The *Romanomermis iyengari* parasite for *Anopheles pseudopunctipennis* suppression in natural habitats in Oaxaca State, Mexico

Alberto Santamarina Mijares,[1] *Rafael Pérez Pacheco,*[2] *Sabino Honorio Tomás Martínez,*[2] *Luis Enrique Cantón,*[2] *and Gonzalo Flores Ambrosio*[2]

ABSTRACT *In September and November 1996* Romanomermis iyengari *Welch, a parasite of larval mosquitoes, was released in 44 natural larval habitat sites of* Anopheles pseudopunctipennis *Theobald in an attempt to reduce the larval populations of this important malaria vector. The selected treatment sites ranged in size from 5 to 500 m^2. The study was carried out in Pochutla District of Oaxaca State, on the Pacific coast of Mexico.*

Chemical pesticides to reduce vector populations have been the principal tool in malaria suppression campaigns. However, the excessive use of these chemicals has created pesticide resistance and other serious collateral problems. Therefore, a biological control project using agents that are pathogens of Anopheles *larvae was initiated in 1996. The principal objective was to establish mass rearing capacities for* R. iyengari. *Detailed methodology for rearing and introducing these nematodes into mosquito larval habitats was established at the National Polytechnic Institute of Oaxaca State. Before application of the parasites to larval habitats, site characteristics were determined, including size, depth, aquatic vegetation, salinity, pH, conductivity, temperature, and pretreatment larval density. With a compressed air sprayer, infective mermithid parasites were released at rates of either 2 000 or 3 000/m^2, and the parasites produced high levels of infection.* Anopheles *populations were sampled 72 h posttreatment, and the larvae obtained were taken to the laboratory and examined through microscopic dissection to determine infection levels and mean parasitism. Nematode parasitism ranged from 85 to 100% at all the treatment sites, even though no previous information concerning field parasitism of* An. pseudopunctipennis *by* R. iyengari *has been reported. In addition, a significant reduction of mosquito larval density at the treatment sites was found five days after the nematode application. Levels of parasitism were indicative of the number of mosquito larvae killed by the treatment since infected larvae never progressed to the pupal stage. Results from sampling nine of the sites 2 months after the initial application of nematodes indicated that a high number of mosquito larvae were infected by parasites that had emerged from eggs previously deposited in the stratum. This work suggests the potential of this mermithid to reduce* An. pseudopunctipennis *populations in Oaxaca State.*

[1] Instituto de Medicina Tropical "Pedro Kouri," Autopista Novia del Mediodía km 6, e/ Autopista Nacional y Carretera Central, La Lisa, C.P. 11500, Ciudad de la Habana, Cuba. Telephone: 53-7-22-0650/-0651. E-mail: santamarina@ipk.sld.cu

[2] Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Oaxaca, Mexico.

Among the states in Mexico with the highest incidence of malaria are Chiapas, Oaxaca, Sinaloa, Michoacán, Guerrero, and Nayarit. *Anopheles albimanus* Wiedeman and *Anopheles pseudopunctipennis* Theobald are the principal vectors of malaria in the country. *Anopheles vestitipennis* Dyar and Knab is considered a secondary vector. Natural populations of *An. pseudopuncti-*

pennis can be found in many malaria-endemic areas of Mexico, and it is the principal malaria vector of the Pacific coastal states of the country. Vector control by the National Campaign to Eradicate Malaria has been very difficult to achieve in these states (*1*).

