TOXORHYNCHITES AMBOINENSIS LARVAE RELEASED IN DOMESTIC CONTAINERS FAIL TO CONTROL DENGUE VECTORS IN A RURAL VILLAGE IN CENTRAL JAVA[1]

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ABSTRACT. The efficacy of *Toxorhynchites amboinensis* larvae for control of dengue vectors in household water storage containers was tested in a rural village in Central Java, Indonesia. Concrete cisterns and clay jars were the most common types of containers used for long-term water storage, although smaller numbers of metal drums were also used. All containers in use in the village received 5–10 second- or third-instar *Tx. amboinensis* larvae biweekly for 7 months. Vector surveillance (adult and larval) was conducted biweekly between treatments. No differences in man-biting rates or larval population indices were noted between the treatment and control areas. It is hypothesized that the multiplicity of larval habitats in this rural area accounted for the lack of impact of predator releases, which were directed solely toward artificial containers.

INTRODUCTION

Dengue fever is a disease typically associated with urban areas, but in recent years it has occurred in rural areas of Java, Indonesia, with increasing frequency (Eram et al. 1979). *Aedes aegypti* (Linn.) and *Aedes albopictus* (Skuse) are common in rural villages in Central Java, and both species can be found breeding in man-made as well as natural containers in and near human habitations (Jumali et al. 1979). In such villages, water for household use is obtained from wells and is stored in several types of artificial containers, including steel drums, clay jars, plastic buckets and "bak mandi" (concrete cisterns approximately 1 m^3). These containers are important sources of dengue vectors.

Methods for the control of *Ae. aegypti* using sequential applications of *Toxorhynchites* Theobald eggs have been described (Gerberg 1985). In a pilot study conducted on the Caribbean island of St. Maarten, a single innundative release of *Tx. brevipalpis* (Theobald) eggs resulted in temporary control of *Ae. aegypti* in water storage containers (Gerberg and Visser 1978).

An advantage to the use of biological agents such as *Toxorhynchites* spp. is that householders may find them less objectionable for use in drinking water than chemical pesticides (Gerberg 1985). We attempted to control dengue vector populations in household water storage containers in a rural village of Central Java using sequential releases of *Tx. amboinensis* (Doleschall) larvae. In a previous study (Annis et al. 1989) using first-instar predator larvae in household water storage containers in Jakarta, Indonesia, the degree of control achieved was poor. This was presumably due, at least in part, to the limited ability of first-instar larvae to withstand periods of starvation. Since later instars are better able to survive longer foodless periods, second and third-instar larvae were used in this study.

MATERIALS AND METHODS

Study area: The treatment area was the village of Susukan in Ungaran, Central Java, Indonesia. Susukan contains 212 houses with a population of approximately 1,500 people and is approximately 2 km^2 in area. The houses are interspersed with large clusters of bamboo and various species of fruit trees, including banana, papaya and mango. The village is surrounded by rice fields and groves of coconut palms.

Houses are of various types of construction including thatch, wood and brick. The great majority of houses are not screened and are open to entry by mosquitoes. The village does not have running water. Water for domestic use is obtained from wells and stored in a variety of containers both in and out of doors.

The village of Mojo, approximately 3 km distant and similar in area and population (228 houses), was used as a control site.

Toxorhynchites *production:* A colony of *Tx. amboinensis* was maintained at the Ungaran

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[1] This study was supported through funds provided by the U.S. Naval Medical Research and Development Command, Navy Department for Work Unit No. 3M161102BS10.AD.410 and by the Indonesian Ministry of Health.

The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the U.S. Navy department or the Naval Service at large and the Indonesian Ministry of Health. Send reprint requests to the Publications Office, U.S. Naval Medical Research Unit No. 2, San Francisco, California 96528–5000 or P.O. Box SC#410, Manila, Philippines.

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laboratory of the Vector Control Research Station of the Department of Health. Larvae were reared individually in 37-ml plastic cups and were fed larvae of *Ae. aegypti* of the same instar daily until pupation. The pupae were rinsed, transferred to beakers and placed inside 61-cm^3 cages in which the adults were kept. Adults were fed a mixture of 10% honey in water from dental wicks suspended from the top of the cage. Black plastic bowls, 10-cm in diam, were placed in the cage for oviposition. Eggs were collected daily and placed singly in wells of 24-well tissue culture plates for hatching. After hatching, larvae were transferred to 37-ml plastic cups.

