AN OUTBREAK OF HISTOPLASMOSIS AT AN ARKANSAS COURTHOUSE, WITH FIVE CASES OF PROBABLE REINFECTION

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An outbreak of histoplasmosis occurred at a county courthouse in Arkansas in July, 1975. Fifty cases were identified by symptoms and positive complement fixation (CF) tests and 18 others by the clinical findings alone. Only three positive CF tests (>1:8) were found in a control group of 55 unexposed persons of the same age. The attack rate for 84 courthouse employees was 52%. 

Histoplasma capsulatum was isolated from the sputum of two cases, and from bird droppings which had been dumped from the roof of the courthouse two weeks before the epidemic’s peak. Window air conditioners apparently helped to distribute infectious particles throughout the building. Nine exposed people had pulmonary calcifications and a negative tuberculin test that were taken as presumptive evidence of previous histoplasmosis. Five (55%) of these became ill. Exposed persons without pulmonary calcifications had an attack rate of 85%. The reinfected cases during convalescence had higher serum CF titers but not larger histoplasmin skin test diameters than the rest of the cases. A microdroplet aerosol of cooking oil was used to simulate air flow patterns during the exposure, and the results agreed with the hypothesized means of infection.

aerosols; fever; fungi; histoplasmosis; immunity; pneumonia

The epidemiology of histoplasmosis was well described during the 1950’s and 1960’s (1-9). Soil enriched with bird feces has been the most common source in epidemics, although bat guano and bird droppings from

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Abbreviation: CF, complement fixation.

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courthouse was underway and excavation had been done, the sera were tested for histoplasmosis. Eight of the 15 employees had complement fixation (CF) titers of 1 to 8 or higher, and a more detailed investigation was begun.

METHODS

The employees of the courthouse were interviewed and blood specimens were taken in early August and again in September. Chest x-rays were offered to all employees on August 21, and histoplasmin and tuberculin skin tests were done on September 10. Epidemiologic information was obtained by means of a form which included a complete explanation of the studies to be done. Written informed consent was obtained from each individual for the interview and the performance of the tests. A control group of individuals on the staff of a local hospital or working in the health department building about a mile from the courthouse also had skin tests, CF titers, and interviews under similar conditions. Samples were taken of the soil surrounding the courthouse and of bird droppings on the tower and roof of the courthouse and several other nearby buildings.

Questionnaires were sent to 1090 individuals who had visited the courthouse to obtain various licenses between July 1 and 18, 1975. One hundred and five of the 451 respondents had two or more of the symptoms on the questionnaire for a period of more than two days, and were considered to be possible histoplasmosis cases. Thirty-one of these had histoplasmin skin tests and gave serum specimens for histoplasma CF tests. A door-to-door search for additional cases was conducted on the city block surrounding the courthouse.

On July 8, 1976, exactly one year after the date of exposure, an oil-droplet generator (Model HCS, MicroGen Equipment Corporation, San Antonio, TX) containing pure vegetable oil (Crisco Brand) was slowly lowered from the roof at the location where histoplasma-containing debris was discovered to have been dumped. The cards were collected about 30 minutes later and the number of droplets on each card was counted the same day with the aid of a dissecting microscope (2X objective and 10X eyepiece). Two fields on each card were counted and averaged to give a single count for each card. The wind on the morning of the epidemic exposure (July 8, 1975) was 0–16.1 kmph (0–10 mph) from the south; when the test was conducted at 5 P.M. on July 8, 1976, the wind was 12.9 kmph (8 mph) from the south-south-west.

The procedure used to determine the presence of Histoplasma capsulatum in the soil specimens has been described previously (13). Portions of the specimens were suspended in physiologic saline that contained 1000 units of penicillin and 1 mg of streptomycin per ml. One ml of the supernatant was then injected intraperitoneally into each of four mice. These were held for four weeks and then killed. Portions of their livers and spleens were inoculated into tubes of a modified Sabouraud's dextrose agar containing 0.05 mg of chloramphenicol per ml. The inoculated tubes were incubated at 25 C and checked for fungal growth for four weeks. All molds suspected of being H. capsulatum were examined microscopically and subcultured for definitive identification and the development of tuberculate macroconidia, smooth microconidia, and conversion to a yeast form.

