# ISOLATION OF *ASCOGREGARINA* SP. (EUGREGARINIDA: LECUDINIDAE) FROM *AEDES HENDERSONI*[1]

EDGAR D. ROWTON,[2,3] ROBERT S. COPELAND[3] AND GEORGE B. CRAIG[3]

Aseptate gregarines found in mosquitoes belong to the genus *Ascogregarina* (Syn. *Lankesteria*; Ward et al. 1982). Elongate, motile gamonts are found in the intestinal lumen of infected 4th instar larvae and small ellipsoid oocysts can be found in the Malpighian tubules, hindgut or rectum of infected pupae or adults. With phase contrast microscopy, intracellular trophozoites are visible in the midgut epithelial cells of 3rd instar larvae. Trophozoites in earlier instars are not easily detected without histological methods. Oocysts are released by fecal contamination or decay of infected adults in water. Young larvae ingest the oocysts while feeding. This life cycle seems well suited to mosquitoes whose larvae develop in tree holes, such as *Aedes triseriatus* (Say), which inhabits both lower and upper level tree holes in hardwood forest habitats (Sinsko and Grimstad 1977) and is commonly infected with *Ascogregarina barretti* (Vavra 1969). However, a sibling species, *Ae. hendersoni* Cockerell, which generally inhabits upper level tree holes has not previously been reported to be infected with gregarines. Larvae of both mosquito species may be found together in the same upper level tree hole. *Ascogregarina* may be pathogenic to mosquito species in which they do not normally develop. We conducted this study to determine if *Ae. hendersoni* from St. Joseph County, IN, were infected with *Ascogregarina*. Also, we wanted to determine if this pathogen is different from *A. barretti* and if it affects *Ae. triseriatus*.

Ten percent of *Ae. hendersoni* larvae (n = 250) collected in the spring and summer of 1983 from tree holes in St. Joseph County, IN, contained motile gamonts resembling *A. barretti*. Subsequently, *Ae. hendersoni* larvae were collected from tree holes which did not contain *Ae. triseriatus*. These larvae were maintained through eclosion in the laboratory. Oocysts were collected from adult mosquitoes and the gregarine was cultured in a laboratory colony of *Ae. hendersoni* as described by Rowton and Munstermann (1984). The infection rate and subsequent parasite development of this new isolate in both *Ae. hendersoni* and *Ae. triseriatus* were compared with those of a sympatric isolate of *A. barretti*. Both mosquito species used in these experiments were colonized from St. Joseph County, IN.

For each mosquito species, four culture dishes were set up containing 100 1st instars in 100 ml of water. Two culture dishes of each mosquito species received $2 \times 10^5$ oocysts of the gregarine isolated from *Ae. hendersoni* and the other two received the same number of *A. barretti* oocysts. The larvae were maintained at 24°C and fed daily. A subsample of 4th instar larvae was dissected and examined for the presence of parasites. Oocysts of the new isolate produced midgut infections in 100% of the *Ae. hendersoni* and 90% of the *Ae. triseriatus* larvae. When the two species of mosquitoes were allowed to feed on *A. barretti* oocysts, the infection rate was 100% for *Ae. triseriatus* and 97% for *Ae. hendersoni* (Table 1).

Although differences between initial infection rates were slight, greater differences were found for the site of trophozoite development within each host, the number of parasites becoming established and the number of oocysts produced. In the mosquito species from which each gregarine was originally isolated, the trophozoites de-

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[2] Department of Entomology, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

[3] Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556.

Table 1. Infection parameters of *Ascogregarina barretti* and the *Aedes hendersoni* isolate in natural and reciprocal hosts.

| Infection parameters | *Aedes triseriatus* | | *Aedes hendersoni* | |
|---|---|---|---|---|
| | *A. barretti* | new isolate | *A. barretti* | new isolate |
| % infection | 100 | 97 | 90 | 100 |
| Location of trophozoites in midgut | anterior | anterior, posterior | anterior, posterior | anterior |
| Gamonts/larva[1] | 114.5 | 33.5 | 36 | 350+ |

[1] Each treatment was replicated twice. Results are expressed as the mean of the medians from the two replicates. Fifteen larvae were examined for each replicate.

veloped in epithelial cells at the anterior portion of the midgut. In cross infections, large numbers of trophozoites were also found in epithelial cells of the posterior portions of the midgut, which might indicate delays in rupture of the oocyst wall and release of sporozoites. In the *A. barretti/Ae. triseriatus* infections, a mean gamont count of 114.5/larva was observed (Table 1) compared to 36/larva when *A. barretti* developed in *Ae. hendersoni.* When oocysts of the newly isolated gregarine were used for infection, a mean gamont count of 350+/larva was observed in *Ae. hendersoni* compared to 33.5/larva in *Ae. triseriatus.*

The only observed pathology in cross infections was occasional clusters of dead, darkly pigmented and hardened gamonts found in the hindgut, Malpighian tubules and/or the posterior midgut of pupae. These clusters, which were rare in normal infections, were found most frequently in dead pupae.

To another experiment, oocysts were introduced as described above, to 1st instars at doses from $2 \times 10^3$ to $32 \times 10^3$/larva. Following emergence of adult mosquitoes, the number of new oocysts produced was determined with a hemocytometer as described by Rowton and Munstermann (1984). Oocysts from all host/parasite combinations were infective to both mosquito species. At all dosage levels, the natural mosquito/gregarine combination produced considerably more oocysts than did the cross infections (Fig. 1).

These data suggest that the gregarine isolated from *Ae. hendersoni* is not *A. barretti,* even though it is similar in morphology and infection rates. It appears to be a distinct species well adapted to its natural host. This conclusion is supported by observed differences in numbers of gamonts and oocysts produced, location of trophozoite development, and the presence of dead, hardened gamonts in cross infections. Although the gregarine isolated from *Ae. hendersoni* may have been responsible for the death of some *Ae. triseriatus* pupae, the level of mortality is too low for this parasite to be an effective biological control agent. However, these gregarines may influence larval competition and habitat selection of these two species of mosquitoes.

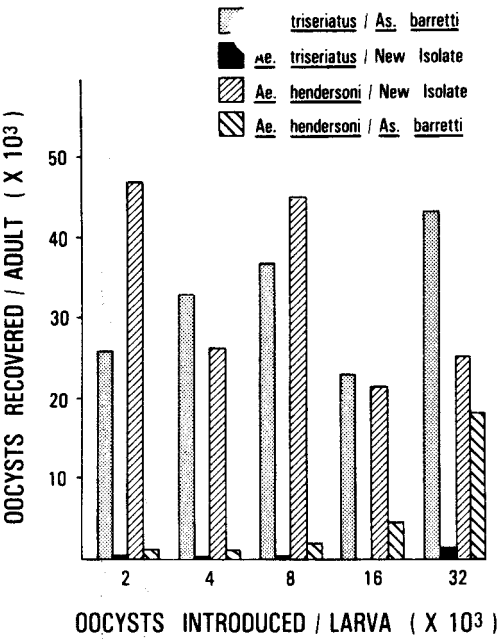


Fig. 1. Number of oocysts produced at 5 dosage levels in *Aedes triseriatus* and *Ae. hendersoni* following infection of 1st instar larvae with *Ascogregarina barretti* and the new gregarine isolated from *Ae. hendersoni.*

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