Applying pesticides for malaria vector and disease suppression has not satisfactorily reduced malaria cases. Such insecticides as DDT, malathion, and Abate have been used in Oaxaca State. However, their use has created serious problems, including the development of pesticide resistance, environmental pollution, the killing of nontarget organisms, alterations to ecosystems, and high expenditures for the newer chemical products. For these reasons alternative methods of vector suppression have been developed. The use of natural pathogenic and parasitic agents of *Anopheles* larvae has been considered. Extensive research on the feasibility of using biological control agents against malaria vectors has shown the potential usefulness of *Romanomermis iyengari* Welch, a mermithid nematode and an obligate parasite of mosquito larvae (*2*). The mermithid *R. iyengari* was originally found as a juvenile parasitic form in the hemocoel of *Anopheles subpictus* Grassi from Bangalore, India (*3*). It was released in rice paddy fields to control mosquito larvae and was observed parasitizing larvae of both *Culex* spp. and *Anopheles* spp. at rates of 92 to 98% (*4*). In waters with *An. albimanus*, *Culex nigripalpus* Theobald, and *Culex quinquefasciatus* Say mosquito larvae, the application of 1 000 parasites/m^2 of surface area resulted in infection levels of 100, 85, and 75% respectively (*5*). Laboratory and field tests demonstrated the pathogenic action of *R. iyengari* in larvae of *Aedes taeniorhynchus* Wiedemann, a salt marsh mosquito. Parasitism levels from 71 to 100% were produced (*6*). Field studies also showed *R. iyengari* became permanently established and continued to reinfect mosquito larvae without additional applications. Safety tests were conducted in the laboratory and under field conditions using single massive doses of *R. iyengari* administered to selected nontarget organisms, both invertebrates and vertebrates. Penetration was attempted but no significant effect on nontarget organisms was observed. *R. iyengari* is suggested to be as safe to humans and other organisms as are the other mermithid nematodes used for vector control (*7*).

This study reports the results obtained in 44 natural larval habitat sites of *An. pseudopunctipennis* with the application of *R. iyengari* cultures produced at the mass rearing unit located in Oaxaca State, Mexico.

MATERIALS AND METHODS

The *R. iyengari* mosquito parasite was mass produced to treat 2 395 m^2 of *An. pseudopunctipennis* breeding area. The basic mass rearing procedure required the exposure of 200 000 second-instar *Cx. quinquefasciatus* larvae to 1 million parasitic nematodes (1:5 ratio) every 10 days for 6 months. This method produced an average of 68.5 g of postparasitic nematodes and a total of 120 cultures over each 10-day period. The desired inoculum of parasitic nematodes (the water from the flooded nematode cultures) was carefully collected in a common container (200 mL). The volume of decanted water containing the parasitic nematodes was then determined by volumetric dilution previously described (*8*). Seven days after exposure, host larvae were removed from each rearing tray (60×40×12 cm), concentrated, washed, and placed in smaller nematode-collecting trays (39.5×27×6 cm). After emergence the nematodes were collected, washed, and placed in plastic containers (18×12×6 cm) containing clean sterile sand (2 cm deep) covered to a depth of 1 cm with distilled water. Then the water was removed after 2 h and the cultures were covered. After about 6 weeks the cultures were used for field applications.

The field applications to evaluate the potential suppression of mosquito larvae populations by *R. iyengari* were conducted from 1 September to 15 November 1996. Forty-four *An. pseudopunctipennis* larval habitats were identified in the San Bernardino and San Isidro localities (between 100 m to 150 m above sea level) and treated with *R. iyengari* at a rate of 2 000–3 000/m^2 of water surface. The bodies of water ranged in area from 5 m^2 to 500 m^2. Pretreatment examination several hours prior to the application of nematodes indicated a high population of *An. pseudopunctipennis* 1st to 4th instars at each site. Numerous insect predators were present in each habitat, including immature and adult stages of Hemiptera, *Belostoma apache* Kirk and *Pelocoris femoratus* Barber; Odonata, suborders Anisoptera and Zygoptera; and Coleoptera, *Thermonectus circumscriptus* Latreille and *Tropisternus lateralis* F. The sites varied greatly in size, shape, depth, and amount of floating and emergent vegetation (filamentous algae). Physical and chemical parameters of the water were calculated, including pH, salinity, conductivity, and temperature.

A total of 370 cultures containing eggs of *R. iyengari* produced at the mass rearing unit were transported to malaria-endemic areas of Pochutla District. Mermithid cultures (240) were flooded with distilled water to obtain parasitic nematodes for field release at the 44 selected sites. The number of infective-stage *R. iyengari* per unit volume of water was determined by volumetric dilution. The cultures provided sufficient inoculum (250 liters) and 69 450 000 preparasites. Final dilutions were made prior to release using water from the test sites. Parasitic larvae were applied directly over the entire water surface using a compressed air sprayer (H. D. Hudson Manufacturing Company, Chicago, IL) at a dosage rate of 2 000 or 3 000/m^2 of surface area and at an approximate pressure of 2 atmospheres, as previously described (*9*). (Larval nematodes were released at a dosage rate of 3 000/m^2 at sites 12, 14, 19, 29, 30, 31, and 32 due to the vegetation present.) The water temperature at the time of application varied from 26 to 33 °C.

