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AN OPERATIONAL EVALUATION OF *BACILLUS THURINGIENSIS* SEROTYPE H-14 AGAINST *ANOPHELES SUNDAICUS* IN WEST JAVA, INDONESIA

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ABSTRACT. A brackish water lagoon (ca. 4.5 ha) was treated 6 times, from the last week in June until the first week in August 1982 with a liquid formulation containing *Bacillus thuringiensis* serotype H-14 toxin. Except during conditions of strong winds, doses of 1.1 to 2.3 kg/ha gave good control of larvae of *Anopheles sundaicus*, the main vector of malaria in the coastal areas of many islands of Indonesia. Adult populations of *An. sundaicus* in nearby hamlets were steadily reduced following successful larvicide treatments but readily increased following a control failure.

INTRODUCTION

Anopheles sundaicus (Rodenwaldt) is the main vector of malaria along the coastal areas of Indonesia (Sundararaman et al. 1957). In southern Java it breeds in brackish water lagoons and in the closed mouths of rivers. Conditions favorable for breeding are 4–30 parts per thousand salinity, exposure to sunlight and the presence of floating algae. The adults have a relatively short flight range (less than 5 km), are readily attracted to man and feed mostly outdoors (S. Kirnowardoyo, unpublished data, 1980).

Bacillus thuringiensis serotype H-14 produces a protein toxin that is highly toxic to mosquito larvae (Goldberg and Margalit 1977) but does not have harmful side effects on fish, insect predators or other nontarget organisms (Miura et al. 1980). The safety of this agent has been thoroughly established and it is fully registered for use in many countries. The toxin must be ingested by mosquito larvae to induce toxicity and it only persists in field waters for a few hours; this results in a need for repeated treatments unless the predator populations build up to effective population densities.

Bacillus thuringiensis H-14 has been evaluated in small-scale field trials in lagoons having a mixed larval population containing *An. sundaicus* (75%) and *An. subpictus* Grassi (10%). A rate of 2.5 kg/ha gave good control even in the presence of large amounts of floating algae; a lower rate of 1.0 kg/ha achieved good control in open water but not when algae were abundant (Sudomo et al.1981).

The objectives of this study were: (1) to evaluate the efficacy of *Bacillus thuringiensis* H-14 against *An. sundaicus*, (2) to determine whether multiple applications of this agent might allow predators to achieve population levels that would provide longer-term control, and (3) to determine whether or not such a larviciding program could be used to reduce the incidence of female mosquitoes landing on outdoor baits in adjacent inhabited areas.

STUDY AREA

The Cibera lagoon is the main production site for *An. sundaicus* near the village of Mekarsari and Karyamukti in the Province of West Java (subdistrict Pameungpeuk, Regency of Garut). Three Kampungs (hamlets) are adjacent to the Cibera lagoon and are well within the flight range of *An. sundaicus*. Kampung Cibera is about 300 m from the edge of the lagoon,

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Kampung Roke 500 m and Kampung Perkebunan Negara within 2 km. Approximately 1000 people live in these three Kampungs.

The Cibera lagoon varies in size and depth with the season and with the tide; its surface can be as little as 39,000 m² or as much as 49,000 m². The lagoon has been treated for larval control each year since 1975. Applications of diesel oil and diesel oil plus spreading agent have not resulted in satisfasctory control. Attempts to reduce or eliminate breeding by physical removal of the algae were also not successful. However, the use of fenthion for larviciding has yielded successful results.

Other *Anopheles* spp. are produced in rice fields in the same general area; these include *An. aconitus* Doenitz, *An. subpictus, An. vagus* Doenitz, *An. annularis* Van der Wulp, *An. tesselatus* Theobald, *An. kochi* Doenitz, *An. barbirostris* Van derWulp and *An. peditaeniatus* (Leicester). Of these, only *An. aconitus* and *An. subpictus* are vectors in this area and they usually are not found in biting mosquito collections taken in the Kampungs Roke, Cibera and Perkebunan Negara.

In this area, malaria is mostly caused by *Plasmodium falciparum* and the peak numbers of cases occur in July and October.

MATERIALS AND METHODS

Larvicide treatments were made by applying either 50, 75 or 100 ml of a liquid formulation of *B. thuringiensis* H-14 (Teknar™) in 8 liters of water to ca. 200 m² of surface area. Spraymen made the applications with Hudson hand sprayers. Only the floating algae was sprayed since very few *An. sundaicus* larvae are found in open water where they are unprotected. The actual dose applied was calculated for each treatment by dividing the kg of material applied by the estimated surface area of algae (see below).

