

Bat and Soil Studies for Sources of Histoplasmosis in Florida

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IN October 1966 a college student contracted histoplasmosis after exploring a cave on a biology field trip in the vicinity of Gainesville, Fla. (1). The cave, in which the infection apparently occurred, was inhabited by bats and contained abundant amounts of guano. Since autochthonous histoplasmosis is rare in Florida, this case inspired a search for the source of the victim's infection. The investigation was subsequently expanded, and bats and guano-enriched soil that had been collected from all bat caves known to us in the State of Florida were analyzed systematically for the presence of *Histoplasma capsulatum*. The results form the basis of this report.

Materials and Methods

The initial studies were made at the suspected point-source of human infection, a sinkhole cave, known as Savior Cave, 10 miles west of Gainesville in Alachua County. This cave was inhabited by a small colony of southeastern myotis bats, *Myotis austroriparius*, and the floor was covered with large amounts of dried guano. Twenty bats and 34 samples of soil were collected from this site.

Approximately 100 yards from Savior Cave is another sinkhole called Grant's Cave. At its base this cave is 40 feet wide and 40 feet long, but it extends farther underground over water. At the proper season, it harbors a maternity colony of several thousand *M. austroriparius* bats (fig.

1). On January 19, 1968, when this site was visited, no bats were in the cave, but 23 samples of soil were collected (fig. 2). The soil contained large amounts of guano and had the consistency of moist sandy loam.

About 8 miles northwest of these caves, in the vicinity of Newberry, Alachua County, are three sinkhole caves, where a total of 98 *Pipistrellus subflavus* (eastern pipistrelle) and four *M. austroriparius* were collected; also 46 specimens of soil: 25 in Jones Cave, 9 in Newberry Bat Cave, and 12 in Seven Sisters Cave.

Other bats from colonies of *M. austroriparius* and *P. subflavus* were collected in caves near Floral City in Citrus County, Marianna in Jackson County, and Ocala in Marion County. The bats were picked up by hand or caught by mist nets. A total of 45 samples of soil were collected in four caves: 10 in No Name Cave, 15 in In-

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dian Cave, 10 in Small Picnic Area Cave, and 10 in Turnley Cave (table 1).

In addition to these cave-dwelling specimens, 104 bats were collected from an attic in Brooksville, Hernando County, a rolled-up awning in Orlando, Orange County, and a storm sewer in Tampa, Hillsborough County. The number and bat species collected (table 2) included 33 *M. austroriparius* and 71 *Tadarida cynocephala*, the Florida free-tailed bat.

The bats were placed in dry ice at -78° C. at the collection site and shipped air express to the laboratory. Autopsies were performed within 6 days after capture, and the lungs, liver, and spleen were cultured. The methods for culturing bat tissue and processing soil have been described previously (2).

In addition, 29 bats that had been sent to the central laboratory of the Florida Department of Health and Rehabilitation Services for rabies examination were studied. They included five *M. austroriparius*, 14 *Dasypterus floridanus* (Florida yellow bat), four *Lasiurus seminolus* (Seminole bat), and six *Nycticeius humeralis* (evening bat). These bats had been submitted from May 24 to September 25, 1968. All had been captured north of Orlando except two yellow bats, which came from Fort Lauderdale. They had been frozen 1 week to 2 months before autopsy—the majority, 4 weeks or less. A total of 366 bats were examined.

H. capsulatum was identified on the basis of its colonial morphology and the characteristic microconidia and tuberculate macroconidia. Representative isolates were converted to the yeast form on brain heart infusion agar enriched with 5 percent whole blood and on Pine's medium (3).

Results

H. capsulatum was isolated from one of the 20 *M. austroriparius* bats captured in Savior Cave (table 2) and from two of the 23 samples of soil collected in adjoining Grant's Cave (table 1). The results obtained with the remaining Florida colonial bats are described according to collection site in table 2. Of the 165 *M. austroriparius* studied, 80 (48 percent) were found to harbor *H. capsulatum*. This fungus was not isolated from the 101 *P. subflavus* or 71 *T. cynocephala* bats. Of the five *M. austroriparius* bats

The following persons assisted in locating and collecting some of the bats used in this study: Dr. James B. Nichols and Dr. E. Charlton Prather, bureau of preventable diseases, and Dr. William L. Jennings, encephalitis research center, Florida Department of Health and Rehabilitation Services, Jacksonville; Dr. Larry N. Brown, department of zoology, University of South Florida, Tampa; Dr. Lyman A. Scribner, Orange County Health Department, Orlando; Steve B. Fickett, Florida Fresh Water Fish and Game Commission, Brooksville; Ole T. Olsen, Marion County Health Department, Ocala; and Rodney Emerson and George Radimer, University of Florida, Gainesville.

submitted for rabies examination, one was found to harbor *H. capsulatum*. This bat had been collected in the vicinity of Leesburg, Lake County, Fla. None of the 18 solitary bats (*D. floridanus* and *L. seminolus*) yielded the fungus.