CF tests against histoplasma mycelial and yeast antigens were performed at the Center for Disease Control Mycology Laboratory (CDC) and at the Arkansas State Department of Health. The results were in general agreement, but CDC titers are given for convenience.
RESULTS

Clinical characteristics

Forty-four (52 per cent) of the 84 employees inside the courthouse developed fever, cough, chest pain, myalgia, and/or laboratory evidence of histoplasmosis during late July and early August. Twenty-four other cases were identified among construction employees outside the courthouse and citizens who visited the courthouse for various reasons during the period of exposure. The clinical features are listed in table 1. Cases in the epidemic consulted 21 different physicians, and no physician saw more than six, making recognition of both the disease and its epidemic nature from the physician’s office difficult. The diagnosis of histoplasmosis was generally made only after reports of the investigation had appeared in the press.

Forty-eight people with fever and at least one other symptom (see table 2) had CF titers for yeast or mycelial histoplasma antigens of ≥ 1:8. *H. capsulatum* was isolated from the sputum of two cases, one of whom had a CF titer of < 1:8. One individual whose serum was anticomplementary had antibodies to histoplasma by the immunodiffusion test. These fifty persons were designated “confirmed cases”. Eighteen other symptomatic individuals with definite exposure were serologically negative (4) or untested (14), and were considered “clinical cases”. Only three of the control group of 55 unexposed persons had CF titers of 1:8 or higher and none exceeded 1:16.

The results of histoplasmin skin tests are shown in table 3. The percentage of positive tests among sick and well persons within the courthouse was approximately the same—97 per cent and 87 per cent—but contrasted sharply with the 35 per cent positivity rate among the 55 controls who did not work at the courthouse. The latter may be taken as an approximation of the skin test positivity rate among the general public of the same age group in the town since both groups averaged 43 years of age and their length of residence in the town was very nearly the same (27 vs 25 years) (table 4).

Six patients described ulcerations of the oral mucosa, none of which were severe; the details were not adequate to distinguish herpes simplex from histoplasmal ulceration. There were nine possible cases of erythema nodosum identified only as “red

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Frequency in fifty serologically confirmed cases (%)</th>
<th>Occurrence in five cases of reinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Fever (°C)</td>
<td>100*</td>
<td>X</td>
</tr>
<tr>
<td>Chills</td>
<td>76</td>
<td>X</td>
</tr>
<tr>
<td>Weight loss (kg)</td>
<td>44†</td>
<td>1.1</td>
</tr>
<tr>
<td>Cough</td>
<td>72</td>
<td>X</td>
</tr>
<tr>
<td>Headache</td>
<td>72</td>
<td>X</td>
</tr>
<tr>
<td>Myalgia</td>
<td>78</td>
<td>X</td>
</tr>
<tr>
<td>Chest pain</td>
<td>74</td>
<td>X</td>
</tr>
<tr>
<td>Arthritis</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Mouth ulcerations (size unknown)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Erythema nodosum: (red swelling on legs)</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Duration of illness (days)‡</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Incubation period (days after July 8)§</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

* Average 39.1°C (102.6°F).
† 1.8 kg to 5.6 kg.
‡ Average 14 days. Excludes cases over one month.
§ Average for A-D, 14 days.
### Table 2

<table>
<thead>
<tr>
<th>Status</th>
<th>Reciprocal of titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unknown *&lt;8 8 16 32 64 128 256</td>
</tr>
<tr>
<td>Cases not known to be reinfections (N = 45)§</td>
<td>1†</td>
</tr>
<tr>
<td>Cases of reinfection (N = 5)§</td>
<td>1‡</td>
</tr>
<tr>
<td>Exposed persons without symptoms (N = 26)</td>
<td>21</td>
</tr>
<tr>
<td>Persons in control group (N = 55)</td>
<td>51</td>
</tr>
</tbody>
</table>

* Highest titer for either mycelial or yeast antigen. Tests performed by Mycology Laboratory, Center for Disease Control, Atlanta, Georgia. Serum samples were taken one to five weeks after illness, before histoplasmin tests were performed.

† Serum anticomplementary. Severe illness, positive immunodiffusion test with mycelial antigen.

‡ H. capsulatum isolated from sputum.

§ Difference between first two groups, p < 0.0016, Mann-Whitney U Test.