Eight sampling stations were marked with stakes along the long edge of the lagoon; additional marking between these stations divide the lagoon into areas of ca. 200 m². One day before treatment, at the time of treatment and 1-day posttreatment, the percentage of the lagoon's surface which was covered by algae (over a 200 m² section) was estimated visually at each of the 8 sampling stations. The numbers of 3rd and 4th instar larvae and pupae were determined by dipping into the floating algae. The number of dips per station were 40 times the percent of the surface area covered by algae; thus, if 50% algal cover was present, 20 dips were taken. The percentage algal cover for all 8 stations was averaged to estimate the overall portion of the lagoon covered by algae.

The numbers of landing mosquitoes in the Kampungs Roke, Cibera and Perkebunan Negara were measured weekly. Four collectors aspirated landing adults from their exposed legs during a 45 min period of each hour from 1800 hr until 2400 hr. Two of the collectors were located inside houses and 2 outdoors but nearby. The captured adults were identified and the ovarian condition (gravid, parous or nulliparous) of each was determined by dissection and microscopic examination.

RESULTS AND DISCUSSION

The Cibera lagoon was treated 6 times with *Bacillus thuringiensis* H-14 (Table 1). One scheduled treatment June 30, 1982 was not made because fishermen had drained most of the water into the ocean during low tide during the night of June 29, 1982.

The actual rate of larvicide applied to the lagoon varies considerably from application to application because of difficulties in spraying. The spraymen move easier and faster in shallow water (up to 30 cm in depth) than in the deeper portions of the lagoon where they are up to their shoulders in the water. Also, the bottom of the lagoon varies greatly from place to place; it is firm in some places and very soft in others and this greatly affects the movement of spraymen. No boats, or other vehicles for use on water, were available.

Table 1. Summary of treatments of the Cibera Lagoon with *Bacillus thuringiensis* H-14 larvicide.

No.	Date (1982)	Dose applied (kg/ha)	No. 3rd, 4th and pupae/dip		Percent reduction	Overall % control[a]
			Pretreatment	Posttreatment		
1	23/6	2.30	4.49	0.044	97.4	99(+)
2	5/7	1.09	0.66	0.048	92.7	98
3	14/7	1.21	2.18	0.48	93.6	97
4	20/7	1.56	0.34	0.061	82.1	90
5	28/7	1.33	0.61	0.23	62.3	70
6	4/8	1.70	0.56	0.064	89.6	95

[a] A subjective total evaluation made in consideration of the reduction of all larval instars.

Relatively good results were achieved except for the treatment on July 28, 1982; the relatively poor results on the latter date appeared to be due to the strong winds present during the application. During conditions of high winds there is considerable loss of spray due to drift. Rather than attempt to improve the calibration of spraying, a dose should be selected that is ample for these variable conditions. It appears that the higher dose of 100 ml Teknar per 8 liters water per spraycan is necessary during windy conditions but that 75 ml is adequate in the absence of such winds.

Even though this highly selective larvicide should allow the predator populations to increase, the formation of the dense algal mats protects large numbers of immature *An. sundaicus* against predation. Thus, regular larvicide treatments were still necessary. Research efforts to find better techniques for reduction or elimination of the algal mats are needed.

Adult collections from the Kampungs Roke, Perkebunan Negara and Cibera are shown in Tables 2–4, respectively. While numbers were initially high, they began decreasing with time and the absence of new adults is reflected by a reduction of nulliparous females. Initially, all adults were dissected to determine ovarian condition but this was too labor intensive. A revised method limited the number of adults to be dissected from each 45 min period to a maximum of 20 from the outdoor and 20 from the indoor collections. This allows one technician to determine the ovarian conditions for the samples from a given night.

On July 28, 1982 there was a large increase in the number of adults at Roke and they were mostly nulliparous. This followed the larvicide application which gave the poorest control. Increases at Perkebunan Negara and Cibera were not yet apparent; Roke is downwind from the lagoon. However by the following week, there were large increases in nulliparous adults at all 3 Kampungs. Thus, the number of new adults can be correlated to the effects, or lack of effectiveness, of the larvicide treatment of July 7, 1982. It is apparent that effective larviciding can achieve reduced biting of adults in these Kampungs.

