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Applied Occupational and Environmental Hygiene

Publication details, including instructions for authors and subscription information:
<http://www.tandfonline.com/loi/uaoh20>

Case Studies

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Published online: 24 Feb 2011.

To cite this article: Dawn Tharr Column Editor & Steven W. Lenhart (1994) Case Studies, Applied Occupational and Environmental Hygiene, 9:4, 230-236

To link to this article: <http://dx.doi.org/10.1080/1047322X.1994.10388303>

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Case Studies

Recommendations for Protecting Workers From *Histoplasma capsulatum* Exposure During Bat Guano Removal From a Church's Attic

Dawn Tharr, Column Editor

Case reported by Steven W. Lenhart Introduction

The National Institute for Occupational Safety and Health (NIOSH) conducted a health hazard evaluation at the request of the chairperson of a church's board of trustees. The request concerned evaluation of the health risks associated with worker exposures to old rock wool insulation and a large accumulation of bat droppings during the removal of these materials from the church's attic.

The main sanctuary of the church was built in 1885, and additions to the original structure were built in 1916 and 1948. During the 1948 construction, rock wool insulation was added to the attics of all three sections, an area of approximately 5000 square feet. In 1992, the church's board of trustees initiated a renovation project that included replacing the old, deteriorated rock wool insulation with new fiberglass insulation. However, upon inspecting the attic, workers discovered that a large accumulation of guano had been created over the past several years by a colony of bats residing there. Guano covered the entire area at a depth of 0.5 to 1 inch. A portion of the attic is shown in Figure 1. Deeper deposits (10 to 15 inches deep) were found under the three major entry/exit locations for the bats (Figure 2) and under frequently used roosting locations. The droppings covered not only the insulation, but also the top of the rectangular duct work of the church's air-handling system (Figure 3). The first corrective measure taken was screening of the major entry/exit locations. NIOSH was then contacted for guidance concerning a personal protective

equipment recommendation for the employees of the insulation removal contractor.

A NIOSH researcher collected 16 samples of bat droppings from the attic which were analyzed for the fungus *Histoplasma capsulatum*. Bat droppings were collected from eight sampling locations in the attic before and after treatment with a 10 percent household bleach solution to investigate the ability of sodium hypochlorite to disinfect material potentially contaminated with *H. capsulatum*. This report presents the results of the analyses of the bat dropping samples and provides recommendations for protecting workers who might be exposed to bat dropping dust during renovation activities.

Background

H. capsulatum is a dimorphic fungus (i.e., exhibits growth in two different forms in different environments); it has a mycelial form at lower growth temperatures (optimal 25°C) and a yeast form when incubated at 35°C on enriched media.⁽¹⁾ The mycelial form is found in nature and is frequently designated as saprobic (i.e., derives its nutrition from dead or decaying organic matter), whereas the yeast form occurs in a host's tissue and is pathogenic. Hyphae, microaleuriospores (microconidia), and macroaleuriospores (macroconidia) are infectious particles of the mycelial form.⁽²⁾ *H. capsulatum* infections in humans result predominantly from inhalation of these aerosolized spores. The spores of *H. capsulatum* are of a respirable size, with 70 to 95 percent reported by one author to have diameters less than 4.8 μm .⁽³⁾

H. capsulatum is the etiologic agent of histoplasmosis, the most common pulmonary mycosis of humans and animals. Forty million people in the United States are estimated to have been infected by *H. capsulatum*, with approximately 500,000 new infections occurring each year.⁽⁴⁾ Asymptomatic or mild infections due to *H. capsulatum* are the rule, whereas the serious chronic or disseminated types are fairly uncommon.⁽²⁾ The extent of acute pulmonary involvement that a person experiences when infected with *H. capsulatum*, whether it be asymptomatic, mild, moderate, or severe, depends on the inoculum dose and the immunologic status of the host.⁽²⁾ Acute, severe pulmonary histoplasmosis usually occurs in small epidemics involving exposure to an aerosol containing numerous spores resulting from the disturbance of highly infected soil. Symptoms of acute respiratory histoplasmosis, including fever and cough, occur within 2 weeks of exposure.⁽⁵⁾ Approximately 95 percent of histoplasmosis cases are inapparent, subclinical, or completely benign. These cases are diagnosed only by X-ray findings of residual areas of pulmonary calcification and a positive histoplasmin skin test. Resolution of the benign form confers a certain degree of immunity to reinfection and, in addition, varying grades of hypersensitivity to the antigenic components of the organism. As a consequence, massive reinfection may result in a fatal acute allergic reaction in a person with highly sensitized lungs.⁽⁶⁾

