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## SIGNIFICANCE AND CONTROL OF FUNGAL DISEASES RELATED TO BIRD ROOSTS

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Certain fungi have been found frequently as saprophytes in areas containing large amounts of bird excreta. These fungi have the ability to survive, multiply, and cause disease once they have entered a host. Two of these are *Crypto-coccus neoformans* and *Histoplasma capsulatum*. Both may easily become airborne and be disseminated throughout an area by the prevailing winds. *C. neo-formans* is commonly isolated from the excreta of pigeon habitats, and in turn has been associated with clinical cases of cryptococcosis, while blackbird roosts, harboring *H. capsulatum*, have been responsible for several outbreaks of histoplasmosis. When either of these fungi have become established in nature, the sites may become foci for infection and epidemics may occur if the sites are disturbed. This has led to investigation of these organisms with respect to: 1) the frequency of isolation of *H. capsulatum* from the soil beneath blackbird roosts in a histoplasmosis endemic area; 2) the infectivity of undisturbed roosts positive for *H. capsulatum*; and 3) the effectiveness of chemical decontamination of areas containing *C. neoformans* or *H. capsulatum*.

### *Blackbird Roost Studies*

In a review of data obtained from the U.S. Fish and Wildlife Service, it was determined that there was a preponderance of blackbird roosts located in the Mississippi River Valley, especially in the states of Arkansas and Missouri. This initiated a survey of the roosts located in these two states to determine the frequency of isolations of *H. capsulatum*. Using as a guide a map showing the approximate location of the winter roosts of 1962-1963, the exact roost location for both states was obtained from the personnel at the Fish and Wildlife Station at Stuttgart, Arkansas, and from state, county, and city officials. An epidemiological form was completed as each roost was located and examined. This form included the type of vegetation, number and type of birds, dates the roost had been used, and whether or not the roost was currently in use.

As each roost was visited, the total area was determined in square yards and from this figure the number of samples to be collected was determined. A minimum of 10, or a maximum of 42 samples, was collected from each roost. To insure that the samples would be evenly distributed throughout the roost, the

area was divided into 7 ft. squares and the samples collected from a random selection of these squares. Each soil sample was collected by scraping away the excrement and surface debris from the square selected and filling an 8 oz. cardboard carton with the top 1 inch of soil. These samples were labeled and mailed to Kansas City where the fungal isolations were performed by a soil dilution, intravenous mouse inoculation method.

Of the blackbirds' roosts (32 roosts were sampled; 16 in each state) nine or 28 percent of these roosts were positive for *H. capsulatum*. We observed that all the positive roosts had been active for 3 years or more. We were unable to isolate the organism from any of the 7 roosts used for 2 years or less.

#### *Infectivity of Undisturbed Roosts*

To determine whether blackbird roosts positive for *H. capsulatum* were responsible for infections of histoplasmosis in human populations surrounding the roosts, skin test surveys were conducted among the school children of grades 1 through 12 in two towns in the state of Missouri which had positive roosts. A control group of children in two nearby towns without bird roosts were also tested. The skin test consisted of the intradermal injection of 0.1 ml of a 1:500 dilution of the filtrate from cultures of *H. capsulatum* grown in a synthetic medium at room temperature. Reactions to the injections of 5 mm or greater indurations at a 48-hour reading indicated past or present infection of the organism.

In towns having positive roosts, a total of 3,643 children were tested. Two thousand fifty-one, or 56.2 percent had positive reactions while at the control towns, 773 of 2,059 or 37.5 percent were reactors. A comparison was made of the elementary school children of Dexter, Missouri, a town with a positive roost, to the children of Maiden, Missouri, a town 20 miles from Dexter without a roost. The children attending Southwest Elementary school located within 1/2 mile of the bird roosts reacted to the skin test at a rate of 56 percent and those at Central School 1 mile away reacted at the rate of 35 percent; both schools showing an overall sensitivity rate of 44.2 percent. At Maiden, the town without a bird roost, the overall sensitivity rate was only 21 percent, indicating that the presence of the roost increased the reaction rate by 23 percent. Further comparison was made relative to the residence of the children within school boundaries near the roost. Students residing within the boundary of the West Side Elementary School (Area 1, nearest school boundary to the roost) had an overall sensitivity rate of 70 percent, while in Area 4 (boundary farthest from the roost) the sensitivity rate was only 51 percent, a decrease of 19 percent.