Three of the soil samples collected in Jackson County's Indian Cave and five of the soils from Turnley Cave in Marion County proved positive for *H. capsulatum*. In total, *H. capsulatum* was isolated from 10 of the 148 soil samples collected in the bat caves (table 1).

Cultures inoculated with bat tissues were kept 6 to 10 weeks before they were discarded. Cultures of the 181 bats collected from Brooksville, Floral City, Ocala, and Tampa (table 2) were kept 12 weeks. The time that macroscopic growth of *H. capsulatum* was first noted is recorded in table 3. Although 62 cultures appeared within the first 3 weeks of incubation, 70 more required 5 weeks of incubation before their de-

Table 1. Recovery of *Histoplasma capsulatum* from soil collected in Florida bat caves

Name of cave and county	Number of samples	Number positive
Grant's, Alachua	23	2
Jones, Alachua	25	0
Newberry Bat, Alachua	9	0
Savior, Alachua	34	0
Seven Sisters, Alachua	12	0
No Name, Citrus	10	0
Indian, Jackson	15	3
Small Picnic Area, Jackson	10	0
Turnley, Marion	10	5
Total	148	10

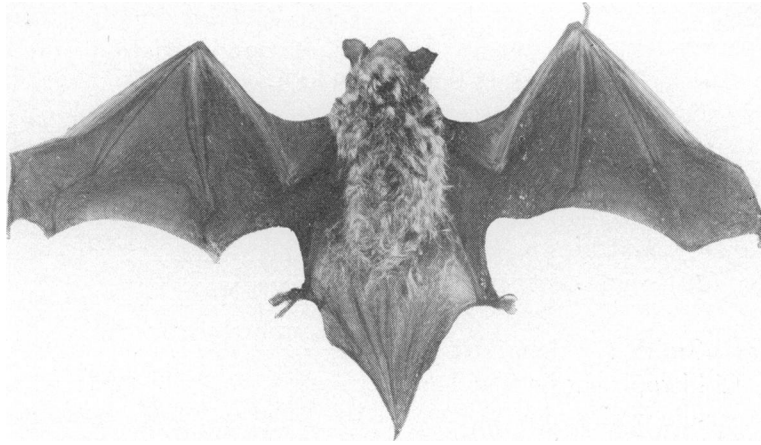


Figure 1. *Myotis austroriparius*, collected in Gainesville, Fla.

velopment could be detected. Fourteen isolates were first noted after 6 weeks and two after 8 weeks of incubation. Holding all negative cultures for 12 weeks failed to give additional



Figure 2. Descending into Grant's Cave, Gainesville, Fla., by collapsible ladder to collect bats and soil

positive cultures. The need to hold culture tubes inoculated with bat tissues for long periods of incubation was emphasized by these observations.

The relative frequency with which *H. capsulatum* was isolated from organ systems is shown in table 4. The spleen was most frequently involved (79 percent), followed by the liver (47 percent) and lungs (28 percent).

For confirmation, we attempted to convert 12 *H. capsulatum* cultures, representative of our isolates, to their yeast form. Only one converted on brain heart infusion agar enriched with 5 percent whole blood; all, however, readily converted on Pine's medium (3).

Discussion

Within the United States many persons consider the endemic area of histoplasmosis to be confined to the Mississippi-Ohio River Valley region. In reality the etiological agent, *H. capsulatum*, occurs far beyond this area. This fungus has been isolated from soils in the States of Connecticut, Georgia, New York, Pennsylvania, Minnesota, Nebraska, and New Mexico (4). In addition a bat survey in Arizona brought to light the existence of histoplasmosis in two species of bats (2).

