HISTOPLASMOsis ASSOCIATED WITH EXPLORING A BAT-INHABITED CAve IN COSTa RICA, 1998–1999

GEORGE M. LYON, ANA V. BRAVO, ARACELLY ESPINO, MARK D. LINDSLEY, ROSA E. GUTIERREZ, ISABEL RODRIGUEZ, ANA CORELLA, FLORA CARRILLO, MICHAEL M. MCNEIL, DAVID W. WARNock, AND RANA A. HAJJE

Mycotic Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Epidemic Intelligence Service, Epidemiology Program OfFice, and Epidemiology and Surveillance Division, National Immunization Program, Centers for Disease Control and Prevention, Atlanta, Georgia; Centro Integrado de Salud de Coronado, San Isidro de Coronado, Costa Rica

Abstract. Between October 1998 and April 1999, 51 persons belonging to two separate groups developed acute pulmonary histoplasmosis after visiting a cave in Costa Rica. The first group consisted of 61 children and 14 adults from San Jose, Costa Rica; 44 (72%) were diagnosed with acute histoplasmosis. The second group comprised 14 tourists from the United States and Canada; 9 (64%) were diagnosed with histoplasmosis. After a median incubation time of 14 days, the last common symptoms were headache, fever, cough, and myalgia. Risk factors for developing histoplasmosis included crawling (odds ratio [OR] = 17.5, 95% confidence interval [CI] = 2.3–802) and visiting one specific room (OR = 3.4, 95% CI = 1.0–12.3) in the cave. Washing hands (OR = 0.1, 95% CI = 0.01–0.6) after exiting the cave was associated with a decreased risk of developing histoplasmosis. Histoplasma capsulatum was isolated from bat guano collected from inside the cave. Persons who explore caves, whether for recreation or science, should be aware of the risk bat-inhabited caves pose for developing histoplasmosis, especially if they are immunocompromised in any way.

INTRODUCTION

Travelers are at risk for a multitude of infectious diseases, which range from the nuisance of traveler’s diarrhea to life-threatening dengue fever, malaria, or hemorrhagic fever.1,2 Endemic mycoses, such as histoplasmosis and coccidioidomycosis, have recently emerged as causing disease in returning travelers.3-7 Histoplasmosis is a systemic disease caused by the dimorphic fungus Histoplasma capsulatum, which exists as a soil saprophyte and grows best in soil enriched with bird or bat guano.8,9 Histoplasmosis occurs throughout the Western Hemisphere, but is highly endemic in the Ohio River and Mississippi River valleys of the United States.8 Most outbreaks occur following activities that disturb the soil of old roosting sites for birds or following a visit to a bat-inhabited cave.10-20 This report describes two outbreaks of histoplasmosis associated with visiting a bat-infested cave in Costa Rica. Histoplasmosis has been described in travelers returning from several Central American, South American, and Caribbean9 countries, but rarely from Costa Rica,20 and the epidemiology of histoplasmosis in Costa Rica is not well understood.

In October, 1998, a group of 61 children and 14 adults from a school in San Jose, Costa Rica traveled to northern Costa Rica. On this annual, two-day trip, the group explored a popular tourist cave. Within two weeks of returning home, several persons reported a febrile, respiratory illness. Initial serologic testing using immunodiffusion showed that two of the five patients’ serum samples reacted with H. capsulatum antigens. After the initial outbreak and despite the Costa Rican government’s recommendation to close the implicated cave to tourists and the posting of onsite warning signs, an adventure tour group from Ohio and New Brunswick, Canada visited this same cave. Within 10 days of returning home, 8 (57%) of the 14 participants developed febrile, respiratory illnesses. This report describes the investigations of these two outbreaks.

MATERIALS AND METHODS

At the request of the Costa Rican Minister of Health, we conducted an outbreak investigation, which is exempt from Institutional Review Board review. Written informed consent was obtained from all persons who completed a questionnaire.

Case definition. A case was defined as a person with laboratory evidence of H. capsulatum infection as determined by 1) a four-fold increase in the complement fixation (CF) titer on testing paired sera, 2) any single serum CF titer ≥ 1:32, 3) an M band seen in serum immunodiffusion testing, or 4) an acute-phase urine antigen with ≥ 1.0 enzyme immunoassay (EIA) units.

Costa Rica cohort study. We conducted a cohort study of the school group from San Jose. Each person completed a self-administered, standardized questionnaire and gave at least one serum sample. Questions included details on past medical history, date of symptom onset and duration, length of time spent in the cave, individual rooms visited, the types of activities performed within the cave, and any potentially protective measures taken. Additionally, clinical data on all ill persons from the cohort were abstracted and summarized from medical records at the Clinica Integrado de Salud de Coronado in Costa Rica, which provides primary medical care for the school and surrounding community of 80,000 people.

