Journal of the American Mosquito Control Association, 11(1):133–135, 1995

OPERATIONAL AND SCIENTIFIC NOTES

COLONIZATION OF *ANOPHELES BARBIROSTRIS* FROM CENTRAL JAVA, INDONESIA[1,2]

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ABSTRACT. The colonization of *Anopheles barbirostris* from Central Java is described. Locally acquired materials and ambient laboratory temperature, humidity, and daylight proved acceptable for continuous rearing. A simple, inexpensive larval diet based on a 10:4 powdered mixture of beef and rice hulls proved advantageous.

Anopheles (*Anopheles*) *barbirostris* Van der Wulp is an important vector of malaria and Brugian filariasis on the islands of Sulawesi, Flores, and Timor in Indonesia (Atmosoedjono et al. 1976, Hoedojo 1983, Lee et al. 1983). In contrast, because of its zoophilic behavior, this species is of little or no medical importance on other islands of the Indonesian archipelago, including Java and Sumatra, or throughout the remainder of its extensive Oriental distribution (Reid et al. 1979).

Past attempts at continuous colonization of *An. barbirostris* from various parts of Indonesia have been unsuccessful. The brief description of procedures by Jayewickrema (1952) in Sri Lanka was found unacceptable for our Indonesian field populations. Choochote et al. (1983) were successful in rearing 2 strains from Thailand for many generations; however, specific information on rearing procedures was not reported. Our general rearing methods follow more or less standard procedures (Gerberg 1970).

The following procedures were initially worked out for a nonvector population from Central Java but have subsequently been found acceptable for a vector strain from the eastern archipelago (Boru, Flores). The Salatiga laboratory of the Vector Biology Research Station contains spacious rearing rooms provided with ample natural light from windows and skylights. General temperature and humidity in the facility were not controlled and were kept at prevailing ambient conditions.

The methods described herein were designed using locally available and inexpensive materials. Only the collapsible aluminum adult cages required purchase outside of Indonesia. *Anopheles barbirostris* were obtained bloodfed or gravid from natural outdoor resting and cattle shelter collections in Jambu subdistrict, Semarang regency, Central Java. Individual females ($n = 150$) were placed in paper cups for oviposition. Each cup was lined with filter paper and one-third filled with nonchlorinated well water. The tops of the cups were covered with fine mesh screen and females were provided with a 10% sugar solution soaked into cotton. Eggs were laid on the water surface and later transferred into white enameled metal pans (25 cm diam × 5 cm depth) containing 500 ml clear (nonfiltered) well water. Four-centimeter-wide filter paper strips were placed along the inner walls of the pans to prevent stranding of eggs. All hatching occurred within 2–3 days.

The following procedures were used for routine colonization: approximately 400 1st-instar larvae were transferred into larger enameled pans (35 × 24 × 5 cm) containing 2 liters of well water. A finely powdered larval food was spread evenly onto the water surface once daily. The amount of food was adjusted to the sizes and numbers of larvae present. A number of different food combinations were tried (Barodji et al. 1985). The one found most acceptable was a mixture (10:4) of low fat (lean) dried powdered beef and ground rice hulls (*bekatul*). Although larval development was delayed 2–3 days compared to food mixtures containing powdered dog food and yeast, larval and adult mortality remained nearly identical between the 2 nutrient combinations. The availability and low cost of the beef:*bekatul* mixture offset the disadvantage of delayed development. This food mixture also reduced maintenance because larval foods containing

[1] The opinions and assertations contained herein are those of the authors and are not to be construed as official or reflecting the views of the U.S. Naval Service or the Indonesian Ministry of Health.

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Fig. 1. Metal enameled pans (35 × 24 × 5 cm) containing well water and clumps of grass (*Paspalum conjugatum*) for larval development.

commercially available dog food were more likely to create unfavorable conditions in the medium. It was found advantageous for larval development and survival to place small clumps of coarse grass (*Paspalum conjugatum*), cleaned of most soil but retaining a root system, into the pans (Fig. 1).

Development to pupae occurred between days 8 and 10. Pupae were transferred into cups and placed into mosquito cages, 45 × 45 × 45 cm and 45 × 45 × 90 cm, to produce 5,000 and 10,000 emergent adults per cage, respectively (Fig. 2). The sex ratio was generally 1:1. Adults were provided 10% sugar solution on cotton pads. Small (500 ml) water-filled clay pots (nonglazed) provided additional humidity. The moist, cool clay surfaces were preferred resting sites for adults. Rearing temperature was dependent on daily ambient conditions, which ranged from 20 to 31°C. Relative humidity varied between 50 and 90%. During hot dry periods of the year, cages were covered with moist toweling. This species rapidly adapted to laboratory rearing despite these humidity and temperature extremes. This strain was free-mating in either cage size. Two- to 3-day-old females readily engorged on restrained guinea pigs. A small (500 ml) wide-mouthed earthen pot lined with filter paper and filled with clear well water and a light grass infusion attractant were placed in the cage for oviposition. Initial

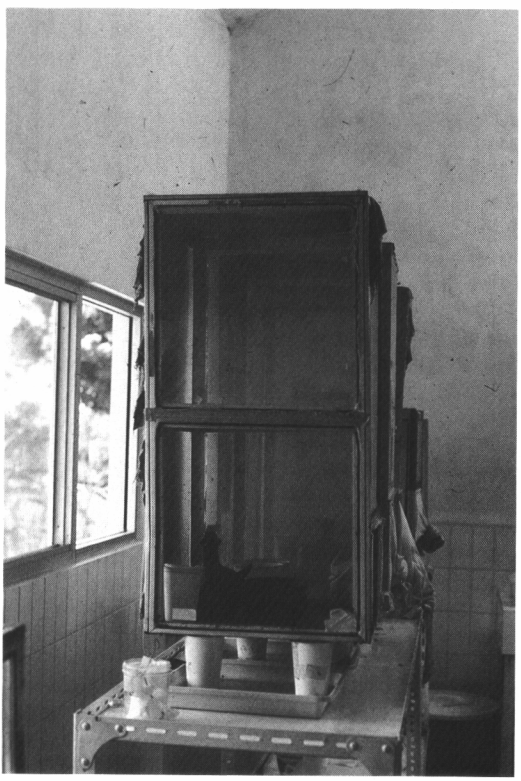

Fig. 2. Collapsible aluminum cages (45 × 45 × 90 cm) for adult mosquitoes containing a fired clay jug for additional moisture and a clay pot for oviposition.

egg laying occurred 2–3 days after a single blood meal.

Mortality of first-generation larvae and pupae from wild-caught adults was high (41%). Adult mortality was also high compared to succeeding generations. Beyond the F_2 generation, the colony rapidly adapted and became synchronized with minimal mortality from immatures to eclosion.

In nature, *An. barbirostris* can occupy a wide range of larval habitats including small pools, swamps, and rice fields. Generally, clear fresh water with emergent, floating, or submergent vegetation are preferred oviposition sites. In Indonesia, large populations are associated with rice cultivation. Rapid success at colonizing this species may be due to the combined use of clear, nonpolluted well water, an acceptable larval food based on rice hulls, the provision of vegetation in the larval medium, and the use of ambient climatic and photoperiod conditions.

Development of routine procedures for continuous colonization of *An. barbirostris* will permit increased laboratory experimentation on the differential vector status of this species, including cross-hybridization and vector competence. Laboratory populations should also prove valuable for evaluating potential control strategies.

This study was supported by funds provided by the Indonesian Ministry of Health and the Naval Medical Research and Development Command, Navy Department for Work Unit No. 3M161102BS13.AD410.

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