On the basis of the chiropteran cases of histoplasmosis discovered in the State, especially by the isolation of *H. capsulatum* from soil, Florida must be considered as one of the endemic areas of the United States. The incidence and prevalence of this disease in the human population, however, is low. Histoplasmin skin-test surveys of lifetime residents have shown that

the prevalence of positive reactors ranges from 2 percent (5) to 8 percent (6). This low prevalence of sensitivity is reflected in the fact that only two autochthonous cases of histoplasmosis have been diagnosed in the State (1, 7). Significantly, both were associated with visits to bat caves. We could not determine whether the cases cited by Meleney (8), Matthiesen (9), and Carmona and Allen (10) were contracted in Florida or elsewhere.

In 1958 Tegeris and Smith (7) described an acute primary case of histoplasmosis that had developed in a 32-year-old white man in 1955 approximately 3 weeks after he had explored a bat cave (exact locality unspecified) near Floral City. Dr. N. F. Conant, professor and chairman of the department of microbiology and immunology, Duke University Medical Center, Durham, N.C., stated in a personal communication (1958) that he had isolated *H. capsulatum* from soil collected in this cave in 1955. During our study, 58 *M. austroriparius* were collected in No Name Cave near Floral City—presumed to be the source of the soil studied by Conant—and 37 (64 percent) were found to be positive for *H. capsulatum*. None of the soils collected from this cave were positive.

The second case involved the college student who had visited Savior Cave in the Gainesville area (1). Tesh and Schneidau (11) reported

Table 3. Relationship of incubation time to isolation of *Histoplasma capsulatum* from bat tissues

Incubation time (weeks)	Total isolations (N=148)	Percent of total
3-----	62	42
5-----	70	47
6-----	14	9
8-----	2	1
12-----	0	0

the isolation of *H. capsulatum* from 80 of 105 *M. austroriparius* (76 percent) captured in northern Florida. In a personal communication of January 18, 1968, Tesh stated that the location was Grant's Cave, adjacent to Savior Cave. In cultures of 20 bats from Savior Cave, one was found to be positive (5 percent). In addition, we recovered *H. capsulatum* from two of the 23 soil specimens collected in Grant's Cave.

On the basis of skin-test surveys, the general population of Florida apparently is not at risk of exposure to *H. capsulatum*. However, our study and that of Tesh and Schneidau (11) point out the potential danger inherent in visits to bat caves in a region generally not considered to be infected with *H. capsulatum*. In Florida apparently little or no fungus exists in the usual

Table 2. Collection sites and results obtained from Florida colonial bats examined for *Histoplasma capsulatum*

Site	Date collected	Species	Number studied	Number positive	Percent positive
Gainesville: Savior Cave.....	Jan. 19, 1968	<i>Myotis austroriparius</i> ¹	20	1	5
Newberry:					
Jones Cave.....	Mar. 16, 1968	<i>Pipistrellus subflavus</i> ²	83	0	0
Jones Cave.....	Mar. 16, 1968	<i>Myotis austroriparius</i>	3	2	66
Seven Sisters Cave.....	Mar. 16, 1968	<i>Pipistrellus subflavus</i>	12	0	0
Bat Cave.....	Mar. 16, 1968	<i>Pipistrellus subflavus</i>	3	0	0
Bat Cave.....	Mar. 16, 1968	<i>Myotis austroriparius</i>	1	0	0
Marianna:					
Indian Cave.....	May 9, 1968	<i>Pipistrellus subflavus</i>	1	0	0
Indian Cave.....	May 9, 1968	<i>Myotis austroriparius</i>	6	4	66
Small Picnic Area Cave.....	May 9, 1968	<i>Pipistrellus subflavus</i>	2	0	0
Small Picnic Area Cave.....	May 9, 1968	<i>Myotis austroriparius</i>	7	4	57
Ocala: Turnley Cave.....	May 21, 1968	<i>Myotis austroriparius</i>	37	23	62
Floral City: No Name Cave.....	May 22, 1968	<i>Myotis austroriparius</i>	58	37	64
Brooksville: attic.....	May 23, 1968	<i>Tadarida cynocephala</i> ³	71	0	0
Tampa: sewer.....	May 24, 1968	<i>Myotis austroriparius</i>	15	9	60
Orlando: awning.....	July 7, 1968	<i>Myotis austroriparius</i>	18	0	0

¹ *M. austroriparius* or southeastern myotis.

² *P. subflavus* or eastern pipistrelle.

