SUSCEPTIBILITY OF *AEDES PSEUDOSCUTELLARIS* AND *AEDES POLYNESIENSIS* TO INFECTION BY *ROMANOMERMIS CULICIVORAX* IN THE LABORATORY

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ABSTRACT. The susceptibility of Fijian *Aedes pseudoscutellaris* and *Ae. polynesiensis* to infection with the mermithid nematode, *Romanomermis culicivorax*, was assessed under controlled laboratory conditions. Both mosquito species were equally susceptible to infection. Infection rates in fourth instar larvae, following exposure to infection as first instars, ranged from 88% to 96% at a treatment ratio (worms:mosquito) of 5:1. Infection rates were 98% in *Ae. polynesiensis* and 100% in *Ae. pseudoscutellaris* at a treatment ratio of 10:1, and 100% in both species at a ratio of 20:1. These are the first data available on the susceptibility of *Ae. pseudoscutellaris* to infection by *R. culicivorax*. Data presented could provide a baseline for determining dosage rates for field trials should attempts be made to control these mosquitoes with *R. culicivorax*.

INTRODUCTION

The mermithid nematode *Romanomermis culicivorax* Ross and Smith has been shown to infect a wide variety of mosquito species either in the field or the laboratory and is generally recognized as being one of the most promising agents for the biological control of mosquitoes (Petersen et al. 1968, Petersen 1973, Petersen and Chapman 1979, Anonymous 1980). Mosquitoes of the *Aedes (Stegomyia) scutellaris* group serve as the primary vectors of subperiodic filariasis on many islands of the South Pacific. The important vectors on Fiji are *Ae. pseudoscutellaris* (Theobald) and *Ae. polynesiensis* Marks (Iyengar 1965). *Aedes polynesiensis* also is a vector of dengue (Rosen et al. 1954) and Ross River viruses (Rosen et al. 1981, Gubler 1981). Ross River virus was responsible for recent outbreaks of epidemic polyarthritis in Fiji, Samoa, and Rarotonga (Rosen et al. 1981). The strain of *Ae. pseudoscutellaris* used in the present study also has been shown to be capable of transmitting Ross River virus (C. J. Mitchell and D. J. Gubler, unpublished data). The importance of developing effective control measures for these 2 mosquito species is obvious. The purpose of this study was to determine the susceptibility of these mosquitoes to infection by *R. culicivorax* under controlled laboratory conditions.

MATERIALS AND METHODS

Eggs of *R. culicivorax* were obtained in moist sand from the U.S. Department of Agriculture (USDA) laboratory, Lake Charles, Louisiana. Mosquitoes were from colonies maintained in the Centers for Disease Control (CDC) insectary in Fort Collins, Colorado. Laboratory facilities and necessary equipment also were provided by CDC.

Two sets of experiments were done. The first (December 1981) resulted in high mortality among early instar mosquito larvae; therefore, a second set of experiments was conducted (January 1982) in which untreated larvae of both mosquito species were included as controls. At the beginning of each set of experiments, about 250 ml of moist sand containing worm eggs were flooded in 500 ml of deionized water. Deionized

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water was used in all phases of the experiments. While worm eggs were hatching (2 to 3 hrs at 27°C), first instar mosquito larvae were sorted into lots of 50 each and placed in 10 to 20 ml of water in 100 ml glass breakers. Then 1 ml of water containing the hatched worms (preparasites) was distributed among 10 wells of a depression slide and the preparasites were counted with the aid of a microscope. Ten ml of water containing the preparasites were treated in this fashion and the average number of preparasites per ml was calculated. Different treatment ratios (worms:mosquito) were used to determine the optimum ratio for achieving 95% or greater infection rates in mosquito larvae surviving to the fourth instar. The desired ratio was achieved by adding appropriate volumes of water containing the preparasites to the lots of 50 mosquito larvae. Beakers containing mosquito larvae and preparasites were then filled to 50 ml and incubated for 24 hr at 27°C without food. At the end of the 24 hr exposure period, each beaker was emptied into individual white enameled pans containing 1.5 liters of water and food (Tetramin® and commercial rabbit chow). Food was provided *ad lib.* until the completion of each experiment.

Infection rates were determined in fourth instar larvae as follows. Larvae were pipetted into a depression slide and examined under the microscope at 7X to 40X magnification. Pupae and patently infected larvae were discarded, and the remaining larvae were returned to their pans and examined again the following day. Pupation was used as the criterion for noninfection; thus, mosquitoes in each pan were examined daily until they had pupated or were removed as visibly infected larvae.

RESULTS

The average number of worm eggs that hatched per ml of water was remarkably constant in the 2 sets of experiments (36 and 37 preparasites/ml, respectively). In the first set of experiments first instar larvae of each mosquito species were exposed to preparasitic worms at ratios of 5:1 and 20:1 (worms:mosquito). Four replicate lots of 50 mosquito larvae of each species were tested at each ratio. In the second set of experiments 3 lots of first instar larvae of each species were exposed to preparasitic worms at ratios of 1:1, 5:1, and 10:1 (worms:mosquito). In addition, 3 lots of each mosquito species were included as untreated controls. Since variation among replicates within the same treatment group was not great, total values for each treatment group are used for purposes of comparison (Table 1).