### Table 3

<table>
<thead>
<tr>
<th>Status</th>
<th>Size of induration (mm) at 48 hours by per cent</th>
<th>Average diameter (mm) of positive tests ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serologically confirmed cases (N = 38)†</td>
<td>3 0 97</td>
<td>16.0 ± 0.6</td>
</tr>
<tr>
<td>Exposed non-ill people (N = 15)</td>
<td>0 7 87</td>
<td>17.5 ± 1.3</td>
</tr>
<tr>
<td>Controls (N = 55)</td>
<td>53 13 35</td>
<td>13.3 ± 1.2</td>
</tr>
</tbody>
</table>

* Approximately one month after illness.

† Twelve cases were not tested.

Swellings on the legs. Thirteen patients complained of pain in various joints, often described as severe pain in the ankles or calcaneus upon first arising in the morning, with improvement later in the day. Several also had pain and/or swelling in the knees, hips, and wrists. Of 38 serologically confirmed cases who had x-rays, 17 (45 per cent) had pulmonary infiltrates or mediastinal lymphadenopathy. Follow-up x-rays of the exposed persons in January, 1976, did not reveal additional cases or undiscovered progressions.

Two patients had progressive disease. One developed a pulmonary cavity in October which slowly responded to amphotericin B therapy. The second had severe emphysema and developed an infiltrate in the right middle lung field about two months after the epidemic which healed without specific therapy. Both of these patients had H. capsulatum isolated from the sputum.

Nine exposed individuals were identified who had calcium deposits in their lungs or pulmonary lymph nodes by chest x-ray, and had negative tuberculin tests. This combination strongly suggests that these individuals had had previous attacks of histoplasmosis in the distant past, since calcification does not occur earlier than many months after infection and the other non-tuberculous causes of pulmonary calcification (coccidiomycosis, blastomycosis) are rare in the area in which the courthouse is located. Five of the nine individuals with evidence of previous infection became ill in this epidemic. One of these was very heavily exposed to the infectious material and became ill with fever, chest pain, and cough only two days later. X-rays eight and 11 days later showed hilar adenopathy and generalized interstitial nodular infiltration in the lungs. He had a CF titer for histoplasmin of 1:32, and a strongly positive histoplasmin skin test.

The other four reinfection cases had symptoms similar to those of the other patients and the onset of disease was not accelerated (table 1). Their convalescent titers were all 1:128 or above, a level
reached by only 17 per cent of the remaining cases (table 2). The average size of the skin test, however, was not significantly different among four sick and four well individuals with previous infection (15.8 mm vs 17.3 mm, \( p > 0.5 \)) and in 29 other infected persons (16 mm, \( p > 0.5 \)).

**Times of onset and attack rates**

The times of onset shown in figure 1 suggest an exposure in early July. Six of the 50 proven cases visited the courthouse for periods ranging from 10 minutes to three hours and only on July 8. One visited only on July 9 and three sometime between July 7 and 10 with the exact day uncertain.

Among 451 replies to the questionnaire sent to license applicants who had visited the courthouse, 105 persons said they had experienced two or more symptoms, such as fever, myalgia, cough, chest pain, chills or headache during the month of July. Thirty-one of these had CF and skin tests. Only two had CF titers greater than 1:8, but both of these had visited the courthouse on July 8. The rest of the 31 “cases” had scattered dates of exposure and only five even had positive histoplasmin skin tests. It was concluded that another respiratory disease was also prevalent at the time and that symptoms alone could not point specifically to histoplasmosis in the general public.

The attack rates for serologically and clinically confirmed cases in the back and front of the courthouse building were 63 per cent and 34 per cent (\( \chi^2 = 5.55, p = 0.02 \)) (figure 2). There was no significant variation from the overall attack rate of 52 per cent on the four main floors of the building, although a licensing office on the ground floor separated from the main building had a rate of 86 per cent.

Both ill and non-ill people in the courthouse averaged 43 years of age, with nearly the same sex ratio, length of residence in Arkansas, percentage who had lived elsewhere, years employed at the courthouse, and percentage of smokers (table 4). The control group resembled the courthouse employees in these details except for having a higher percentage of females, and a greater percentage who had lived out of state (table 4).

**The environment**

The four-story brick courthouse was built in 1906. In the past several years pigeons and possibly other birds roosting on the roof of the courthouse and in the trees on the front lawn had been a problem and a poison bait had been placed on the roof in order to control the birds.

