Histoplasma capsulatum Recovered from Bat Tissues

Abstract. *Histoplasma capsulatum* was recovered from the liver and spleen tissues of a species of predominantly insectivorous bats as well as from soil collected in a man-made harborage at Madden Air Field in the Republic of Panama.

Although the recovery of the fungus *Histoplasma capsulatum* from soils enriched with bat or bird guano has been documented (*1*), successful attempts to recover it from the tissues of bats have not been reported.

More than 100 bats were collected from a building adjacent to the air strip of the now-abandoned Madden Air Field, Republic of Panama. Within the building, the roosting habits of the bats were such that the guano collected could be related to the predominant bat using that particular roost. Three species of bats were recovered, *Chilonycterus rubiginosa fusca, Carollia perspiculata azteca,* and *Phyllostomus hastatus panamensis.* The latter species, an omnivore, was not examined for the presence of *Histoplasma capsulatum.* In addition to the bats and guano collected within the building, soil samples were taken at the entrance, under the eaves, and at the rear of the building.

Thirty *Chilonycterus rubiginosa fusca* were killed in groups of five; livers and spleens were pooled and made into a homogeneous suspension with the aid of sterile sand, mortar, and pestle. The supernatant from this suspension was then inoculated onto two blood-fortified mycosel (*2*) plates and into each of five mice (0.2 ml intraperitoneally). Forty-five *Carollia perspiculata azteca* were processed in similar fashion, after a holding period of 22 days at the Corozal Veterinary Quarantine Station. Thus a total of six pools of *Chilonycterus* and nine pools of *Carollia* liver-and-spleen suspensions were sampled for the presence of *Histoplasma capsulatum.*

None of the plates inoculated directly with the suspensions of liver and spleen revealed the presence of *H. capsulatum.* In addition, none of the mice inoculated with the tissues of *Carollia perspiculata azteca* yielded *Histoplasma capsulatum* when killed at varying times after inoculation. In contrast, two of the *Chilonycterus* pools (A445 and A447) yielded positive cultures. The details of these recoveries are of interest: of the five mice inoculated with pool A445, two were sacrificed on the 36th, two on the 37th, and one on the 38th day after inoculation. Liver and spleen suspensions were prepared and inoculated onto two blood-fortified mycosel plates which were then incubated at 26°C for approximately 15 days. Each of the two plates prepared on the 36th day after inoculation yielded *Histoplasma capsulatum.* One mouse of the five inoculated with pool A447 died on the second day after inoculation, and it was discarded. The remaining four mice were killed in two groups on the 36th and 37th days after inoculation. Each of the four plates prepared after their sacrifice yielded *H. capsulatum* after 2 weeks of incubation at 26°C.

The soil and guano specimens were placed at −20°C for 24 hours before processing to reduce the activity of minute fauna. Each 2-g sample of soil or guano was suspended in saline containing penicillin, streptomycin, and Achromycin (*3*) to a final concentration of 400 units, 400 and 40 μg/ml, respectively, and then shaken vigorously for 5 minutes. After 2 hours of settling, the supernatant was decanted and used to inoculate each of five mice intraperitoneally with 0.5 ml. All mice inoculated with supernatants from guano speciments died within 24 hours, despite prior treatment with antibiotics. Repeat isolation attempts from these materials, with more dilute inoculum, were unsuccessful, since the toxic principle in the guano persisted even after dilution.

Mice inoculated with the four soil samples were sacrificed at varying intervals of time after inoculation. Pools of liver and spleen from mice inoculated with soil samples collected at the entrance to the building were prepared on the 35th (two mice), the 37th (two mice), and the 41st day (one mouse) after inoculation. Each pool was inoculated onto duplicate blood-fortified mycosel plates and incubated at 26°C. When examined 15 days later, all six plates revealed the presence of *H. capsulatum.*

Thus, from one site in Panama, *Histoplasma capsulatum* was recovered from the tissues of two pools of bats and from the soil at the entrance to the artificial harborage. The multiple recoveries from bat pools, the recovery of the organism from mice inoculated with soil, and the numerous negative findings from similar materials processed at the same time substantiate the validity of these findings.

Three months later (3 October 1961) a second collecting trip to this same site yielded 14 specimens of *Chilonycterus rubiginosa fusca* which were brought to the laboratory and sacrificed immediately for inoculation of liver and spleen suspensions onto blood-fortified mycosel media and into mice. From these direct inoculations 9 of the 14 bats examined yielded positive cultures for *Histoplasma capsulatum.*

The role of bats in the dissemination of *H. capsulatum* in nature remains to be determined. Whether they are able to disseminate the organism in their excreta or whether they experience overt disease cannot be stated at this time. However, the association of the organism with bat guano suggests this animal may eventually be implicated as one of the reservoirs in nature.

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References and Notes

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2. Ten percent citrated human blood, obtained from Baltimore Biological Laboratories.
3. Achromycin is Lederle Laboratories' brand of tetracycline hydrocholoride.

20 October 1961

Irrigation and Nitrogen Effects on Sweet Corn Row Numbers at Various Growth Stages

Abstract. Kernel rows per ear of a hybrid of *Zea mays L.* were found to be affected by environmental conditions during the first 7 weeks after planting. Increased row numbers were evident on harvested ears if as little as 25 pounds per acre of nitrogen fertilizer was added at planting.

This investigation was conducted on Superstition loamy fine sand at Yuma, Arizona, in 1959 as part of a much larger 3-year study, 1957–1959 (*1*). Sweet corn, *Zea mays L.*, certified Golden Cross Bantam, was grown to determine the effect of quantity and timing of irrigation and nitrogen on the several basic components of yield: ears per plant, rows per ear, kernels per row, and weight of individual kernels. Three growth stages were defined and used