Costa Rica case-control study. To identify particular group activities during the school trip that may have been associated with an increased risk of H. capsulatum infection, we compared the entire cohort from the October 1998 visit with a convenience sample of 43 children at the same school who had visited the same cave on previous trips. The questionnaire administered to the control group was an abbreviated version of the questionnaire completed by the case-cohort.

United States/Canada cohort study. All 14 members of the United States/Canada adventure-tour group completed an abbreviated version of the original self-administered questionnaire and provided a single serum sample for analysis.

Serologic testing. All serum samples were transported to and evaluated at the Centers for Disease Control and Prevention (CDC) (Atlanta, GA). Immunodiffusion and CF tests for specific antibodies to H. capsulatum were performed on each serum sample.22 Tests were conducted at CDC using antigens that were produced at CDC using the microimmu-
noduleffusion and the microcomplement fixation protocols as previously described.\textsuperscript{23,24} Urine samples were sent to the Indiana University School of Medicine (Indianapolis, IN) to test for \textit{H. capsulatum} antigen using an EIA.\textsuperscript{25}

**Environmental survey.** The cave has two sections, each with three rooms. (Figure 1). One section is relatively dry and the other has a stream flowing through all three rooms. The second and third rooms on the wet side have openings to the outside through which bats freely enter and exit the cave. Bats and bat guano are found throughout the cave. Environmental samples were obtained from 5 (83\%) of the 6 rooms in the cave and cultured for \textit{H. capsulatum} using a mouse intraperitoneal method.\textsuperscript{26} Briefly, approximately 10 grams of bat guano was collected from each room. The guano was agitated with sterile saline, then allowed to settle. The supernatant was injected into the peritoneum of mice; six weeks later the mice were killed, and the spleens were homogenized and cultured on Sabouraud’s dextrose agar plates at 30°C. Colonies of \textit{H. capsulatum} were identified by their characteristic morphology and flat, gray colony appearance on Sabouraud’s dextrose agar and by growth in the yeast phase at 37°C on brain-heart infusion agar.

![Figure 1](image-url)

* - Positive culture  
† - Negative cultures

**RESULTS**

**Patient characteristics.** The demographics, serologic results, and symptoms of the cohort studied are summarized in Table 1. Acute sera were obtained from 54 persons from the Costa Rica cohort in November 1998. Convalescent sera were again collected from 55 persons from this cohort in February 1999. Sixty-nine persons provided at least one serum sample; 50 (72\%) showed serologic evidence of recent infection with \textit{H. capsulatum}. Questionnaires were completed for 62 (83\%) of the 75 persons who went on the school field trip. Our cohort analysis involved only those 56 (75\%) persons who had both a completed questionnaire and at least one serum specimen. Six (86\%) of 7 adults and 38 (76\%) of 49 children met the case definition for a total of 44 (79\%) patients. The median age was 14 years (range = 9–48); 34\% were female. Thirty-six (82\%) of the cases were symptomatic; for these, the median incubation period was 14 days (range = 8–18). The most common symptoms were headache, fever, cough, myalgias, chest pain, nausea, and dyspnea. The duration of symptoms in patients ranged from 1 to 45 days with a median of 4 days. Risk factor analysis of the cohort showed no exposures or activities to be significantly associated with an increased risk of developing histoplasmosis. Exposure to live chickens at home was associated with a decreased risk of developing histoplasmosis (relative risk \[RR\] = 0.5; 95\% confidence interval \[CI\] = 0.2–1.0) (Table 2). Most persons (77\%) in this group had no prior knowledge of histoplasmosis. However, this was not associated with an increased risk of developing histoplasmosis.

**Case-control study.** The case group comprised the 62 persons from the October 1998 trip who completed a questionnaire, and the control group had 43 students who had visited the cave in previous years. Persons who visited the cave in 1998 were more likely to have crawled while inside the cave (OR = 17.5, 95\% CI = 2.3–802) and to have visited wet room 3 in the cave (OR = 3.4, 95\% CI = 1.0–12.3). Washing hands after exiting the cave but before eating was associated with a decreased risk of histoplasmosis (OR = 0.1, 95\% CI = 0.01–0.6) (Table 2).