Several months before the epidemic, construction began of a number of new rooms on the west end of the courthouse. Major excavation was done at that time, but further minor excavations had been done on July 10 and 14 at the rear of the courthouse building. Careful inspection of the back of the courthouse showed that a layer of what appeared to be bird droppings lay on top of piles of dirt which had been placed there by the contractor in April or May. A number of bird bones were found in this debris, which apparently had been dropped from

### Table 4

**Characteristics of ill and well people and controls in an outbreak of histoplasmosis at an Arkansas courthouse in July, 1975**

<table>
<thead>
<tr>
<th>Status</th>
<th>Average age (years)</th>
<th>% males</th>
<th>Years lived in Arkansas</th>
<th>% ever lived outside Arkansas</th>
<th>Years employed at courthouse</th>
<th>% smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ill people (N = 46)*</td>
<td>42.6</td>
<td>51</td>
<td>26.4</td>
<td>43</td>
<td>5.2</td>
<td>63</td>
</tr>
<tr>
<td>Well people (N = 26)</td>
<td>43.2</td>
<td>62</td>
<td>25.0</td>
<td>42</td>
<td>5.7</td>
<td>52</td>
</tr>
<tr>
<td>Controls (N = 55)</td>
<td>41.7</td>
<td>25</td>
<td>24.7</td>
<td>68</td>
<td>58</td>
<td></td>
</tr>
</tbody>
</table>

* Information incomplete for four other cases.
the roof to the ground behind the building. Further inquiries revealed that the tower on the roof of the courthouse had been cleaned and the roof repaired in early July. Records of the roofing company showed that two men had cleaned the tower on July 8 and 9, with one of them completing the job on July 10. The two men came from another part of the state. They described the tower of the courthouse as a roosting place for pigeons, and the catwalk around the tower as covered by old bird droppings a foot deep interspersed with bones and feathers. On July 8, these droppings had been cleaned off with a shovel by one of the roofers and lowered in a bucket to the roof. His partner had then dumped the contents of the bucket from the roof to the ground in the back of the courthouse. Both roofers developed histoplasmosis, the one most exposed being the earliest of the cases of apparent reinfection.

In the 13 days prior to July 8, only 2.5 mm (0.1 in) of rain had fallen, and on July 8 the wind was blowing toward the back wall of the building at 0–16.1 kmph (0–10 mph). Daily high temperatures had been over 32.2 C (90 F) for a number of days. *H. capsulatum* was isolated from two of the 13 soil samples tested. The two positive samples were from the bird droppings found under the windows in the middle of the building’s south wall where the roof material had been dumped. The bones found in this material have been identified by Dr. A. C. Ziegler, Bishop Museum, Honolulu, HI, as those of the domestic pigeon (*Columba livia*). The remaining soil samples, including several from the soil excavated on July 10 and 14 did not yield *H. capsulatum*.

The microdroplet aerosol of oil apparently reproduced the pattern of air flow during the epidemic (figure 2). The droplet counts ranged from three to 149 (average of two counts each per 0.29 cm² area). The average count for rooms along the back wall of the building was 61 and for other rooms 43, the two figures having a ratio (1.42:1) resembling the ratio of attack rates in these
FIGURE 2B

4th FLOOR
ROOM CENTRALLY
AIR CONDITIONED

43

FIGURE 2. Cases of histoplasmosis and oil droplet counts in various rooms on the south (Figure 2A) and north (Figure 2B) sides of an Arkansas courthouse, July, 1975. Not all cases are shown in these two views. Average oil droplet counts per microscopic field are shown as numbers. ● = case (symptomatic, with or without laboratory confirmation). ○ = non-case. ☒ = window air conditioner.
two areas (63%:34% = 1.85:1), but the difference was not statistically significant \((p \approx 0.16, \text{ Student's } t \text{ test})\).

The cases and non-cases were compared by calculating for each person the average droplet count in the room where he worked. The averages of these figures for cases and non-cases were 63.2 and 52.9, respectively \((p \approx 0.19 \text{ by Student's } t \text{ test})\).