A small percentage of histoplasmosis cases may have a chronic progressive lung disease, a chronic cutaneous or systemic disease, or an acute fulmi-



FIGURE 1. Bat droppings covering a portion of the attic.

nating, rapidly fatal, systemic infection. The latter form is particularly common in children.⁽⁶⁾ In the United States, 1500 to 4000 hospitalizations and 25 to 100 deaths occur annually due to histoplasmosis.^(4,4) These estimates were made before 1980 and do not include the increasing incidence of opportunistic histoplasmosis in patients with acquired immunodeficiency syndrome (AIDS).⁽⁵⁾ In addition to AIDS, a rapidly progressive opportunistic infection occurs in some patients with the lymphoma-leukemia-Hodgkin's group of diseases, or those on steroid therapy or other immunosuppressive agents.⁽⁶⁾ *H. capsulatum* is now considered a regularly encountered opportunist in these circumstances and appears to be involved in opportunistic infections more often than the other "true" pathogenic fungi.⁽⁶⁾

For many years, only the severe disseminated form of histoplasmosis was recognized, and the disease was thought to be uniformly fatal. However, in the mid 1940s it was shown that histoplasmin skin reactivity was common in asymptomatic individuals. The skin test antigen, histoplasmin, is a valuable epidemiologic tool.⁽⁶⁾ However, a positive histoplasmin test merely indicates that a person has probably lived in an endemic region of the United States at one time, and the test by itself has limited diagnostic value.^(4,6) In addition, the prevalence of histoplasmin reactivity at any given time underestimates the prevalence of all past and present infections, since the skin test may revert to negative over a period of time with no exposure.⁽⁹⁾ The overall incidence of histoplasmin sensitivity in the United States

is about 22 percent.⁽⁹⁾ However, the risk of infection is not uniform, but varies from location to location. The region with the highest level of reactivity is the central United States, along the valleys of the Ohio, Mississippi, Missouri, St. Lawrence, and Rio Grande rivers.⁽⁶⁾ In a series of studies conducted in the highly endemic area of Kansas City, it was found that, by age 20, between 80 and 90 percent of the population had a positive histoplasmin skin test. The same is true in the Cincinnati-southern Ohio and southern Indiana region, southern Illinois, central Missouri, and areas of Kentucky, Arkansas, and Tennessee. The first documented human case of histoplasmosis in the United States was reported in Tennessee, in 1932, and epidemiologic surveys have implied that a positive histoplasmin skin test will be found in over 60 percent of the residents of this state.⁽⁷⁾ Focal areas of high endemicity also occur in Michigan, Wisconsin, Minnesota, Georgia, and Louisiana.⁽⁶⁾

The largest outbreak of acute respiratory histoplasmosis occurred in Indianapolis, Indiana, between September 1978 and August 1979.⁽⁸⁻¹⁰⁾ Over 100,000 people were estimated to have been infected during this period, and over 300 people were hospitalized. Forty-six patients had progressive disseminated histoplasmosis, and 15 deaths were directly or indirectly related to histoplasmosis.⁽⁸⁾ On the basis of epidemiologic data, the site where an abandoned amusement park had been dismantled was suspected as the envi-



FIGURE 2. Accumulation of bat droppings below a major entry/exit location.



FIGURE 3. Accumulation of bat droppings under a frequently used roosting location.

ronmental source of this outbreak. However, *H. capsulatum* was not recovered from any of the soil samples collected there.

While an *H. capsulatum* infection is most often a pulmonary disease or a systemically disseminated disorder, a multifocal choroiditis (inflammation of the vascular coat of the eye), termed "presumed ocular histoplasmosis" has been described by many investigators.⁽⁷⁾ This disease was called ocular histoplasmosis throughout the early 1960s even though evidence for ocular histoplasmosis was circumstantial, since *H. capsulatum* has not been recovered from eye lesions, cultured, and recovered in an animal model.⁽⁸⁾ Although structures suggestive of an organism have been found in such lesions, the identity of the fungus has been difficult to demonstrate.⁽⁷⁾ A correlation between exposure to *H. capsulatum* and ocular abnormalities has been suggested from the results of epidemiologic studies, but the characteristic multifocal choroiditis has rarely been reported in patients who have the typical forms of this disease.⁽⁷⁾ While the results of laboratory tests suggest that presumed ocular histoplasmosis is associated with hypersensitivity to *H. capsulatum*,⁽⁹⁾ the incident that converts asymptomatic to symptomatic presumed ocular histoplasmosis remains unknown.⁽⁷⁾

