THE USE OF FORMALIN TO KILL HISTOPLASMA CAPSULATUM AT AN EPIDEMIC SITE


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Early attempts to eliminate Histoplasma capsulatum from known positive sites were either unsuccessful or rendered the soil unfit for growth of vegetation (1, 2). Recently, formaldehyde solution (formalin) and cresol compound have been shown to be effective decontamination agents in the laboratory and in field experiments under natural conditions (3).

The promising results with formaldehyde solution led to its large-scale field trial to decontaminate a 5-acre site in Mason City, Iowa, which had been the source of 2 epidemics of histoplasmosis (4, 5). The present paper describes the methods used and the results of the decontamination project.

MATERIALS AND METHODS

Description of site. The area to be decontaminated in Mason City was located along Willow Creek near the downtown business district. Before July 1962, the area along the creek had been covered with underbrush and large trees, where thousands of starlings had roosted each summer for several years. The ground under the roost had become covered with a thick carpet of bird excreta. In July of 1962, the creek was deepened and widened here, and debris from the creek was piled along the south bank. At the same time, most of the trees on the site were sawed down, and the area was partially cleared by a bulldozer. The work on this site resulted in an epidemic of histoplasmosis (4). In 1964, the remaining trees were cut down, sawed, and hauled to the city dump. A second epidemic of histoplasmosis resulted (5).

When decontamination of the site was undertaken in October 1964, the area was very rough (figure 1). The soil was mostly clay but in places contained large pieces of concrete and variable amounts of gravel, cinders, broken glass, humus and black soil. There were also a few tree stumps on the site.

Application of chemical. The city purchased 8,000 gallons of saturated solution of formaldehyde (37 per cent formaldehyde gas by weight), to be brought to a siding in the area by railway tank car. A city street-washing truck with a capacity of 1,500 gallons was made available, and several men from the street department were assigned to the project. To prepare the 3 per cent formalin8, 45

8Three per cent formalin contains 1.1 per cent formaldehyde gas by weight.
gallons of the 37 per cent formaldehyde solution from the tank car were pumped into the truck and the truck filled with water to the 1,500 gallon capacity. The city provided a gasoline engine-driven pump, to which a meter had been added to measure the saturated solution of formaldehyde as it was pumped from the railway tank car. A fire hose ran from a nearby fire hydrant to a fitting on the bottom of the truck. Formaldehyde solution was pumped from the railway tank car into an opening on the top of the truck, and water was added. The force of the water entering the truck from the fire hydrant mixed the formaldehyde and water thoroughly.

The truck was driven to the site to be treated; 2 hoses from the truck sprayed the solution as evenly as possible over a designated area. A spray bar attached to the rear of the truck was used in areas where the truck could be driven. The spray bar consisted of a pipe 2 inches in diameter, approximately 10 feet long, and closed at each end. There were holes $\frac{3}{8}$-inch in diameter located 3 inches apart along the bottom of the pipe; a fitting near the center of the pipe was used to attach one of the hoses from the truck. The truck could be emptied in approximately 7 minutes through the spray bar and the second hose.

The plan was to apply 36 gallons of 3 per cent solution per 100 square feet of area each day for 2 days, then level the area with a bulldozer before a third application of the formalin. The street-washing truck contained enough solution to cover approximately 4,100 square feet. The city engineer measured the entire area, divided it into plots delineated by stakes, and prepared a blueprint showing the area of each plot. The number of truck loads of solution to be applied to each plot was calculated.
The operation went rather slowly the first day, Monday, as it was necessary to determine if the fumes from the formalin would require spraymen to wear gas masks. Also, observations were made to determine if the fumes would be offensive to people who worked or lived nearby. The fumes were no problem unless one walked in the area immediately after spraying, because then the fumes made eyes burn and irritated the mucous membranes of the nose. These effects cleared in a few minutes once the person had left the area. To avoid contact with fumes, the spraymen rode on the tops of the trucks. The area being treated was along a small creek, and stream contamination had to be kept to a minimum. The stream was frequently inspected for evidence of toxicity to fish.

By the afternoon of the first day, it had become obvious that the project could not be completed in one week with only one truck. Arrangements were made to borrow a Civil Defense fire truck with a capacity of 2,000 gallons. Both trucks were used to complete the project. Two applications of solution had been made to most of the area by Wednesday afternoon. This amount of solution is approximately equivalent to an inch of rainfall.