Mosquito larvae (1st to 4th instars) were sampled 72 h posttreatment from the 44 treated sites (100 larvae per site) and examined under a compound microscope to determine infection levels and mean parasitism. Two control sites were established to compare the results. In addition, mosquito larvae abundance was determined at the sites 5 days after the first application using a larval net 20 cm in diameter and 20 cm in depth with a handle of 2 m (*10*).

Data obtained did not show a normal distribution, so prior statistical analysis data were transformed by the square root of x. Analysis of variance (ANOVA) and the Duncan multiple-range test were used to compare mean infections. The percentage reduction in mosquito larvae density at the 44 sites was evaluated through the following formula (*11*) where:

$$\% \text{ reduction} = 100 - ((T_2/T_1 \times (C_1/C_2) \times 100)$$

and where C_1 and T_1 and C_2 and T_2 are the abundance of larvae in the control (C) and treated (T) sites before ($_1$) and after ($_2$) applications, respectively.

TABLE 1. Ecological characteristics of 44 natural mosquito larval habitat sites treated with *Romanomermis iyengari* and two untreated control sites, Oaxaca State, Mexico, 1996

Site	Area (m^2)	Depth (cm)	Vegetation	pH	Salinity (mol)	Conductivity (ms/cm)	Temp (°C)
1	12	25	algae	7.6	0.40	1	26
2	12	27	algae	7.7	0.40	1	26
3	42	32	algae	7.7	0.40	1	26
4	18	29	algae	7.7	0.40	1	26
5	40	32	algae	7.8	0.38	1	32
6	46	38	algae	8.0	0.39	1	32
7	11	34	algae	8.6	0.24	1	32
8	114	68	algae	8.5	0.25	1	31
9	62	55	algae	8.7	0.24	1	32
10	75	59	algae	8.0	0.30	1	33
11	26	35	algae	7.8	0.28	1	28
12	11	29	algae	7.2	0.28	1	29
13	10	19	algae	8.7	0.21	1	28
14	28	22	algae	8.2	0.22	1	28
15	56	44	algae	7.7	0.23	1	30
16	24	19	algae	7.8	0.22	1	27
17	62	45	algae	8.2	0.22	1	27
18	80	75	algae	7.9	0.23	1	27
19	6	16	algae	8.1	0.23	1	28
20	8	17	algae	7.6	0.23	1	28
21	216	84	algae	8.9	0.30	1	29
22	19	29	algae	7.8	0.5	1	28
Control	42	31	algae	7.7	0.35	1	28
23	5	17	algae	8.7	0.27	1	29
24	30	38	algae	7.5	0.32	1	30
25	56	44	algae	7.4	0.33	1	30
26	36	33	algae	7.5	0.33	1	32
27	60	49	algae	7.5	0.33	1	31
28	27	25	algae	7.6	0.40	1	30
29	40	37	algae	7.4	0.82	1	31
30	10	15	algae	6.9	0.81	1	31
31	36	29	algae	7.6	0.75	1	30
32	72	68	algae	7.7	0.79	1	31
33	11	18	algae	7.4	0.68	1	30
34	16	19	algae	7.0	0.68	1	31
35	15	29	algae	7.4	0.67	1	30
36	19	39	algae	7.2	0.68	1	30
37	36	49	algae	7.2	0.67	1	30
38	78	70	algae	7.4	0.65	1	30
39	12	65	algae	7.4	0.58	1	30
40	44	59	algae	7.4	0.61	1	30
41	38	48	algae	7.2	0.60	1	29
42	30	65	algae	7.9	0.63	1	28
43	50	58	algae	7.9	0.68	1	29
44	500	82	algae	8.5	0.64	1	30
Control	38	47	algae	8.2	0.63	1	30