A primary source of *H. capsulatum* is soil, especially in regions of bird or bat habitats. While wind is probably the most important means of disseminating *H. capsulatum*, the fungus can survive and be transmitted from one location to another on the feet of both birds and bats.⁽⁶⁾ The organism thrives in humid areas where large numbers of birds have roosted over a period of several years. It is found in association with old or unused chicken houses and under blackbird and starling roosts. Bird excreta provide nutrients that promote the growth of the organism in the soil, although the requirements for growth are not precisely defined. Caves sheltering bats, and soil at the base of buildings fertilized by droppings from bats inhabiting the buildings, also often provide environmental condi-

tions suitable for the existence and propagation of the fungus.⁽³⁾ Unlike birds, bats can become infected with *H. capsulatum* and consequently may excrete the organism in their feces.⁽⁵⁾ *H. capsulatum* has been isolated from bat guano collected from around the world, and by 1970, 25 bat species had been reported to harbor this organism. Isolations of *H. capsulatum* from bats captured in the United States have been extensive.⁽¹⁴⁾

While accumulations of bat droppings alone have been shown to be contaminated with *H. capsulatum*,⁽¹⁴⁻²²⁾ similar results have been reported far less frequently for samples taken from accumulations of bird droppings.⁽²³⁾ In avian habitats, the organism seems to grow preferentially where the guano is rotting and mixed with soil rather than in nests or fresh deposits.⁽⁶⁾ Attempts to demonstrate the presence of *H. capsulatum* in the organs and excreta of birds have never proven them to be carriers of the organism.⁽²⁰⁾ It has been suggested that birds do not harbor *H. capsulatum* because the organism does not survive at elevated avian body temperatures of 41° to 42°C.⁽⁵⁾ However, the same temperature has been recorded in certain bats (*Molossus major*) for which *H. capsulatum* was demonstrated from cultures of their internal organs.⁽²⁰⁾

Exposure to accumulations of bird droppings alone cannot be assumed to be risk-free because disturbance of bird habitats is associated with a risk of infection by *Cryptococcus neoformans* and the development of cryptococcosis.⁽²⁴⁾ *C. neoformans*, an encapsulated yeast, is ubiquitous in the soil and avian fecal material, such as pigeon droppings, which apparently provide a reservoir of organisms.⁽²⁵⁾ *C. neoformans* has the ability to use the creatine found in avian feces as a nitrogen source. There, it gains a competitive advantage over other microorganisms and multiplies exceedingly well in bird droppings.⁽²⁴⁾ *C. neoformans* has also been recovered from bat droppings and associated dusts during studies for which samples were also found to contain *H. capsulatum*.^(17,18,22) Unlike outbreaks of other mycoses, outbreaks of

cryptococcosis traced to environmental sources have not been described, and it is presumed that most people can mount adequate defenses when exposed to the organism. However, as with histoplasmosis, the prevalence of cryptococcosis is markedly increased among immunocompromised patients.⁽²⁶⁾ More detailed information on *C. neoformans* and cryptococcosis is available elsewhere.⁽²⁵⁻²⁷⁾

Methods

Sixteen samples of bat droppings were collected from 8 sampling locations in the attic of the church and were analyzed for *H. capsulatum*. Samples were collected from 4 locations of the attic above the main sanctuary, from 2 locations of the 1916 addition, and from 2 locations of the 1948 addition. Each sample was collected in a sterile, non-pyrogenic plastic 50-ml centrifuge tube. The volume of droppings collected at each sampling location ranged from 20 to 50 ml (approximately 7 to 17 g). While collecting samples, the NIOSH investigator wore a NIOSH/Mine Safety and Health Administration (MSHA)-approved full-facepiece powered air-purifying respirator with high-efficiency filters, disposable protective clothing with a hood, disposable latex gloves, and disposable shoe coverings.

After the collection of a sample from each of the eight sampling locations was completed, the remaining bat droppings at each sampling location were soaked with a bleach and water solution prepared by adding 7 oz of Chlorox® bleach to 2 qt of tap water. The resulting 10 percent bleach solution, containing approximately 5000 ppm of sodium hypochlorite, was sprayed at each sampling location. Eight additional samples of bat droppings were collected from the same sampling locations the next day to permit sufficient contact time between the hypochlorite solution and any microbial contamination.