On Thursday morning, the area was leveled. During the leveling operation, water was sprayed around and in front of the bulldozer to prevent spores of the fungus from becoming airborne. The fire truck was used to spray water the same day and part of the next until work with the bulldozer was finished. The other truck proceeded with spraying formalin after dispensing 2 loads of water on Thursday. After the area had been leveled with the bulldozer, a third application of formalin was made over the entire area. The area of the old starling roost received a fourth application. The project was completed by mid-afternoon Saturday, October 31, 1964.

Collection of soil samples. Immediately preceding the work on the property, 20 soil samples were collected in half-pint cardboard cartons from the surface of the area known to be most heavily contaminated with the fungus. The site of each collection was marked with a stake. After two applications of the formalin, the same 20 sites were again sampled. After the area had been leveled, 39 samples were obtained at random from the area that had been sampled before bulldozing. Each of these 39 new sites was marked. Then the third application of formalin was made. Some of the area received a fourth application. Soil samples were collected from the 39 designated areas at 1 week, 2 weeks, 3 weeks and 4 weeks after the last application. From December 1964 through October 1965, the 39 sites were sampled monthly, except in February 1965 when the area was covered with snow and the ground was frozen solid.

Samples of soil were collected at a depth of approximately 6 inches from each of the 39 designated sites in January 1965 and again in September 1965. The soil samples were mailed to the Kansas City Field Station Laboratory, where fungal cultures were performed. The procedures used to isolate *H. capsulatum* from soil were:

One hundred grams of soil were placed in a 250 ml flask, and physiological saline (200 ml), penicillin (250,000 units) and streptomycin (125 mg) added; the mixture was shaken vigorously on a shaker for 15 minutes and allowed to stand for 20 to 30 minutes. Ten ml of fluid from the interface of the sediment and supernatant were drawn off with a pipette and put into a screw-cap tube. One ml of this material was injected into
Earlier studies have shown that physical changes in an area and the passage of time are not sufficient to eliminate the fungus from a positive site (1). Chemical agents must therefore be used to decontaminate positive sites which have been responsible for epidemics and are a potential source of future infections. The ideal agent has been described as one that kills *H. capsulatum*, is relatively non-toxic to human beings, animals and vegetation, and is relatively cheap and easy to apply (1). Formalin solution comes very close to meeting these requirements. A 3 per cent solution of formalin kills the fungus, including spores, after only a few minutes of contact (3). The chemical is relatively non-toxic to human beings unless ingested, although repeated contact can produce a contact dermatitis. As demonstrated in this project, the chemical is relatively easy to use and causes no difficulty among workers. There were no complaints from people working or living near where the formalin was used. The chemical killed grass and small vegetation; however, the solution did not penetrate deep enough to damage small trees and bushes on the treated site. The active ingredient, formaldehyde gas, evaporates or is inactivated within a few days after it is applied, leaving no undesirable residual effects; weeds and grass entirely covered the site in Mason City during the summer of 1965. Frequent observations of the stream revealed no dead fish during the treatment days. The chemical is not overly expensive; it costs less than 40 cents a gallon when purchased in very large quantities. The cost of decontaminating the 5-acre site in Mason City was estimated to be $4,000.

This study has shown that 3 per cent formalin can be used to decontaminate the surface of large areas harboring *H. capsulatum*. A problem still to be solved is that of getting the solution deep enough into the soil to eliminate viable particles of the fungus present six inches or more below the surface.

**Summary**

Laboratory and field studies on chemical decontamination of soil containing *Histoplasma capsulatum* revealed that 3 per cent formalin was effective in destroying this fungus. These results led to a large-scale trial of the chemical on an urban starling roost which had been the source of 2 epidemics of histoplasmosis in Mason City, Iowa.

In October 1964, 3 (in some parts, 4) applications of 3 per cent formalin were made to the 5-acre site. Soil samples for culture for *H. capsulatum* were collected from the surface of the area before spraying, after the second application, and at weekly or monthly intervals for 1 year after the final application of formalin. Also, samples of soil were collected at a depth of six to eight inches in January and September 1965. Before treatment, 13 of 20 surface samples were positive for *H. capsulatum*. After treatment, 1 of 572 surface samples and 9 of 78 depth samples were positive. These data show that 3 per cent formalin is an effective chemical for destroying *H. capsulatum* on the surface of natural sites. The fungus has not been recovered from the surface of the treated site during 10 months of follow-up but whether or not decontamination is permanent is not yet known.

**REFERENCES**

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