³ *T. cynocephala* or Florida free-tailed bat.

avian habitats known to favor its development elsewhere (12). It would seem that the chances of becoming infected by *H. capsulatum* are greater following visits to bat habitats, especially caves, than in daily life outside of such habitats. The validity of this hypothesis is strengthened by finding that among a group of Florida speleologists 71 percent, or 10 of 14, had positive reactions to histoplasmin (1).

Distribution of *M. austroriparius* in Florida is known to be limited to the area north of Tampa, and individual bats of this species have a range of only 10.6 miles (13). It has been suggested that the range of infected bats may also be a factor in the prevalence of histoplasmosis in the human population if the bat is considered responsible for the dissemination of *H. capsulatum* (14). The precise role played by bats in the epidemiology of histoplasmosis remains unknown. Carefully controlled studies of the type initiated by Shacklette and Hasenclever (15) are needed to determine whether these interesting mammals actually seed caves and other areas with *H. capsulatum* or whether they are merely chance victims of this fungus.

The paucity of clinical cases of histoplasmosis in Florida may be influenced by the habits of *M. austroriparius*. This chiropteran species is known to prefer caves with permanent pools of water for its maternity colony (13). These pools are used as a source of drinking water and

to maintain the relative humidity of the harbor- age at 99 or 100 percent. The water also serves to prevent predators from climbing the walls and capturing the young bats. Most sinkhole caves in Northern Florida contain natural pools of water.

Shacklette and Hasenclever (15) studied the effect of flooding in a cave known to be contaminated with *H. capsulatum*. They observed that after water covered the floor of the cave, the number of isolations of viable particles of the organism were decreased in soil, water, and air samples and in sentinel animals. This may explain the low frequency of spore dispersal in Florida.

Additional evidence to support this hypothesis is a comparison of the lung infections of bats in Florida and Arizona. Only 28 percent of infected bats from the wet Florida caves yielded *H. capsulatum* from the lungs, while this pathogen was isolated from the lungs of 66 percent or 10 of 15 of the positive bats captured in the hot, dry, dusty harborages of Arizona (2).

Of the colonial species of bats studied (*M. austroriparius*, *P. subflavus*, *T. cynocephala*, and *N. humeralis*) only *M. austroriparius* proved to be infected by *H. capsulatum*. Eighty-one or 48 percent of the 170 specimens of this species collected were positive. Tesh and Schneidau (11) collected *M. austroriparius* in only three Florida caves and found 66 percent to be infected. *H. capsulatum* was not isolated from any of the 101 *P. subflavus* studied, yet these bats were captured in the same caves that harbored infected *M. austroriparius* (table 2). *P. subflavus* has not been reported to be infected with this fungus. Not one of the solitary bats cultured by us (*D. floridanus* or *L. seminolus*) yielded *H. capsulatum*. None of the 337 bats captured during this study demonstrated overt signs of clinical illness at the time of capture.

We cannot account for the fact that only one of the six bat species collected in Florida was infected with *H. capsulatum*. This finding probably was not due to a differential in susceptibility of the species because *N. humeralis* has been found to be infected elsewhere by *H. capsulatum* (11), as has *T. cynocephala* (2, 16, 71), considered by some mammalogists to be co-specific with *Tadarida brasiliensis*.

Table 4. Frequency of isolation of *Histoplasma capsulatum* from *Myotis austroriparius*, by organ system

Collection site	Number of positive bats	Number of cultures, by organs ¹		
		Lung	Liver	Spleen
Savior Cave.....	1	1	0	1
Jones Cave.....	2	0	0	2
Indian Cave.....	4	2	3	1
Small Picnic Area Cave.....	4	2	1	1
Ocala.....	23	6	7	20
Floral City.....	37	7	21	33
Tampa.....	9	5	5	6
Leesburg ²	1	0	1	0
Total.....	81	23	38	64

¹ 6 cultures per bat, 2 per organ.

² Leesburg specimen was 1 of 5 previously forwarded to laboratory for rabies examination.

None of the 161 *T. brasiliensis* bats collected in attics by Tesh and Schneidau (11) in Louisiana, Mississippi, and Texas proved to be infected by *H. capsulatum*. Therefore these investigators speculated that "habitat rather than species may be the major factor in determining whether or not a bat becomes infected." They suggested that the "conditions influencing the growth and survival of the fungus (temperature, humidity, pH, and composition of soil), are quite different in a cave and a hot, dry attic." In a Trinidad study, Ajello and co-workers (18) considered attics inimical to the survival of *H. capsulatum*. However, this line of reasoning appears to be invalid. Tesh and Schneidau (11) isolated *H. capsulatum* from two of 37 *N. humeralis* bats collected in a Mississippi attic. Ajello and co-workers (16) also found 17 percent of the *T. cynocephala* collected in an Alabama attic to be infected. In addition, they isolated *H. capsulatum* from the bat guano that had accumulated in this attic. The basis for these discrepancies undoubtedly lies in the environmental variations that exist in attics.

