Occurrence of Dermatophytes in Fresh Bat Guano

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ABSTRACT

KAJIHIRO, EDWIN S. (Wayland Baptist College, Plainview, Tex.). Occurrence of dermatophytes in fresh bat guano. Appl. Microbiol. 13:720-724. 1965.—Evidence is presented in support of the hypothesis that fresh bat guano serves as a means of pathogenic fungi dissemination in caves. A total of 371 guano samples were collected from caves in southeastern New Mexico. Each sample was agitated in sterile saline and sand. The supernatant fluid was treated with an antibiotic and streaked on differential media. Cultures were incubated at 25 and 37 C and examined at intervals over a 4-week period. For animal inoculation, highly concentrated inoculum was injected intraperitoneally into white Swiss mice. Animals were sacrificed 4 weeks later, and portions of their lung, liver, and spleen were cultured on selective media, incubated at 25 C, and examined at intervals over a 4-week period. Microsporum gypseum was isolated at all 10 collecting stations with an incidence of 22.4%, Trichophyton mentagrophytes at 7 stations with an incidence of 5%, T. rubrum at 3 stations with an incidence of 3%, and T. terrestre at 1 station with an incidence of 0.5%. From a total of 60 pools of liver-spleen-lung suspensions, 6 pools yielded positive cultures of Histoplasma capsulatum and 1 pool yielded T. mentagrophytes. No significant difference was found among the different selective media with respect to recovery of dermatophytes. Among the human pathogenic fungi isolated were Candida sp., Cladosporium sp., Coccioides immitis, Cryptococcus neoformans, H. capsulatum, M. gypseum, T. mentagrophytes, T. rubrum, T. terrestre, and Sporotrichum sp.

The dermatophytes discussed in this paper were isolated during a search for Histoplasma capsulatum in fresh bat guano. The findings provide information on their distribution and incidence in caves infested with bats. The first report of a dermatophyte growing saprophytically in nature was of one isolated from the mud of watercourses in the park of the University of Peco (Szathmary, 1936). One year later a colony of Trichophyton sp. was found growing on horse dung (Munende and Webb, 1937). While investigating the cause of “Cave Disease,” T. mentagrophytes (asteroids type) was isolated from the soil of Johnson’s Pothole and Makapan caves (Lurie and Borok, 1955). This was believed to be the first record of the isolation of T. mentagrophytes from soil. T. mentagrophytes and Microsporum gypseum were isolated from the atmosphere of the Makapan caves by exposure of laboratory animals (Lurie and Way, 1957). Cultures of the livers and spleens of some of the sacrificed mice resulted in the growth of dermatophytes. This was believed to be the first record indicating that the spores of these fungi may be found in the atmosphere.

In April, 1963, the workers at Carlsbad Caverns were reported as being predominantly reactors to histoplasmin sensitivity tests. With the knowledge that environmental factors may intensely affect the occurrence of human pathogenic fungi (Zeidberg and Ajello, 1954), attempts were made to explore the relationship of H. capsulatum to its environment of Carlsbad Caverns. In view of the recent reports (Emmons, 1958, 1961; McDonough et al., 1961) of the isolation of H. capsulatum from soils enriched with bat or bird guano, samples of fresh bat guano from Carlsbad Caverns were examined for the presence of H. capsulatum. A large number of dermatophytes were found, and, since the recovery of these organisms from bat guano has not been reported, an investigation of the occurrence of dermatophytes in bat guano resulted. The description of the isolation methods used and the occurrence of dermatophytes isolated from fresh bat guano from four caves in the southeastern region of New Mexico are reported in this paper.

MATERIALS AND METHODS

The mycological study of fresh bat guano was begun on 6 July 1964. A total of 371 fresh guano
samples were collected on three occasions (July, August, and October, 1964) from Carlsbad, Sitting Bull, McKittrick, and Cottonwood caves, all of which were infested with bats. Due to road hazards, it was not possible to visit Mudgetts Cave.

The samples were scooped up directly into sterile 8-oz screw-cap bottles and were placed in a dry-ice chest to reduce the activity of the microflora. Immediately upon return to the laboratory, 1 g of each sample was suspended in 100 ml of sterile physiological saline solution with sterile sand and vigorously agitated for 30 sec. After several hours of settling at room temperature, 10 ml of the supernatant fluid were pipetted into a test tube. For each milliliter of the supernatant fluid, 0.25 ml of an antibiotic solution (2 mg of streptomycin and 5 mg of penicillin per ml of water) was added; 1 ml of this inoculum was streaked on two plates, each composed of Sabouraud Dextrose, Mycobiotic, Littman Oxgall, and Dextrose (all Difco products) agar medium. One set of the cultures was left at room temperature (25°C), and the duplicated set at incubated temperature (37°C). The culture plates were examined at intervals over a 4-week period. For selective isolation of dermatophytes and other keratinophilic fungi, the hair-bait technique was also used (Vanbreuseghem, 1952). Sterile petri dishes of moistened guano were baited with short strands of sterilized human hair and incubated at room temperature. Mycelium growth on hair filaments was examined microscopically in lactophenol cotton blue and also cultured on Sabouraud Dextrose medium containing streptomycin and penicillin. For animal inoculation, 1 ml of the antibiotic-treated inoculum was injected intraperitoneally into white Swiss mice. Animals were sacrificed 4 weeks later, and the lungs, livers, and spleens of five mice were pooled and made into a homogeneous suspension with the aid of sterile sand, mortar, and pestle. The supernatant fluid from this suspension was then inoculated onto five plates of Brain Heart Infusion Dextrose (Difco) agar with 6% human blood, 20 units of penicillin, and 40 units of streptomycin per ml. The petri plates were incubated at 37°C and examined at intervals over a 4-week period. The remainder of the original guano suspension was kept at room temperature for further examination if necessary.