Bat dropping samples were analyzed for *H. capsulatum* at the University of Cincinnati Medical Center in Cincinnati, Ohio. One-half gram of the material from each sample was diluted

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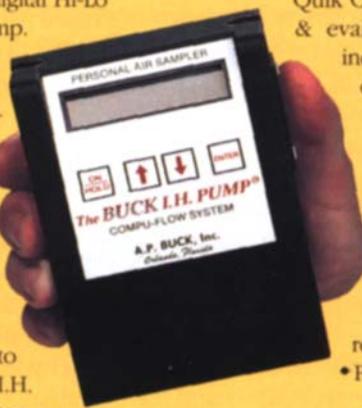
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1:20 (w/vol) and 1 ml was injected intraperitoneally into mice. Four mice were tested for each of the 16 bat dropping samples. The mice were observed for 4 weeks and then killed. The spleens were removed from the mice, homogenized, and the homogenate was streaked onto brain-heart infusion agar plates supplemented with sheep erythrocytes, glucose and L-cysteine.

In addition, 6 samples (3 samples collected before and 3 samples collected after treatment with the bleach solution) were subjected to direct plating by adding 100 μ l of the diluted material to culture plates that contained the same agar as described above. All plates were observed for growth for 4 weeks.

Results

H. capsulatum was isolated from all 4 mice inoculated with material from 1

of the 2 bat dropping samples collected at the base of the back wall of the original 1885 building. *H. capsulatum* was not isolated from any of the other inoculated mice, and no mold growth was found on any of the agar plates that were direct-plated. Although the sample containing *H. capsulatum* was collected after treatment with a 10 percent bleach solution, a conclusion on the effectiveness of such treatment cannot be reached based on the sampling results of this study.

Discussion

Mice are extremely susceptible to infection with *H. capsulatum* spores, and infection of mice inoculated with single spores has been demonstrated experimentally.⁽²⁸⁾ However, while mouse inoculation is the most reliable method for detecting *H. capsulatum* in environmental samples such as the bat droppings collected during this study,

the method has a disadvantage of requiring several weeks before results are available. The method also has the limitation of using only a very small portion of a sample. This limitation might explain why *H. capsulatum* was isolated from a sample collected at the same location from which a negative sample was collected on the previous day. Nevertheless, the laborious and time-consuming procedure required for the isolation of this fungus from its natural sources remains the important factor that restricts more extensive investigation into ecological relationships. The expense, space, and personnel required for large-scale studies are also important limiting factors.⁽¹³⁾ Direct isolation of *H. capsulatum* in culture from soil samples has been accomplished,⁽²⁹⁾ but the sensitivity of the method is inferior to mouse inoculation.⁽²⁰⁾

To overcome the disadvantages associated with mouse inoculation, de-

velopment of a simple and reliable technique is necessary for the detection of *H. capsulatum* in samples collected from its natural environment.⁽³⁾ Researchers have successfully identified pathogenic fungi in clinical samples using polymerase chain reaction (PCR) probe detection systems and chemiluminescent DNA probe assays.⁽³⁰⁻³³⁾ PCR probe systems have an advantage over DNA probe assays in that identification of pathogenic fungi in samples can be accomplished directly, without the need to wait for the growth of isolates from culture. A PCR probe system would also be capable of analyzing a larger portion of a sample of material at one time than the very small portion used with the mouse inoculation method. More important development of a PCR probe system for the analysis of *H. capsulatum* in environmental samples would reduce the time presently necessary for analysis using mouse inoculation from weeks to only a few days.

Disinfection of soils contaminated with *H. capsulatum* has been tried with various chemicals. Formaldehyde has fungicidal properties, and it has been shown to be the most effective of the chemical agents tried based on the performance of pretreatment and posttreatment sampling for *H. capsulatum*.⁽²⁾ A 37 to 40 percent solution by weight (formalin) stabilized with 10 to 15 percent methanol has been the basic formulation used. For decontamination procedures outdoors, a 3 percent formalin solution has been found to be effective.^(24,34,35) However, exposures to formaldehyde during soil disinfection operations have been reported to cause adverse health effects among applicators. Workers at one site reported burning eyes and mucous membrane irritation,⁽³⁴⁾ while workers at another site reported nausea with vomiting.⁽³⁵⁾

In addition to soil disinfection, formaldehyde has also been reported to be effective for disinfecting *H. capsulatum*-infected accumulations of bat droppings in the attics of buildings, using formalin concentrations of 3⁽¹⁶⁾ and 4 percent.⁽²²⁾ Formaldehyde solutions should be used with caution

since this chemical may cause adverse health effects following exposure via inhalation, ingestion, or dermal or eye contact.⁽³⁶⁾ Mild to unpleasant eye irritation occurs at 2 to 10 ppm, and intolerable irritation (tissue damage possible) occurs at levels above 25 ppm.⁽³⁶⁾ Workers exposed to 0.3 ppm of formaldehyde have reported symptoms of upper respiratory and acute bronchial irritation during a work shift.⁽³⁷⁾ There have also been reports of primary skin irritation and allergic dermatitis as a result of skin contact with water solutions of formaldehyde.