Larvae to be released in the field were fed *Ae. aegypti* and reared to the second or third stadium in the laboratory. Larvae were transported to the field in 37-ml cups with snap-on lids.

Vector surveillance and predator release: Prior to initiation of the study, all houses in the treatment and control villages were examined to determine the types and quantities of water storage containers present. Vector surveillance was conducted biweekly and consisted of landing-biting counts and a larval survey. Landing-biting counts were performed by 2 collectors collecting indoors for 10 min in each of 6 randomly selected houses per day, 4 days each sampling week. Landing mosquitoes were aspirated from exposed lower legs. Collections were made between 1000 h and noon. The mean number of bites per man-hour was calculated.

Larval surveillance was done in all containers in use in the village at the time of the survey. Containers were recorded as being positive or negative for *Aedes* larvae. No attempt was made to distinguish between *Ae. aegypti* and *Ae. albopictus* in this study.

To establish baseline data, surveillance was conducted on 4 occasions between September 22 and November 2, 1987. Treatment was begun on November 10. Releases were made biweekly in the weeks between surveillance activities. All containers in use in the treatment village at the time of release were treated. Each container, depending on its size, received 5–10 second or third-instar *Tx. amboinensis* larvae. Treatment was carried out until May 23, 1988, and the final vector survey was conducted on May 30.

In order to reduce accidental removal of predator larvae by water consumers, a public education program was instituted in the treatment village. Technicians visited each family and alerted household members to the presence of the predators, taught them to distinguish predators from prey larvae, and briefed them on the objectives of the project and the role of predators in biological control. Community participation in predator conservation was encouraged.

RESULTS AND DISCUSSION

The types and quantities of water storage containers used in the study areas are shown in Table 1. Clay jars predominated in the control village while jars and bak mandi were nearly equally abundant in the treatment village. With the exception of steel drums, the majority of containers were kept indoors.

At the initiation of the study, container indices in the treatment and control areas were similar (Fig. 1), while the biting rate was considerably higher in the treatment area (Fig. 2). The higher biting rate in the treatment area may have been due to the greater abundance of bamboo. Bamboo stumps are sources of *Ae. albopictus*, and to a lesser extent, *Ae. aegypti*, in other rural villages in Central Java (Jumali et al. 1979). *Aedes* population trends were similar in both villages. The container index showed an overall decline in both areas as the study progressed. By comparing the percent change in the container indices from the pretreatment baseline, the impact of treatment can be estimated. It can be seen in the lower portion of Fig. 1 that population changes were very similar in the treatment and control areas, and that the degree of decline was actually slightly greater in the control area.

A similar trend is apparent in the adult population surveillance data (Fig. 2). After an initial increase in November, biting rates decreased in both areas as the study progressed. In absolute terms, the amount of increase in the biting rate in the second month was quite similar in the 2 villages. However, the relative increase in the control area was considerably higher. This might indicate some population suppression due to

Table 1. Types and quantities of water storage containers used in 2 villages in Central Java, Indonesia

	Control village			Treatment village		
Type	Indoor	Outdoor	Total	Indoor	Outdoor	Total
Bak mandi	18	14	32	93	27	120
Clay jar	198	36	234	132	13	145
Drum	1	13	14	10	21	31
Other	49	1	50	73	11	84

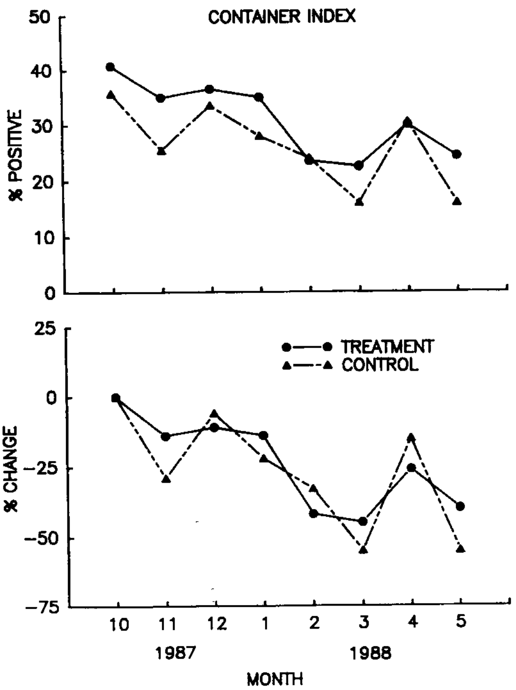

Fig. 1. Container indices and percent change in control and treatment villages.

