperature. These cultures were examined at weekly intervals for 6 weeks; any fungus seen

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on these media was subcultured and its identification attempted.

One hundred and twenty-five of these soil samples were studied for dermatophytes by the hair baiting method of Vanbreuseghem (*2*) as modified by Ajello (*3*). Sterile petri dishes were half filled with a soil sample, moistened with sterile distilled water, and incubated at room temperature in the dark for 6 weeks. These plates were examined periodically for the presence of mycelial growth on the hairs; positive cultures were subcultured on Mycosel agar for identification.

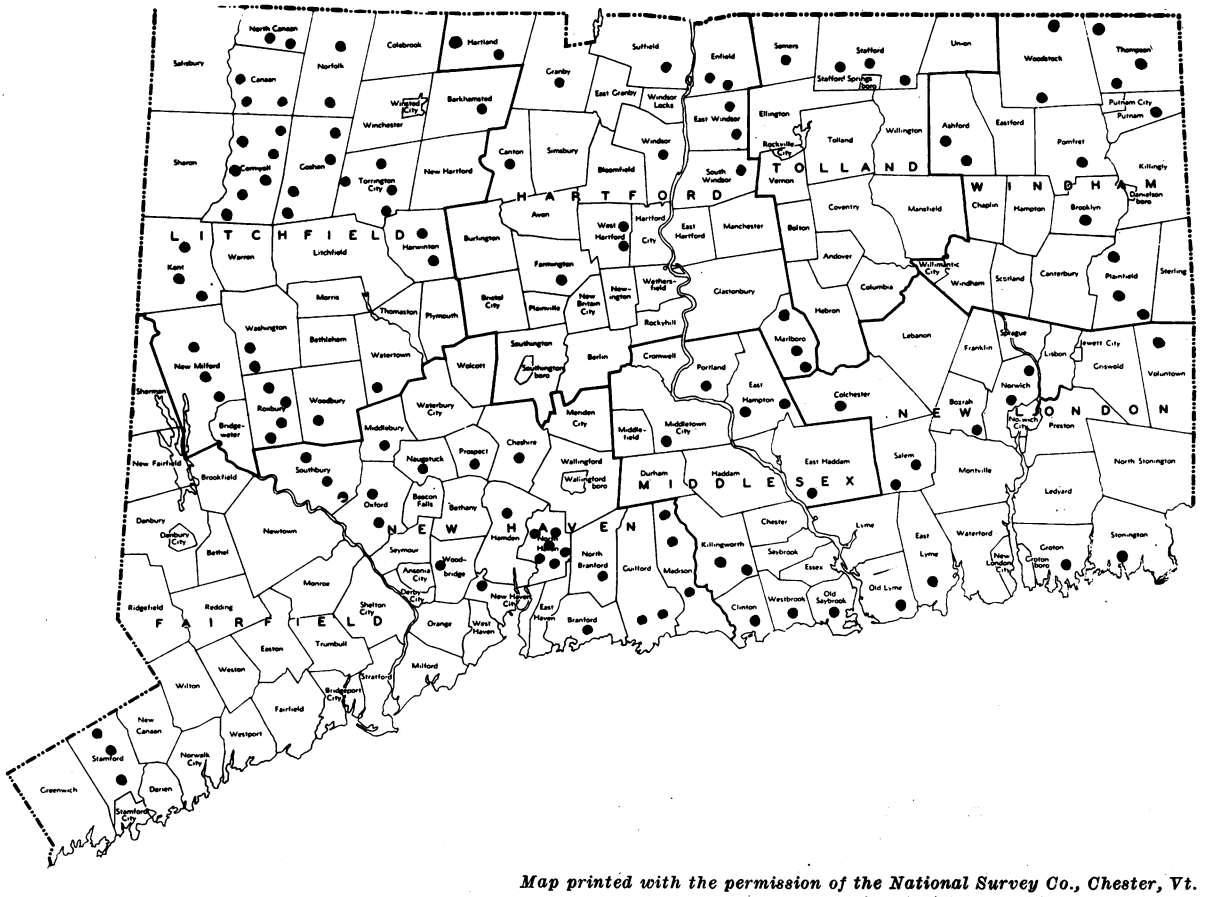
The strains of *C. neoformans* were identified by carbon assimilation studies, ability to grow at 37° C., morphological appearance, and their pathogenicity for mice. The dermatophytes were identified on the basis of pigment production, and gross and microscopic characteristics as were the strains of *Sporotrichum*.