Identification of fungi recovered was made by direct examination of the colony characteristics (Fig. 1) and microscopic examination of the spores.

**Results**

In July, August, and October, 1964, 371 fresh bat guano samples were collected from four caves in the southeastern region of New Mexico. The dermatophytes isolated from specific habitats are listed in Table 1. The number of stations, sample per station, and per cent isolation are also cited. Table 2 presents the method of isolation, number of isolations, and total per cent isolation.

All samples were collected from just below the top of the fresh guano piles, thus eliminating as much contamination from the atmosphere as possible.

Two hundred samples were taken from the bat cave of Carlsbad Caverns. This cave extended some 548.8 m, with a ceiling of around 60.9 m. Some 300,000 Mexican freetail bats [Tadarida mexicana (Saussure)] were estimated to be roosting during the period of collection. Guano piles were found as high as 1.5 to 1.8 m approximately every 15.2 m. Samples were collected from five stations within a distance of 304.9 to 533.5 m from the main entrance of the bat cave. *M. gypseum* was isolated from 16 of 200 samples from all five stations. Nine samples from three stations were recovered with *T. mentagrophytes*. *T. rubrum* was found in four samples at two stations.

Samples (117) were taken from McKittrick cave which, from a narrow passage, led directly into a large central room with numerous adjoining caves. Some 3,000 Mexican freetail bats were estimated in the area of 60.9 to 91.5 m from the central room. Samples were taken from three stations where guano was found covering the floor from 5.1 to 14.7 cm. Samples taken from all three stations were recovered with *M. gypseum* and *T. mentagrophytes*. Of 117 samples, 29 showed isolates of *M. gypseum*, 7 samples were recovered with *T. rubrum*, and 2 samples with *T. terrestris*.

Thirty-six samples were taken from Sitting Bull cave. This cave is a small oblong structure 15.2 m long, with a ceiling of about 2.1 m. Only a small young colony (about 100 to 300) of Mexican freetail bats was found just 6.1 m inside the main entrance, which is concealed by a waterfall. *M. gypseum* was recovered from two samples and *T. mentagrophytes* from one sample.

Only 18 samples were taken from Cottonwood cave. This huge cave is located about 121.8 m down along the slope of Frank's Canyon. A small roosting area of about 3,000 to 4,000 Mexican freetail bats was found about 609.8 m from the main entrance of the cave. Due to the rapid sloping nature of this cave, search for other roosting areas of bats was not made. *M. gypseum* was the only organism isolated from two samples.

**Discussion**

The recovery of *M. gypseum*, *T. mentagrophytes*, *T. rubrum*, and *T. terrestris* during this study reveals that dermatophytes do exist as saprophytes in fresh guano of bats.

The importance of isolation techniques was demonstrated in the results obtained with dermatophytes and other human pathogenic fungi isolated during this investigation.
Fig. 1. Dermatophytes grown on Mycobiotic agar for 4 weeks at 25 °C. (a) Microsporum gypseum; (b) Trichophyton mentagrophytes; (c) T. rubrum; (d) T. terrestre.
From a total of 10 collecting stations, 371 samples yielded 112 positive samples with a 30.2% total isolation. Of 371 samples collected, 83 of these were shown to contain *M. gypseum* by use of the direct culture method at 25 C, and 2 at 37 C. Of these organisms, 78 were recovered by use of the hair-bait technique, and 3 were from tissue pools of mice. Of 371 samples, 17 yielded isolates of *T. mentagrophytes* by use of direct culture at 25 C, and 1 was from tissue pools of mice. Direct culture at 37 C and hair-bait culture failed to yield this fungus. Of 371 samples, 10 were recovered with *T. rubrum* by use of direct culture at 25 C. Two samples from direct culture at 25 C were recovered with *T. terrestre*, *T. rubrum* and *T. terrestre* were not cultured at 37 C by use of direct culture or by use of the hair-bait method.

The human pathogenic fungi isolated were: *Candida* sp., *Cladosporium* sp., *C. immitis*, *C. neoformans*, *H. capsulatum*, *M. gypseum*, *T. mentagrophytes*, *T. rubrum*, *T. terrestre*, and *Sporobolichum* sp. Except for *C. immitis*, all of these fungi are not known to be endemic in the southeastern region of New Mexico. It is believed that this is the first record of the isolation of these organisms from fresh guano of Mexican freetail bats.

Despite the many reported isolations of systemic pathogenic fungi associated with excreta of pigeons (Emmons, 1955; Littman and Schneier- son, 1958) and decayed bat guano in caves (Alarcon, 1957; Campins et al., 1955; Gonzalez, 1959; Lazarus and Ajello, 1958, Montemayor, Heredia, and Pietri, 1958), little or no knowledge has been reported on the isolation of dermatophytes from excreta of birds or mammals.

It is probable that if adequate samples were collected further association of dermatophytes and other pathogenic fungi in fresh guano of bats would have occurred. Owing to the many hazards involved in the search for bats, especially in these caves with little or no information as to their structural formation, it was difficult at times to collect samples of significant number. However, only by testing guano samples from as many different species of bats would the significant occurrence of these fungi be revealed. Consequently, to evaluate the significance of these fungi in fresh bat guano, samples will be collected from caves at Big Bend National Park and possibly farther south in central Mexico, where
many species of bats from New Mexico and West Texas are known to migrate in late fall and early winter of each year.

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Literature Cited