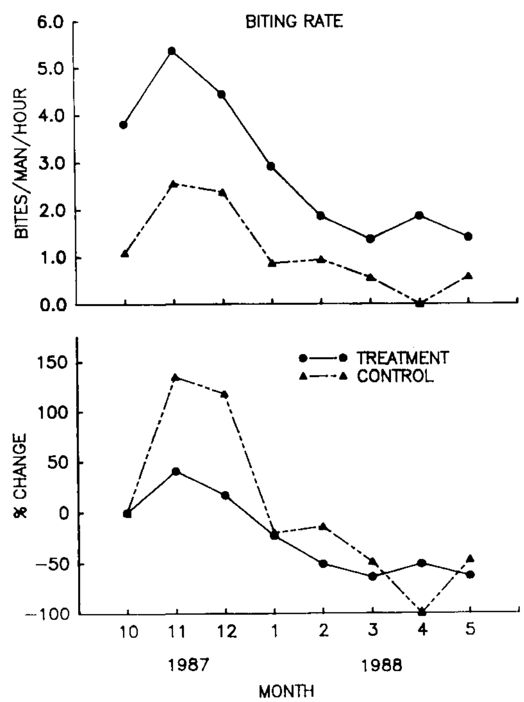

Fig. 2. Man-biting rates and percent change in control and treatment villages.

treatment, but it is quite clear that over the course of the entire study period the degree of change was similar in the treatment and control villages. Therefore, we feel that changes in *Aedes* populations in both areas were mediated primarily by environmental or biotic factors not associated with treatment, and that the impact of released *Tx. amboinensis* larvae was insignificant.

The results of this study were disappointing in light of our previous experience with releases of first-instar larvae in an urban neighborhood of Jakarta (Annis et al. 1989). In that study, in which each container in the treatment area was treated with a single first-instar larva about once every 2.5 weeks, some suppression of the *Ae. aegypti* population was evident in both adult and larval population indices. This study was designed to improve the degree of control by overcoming 2 of the major problems presumed to have hampered the first trial, e.g., losses of predators due to starvation, and their accidental removal as water was consumed. Hence, the more starvation-resistant second- and third-instars were used, and an intensive public education and community participation effort was initiated. In addition, the number of predators per container was increased. Yet no effect due to treatment was detected.

Focks et al. (1980) stated that a computer simulation model predicted that a single *Tx. rutilus rutilus* (Coquillett) larva in 80% of containers in an area would reduce adult prey density by 75% in about 20 days. In field trials, adult emergence was reduced an average of 74% in containers receiving 1 or 2 first-instar *Tx. rutilus rutilus* larvae every 10 days for 50 days (Focks et al. 1982).

A probable explanation for our failure to see significant reductions in adult vector populations is that outdoor breeding in natural containers remained unaffected by treatment and was a more important source of vectors than domestic containers. However, Jumali et al. (1979) concluded that natural containers were relatively unimportant sources of *Ae. aegypti* compared to water storage containers. Our landing-biting collections were performed indoors and would have reflected changes in the *Ae. aegypti* population, since *Ae. albopictus* is rarely collected indoors (Gould et al. 1970, Jumali et al. 1979). Therefore, any impact of treatment of household containers should have been detected by adult surveillance and not have been masked by breeding of *Ae. albopictus* in natural containers.

Control efforts directed solely towards water storage containers may be inadequate in rural situations where a significant amount of breeding occurs in natural containers. Predator releases in households may be more effective when used as an adjunct to outdoor control activities. To examine this question, predator introductions should be compared with a method known to provide complete control in water storage containers, such as larviciding with temephos, as a component of a more comprehensive control program including outdoor control measures.

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