RESULTS

Ecological characteristics of the 44 study sites are shown in Table 1. In this study *An. pseudopunctipennis* was found to be susceptible to infection by *R. iyengari* when application rates were either 2 000/m^2 or 3 000/m^2. After applications of infective parasites at a dosage rate of 2 000/m^2, 21 of the 44 sites (11, 13, 15, 16, 17, 18, 21, 22, 23, 24, 25, 26, 27, 33, 35, 36, 38, 39, 40, 41, 42, and 44) produced *An. pseudopunctipennis* with mean levels of infection ranging from 3.2 to 9.8 and 100% parasitism (Table 2). Although all instars (1st through 4th) were present at these sites, the majority of larvae collected were 1st and 2nd instars, and the highest mean incidence of parasitism occurred in these instars. Parasitism was somewhat lower—85 to 94%—at a group of 16 other sites (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 22, 28, 34, 37, and 43), with mean infections of 1.2 to 2.8 (Table 2). The older larvae of *An. pseudopunctipennis* in these 16 sites proved to be less susceptible than younger larvae, although these larvae (last instars) also became infected. A comparison of mean values of infection by one-way ANOVA and the Duncan multiple-range test showed differences between those two groups of sites ($F = 465.2$; $P < 0.005$), with the highest infection means occurring in the first group of sites.

At a third group of 7 sites (12, 14, 19, 29, 30, 31, and 32) dosage rates of 3 000/m^2 were used because aquatic

TABLE 2. Mean infections and percentage of parasitism of *Anopheles pseudopunctipennis* larvae at 44 sites treated with *Romanomermis iyengari*, Oaxaca State, Mexico, 1996

Site number	Mean infections[a]	Parasitism (%)
1	1.7 b	92
2	2.5 b	90
3	2.8 b	90
4	2.0 b	88
5	1.7 b	90
6	1.8 b	90
7	1.5 b	85
8	1.2 b	85
9	1.2 b	85
10	2.6 b	94
11	3.2 a	100
12	— c	—
13	6.8 a	100
14	— c	—
15	3.5 a	100
16	4.3 a	100
17	4.0 a	100
18	3.4 a	100
19	— c	—
20	2.5 b	89
21	3.4 a	100
22	2.2 b	94
23	9.8 a	100
24	5.0 a	100
25	4.8 a	100
26	3.2 a	100
27	6.0 a	100
28	2.5 b	92
29	— c	—
30	— c	—
31	— c	—
32	— c	—
33	4.2 a	100
34	2.0 b	90
35	8.8 a	100
36	3.8 a	100
37	2.4 b	88
38	6.0 a	100
39	5.4 a	100
40	4.7 a	100
41	9.2 a	100
42	3.2 a	100
43	2.4 b	88
44	3.9 a	100

[a] Means of a first group (those followed by the letter "a") were significantly different ($P < 0.005$) from means of a second group (those followed by the letter "b"). At a third group of sites (those identified with the letter "c"), an application rate of 3 000/m^2 had been used, and mosquito larvae were completely eliminated 72 h posttreatment.

vegetation (algae) was prominent. At those 7 sites mosquito larvae populations were found to be completely eliminated 72 h after the release of the infective parasites (Table 2). The high mortality of the *Anopheles* larvae is believed to result from multiple infections, which produce the early death of the hosts and parasites, especially in early instars. This reduces the chance that the parasites will become established.

All the 44 sites produced high levels of infection at both doses (2 000/m^2 and 3 000/m^2) though the host populations were very different at the time of treatment. The results demonstrated that *R. iyengari* parasites efficiently locate and parasitize *Anopheles* larvae, that the amounts of vegetation (algae) had little influence on the levels of parasitism when the dosage rate was 3 000/m^2, and that levels of parasitism can be increased by increasing the dose of nematodes. The data also suggest that doses in excess of 3 000/m^2 may produce many cases of multiple infections, which result in early death of both hosts and parasites.

A small increase in mosquito larvae density was observed at the two control sites (Table 3). Nontarget predator populations were sampled 72 h posttreatment by collecting several samples from each treated site. Large concentrations of insect predators were found. Results from sampling nine of the sites (11, 13, 15, 16, 18, 23, 25, 26, and 33) 2 months after treatment indicated that a high number of mosquito larvae were infected by parasites that had emerged from eggs previously deposited in the stratum. This demonstrated that *R. iyengari* was established (data not reported numerically).