#### *Positive Site Decontamination*

The effectiveness of 3 percent solutions of two chemicals, cresol compound and concentrated formaldehyde, was evaluated in natural sites located at Leavenworth, Kansas, and Mexico, Missouri, containing either *C. neoformans* or *H. capsulatum*.

An 11-acre site at Mexico, Missouri, 5 acres of which had been an old blackbird roost containing *H. capsulatum*, was selected to evaluate the 3 percent solution of formalin. The area was staked off into plots measuring 5,000 or 10,000 square feet per plot. To these plots, 36 gallons per 100 square feet of the 3 percent formalin solution were applied at each of three applications. Each application of the solution was sprayed onto the surface of the soil from trucks equipped with tanks and power sprayers. The application of the solution was accomplished over a period of 1 week. Soil samples were collected from permanent random sampling sites throughout the entire area prior to treatment and for 3 consecutive months post treatment, then yearly. The results of the soils collected from the site from 1959 through 1968, including the decontamination, are shown in Table I. During the years prior to the decontamination in March 1966, the percentage of isolation of *H. capsulatum* from the soils ranged from 13 percent in 1966 to a high of 67 percent in 1961. The lower isolation percentages of 1964 and 1966, prior to decontamination, were due to the sampling of the entire 11-acre area, not just the 5 acre bird roost site. As can be seen from Table 2, after the application of the formalin, including the samplings made in 1968, *H. capsulatum* has not been isolated from any of the 164 surface or 45 depth samples collected.

To evaluate the effectiveness of the 3 percent solution of cresol compound, two buildings, at Leavenworth, Kansas in which 300 or more pigeons had been roosting for a period of 5 to 15 years, was selected. The two buildings were scheduled to be razed and *C. neoformans* had been isolated on several occasions from samples of excreta. Building owners had expressed a desire to decontaminate prior to razing to prevent possible infections of cryptococcosis in the surrounding population. Three percent cresol was applied to the ceilings, walls, and floors of each building at the rate of 1 gallon per 6 feet of floor space. This amount was sufficient to completely saturate the layer of fecal material covering the floor. The solution was mixed in a 200-gallon tank and applied with a power operated sprayer. To prevent the solution from running off before saturation could occur, the application was carried out over a period of 2 days.

Samples were collected and processed for the isolation of the organism by direct and mouse inoculation techniques. Samples were collected prior to treatment, and 1 week and 1 month after the treatment. Both buildings were razed between the 1st and 2nd months after the treatment. After the cresol was applied, the openings in each building were closed to prevent the pigeons from utilizing the roost and possibly reintroducing the organism.

The isolations of *C. neoformans* from the buildings prior to and after treatment with the cresol are shown in Table 3. Prior to the treatment, the organism was isolated from 7 of 13 samples collected from Building Number 1, and from 3 of 14 samples collected from Building Number 2. The organism was isolated by both isolation techniques. After treatment, and until the buildings were razed 2 months later *C. neoformans* was not isolated by either method from 36 samples collected from the 2 buildings.

Table 1. NUMBER OF SOIL SAMPLES AND RESULTS OF CULTURES BY DATE OF COLLECTION, MEXICO, MO., 1959-1968

DATE COLLECTED	NUMBER COLLECTED	POSITIVE BY CULTURE	
		NUMBER	PERCENT
1959	98	56	57
1960	106	68	64
1961	178	119	67
1962	164	78	48
1963	160	77	48
1964	35*	9	26
1966	32*	4	13
Mar. 1966-300,000 Gallons of 3% Formalin Applied			
1966	122	0	0
1967	53	0	0
1968	24	0	0

\*11-acre site sampled; '59-'63 samples were from 5-acre roost area only.

