# TRANSMISSION OF *PLASMODIUM GALLINACEUM* BY ADULT *AEDES AEGYPTI* INFECTED AS LARVAE

A. BURNS WEATHERSBY[1] AND GREGGORY K. STOREY[2]

*Department of Entomology, University of Georgia, Athens, GA 30602*

ABSTRACT. Transmission of *Plasmodium gallinaceum* to chickens by adult mosquitoes eclosed from larvae that consumed infected adult mosquitoes was investigated. No malarial infections were observed in chicks fed on by mosquitoes that eclosed from larvae that had consumed crushed infected adult mosquitoes. Where cadavers with noncrushed thoraces were used, 3 of 4 chicks fed on by adult mosquitoes developed parasitemias ranging from 6 to 11% infected erythrocytes.

Sergent (1937) demonstrated that *Culex pipiens* Linn. adults could infect canaries with *Plasmodium relictum*, if, as larvae, they had ingested adult mosquito thoraces containing sporozoites. A single canary was infected after being bitten by a female *Cx. pipiens* eclosed from a larva fed 2 *Cx. pipiens* thoraces containing live sporozoites 29 days previously. A low-level parasitemia was evident in the bird 12 days after the feeding and persisted for 5 days. This mode of transmission by ingestion of infected mosquitoes by larvae has yet to be confirmed with adequate controls and larger sample sizes. Therefore, the objective of our study was to conduct experiments similar to those of Sergent, but with larger numbers of mosquitoes, in order to evaluate this mechanism of transmission.

The NMRI strain of *Aedes aegypti* (Linn.) was reared in the laboratory and maintained at 27°C and 70% RH. The larvae were fed a diet of equal parts of ground Purina Dog Chow (100 mesh), lactalbumin and brewer's yeast. The 8A strain of *P. gallinaceum* was maintained by alternate passage from mosquitoes to white rock (Peterson-Albraker) cockerels. Parasitemias (10–30%) in the chicks were assessed routinely by blood examinations and infections in the mosquitoes by examining salivary glands for sporozoites (30 sporozoites per 10 microscope fields; 400×).

Fifty female third instar *Ae. aegypti* larvae were transferred to a 4- × 12-cm porcelain container. Within 3 days, all larvae had molted to the fourth instar. The larvae were transferred to a clean container without food. *Plasmodium gallinaceum* infected adult mosquitoes were killed by freezing at −20°C for 15 min. The legs and wings of the adults were removed and 50 cadavers, either with whole or crushed thoraces, were placed in the container of larvae. Fourth instar larvae fed upon the infected adult mosquitoes placed on the water surface and consumed all the cadavers. After 24 h, the larvae were returned to their normal diet. Pupation occurred within 2 days, and adults were collected upon eclosion.

Where infected cadavers with crushed thoraces were used to feed larvae, a single 10-day-old chick was exposed to ca. 25 of the adult *Ae. aegypti* females for 15 min 4 days posteclosion. Additionally, 4 chicks were each injected with 0.2 cc of a Hank's balanced salt solution homogenate of 10 of the eclosed mosquitoes. Infection of the chicks was determined by microscopic observations of Giemsa stained blood smears at 7 and 14 days postexposure.

In the second experiment using cadavers with noncrushed thoraces, the infectivity of the eclosed mosquitoes was evaluated as described above. The experiment was replicated 4 times. No malarial infections were observed in chicks fed on by mosquitoes that eclosed from larvae that had consumed crushed infected adult mosquitoes. Likewise, there were no infected chicks among those injected with homogenates of female mosquitoes eclosed from larvae that had consumed crushed infected adult mosquitoes. This was probably because sporozoites lose their infectivity upon exposure to water within the ruptured thoraces (Martin D. Young, Department of Infectious Diseases, University of Florida; personal communication).

Where cadavers with noncrushed thoraces were used, 3 of 4 chicks fed on by adult mosquitoes developed parasitemias ranging from 6 to 11% infected erythrocytes. All 16 chicks injected with homogenates of 10 mosquitoes derived from larvae that had fed on noncrushed infected mosquitoes developed parasitemias which ranged from 6 to 13% infected erythrocytes. No information was collected on the presence of sporozoites in the salivary glands of the eclosed adults.

This study demonstrates that mosquito larvae that fed on infected adult mosquitoes were infective as adults, as reported by Sergent (1937). Although no data was collected on the actual route of transmission of the sporozoites follow-

[1] Deceased.

[2] Current address, University of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850.

ing ingestion, it is presumed that the sporozoites entered the hemocoel through the midgut and invaded the salivary glands. More research must be conducted to determine the actual route of transmission of the sporozoites following ingestion.

Weathersby (1960) reports that *Ae. aegypti* injected with exoerythrocytic stages of the parasite are capable of transmitting *P. gallinaceum* to chicks. Weathersby et al. (1971) also reported that *P. gallinaceum* can be acquired by adult *Ae. aegypti* feeding on engorged cage mates. It seems unlikely though that plasmodia could be perpetuated solely by the mechanisms listed here, since no evidence of replication of the parasite in the mosquito has been observed in any of the studies.

We are grateful to Kenneth Holm for his assistance with mosquito cultures and related techniques, Seaboard Farms of Athens Inc. for providing chickens and Arden Lea, Martin Young and Clayton McCoy for a critical review of the manuscript.

## REFERENCES CITED

Sergent, E. 1937. Transmission de *Plasmodium relictum* selon des modes non habituels. Arch. Inst. Pasteur Algier. 15:11–17.

Weathersby, A. B. 1960. Experimental infections of *Aedes aegypti* with exoerythrocytic stages of *Plasmodium gallinaceum*. Exp. Parasitol. 9:334–337.

Weathersby, A. B., H. Ah and J. W. McCall. 1971. Mosquitoes feeding on engorged mosquitoes. Mosq. News 31:110–111.