Results

The table gives the counties from which soil samples yielded pathogenic fungi. *C. neoformans* was isolated from two soil samples and from two samples of guano taken from under a pigeon roost. *M. gypseum* was isolated from three garden samples and from the yards of two families who had children with tinea capitis caused by this fungus. *Microsporum cookei* was isolated from one of the yards yielding *M. gypseum*. *Keratinomyces ajelloi* was isolated from samples obtained from two yards, one garden, and one chicken farm. *H. capsulatum* and *Blastomyces dermatitidis* were not isolated.

Six isolations were made of a fungus which we thought to be a dermatophyte but were unable to identify. One of these isolates was sent to Dr. Lucille Georg of the Communicable Disease Center who in turn sent it to Dr. J. W. Carmichael, Edmonton, Alberta, Canada. Dr.

Collection sites of soil samples in Connecticut



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Pathogenic fungi isolated from Connecticut soils

County	Number sites	Number samples	*Cryptococcus neoformans*	*Microsporum gypseum*	*Microsporum cookei*	*Keratinomyces ajelloi*
Litchfield	39	55	1			
Hartford	14	24	1			
Fairfield	3	3				1
New Haven	25	40	2	5	1	3

Carmichael stated that the fungus was a *Trichophyton* but not one of the group of recognized pathogens. He thought it might be related to *Trichophyton terrestre* Durie and Frey.

Ten isolations of a *Sporotrichum* species were also made. This fungus failed to grow at 37° C. and did not cause infections when inoculated into the foot pads of mice. The organisms were able to survive at 37° C. for some time since they were recovered from liver and spleen cultures. One of these isolates was forwarded to Dr. Chester Emmons, National Institute of Allergy and Infectious Diseases, who agreed that it was a *Sporotrichum* species. This species of *Sporotrichum* has been isolated occasionally from sputum specimens in our mycology laboratories without any evidence of its being pathogenic.

Discussion

The recovery of *C. neoformans* from some of our samples is not unexpected. Emmons (*4*) and others have demonstrated repeatedly its presence in soil. Emmons has pointed out that soil reservoirs might be the source of the organism in cryptococcal meningitis in instances where primary pulmonary lesions are demonstrated. It should be noted that these lung infections may be subclinical and undetected.

Although we did not isolate *H. capsulatum* in this study, Kaplan and associates (*5*) recently reported the isolation of this organism from Connecticut soil. The fungus was obtained from the soil of a potted poinsettia prepared by a florist in North Haven. Investigation of the soil used revealed that it was mixed with soil from an orchard where chickens had been raised. The incidence of *H. capsulatum* in soil of this State is probably not high; Manos and associates (*6*) reported a histoplasmin sensitivity of 2–10 percent in northwestern Connecticut and 0–2 percent in the remainder of the State. These findings were based upon the testing of 111 young adults who had not resided outside of the area.

The failure to isolate *B. dermatitidis* from the soil samples may be due to the technique used in this study. Denton and associates (*7*) recovered *B. dermatitidis* from 1 of 54 soil samples by a new technique. The samples were prepared in much the same manner as was done in this study; then 1 ml. of the specimens was mixed with 0.2 ml. of a suspension of *Mycobacterium fortuitum;* 0.5 ml. was inoculated intravenously into each of five white, Swiss mice. The animals were killed in 3 weeks and liver, spleen, and lungs were cultured. Two of five mice inoculated with the sample containing *B. dermatitidis* yielded the organism from lung cultures. Liver and spleen cultures were negative.

The presence of *M. gypseum* and *M. cookei* in soil has been demonstrated by Ajello (*8, 9*). He has emphasized the fact that these organisms are primarily saprophytes although *M. gypseum* has the ability to parasitize man and animals; the pathogenicity of *M. cookei* is not known since the numerous animals from which it has been isolated, with the possible exception of one dog, have had no lesions.

K. ajelloi has been recovered from soils by Vanbreuseghem (*9*) and by the mycologists of the Communicable Disease Center. Georg and her associates (*10*) confirmed its pathogenicity for animals. No infection of man due to this organism has been reported.

Summary

Two hundred and four soil samples from 119 sites in Connecticut were studied for the pres-

ence of pathogenic fungi. *Cryptococcus neoformans* was isolated from four samples, *Microsporum gypseum* from five, *M. cookei* from one, and *Keratinomyces ajelloi* from 4 samples. Ten isolates of *Sporotrichum* and five isolates of a species of *Trichophyton* believed to be closely related to *T. terrestre* were also isolated.

Histoplasma capsulatum was not isolated during this study possibly because of the type of soil examined rather than the absence of the organism. *Blastomyces dermatitidis* was not isolated; it is suggested that this may be because of the technique employed in this study.

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