Table 2. RECOVERY OF *H. CAPSULATUM* FROM A SITE BEFORE AND AFTER TREATMENT WITH FORMALIN (1966-1968)

	SURFACE	DEPTH
PRETREATMENT	4/32*	
POST TREATMENT		
1 day	0/8	0/8
1 month	0/32	-
2 months	0/32	0/9
3 months	0/31	0/12
1 year	0/41	0/12
2 years	0/20	0/4
TOTAL	0/164	0/45

\*Number positive samples/number samples collected.

Table 3. RECOVERY OF *C. NEOFORMANS* FROM TWO BUILDINGS BEFORE AND AFTER TREATMENT WITH CRESOL

	BUILDING #1	BUILDING #2
PRETREATMENT	7/13*	3/14
POST TREATMENT		
1 week	0/10	0/8
1 month	0/10	0/8
2 months	Building Destroyed	Building Destroyed
TOTAL	0/20	0/16

\*Number positive samples/number samples collected.

These investigations have indicated that blackbird roosts are a source of endemic infection of histoplasmosis although the roosts may not have been disturbed. Infections that occur from these roosts as well as those that may occur from areas which contain *C. neoformans* may be controlled by the application of 3 percent solutions of cresol compound or formalin.

Of the sites utilized for 3 years or more as blackbird roosts, 36 percent were found to be positive for *H. capsulation*, however, there were several negative roosts which had been utilized for periods ranging from 10 to 28 years. So, age does not seem to be the only factor which influences whether a roost will be positive. The number of birds or the physical size of the roost seems to have little correlation to the presence or absence of the organism. Other physical and chemical factors were considered as reasons for the roosts to be positive but again, the values for both positive and negative roosts were similar.

Although there seems to be no particular reason for a bird roost to be positive except the age of the roost, a positive roost does increase the number of infections that may occur in the human population. This is evidenced by the comparison of the overall skin test sensitivity rate in the school children in comparable towns within a few miles of each other. The sensitivity rate was 18 to 20 percent greater in the towns having positive bird roosts. Children living closer to the roosts also had higher sensitivity rates than those who lived a greater distance from the roost. Decontamination of these sites containing *H. capsulatum*, as well as those which contain *C. neoformans* may be accomplished by applications of cresol or formalin. Three percent solutions of cresol compound eliminated *C. neoformans* from two buildings from which the organism had been isolated. The buildings remained negative for the organism until they were torn down. During and after the time the buildings were being torn down, there were no apparent infections to the workers or the population in the surrounding area. *H. capsulatum* has been eliminated from a positive site by the application of 3 percent formalin solution and the site has remained negative for the organism for 2 years after the application.

The inclination of various birds to roost in large numbers near population centers and the fact that pathogenic fungi may be readily isolated from the material from beneath these areas indicate that steps should be taken to determine whether these fungi are present before initiating any work in the site. Men working on a site known to be contaminated with *C. neoformans* or *H. capsulatum* may protect themselves from the airborne spores by implementing dust control measures and the use of face masks designed to filter out small particles, but this does not eliminate the organisms or protect the population in the surrounding area. Therefore, to prevent the possibility of an epidemic and to protect the workmen, chemical decontamination should be accomplished on the positive sites by the application of 3 percent solutions of either cresol compound or formalin.

#### DISCUSSION:

QUESTION: This 56% sensitivity you referred to-would you explain just what you mean by that?

WEEKS: Fifty-six percent of the school children tested had a positive reaction to histoplasmin.

QUESTION: Do they have histoplasmosis?

WEEKS: They have had the disease at one time or another.