During our Florida study, we found that long periods of incubation were needed to isolate *H. capsulatum* from bat tissues. During the first 3 weeks of incubation of one group of bat tissues, only 42 percent of the positive cultures grew. Forty-seven percent appeared after 5 weeks. An additional 9 percent was obtained 6 weeks after inoculation. Holding the negative cultures 6 weeks longer (total of 12 weeks incubation) only yielded two more positive cultures (1 percent), which occurred at the end of 8 weeks.

We do not feel that bat isolates of *H. capsulatum* are intrinsically difficult or impossible to convert to the yeast form by *in vitro* procedures, as implied by other investigators (11). Persistence and the choice of media are critical factors in achieving success.

Careful examination of all the primary isolates of *H. capsulatum* obtained in this and the Arizona (2) study showed that all isolates formed white colonies with aerial mycelium, which turned golden brown as tuberculate macroconidia formed. The brown mutant form of *H. capsulatum* described by Berliner (19) was not noted in primary isolation tubes or on subculture.

Summary

An autochthonous case of histoplasmosis in a college student in Florida, the second case on record in the State, was the impetus for an investigation of the distribution of *Histoplasma capsulatum* in bats and their environment. The patient had explored a sinkhole cave that was inhabited by bats.

H. capsulatum was isolated from one of 20 *Myotis austroriparius* collected from this cave. Examination of cave dwelling bats demonstrated this fungus to be present in 48 percent of the 170 bats of this species only. Bats from which *H. capsulatum* was isolated were collected in the vicinity of Floral City, Gainesville, Marianna, Newberry, Ocala, and Tampa, Fla. *H. capsulatum* was not isolated from the 101 *Pipistrellus subflavus* or 71 *Tadarida cynocephala* specimens studied.

H. capsulatum was also isolated from 10 of 147 guano-enriched soil samples (6.8 percent) collected in nine bat caves. *M. austroriparius*, the only species found to be infected with *H. capsulatum* in Florida, is known to prefer caves with permanent pools of water and a relative humidity of 99 to 100 percent. It is suggested that the wet caves may tend to inhibit spore dispersal and thereby account for the low rate of histoplasmin sensitivity and the rarity of clinical histoplasmosis among native Floridians.

Prolonged incubation up to 8 weeks increased the yield of *H. capsulatum* from bat tissue with 10 percent of the isolates not appearing until the fifth to eighth week.

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Tearsheet Requests

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Effective Therapy for Rickets Resistant to Vitamin D

The first effective treatment for vitamin D resistant rickets has been announced at the University of Wisconsin. The active form of vitamin D, isolated at Wisconsin by biochemist Hector F. DeLuca, caused dramatic improvements in patients suffering from this rare form of bone disease. Clinical tests were conducted by Dr. J. Rodman Seely at the University of Oklahoma Medical Center.

Persons with vitamin D resistant rickets are not responsive to the usually effective vitamin D therapy because they cannot change enough of the vitamin they receive into its health-supporting active form to satisfy their bodies' needs. This active form, discovered by Dr. DeLuca and his research team, allows the body to utilize calcium in building strong bones and aids muscle action.

Typical of the series of successful treatments was that of a 44-year-old invalid housewife

suffering such severe bone pain that she was confined to a wheelchair. She was given the active vitamin D form, called 25-hydroxy-cholecalciferol or 25-HCC, in therapeutic amounts over a 10-month period. Subsequent X-rays showed complete healing of old fractures and overall strengthening of her bones. In time, she was able to walk again.

Although relatively large healing doses of 25-HCC are necessary, Dr. DeLuca says that no undesirable side effects or toxic reactions have been noted among the five patients treated in the current study. He noted that the large amounts of 25-HCC may be necessary to overcome a hereditary defect in either the body's transport system for vitamin D and its active form or the body's vitamin D metabolism.

The joint Wisconsin-Oklahoma research team is supported by the Public Health Service.