Mortality rates in early instar larvae were determined retrospectively when fourth instar larvae and pupae were counted. Mortality rates were consistently higher in *Ae. polynesiensis* at all treatment ratios (28% to 75% mortality) as compared to *Ae. pseudoscutellaris* (18% to 41%). This also was the case among untreated controls (63% for *Ae. polynesiensis* and 31% for *Ae. pseudoscutellaris*).

Romanomermis culicivorax infections in fourth instar larvae usually were determined by observing the worms coiled in the thoraces of the larvae. Occasionally, postparasitic worms were observed emerging from larvae (Fig. 1). Infection rates in fourth instar larvae were quite similar in the 2 mosquito species except at the 1:1 treatment ratio (38% in *Ae. pseudoscutellaris* and 63% in *Ae. polynesiensis*). Infection rates in both species ranged from 88% to 100% at treatment ratios of 5:1 and 10:1, and were 100% in mosquitoes treated at a ratio of 20:1.

DISCUSSION AND CONCLUSIONS

The cause of excessive mortality among early instar larvae of *Ae. pseudoscutellaris* and *Ae. polynesiensis* in both treated and untreated groups is not apparent. There is a marked species difference with *Ae. polynesiensis* being most affected. Perhaps starving the first instar larvae during the 24 hr exposure period was detrimental. In any event, infection rates in fourth instar

Table 1. *Romanomermis culicivorax* infection rates in *Ae. pseudoscutellarsis* and *Ae. polynesiensis* fourth instar larvae and mortality rates in early instar larvae.

Species	Treatment ratio (worms: mosquito)	Early instar mortality rates		Fourth instar infection rates	
		No. tested	% mortality	No. examined	% positive
Ae. pseudoscutellaris	Untreated control	150	31	103	0
Ae. polynesiensis		150	63	56	0
Ae. pseudoscutellaris	1:1	150	18	123	38
Ae. polynesiensis		150	59	61	63
Ae. pseudoscutellaris	5:1*	200	21	157	96
Ae. polynesiensis		200	28	143	93
Ae. pseudoscutellaris	5:1	150	26	111	90
Ae. polynesiensis		150	36	96	88
Ae. pseudoscutellaris	10:1	150	41	88	100
Ae. polynesiensis		150	60	60	98
Ae. pseudoscutellaris	20:1*	200	41	117	100
Ae. polynesiensis		200	75	49	100

* These data from first set of experiments (December 1981); all others from second set (January 1982).

larvae are unlikely to be greatly biased by earlier deaths that were due to extraneous causes. Although some mortality among early instar larvae in the treated groups may have been due to excessive worm burdens, it seems unlikely that this was a major factor since mortality rates among the untreated controls fall within the mid-range of mortality rates in the treated groups.

Aedes pseudoscutellaris and *Ae. polynesiensis* had similar infection rates at each treatment ratio except at the 1:1 ratio. Additional experiments would be required to determine whether this is a consistent difference. Both mosquito species were quite susceptible to infection; rates ranged from 98% to 100% at treatment ratios of 10:1 or greater. Petersen and Chapman (1979) classified *Ae. polynesiensis* as being moderately susceptible (numerical value of 3 on a scale of 1 to 5) to infection by *R. culicivorax* in comparison to other mosquito species. We are not aware of any previous study on the susceptibility of *Ae. pseudoscutellaris*. The fact that this species is very similar to *Ae. polynesiensis* in its susceptibility to infection by *R. culicivorax* is not surprising in view of the close taxonomic relationship of these mosquito species.

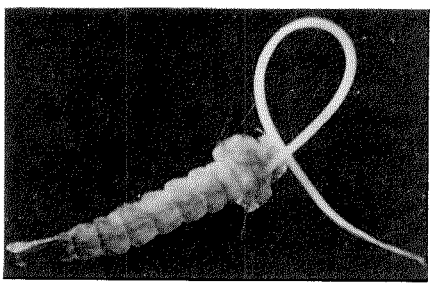

Fig. 1. Postparasitic *Romanomermis culicivorax* emerging from fourth instar *Aedes polynesiensis* larva.

The susceptibility of *Ae. pseudoscutellaris* and *Ae. polynesiensis* to infection by *R. culicivorax* in the laboratory suggests that these mosquitoes might be amenable to control by this parasite under field conditions. Our data provide a baseline for determining dosage rates for field trials. A major limiting factor may be the difficulty involved in treating the extremely wide range of breeding habitats of these species. These mosquitoes utilize treeholes, coconut shells and husks, canoes, artificial containers and crabholes

as oviposition sites (Belkin 1962). However, the problems involved in treating breeding sites with *R. culicivorax* would not be expected to differ significantly from those involving treatment with a chemical larvicide. Alkalinity of tree hole water and the salinity of habitats such as crabholes may be limiting factors in some areas. Petersen (1981) showed that *R. culicivorax* was adversely affected in dilutions of sea water with conductivity of 1500 μmho/cm and no infections were observed at concentrations above 3000 μ mho/cm. Also, *R. culicivorax* failed to infect mosquitoes in dilutions containing 25% or more of tree hole water. However, tree hole water on Pacific islands may differ from that found in northern temperate woodlands. Laird et al. (1982) found that rainwater trapped in man-made tree holes in coconut palms is near neutrality and of low electrical conductivity. They demonstrated the establishment and long term survival of *R. culicivorax* in this important habitat of *Ae. polynesiensis* in the Tokelau Islands.

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