JACKSON: It might have been in a subclinical form; it might have been inapparent, but there was a physiological reaction within the body.

QUESTION: If a roost is broken up, how long will these organisms remain active?

WEEKS: We don't know. At Mexico, Missouri where we decontaminated a couple of years ago, we had been isolating the organism since 1959. The roost had been completely destroyed in 1960; it was completely burned. At another site in Iowa, a chicken house which had been torn down, we have been isolating histo since 1952. At one site in Clarksburg, Maryland, the NIH people have been isolating it from around the foundation of a house which bats had been going in. The house since has been burned down, but they can still isolate the organisms. We've isolated it from chicken houses which have been burned down two or three years ago.

SEUBERT: I might say that we've (Fish and Wildlife Service) been working very closely with Bob; we're very concerned with the health of our employees when they go in to a roost to work. There might be a benefit, Bob, if you explained to these fellows that there is a lot of confusion about histo on the part of the medical profession. You might give them some guidelines.

WEEKS: This is a problem. We in the Public Health Service have been trying to educate the physicians on recognizing and diagnosing the fungal diseases, especially histo, which we're quite concerned with. Presently, unfortunately, there's no standard skin test antigens, or this type of thing. The physician, unless he lives in Missouri, Arkansas, or close proximity to our place, in all probability has very little knowledge of the fungal diseases. In most medical schools they get two or three weeks studying fungus.

Before anyone goes into a bird roost, he should have a skin test. This skin test with histoplasmin is very simple. There is one commercial producer of histoplasmin—I think it's Parke-Davis. It does work; it's sort of an all or none reaction. If you have a reaction of 5 mm or greater, then this means you have the disease or have had the disease. To follow this up they should probably take a blood sample and run a complement fixation test. A lot of state health departments can do this (sometimes they are the only ones); we do these things for people, if we receive a sample and a letter. The report we send out to the physician indicates whether you have a titre or not; and this titre should be interpreted by someone trained in the fungus field, a physician, or maybe one of our people.

JACKSON: I think Charlie McGriff has a question back here.

McGRIFF: What I'm interested in is along this same line. We have occasion to go into a number of bird roosts. What precautions should we take or what should we do as workers before we go in to these bird roosts?

WEEKS: Well, the best precaution to take would be to have a skin test. If you're positive, go ahead, go right into the bird roosts. If you're negative, stay out of them. Simple as that. This is what we recommend at our place. People who we hire to work in this particular field, whether sampling chicken houses or bird roosts, are hired contingent to a positive skin test. If they don't have it, we don't want them.

JACKSON: This sounds like discrimination!

LIEB: Are the symptoms of this quite similar to tuberculosis?

WEEKS: Very decidedly they can be confused. Fortunately histo is sort of a benign disease. Ninety percent (and don't quote me on these figures) or thereabouts of the people infected with histo have a subclinical illness; you might think that you have the flu or something like this, be sick for a few days, recover and no problems whatsoever. On the other end of it, about 10% of the people have a disease, illness in which they are sick, go to bed and really feel down and out. About 1% of these people require hospitalization and treatment. People may die from this disease, too.

LIEB: My reason for asking was that my mother grew up in an area where they had lots of chicken coops and she was in them a lot. They sent her to a TB hospital three different times thinking she had TB. She never reacted to TB treatment, so they finally sent her home and told her there was nothing they could do.

WEEKS: I think Dr. Furculow, who has retired from our place now, has some figures along this line. His figures state that 8% or thereabout of the people admitted to TB sanatoria have fungal diseases and not TB. This very easily happens.

QUESTION: You mentioned that when you are positive you can go into these areas. Does this mean that when you are positive you have built up an immunity to the fungus?