**United States/Canada cohort.** All 14 participants in this group completed a questionnaire and provided a single serum specimen for testing; 9 (64\%) met the case definition (Table 1). All cases visited the cave, whereas only 1 (33\%) of 3 non-cases visited the cave \(\chi^2 = 7.2, P = 0.007\). Seven (78\%) of the case-patients were symptomatic; the most common symptoms were cough, myalgias, fever, sweats, chest pain, and arthralgias. Ten (71\%) had no prior knowledge of histoplasmosis before visiting the cave in Costa Rica. Because of the previous outbreak and posted warnings, they were provided paper masks that most (91\%) wore. However, these loose-fitting masks did not provide any protection against histoplasmosis (\(RR = 0.9, 95\% CI = 0.7–1.1\)) (Table 2).
Environmental investigation. *Histoplasma capsulatum* was isolated only from the guano specimen taken from wet room 3 (Figure 1).

**DISCUSSION**

These investigations highlight the risk of acute histoplasmosis while visiting bat-inhabited caves in the Americas and the lack of awareness of this disease among travelers. Ashford and others found crawling beyond a specific point of a cave to increase the risk of histoplasmosis.18 Likewise, we found visiting the third and final room on the wet side of the cave to be associated with an increased risk of histoplasmosis. This is the same room from which *H. capsulatum* was cultured. We also found that crawling increased one’s risk of becoming infected with *H. capsulatum*. We hypothesize that this activity results in increased aerosolization of spores in the soil or guano, and brings one’s mouth and nose closer to ground sources of guano. Prior to these outbreaks, this cave was popular with tourist, averaging about 600 visitors per month. Despite these outbreaks and posting of warning signs, a recently conducted internet search showed greater than 10 tour operators who offer tours of this cave (http://www.google.com). It is unclear why outbreaks have not been associated with this cave prior to October 1998. It is hypothesized that *H. capsulatum* was introduced into the bat population of this cave soon before the October 1998 visit. The new *H. capsulatum*-infected bat(s) likely entered the cave through the large opening in wet room 3, which would explain why *H. capsulatum* was cultured from this room.

We identified two factors associated with a decreased risk of histoplasmosis, prior exposure to live chickens and washing one’s hands after leaving the cave. It is known that *H. capsulatum* grows better in soil enriched with bird droppings. 9 Therefore, one could hypothesize that prior exposure to

**Table 1**

Demographic, serologic, and clinical data from both the Costa Rican and United States/Canada cohorts*

<table>
<thead>
<tr>
<th>Factor</th>
<th>Costa Rica cohort risk ratio (95% confidence interval)</th>
<th>Costa Rica case-control cohort odds ratio (95% confidence interval)</th>
<th>United States/Canada cohort risk ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total members</td>
<td>76 (91)</td>
<td>14 (100)</td>
<td></td>
</tr>
<tr>
<td>Questionnaires completed</td>
<td>62</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>One or more serum samples</td>
<td>69 (91)</td>
<td>14 (100)</td>
<td></td>
</tr>
<tr>
<td>Acute sera</td>
<td>54 (71)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Convalescent sera</td>
<td>55 (72)</td>
<td>14 (100)</td>
<td></td>
</tr>
<tr>
<td>Met case definition</td>
<td>44 (79)</td>
<td>9 (64)</td>
<td></td>
</tr>
<tr>
<td>Four-fold increase in titer</td>
<td>15</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Single CF titer ≥ 1:32</td>
<td>38</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>M band on immunodiffusion</td>
<td>35</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Urine EIA ≥ 1.0 unit</td>
<td>2</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Questionnaire + ≥1 serum sample</td>
<td>56 (75)</td>
<td>14 (100)</td>
<td></td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>14 (9–48)</td>
<td>56 (36–69)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>15 (34)</td>
<td>8 (57)</td>
<td></td>
</tr>
<tr>
<td>Prior knowledge of histoplasmosis</td>
<td>14 (23)</td>
<td>4 (29)</td>
<td></td>
</tr>
<tr>
<td>Symptomatic</td>
<td>36 (82% of cases)</td>
<td>7 (78% of cases)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2**

Selected risk factors from the Costa Rica cohort, the Costa Rica case-control study, and the United States/Canada cohort*