The data in Tables 2–4 indicate that there is no relationship between ovarian condition and collection place (indoor or outdoor). For statistical analysis it was first necessary to transform the parity data (since they are based on proportions) with arc-sine transformations. Subsequent evaluation by analysis of variance shows a lack of interaction ($P>.05$) between parity and collection place. Thus, adults move inside houses irrespective of ovarian condition but most biting activity is outdoors.

No *Anopheles* females except *An. sundaicus* were ever found in these collections throughout the study period. Care was taken to determine if *An. aconitus* was present because of the local rice fields. Adults were collected from water buffalo sheds (Kandangs) on June 12, 1982 near Pameungpeuk and on July 16, 1982 near Kampung Cibaregbeg; in the first case only *An. vagus* were present and for the latter there were 89% *An. vagus* and 11% *An. sundaicus*. Thus during this period, which coincided with an unusually severe dry season, *An. sundaicus* was the only vector species that was present. There were 32 confirmed cases of malaria in this locality in July (including our driver); 1 introduced case was due to *P. vivax* and the remain-

Table 2. Collections of landing *An. sundaicus* adults at Kampung Roke from 1800–2400 h.

Date (1982)	Collection place	No. females collected	No. dissected	Ovarian condition (%)		
				Gravid	Parous	Nulliparous
16/6	Outdoors	39	39	2.6	43.6	53.8
	Indoors	3	3	0.0	100.0	0.0
23/6	Outdoors	308	308	2.6	48.4	49.0
	Indoors	68	68	8.8	50.0	41.2
30/6	Outdoors	399	399	3.2	85.5	11.3
	Indoors	24	24	4.1	79.2	16.7
5/7	Outdoors	52	52	7.7	69.2	23.1
	Indoors	20	20	5.0	70.0	25.0
14/7	Outdoors	23	23	13.0	56.5	17.4
	Indoors	3	3	33.3	66.7	0.0
20/7	Outdoors	37	37	0.0	89.2	10.8
	Indoor	18	18	16.7	77.8	5.5
28/7	Outdoors	129	90	10.0	35.5	54.5
	Indoors	41	41	12.2	19.5	68.3
4/8	Outdoors	411	120	10.8	25.8	63.4
	Indoors	76	71	15.5	31.0	53.5

Table 3. Collections of landing *An. sundaicus* adults at Kampung Cibera from 1800–2400 h.

Date (1982)	Collection place	No. females collected	No. dissected	Ovarian condition (%)		
				Gravid	Parous	Nulli-parous
15/6	Outdoors	14	14	0	57.1	42.9
	Indoors	0	0	—	—	—
22/6	Outdoors	88	88	4.5	35.3	60.2
	Indoors	2	2	0	50.0	50.0
29/6	Outdoors	107	87	5.7	75.9	18.4
	Indoors	1	1	100	0	0
6/7	Outdoors	28	28	10.7	85.7	3.6
	Indoors	0	0	—	—	—
13/7	Outdoors	65	58	13.8	58.6	27.6
	Indoors	0	0	—	—	—
19/7	Outdoors	5	5	0	75.0	25.0
	Indoors	1	1	—	100	—
27/7	Outdoors	57	57	15.8	66.7	17.5
	Indoors	0	0	—	—	—
5/8	Outdoors	190	112	10.7	34.8	54.5
	Indoors	3	3	33.3	33.3	33.3

Table 4. Collections of landing *An. sundaicus* adults at Kampung Perkebunan Negara from 1800–2400 h.

Date (1982)	Collection place	No. females collected	No. dissected	Ovarian condition (%)		
				Gravid	Parous	Nulli-parous
14/6	Outdoors	8	8	0	50.0	50.0
	Indoors	0	0	—	—	—
21/6	Outdoors	26	26	23.0	46.2	30.8
	Indoors	2	2	0	50.0	50.0
28/6	Outdoors	49	49	2.0	44.9	53.1
	Indoors	6	6	0	16.7	83.3
7/7	Outdoors	17	17	11.8	52.9	35.3
	Indoors	2	2	0	100	0
12/7	Outdoors	8	8	0	87.5	12.5
	Indoors	1	1	0	100	0
18/7	Outdoors	1	1	100	—	—
	Indoors	1	1	—	100	—
26/7	Outdoors	16	16	6.2	62.5	31.3
	Indoors	0	0	—	—	—
2/8	Outdoors	158	98	8.2	25.5	66.3
	Indoors	7	7	14.3	28.6	57.1

der were due to the transmission of *P. falciparum*.

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