Two of the lowest counts—13 and 25—were obtained in rooms on the northwest corner of the first floor occupied by six persons who were not ill (figure 2). A card placed on the windowsill outside one of these rooms gave a count of only four. The room immediately between the two disease-free rooms had a count of 89 and three of eight persons employed there became ill. This center room, unlike the other two, had a door leading to the hallway which was frequently opened by people having business in the office. Thus, although the aerosol counts did not correlate precisely with attack rates, there was a tendency in this direction, and the aerosol method did explain several unusual features of the epidemic when the distribution of cases was scrutinized closely. Since almost all of the non-ill persons had positive skin tests and may have been partially immune, the location of susceptibles within the building may have been more important than particle counts in determining case location.

**Control measures**

It was concluded that infection had occurred on July 8 when numerous buckets of composted bird droppings were thrown from the roof to the ground past the back wall of the courthouse.

The contractor was advised to soak the infectious material with 3 per cent formalin on three different occasions and then bury it in a location safe from further excavation. This was accomplished in mid-August and the filters in all air conditioners in the building were changed by a workman who was histoplasmin positive and who was wearing a protective mask.

A circular was prepared and sent to contractors throughout the state, describing the epidemic and advising control measures such as wetting down dry bird droppings before removal. Follow-up of the affected individuals by periodic chest x-rays was continued for a year. No further cases were found after the initial epidemic subsided.

**Discussion**

Many other epidemics of histoplasmosis are described in the literature. Forty-one epidemics of histoplasmosis were mentioned in a review by Lehan and Furcolow (1) in 1957, and others have been described since then (2-9). The number of persons involved has varied from two to an estimated 8400 (5), and the usual mode of exposure has been inhalation of dust from soil which has been fertilized with bird droppings or in some cases from caves containing deposits of bat guano. Since the birds themselves had not been shown to be infected with *H. capsulatum* it has been proposed that bird droppings, perhaps through their salt content, suppress the growth of other fungi and bacteria and allow *H. capsulatum* to proliferate (14). Most epidemics have been due to material in contact with the ground. However, at least four epidemics have been described in people who cleaned out belfries, towers or buildings above ground level (1). In the case of the present epidemic, the deposits of droppings had apparently built up on the roof for many years and through composting had essentially become deposits of soil. Unfortunately, all deposits had been removed and the roof had been thoroughly tarred before the investigation began, and through a laboratory accident the few remaining samples obtained from the roof were discarded. Despite this, it appears virtually certain that the droppings dumped from the roof on July 8 were the source of the exposure.

Each office in the courthouse had a separate window air-conditioner except for the fourth floor which was centrally air-condi-
tioned but acquired its intake air from the lower three floors via the stairwell. Most of the window air-conditioners were set to recirculate inside air, but many appeared quite efficient in sucking aerosols (and infectious particles) into the building. The higher attack rate among people working in the back of the building where the dust was created, and the higher aerosol droplet counts in this area suggest that this was the case.

The aerosol method proved quite effective in quantitating the movement of air. In contrast to the more commonly-used smoke bomb method, semi-quantitative measurements of air flow can be taken at many points simultaneously at very little expense. There was reasonable correlation between the attack rates and the droplet counts in this epidemic. Other factors than exposure to the infectious material played a role, however, and the correlation might have been more striking had not nearly all the susceptibles in the building been infected.

The question of immunity to histoplasmosis and the amount of protection conferred by previous infection is a matter of dispute. Cases of presumed reinfection have been described in some epidemics (7, 12) and Powell et al. (11) described six cases of symptomatic infection in people with previously positive skin tests or a combination of pulmonary calcifications and a negative tuberculin test. CF titers in his cases ranged from 1:8 to 1:256. There is evidence that reinfection is more likely to have a short incubation period, perhaps due to hypersensitivity (12). This was strikingly demonstrated in one of our cases, with the onset of illness only two days after exposure, but the other four cases had "normal" incubation periods.

In the present epidemic the fact that 87.5 per cent of those who escaped illness had positive skin tests for histoplasmosis suggests that previous infection may have afforded some immunity. Since five persons with good evidence for a previous histoplasma infection were infected during this exposure, however, immunity appears to be only partial.

Those who were reinfeiced had considerably higher convalescent titers than the average case, raising the possibility that a high titer may indicate reinfection. Reinfection could not be confirmed or excluded in several others in the epidemic with high titers, because positive tuberculin tests obscured the meaning of calcium deposits on their chest x-rays. The skin test was not larger in reinfected individuals than in others in the epidemic, suggesting either that cellular immunity is not further stimulated by reinfection or that primary infection produces the maximum possible stimulation.

REFERENCES