<table>
<thead>
<tr>
<th>Factor</th>
<th>Costa Rica cohort risk ratio (95% confidence interval)</th>
<th>Costa Rica case-control cohort odds ratio (95% confidence interval)</th>
<th>United States/Canada cohort risk ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>0.9 (0.6–1.2)</td>
<td>0.8 (0.4–1.6)</td>
<td></td>
</tr>
<tr>
<td>Time in cave &lt; 2 hours</td>
<td>1.3 (0.9–1.8)</td>
<td>1.1 (0.9–1.4)</td>
<td></td>
</tr>
<tr>
<td>Wore mask</td>
<td>0.8 (0.5–1.5)</td>
<td>3 (0.6–30.4)</td>
<td>0.9 (0.7–1.1)</td>
</tr>
<tr>
<td>Crawling†</td>
<td>17.5 (2.3–802)</td>
<td></td>
<td>0.8 (0.6–1.2)</td>
</tr>
<tr>
<td>Contact with bat guano</td>
<td>1.2 (0.7–2.1)</td>
<td>2 (0.6–6.6)</td>
<td></td>
</tr>
<tr>
<td>Crawled over guano</td>
<td>1.0 (0.7–1.4)</td>
<td>0.6 (0.2–2.0)</td>
<td></td>
</tr>
<tr>
<td>Wiped face or nose with guano on hands</td>
<td>1.1 (0.8–1.6)</td>
<td>0.7 (0.2–2.3)</td>
<td>1.3 (0.8–1.9)</td>
</tr>
<tr>
<td>Visited third wet room</td>
<td>1.1 (0.6–1.8)</td>
<td>3.4 (1.0–12.3)</td>
<td></td>
</tr>
<tr>
<td>Washed hands after leaving cave but before eating</td>
<td>0.8 (0.6–1.1)</td>
<td>0.1 (0.01–0.6)</td>
<td>0.9 (0.7–1.1)</td>
</tr>
<tr>
<td>Exposed to live chickens at home</td>
<td><strong>0.5 (0.2–1.0)</strong></td>
<td>1.1 (0.3–3.6)</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in **bold** indicate a statistically significant association with acquiring histoplasmosis.
† All members of the group reported exposure to this factor.
‡ No members in the group reported this exposure.
chickens may have imparted some immunity. However, our case definition captured individuals with prior immunity as cases. Thus, it appears that prior chicken exposure is either a spurious finding or a marker for some other factor. Washing one’s hands after leaving the cave likely decreases the likelihood of depositing bat guano in or around the nose and mouth, as well as being a marker for improved hygiene. It is interesting that the use of paper masks in the United States/Canada cohort failed to protect against developing histoplasmosis.

Endemic mycoses had not been previously considered as a routine concern in the returning traveler. Two recent reports have described coccidioidomycosis occurring in traveling church-group members. In 2000, Cairns and others published a report of 21 cases of coccidioidomycosis occurring among a group of 126 people returning from northern Mexico. Similarly, another report from CDC described 8 cases of coccidioidomycosis among 30 persons returning from northern Mexico. While coccidioidomycosis has been known to occur among non-immune travelers to endemic regions, histoplasmosis has been principally described occurring among residents of endemic regions and posed little risk to visitors unless they explored a cave. However, in the spring of 2001 a large outbreak of histoplasmosis occurred among college students who had traveled to Acapulco, Mexico for spring break. As international travel becomes easier and more commonplace, travelers and physicians who care for them need to be aware of clinical manifestations, risk factors, prevention measures, and treatment of fungal diseases. This is especially true for immunocompromised individuals because endemic fungal diseases are more likely to disseminate and be fatal in this patient population. Recently, coccidioidomycosis and histoplasmosis have been included in the CDC Health Information for International Travel.

Despite several limitations, such as recall bias and a small number of controls, this study highlights the increased risk of histoplasmosis associated with exploration of bat-inhabited caves, including frequently visited caves. Persons who wish to explore these caves should be informed about histoplasmosis and how it is contracted. Like workers removing bird or bat guano from buildings, persons who explore caves should use personal protective equipment such as tight fitting masks to help prevent infection.

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Authors’ addresses: George M. Lyon, Department of Medicine, Infectious Diseases Division, Emory University School of Medicine, 1639 Pierce Drive, Woodruff Memorial Building, Room 2010 Atlanta, GA 30322, Telephone: 404-712-2051, Fax: 404-712-2278, E-mail: gmlyon@emory.edu. Ana V. Bravo, Araely Espino, Rosa E. Gutierrez, Isabel Rodriguez, Ana Corella, and Flora Carrillo, Centro Integrado de Salud de Coronado, San Isidro de Coronado, Costa Rica. Mark D. Lindsley, David W. Warnock, and Rana A. Hajjeh, Mycotic Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333. Michael M. McNeil, Epidemiology and Surveillance Division, National Immunization Program, Centers for Disease and Prevention, Atlanta, GA 30333.

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