# ADDITIONAL FIELD TESTING OF THE MERMITHID PARASITE ROMANOMERMIS CULICIVORAX AGAINST ANOPHELES ALBIMANUS IN EL SALVADOR

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ABSTRACT. Twenty-two breeding sites of Anopheles albimanus in the coastal area of El Salvador were treated with the mermithid nematode Romanomermis culicizorax at dosages ranging from 2400 to 4800 preparasites/sq m of surface water. A mean parasitism of 96% was obtained for all of the treatments. No statistical differences were apparent between dosages or instars and levels of parasitism. Also 2 early evening treatments of the anopheline

breeding area (1.2 ha) of Lake Apastepeque in November, 1977, with the nematode produced an average 96% parasitism. It was concluded that the increased time between the early evening treatments and wave action the next day allowed greater contact between the parasite and host and was responsible for the much higher level of parasitism than previously observed following midday treatments.

A pilot test conducted in early 1977 against Anopheles albimanus involved the in vivo rearing of 6392 g of postparasites of the mermithid Romanomermis culicivorax and the production of sufficient cultures to treat the breeding area (1.2 ha) of an isolated site (Lake Apastepeque) 11 times over a 7-week period with a total of 393 X 10<sup>6</sup> preparasites. Parasitism averaged 58% for the 11 treatments, and the anopheline population was reduced 94% between the beginning and the end of the treatments (Petersen et al. 1978a & b).

To determine the efficacy of this nematode against larvae of *An. albimanus* in the more typical breeding sites in the coastal area of El Salvador, treatments were made in late 1977 and in 1978. Also, because our observations showed a much higher level of parasitism in Lake Apastepeque when treatments were made during evening hours when wave action was low, the area was retreated twice in November, 1977. The results of both of these studies are reported herein.

## MATERIALS AND METHODS

Cultures of the nematode were produced at the Gulf Coast Mosquito Research Laboratory, Lake Charles, Louisiana, and shipped air freight to El Salvador. The method of hatching, de-

termination of the number of nematodes by volumetric dilution, transport to the field, and method of application were similar to those reported by Petersen et al. (1978a & b). Treated areas in the coastal area of El Salvador were sites such as flooded pastures, roadside ditches. ponds, and swamps that contained larval populations of An. albimanus. Vegetation at these sites was minimal to moderate, and plot size ranged from 21 to 450 m<sup>2</sup>. Dosages used ranged from 2400 to 4800 preparasites/sq m of surface breeding area. In the second test the breeding margin of the isolated Lake Apastepeque in inland El Salvador was treated twice at a rate of 3600 preparasites/sq m of surface area.

Larvae from each site were collected at 24 hr post-treatment, returned to the laboratory, identified to species, separated to instar, dissected, and examined microscopically for the presence of nematodes.

### RESULTS AND DISCUSSION

The results of treating 22 sites in the coastal area of El Salvador are given in Table 1. All treatments gave excellent parasitism of *An. albimanus*, regardless of the dosage or instar. Multiple parasitism of larvae was common. Overall parasitism following the treatments averaged 96%,

Table 1. Parasitism of Anopheles albimanus by released Romanomermis culicivorax in coastal breeding sites and of An. albimanus and An. p. pseudopunctipennis in Lake Apastepeque.

Treatment date	No. of plots	Dosage (preparasites/sq m)	Percent infection (range)
		Coastal sites	
Nov. '77	2	4800	99 (98-100)
June '78	7	3600	89 (74–100)
Dec. '78	5	3600	96 (93–100)
Dec. 78	8	2400	98 (94–100)
		Lake Apastepeque	
March '77 <sup>1</sup> Nov. '77	1	3000	92
	i	4800	87
	1	4200	90
	1	3600	96
	1	3600	95

<sup>&</sup>lt;sup>1</sup> Data from Petersen et al. 1978b (treatments 9-11).

and mean parasitism for 1st to 4th instars was 95, 95, 98, and 95%, respectively.

Two treatments made just prior to a heavy rain in June, 1978, produced infection levels of only 74 and 89% and were responsible for the lowest parasitism (89%) recorded for all groups of treatments in the coastal areas.

The levels of parasitism presented herein are undoubtedly lower than those actually obtained in the treatments because of several factors. Multiple parasitism is often lethal to first instars; thus larvae that are killed are unavailable for post-treatment collections. Also, because of the small size of the day-old preparasites, it is possible to overlook them in dissections of larvae, particularly in 4th instars.

Occasionally larvae of *Uranotaenia lowii* and *Culex erraticus* were present and infected.

The 2 early evening treatments at Lake Apastepeque in November of 1977 produced an average parasitism of 96% (Table 1). When this figure is combined with the figure for the 3 early evening treatments of the lake in March, 1977 (Petersen et al, 1978b), 92% of the larval populations of An. albimanus and An. p. pseudopunctipennis were parasitized. This is much higher than the 58% parasitism achieved during the 11 treatments in early 1977 (Petersen et al. 1978b) and exactly double the 46% parasitism for the

8 midday treatments made during the same study. We observed that about 2 hr usually elapsed between the time of the midday treatments and the start of the wind and wave action, which then lasted about 4 hr. In contrast, there were about 20 hrs between the early evening treatments and the beginning of turbulent water the next day. Undoubtedly, this increased interval of quiet water allowed longer contact between nematodes and host larvae and resulted in much higher parasitism.

These results indicate that this nematode was extremely effective against all instars of anopheline larvae that were present in many kinds of freshwater habitats.

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