DISCUSSION

Many authors have demonstrated high levels of parasitic infection by *R. iyengari* of such mosquito larval species as *Aedes* spp., *Anopheles* spp., and *Culex* spp (*12*). However, previous information concerning field parasitism of *An. pseudopunctipennis* by *R. iyengari* has not been reported. This field test data demonstrated that *R. iyengari* is a potential natural parasitic agent able to reduce *An. pseudopunctipennis* larval populations in Oaxaca State. Results of treatment at all 44 sites showed that larval populations of *An. pseudopunctipennis* could be substantially reduced. At 7 of the sites (12, 14, 19, 29, 30, 31, and 32) no larvae could be found 72 h after treatment. In these sites dense vegetation (algae) contributed to a decreased contact between parasites and mosquito larvae, so a higher dosage rate of 3 000/m^2 was used. The reduction in host numbers posttreatment was attributed to the early death of many 1st- and 2nd-instar larvae due to multiple infections by the nematodes. These results suggest that a higher percentage of parasitism and subsequent host mortality could be achieved by simply increasing the number of nematode larvae released. Nevertheless, this high level of infection at the 7 sites is presumed to be unfavorable for nematode establishment, since host populations were drastically reduced.

The high susceptibility of *An. pseudopunctipennis* larvae to infection by mermithid larvae can be partially explained by the fact that mosquito larvae adopt a horizontal position below the water surface, thereby facilitating their invasion by the preparasite mermithid larvae, which exhibit negative geotropism (*13*).

Differences in the level of parasitism between the first and second groups of sites were attributed primarily to the higher populations of younger-instar *An. pseudopunctipennis* in the first group. Levels of infection were lowest in the 3rd and 4th instars at the second group of sites. This finding is supported by laboratory data that observed that early instars were more susceptible than older instars (*2, 6, 14*). This phenomenon seems to be related to the thickness of the mosquito larvae cuticle (*15*). Probably the lower infection levels in the second group of sites were also due to the water current at these sites, whose velocity was 1 m/4.84 seconds.

When the population of insect predators at the sites treated at a rate of 2 000/m^2 was sampled 72 h posttreatment, the predators were still

TABLE 3. Percentage reduction in *Anopheles pseudopunctipennis* larval populations 5 days posttreatment, Oaxaca State, Mexico, 1996

Site number	Larval density pretreatment per m^2	Larval density posttreatment per m^2	% reduction
1	261	20	94
2	370	25	95
3	244	28	91
4	244	40	87
5	178	18	92
6	130	15	90
7	71	14	86
8	152	25	87
9	150	27	85
10	144	8	95
11	148	6	98
12	52	0	100
13	182	4	98
14	326	0	100
15	181	4	98
16	223	2	99
17	479	5	99
18	563	5	99
19	1 053	0	100
20	132	15	90
21	149	2	98
22	397	23	95
Control	355	371	—
23	895	2	100
24	643	3	100
25	1 496	4	100
26	6 105	3	100
27	849	5	100
28	792	40	96
29	189	0	100
30	676	0	100
31	248	0	100
32	181	0	100
33	981	8	99
34	2 128	8	100
35	1 551	7	100
36	3 366	2	100
37	599	75	90
38	1 782	2	100
39	726	6	100
40	709	8	99
41	338	5	99
42	302	4	99
43	237	18	94
44	249	7	99
Control	340	395	—

there and were not parasitized or affected by the nematode larvae. Examination 72 h posttreatment of the sites treated at a rate of 3 000/m^2 also indicated the presence of a population of nontarget predators.

The results indicated that the water quality in the test sites was within the acceptable ranges for use of this parasite and that such factors as the levels of temperature, pH, salinity, and conductivity had no detectable adverse effects on the nematode-egg hatch or on the viability and infectivity of the nematode larvae. Moreover, the size of the treatment sites did not have a negative effect on the levels of infection.