WEEKS: It seems that if you're positive or have been infected then you have somewhat of an immunity to it. Now we don't know what's going to happen to an individual when he's 50 or 60 years old and has been working in this bird roost. This is what my boss tells me, "Take some precautions, Bob. In three or four years you might have a case of histo." He has some data from Mason City, Iowa, in which they had two epidemics of histo. He has nine cases which he thinks were reinfections from the same bird roost site. These people had been infected in 1962; 2 years later they were reinfected from the same site due to inhaling a large number of spores. Today they're in pretty bad shape; the treatment doesn't work.

BRINK: I wonder if with all the incriminating evidence against the starling and blackbird roosts, is there any one single agency spearheading any effort—is there any central agency concerning itself with eliminating these pest birds?

WEEKS: I know we're not. As Dr. Seubert said, we work closely with them, hoping that they eliminate them.

QUESTION: According to Dr. Scott's circular on pigeon control, he has a setup for going into pigeon roosts with respirators on. Don't you use that instead of just relying on being positive?

WEEKS: Respirators would be fine, but some of the places I have personally been in blackbird roosts, if I had had a respirator on, I'd probably still be floundering around in there. You have to temper what you're going to use with what you're going to do. In the buildings at Leavenworth that I went into, I took no particular precautions primarily because cryptococcosis is a disease we don't know that much about yet, so I wasn't afraid of it!

STECKEL: These materials you used for decontaminating are they fungicides?

WEEKS: They're common disinfectants that you find in a laboratory.

STECKEL: They don't react then as a fungicide?

WEEKS: No, they act as a decontamination agent. They will kill all organisms that are there.

STECKEL: What is the length of time you can expect decontamination to remain?

WEEKS: The reason you decontaminate an area is to remove the birds. Once you have moved the birds, you are altering the environment for the establishment of histo. We think that after removing the birds and decontaminating that histo will not be reestablished in this site unless the birds come back. This doesn't imply that the birds carry the histo or anything about it. We don't know how it gets in a roost. You're chopping down the trees and letting the ultra-violet light from the sunlight irradiate the area, so this tends to prevent histo from reestablishing itself there. We've tried cresol on histo and crpto sites.

SEUBERT: One point I think we should talk about a little bit and that is the advice you hear from people who have to work in blackbird roosts and it goes back to the adage: the more knowledge we have, the more cautious we become. Ten years ago our people would go into blackbird roosts and never give histo a thought. Then with more information gained about histo we become increasingly concerned over the safety of our employees. What we do is have Bob's group determine for us whether it is a positive or negative roost. If it's a positive roost we try to bypass it

as an experimental site. If it's a negative roost, well we have no problems. We have people working in the roost who are positive reactors and we would have these people go in the roost instead of those who are negative reactors. Then you recommend respirators for both positive and negative reactors. Then we tell our people what the symptoms of the disease are and the treatment because some of the local people don't know this.

WEEKS: This is right.

WINTERS: I had a blood test here a couple of weeks ago. Now is this better than the skin test?

WEEKS: Well, no, not necessarily. You should have a blood test in conjunction with a skin test. You can have a sera test and it can be negative, but this doesn't say whether you have been infected with the organism or not. It means that you have no antibodies to the organism at this time. The skin test is an all or none thing; if you're positive it means that you have the disease now or have had it at one time. They use the blood test in conjunction with X-ray pictures, if you have a titre, or in conjunction with your skin test. Actually the higher the titre the more likelihood that you have been infected with the organism recently. This is something which should actually be interpreted by a physician or someone trained in serology.

COMMENT: Another thing, we did a survey last winter and found out there were 169 such roosts in Ohio alone. This is a big problem.

WEEKS: Yes, it's a big problem, but how many of these roosts have been used for three years or more.

COMMENT: All of them.

WEEKS: Then it gets down to this - I'm glad I'm not working here. How many of these are over water or marshy areas? These we can't sample very well. We found it to be generally true that roosts over marshes which are covered with water a good portion of the time tend to be negative. The ones along the Mississippi River which are flooded in the spring only can very well be positive.