Although a threshold for the development of these skin conditions has not been clearly defined, it is estimated to be a water solution containing less than 5 percent formaldehyde.⁽³⁸⁾ Based upon the results of laboratory tests that have demonstrated carcinogenic and mutagenic activity of formaldehyde in animals, NIOSH and the Occupational Safety and Health Administration (OSHA) recommend that formaldehyde be handled in the workplace as a potential occupational carcinogen.^(39,40) NIOSH recommends that occupational exposures to formaldehyde be controlled to the lowest feasible limit.⁽³⁹⁾

Conclusions and Recommendations

H. capsulatum was isolated from 1 of 16 samples of bat droppings collected in the church's attic. Therefore, precautions were recommended for protecting workers from inhalation exposure to dust disturbed during the removal of the rock wool and bat droppings. Samples of bat droppings were collected and analyzed primarily to investigate the effectiveness of a bleach solution to disinfect the bat droppings. Because of the large accumulation of bat droppings in the church's attic and because the church is located in an endemic region for *H. capsulatum*, it would have been prudent to assume that a health risk existed if none of the samples was positive, or even if no samples had been collected and analyzed.

The health risks associated with exposure to *H. capsulatum* were communicated before the start of removal activities to each worker who might be exposed to bat droppings during the course of the project. Individuals with compromised cell-mediated immunity are at greater risk of clinical histoplasmosis should infection occur, so such workers should avoid exposure to all materials potentially contaminated with *H. capsulatum*.

To reduce the potential for aerosolization of both rock wool dust and bat dropping dust, workers sprayed these materials with water. Then, the dampened materials were collected in heavy-duty trash bags, and immediately disposed of at a landfill. Because the water evaporated over the course of the removal operation, additional water was sprayed as needed. The addition of a surfactant (wetting agent), such as a small amount of detergent, to the water may have improved the dust suppression ability of the water alone. After removal of the bulky material, dust remaining in the attic was removed with an industrial vacuum cleaner equipped with a high-efficiency particulate air filter.

Workers wore personal protective equipment while spraying water on the rock wool and bat droppings, and while collecting these materials in plastic bags. A NIOSH/MSHA-approved full-facepiece powered air-purifying respirator with high-efficiency filters, disposable protective clothing with a hood, disposable latex gloves under cotton work gloves, and disposable shoe coverings was expected to provide adequate protection. Respirators were used in accordance with the regulations of OSHA⁽⁴¹⁾ and the recommendations of NIOSH.⁽⁴²⁾ Since the recommended ensemble of disposable personal protective equipment is more insulating than normal work clothing, sweat evaporation was anticipated to be impeded during removal activities. Therefore, precautions were taken during these activities to reduce the risk of heat stress-related illnesses, and removal activities were scheduled when temperatures in the attic were relatively cool.

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Health risks are associated with exposures to even low air concentrations of formaldehyde.⁽³⁸⁾ Therefore, alternative chemicals should be used to disinfect those materials for which removal is impractical, such as a large volume of contaminated soil. Household bleach is one possible alternative since it contains sodium hypochlorite, which has bactericidal and sporicidal properties. Household bleach also has the practical advantages of being readily available and less expensive than most other chemical bactericidal and sporicidal agents. However, a disadvantage of hypochlorites is that their activity is greatly reduced in the presence of organic matter.⁽⁴³⁾ Because of the limited number of positive samples collected during this study, the effectiveness of bleach solutions to disinfect bat droppings containing *H. capsulatum* could not be evaluated. The effectiveness of bleach solutions or other disinfectants

should be documented before their use is recommended for decontaminating environmental materials containing *H. capsulatum*.

Acknowledgments

Appreciation is extended to Millie Schafer, Ph.D, for her invaluable editorial and technical guidance and to George S. Deepe, Jr, of the University of Cincinnati College of Medicine for his expertise in conducting sample analyses.

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Editorial Note: Steve Lenhart is with the Hazard Evaluation and Technical Assistance Branch of NIOSH. More detailed information on this evaluation is contained in the Health Hazard Evaluation Report No. 92-0348-2361 available through NIOSH, Hazard Evaluation and Technical Assistance Branch, 4676 Columbia Parkway, Cincinnati, OH 45226; or by telephoning 1-800-35-NIOSH.