A marked reduction in mosquito larvae density was observed at all the sites treated with *R. iyengari* nematode larvae. Data obtained from field bioassay as well as other reported data (*16*) have indicated that the small increases in the mosquito larvae populations at the two control sites was due to the continual oviposition of eggs and the hatching of new larvae. The examination of mosquito larvae from 9 of the sites 2 months after the initial nematode applications showed the capacity of *R. iyengari* to become established in this field situation (*17*). The natural populations of *An. pseudopunctipennis* larvae were drastically reduced or even eliminated entirely within 72 h when the application rate was 3 000/m^2.

Among the other conclusions that can be drawn from this research are:

a) *An. pseudopunctipennis* is highly susceptible to parasitism by *R. iyengari* under natural field conditions and that natural populations of these larvae, especially younger larvae, can be reduced.
b) Natural populations of *An. pseudopunctipennis* larvae can be completely killed within 72 h by increasing the dose of nematodes to 3 000/m^2.
c) Older larvae of *An. pseudopunctipennis* exposed to *R. iyengari* were infected but proved to be less susceptible to parasitism than younger larvae.
d) The levels of water temperature, pH, salinity, conductivity, depth, and vegetation which were measured during this study were all within the acceptable ranges for the use of this parasite.
e) The nematode had become established at 9 sites monitored 2 months after the initial applications.

Acknowledgments. The authors wish to recognize the financial support of the Sistema de Investigación Benito Juárez.

REFERENCES

1. Rodríguez López MH. *Enfermedades tropicales en México. Diagnóstico, tratamiento y distribución geográfica*. México, D.F., México: Instituto Nacional de Diagnóstico y Referencias Epidemiológicas en México; 1994:267–277.
2. Santamarina AM. Actividad parasitaria de *Romanomermis iyengari* (Nematoda: Mermithidae) en criaderos naturales de larvas de mosquitos. *Miscelánea Zoológica* 1994;17:59–65.
3. Welch H. *Romanomermis iyengari* species nov. Nematoda: Mermithidae Braun, 1883. *Pilot Register of Zoology*, Card no. 209.
4. Chandrahas RK, Rajagopalan PK. Mosquito breeding and the natural parasitism of larvae by a fungus Coelomomyces and mermithid nematode *Romanomermis* in paddy fields in Pondicherry. *Indian J Med Res* 1979; 69:63–70.
5. Santamarina AM, García IA, González RB. Valoración de la capacidad infectiva del nemátodo parásito *Romanomermis iyengari* (Nematoda: Mermithidae) en criaderos naturales de larvas de mosquitos. *Rev Cub Med Trop* 1993;45:128–131.
6. Santamarina AM, García IA, Rosabal JR, Solís AM. Release of *Romanomermis iyengari* (Nematoda: Mermithidae) to control *Aedes taeniorhynchus* (Diptera: Culicidae) in Punta del Este, Isla de la Juventud. *J Med Entomol* 1996; 33:680–682.
7. Poinar GO. *Entomogenous nematodes, a manual and host list of insect nematode associations*. Leiden, Netherlands: E.J. Brill; 1975.
8. Petersen JJ, Willis OR. Procedures for the mass rearing of a mermithid parasite of mosquitoes. *Mosquito News* 1972;32:226–230.
9. Levy R, Murphy LJ Jr, Miller TW Jr. Effects of a simulated aerial spray on a mermithid parasite of mosquitoes. *Mosquito News* 1976;36: 498–501.
10. Dubitskij AM. Métodos de control biológico de los dípteros hematófagos en la URSS. T. 1. Acad. Csi. Kaz. SSR. Alma Ata, Ed. 11265 HC. 1978.
11. Lacey LA, Mulla MS. Field evaluation of difluobenzurón (Dimilin) against *Simulium* larvae. *Mosquito News* 1979;39:86–90.
12. Gajanana A, Kazmi SJ, Bheema Rao US, Sujuna SG, Chandrahas RK. Studies on a nematode parasite (*Romanomermis* sp.: Mermithid) of mosquito larvae isolated in Pondicherry. *Indian J Med Res* 1978;68:242–247.
13. Rojas W, Northup J, Gallo O, Montoya AE, Restrepo F, Nimnich M, et al. Reduction of malaria prevalence after introduction of *Romanomermis culicivorax* (Nematoda: Mermithidae) in larval *Anopheles* habitats in Colombia. *Bull World Health Organ* 1987;65(3):331–337.
14. Santamarina AM, Pérez RP. Efecto patogénico del nemátodo parásito *Romanomermis iyengari* (Nematoda: Mermithidae) en larvas del mosquito *Aedes aegypti* (Diptera: Culicidae) en condiciones de laboratorio en el Estado de Oaxaca, México. *Rev Cub Med Trop* 1997;49: 93–97.
15. Petersen JJ. Comparative susceptibility of larval mosquitoes exposed by instar or in mixed populations to the nematode *Romanomermis culicivorax*. *Journal of Nematology* 1981;13: 228–229.
16. Petersen JJ, Willis OR. Experimental release of a mermithid nematode to control *Anopheles* mosquitoes in Louisiana. *Mosquito News* 1974;34:315–319.
17. Petersen JJ, Willis OR. Establishment and recycling of a mermithid nematode for the control of mosquitoe larvae. *Mosquito News* 1975; 35:526–532.

Manuscript received on 8 August 1997. Revised version accepted for publication on 7 December 1998.

RESUMEN

El parásito *Romanomermis iyengari* para la supresión de *Anopheles pseudopunctipennis* de sus hábitat naturales en el estado de Oaxaca, México

En septiembre y noviembre de 1996, *Romanomermis iyengari* Welch, parásito de larvas de mosquitos, fueron liberados en 44 lugares que servían de hábitat natural a larvas de *Anopheles pseudopunctipennis* Theobald con objeto de reducir las poblaciones larvarias de este importante vector de la malaria. Los lugares elegidos para el tratamiento variaron en tamaño de 5 a 500 m^2. El estudio se llevó a cabo en el distrito de Pochutla en el Estado de Oaxaca, sobre la costa pacífica de México.

Los plaguicidas químicos que reducen las poblaciones del vector han sido el instrumento principal en las campañas para la supresión de la malaria. No obstante, el uso excesivo de estas sustancias químicas ha dado origen a resistencia y a otros problemas serios. Por consiguiente, en 1996 se inició un proyecto de control biológico basado en el uso de agentes que son patógenos para las larvas de *Anopheles*. El objetivo principal fue establecer un sistema para criar *R. iyengari* en grandes cantidades. En el Instituto Politécnico Nacional del estado de Oaxaca se estableció un método detallado para criar estos nematodos e introducirlos en los hábitat de las larvas de mosquitos. Antes de colocar los parásitos dentro de los hábitat de las larvas, se determinaron las características de los lugares, entre ellas su tamaño, profundidad, vegetación acuática, salinidad, pH, conductividad, temperatura y densidad larvaria antes del tratamiento. Con un fumigador por compresión de aire se liberaron parásitos de los nematodos infectantes a razones de 2 000 ó 3 000 por m^2 y los parásitos produjeron tasas de infestación elevadas. A las 72 horas de aplicarse el tratamiento se tomaron muestras de las poblaciones de *Anopheles* y las larvas así obtenidas se llevaron al laboratorio, donde se sometieron a disección y examen microscópico para determinar los niveles de infestación y la carga parasitaria promedio. La carga de parásitos osciló entre 85 y 100% en todos los lugares donde se aplicó el tratamiento, a pesar de que no se habían notificado datos previamente sobre la carga parasitaria de *An. pseudopunctipennis* por *R. iyengari* en campo abierto. En los lugares tratados también se detectó una notable reducción de la densidad de las larvas de mosquitos a los 5 días de aplicarse los nematodos. La carga parasitaria fue indicación del número de larvas de mosquitos que murieron como consecuencia del tratamiento, ya que las larvas infestadas nunca llegaron a formar crisálidas. A juzgar por los resultados del muestreo de nueve de los lugares tratados 2 meses después de la aplicación inicial de nematodos, un gran número de las larvas de mosquitos fueron infectadas por parásitos que habían nacido de huevos que ya estaban depositados en la tierra. Este trabajo sugiere que este nematodo puede reducir las poblaciones de *An. pseudopuctipennis* en el Estado de Oaxaca.