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Reduction of malaria prevalence after introduction of *Romanomermis culicivorax* (Mermithidae: Nematoda) in larval *Anopheles* habitats in Colombia

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The worldwide resurgence of malaria has become a major public health problem. New methods of controlling the vectors of the disease are required, and we therefore studied the biological control of Anopheles albimanus *by* Romanomermis culicivorax *in Colombia. The investigation was carried out in El Valle and Nuquí, two towns on the northern Pacific coast of the country. All of the mosquito larval habitats surrounding El Valle were seeded with the eggs and adults of* R. culicivorax. *The nematode established itself in the new habitat and recycled over 27 months. The larval population of* A. albimanus, *the only malarial vector detected in the two towns, decreased in El Valle. In contrast, no change in the larval populations of the vector was detected in ponds located near Nuquí, the untreated control town. A rapid and progressive decrease of the prevalence of malaria among schoolchildren in El Valle was observed during the 2-year evaluation period.*

The worldwide resurgence of malaria has become a major public health problem owing to the increased resistance of the *Anopheles* vector of the disease to insecticides (e.g., DDT), the high cost of new insecticides, the appearance of drug-resistant strains of *Plasmodium falciparum*, the complexities of maintaining house-spraying programmes, and the behavioural changes of some species of *Anopheles* that have become exophilic. Statistical surveys indicate that there were 300 million new cases of the disease in 1984 with more than 1 million malaria-related deaths in tropical Africa in the same year (*1*). In 1961 there were 16 974 registered cases of malaria in Colombia, but this had risen to 90 730 registered cases by 1986; however, since the registration procedure is inefficient it is estimated that the total number of cases for 1986 in Colombia must have been over 400 000.[a] Additional and innovative vector control methods are therefore required, either alone or in combination with traditional approaches, to decrease the transmission of the disease.

The use of microbiological agents that are pathogenic to *Anopheles* larvae has recently been considered for the biological control of vectors. In this respect WHO has produced an inventory of pathogens of mosquito larvae and periodically reviews published data in this area.[b-f]

An ideal biological system for control of *Anopheles* must satisfy three basic criteria:

— be safe for humans and for the environment;

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[a] Servicio Erradicación de la Malaria (SEM), Bogotá. Report, 1987.

[b] ROBERTS, D. W. ET AL. *Biography of pathogens of medically important arthropods.* Unpublished document, WHO/VBC/83.1, 1983.

[c] WESTERDAHL, B. B. ET AL. *Successful establishment and subsequent recycling of* Romanomermis culicivorax *in a California rice-field following postparasite application.* Unpublished document, WHO/VBC/81.826, 1981.

[d] *Protocol for field testing of nematodes against mosquitos.* Unpublished document, WHO/VBC/82.3, 1982.

[e] *Data sheet on biological control agents.* Unpublished document, WHO/VBC/80.09, 1980.

[f] WEISER, J. *Guide to field determination of major groups of pathogens affecting arthropod vectors of human diseases.* Unpublished document, WHO/VBC/82.860, 1982.

—be effective in reducing vector populations and transmission; and
—be amenable to local production and maintenance.

Extensive research on the feasibility of using biological control agents against malaria vectors has demonstrated that *Romanomermis culicivorax*, a mermithid nematode and an obligate parasite of mosquito larvae, could potentially fulfil these conditions (*2–6*). It has no known hazardous effect on humans, domestic animals, fish, or wildlife, and is registered by the United States Department of Agriculture for use in the control of mosquitos (*7*). The nematode infects no organisms other than aquatic diptera larvae.[g] In 1978 Peterson demonstrated its effect in controlling *Anopheles albimanus* larvae in field tests in El Salvador (*8*). Furthermore, ongoing populations of *R. culicivorax* have been established by Washino in rice-fields in California (*9*), and the nematode is routinely used by the Yuba City mosquito abatement district in the rice-field area of north-central California. Methods for mass production of the parasite in the laboratory are well established.

We report here the results of a study of the use of *R. culicivorax* as a biological agent to control malaria vectors in an area of the northern Pacific coast of Colombia.

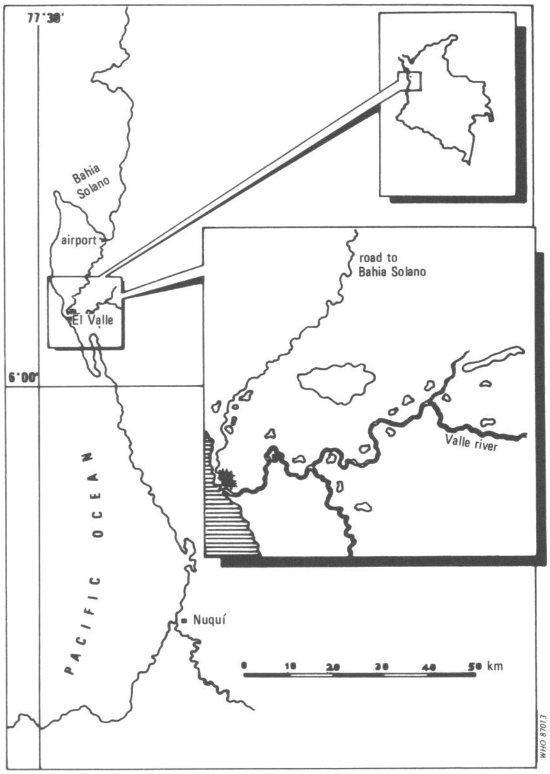

Fig. 1. Location of the experimental area in Colombia.

MATERIALS AND METHODS

Experimental area

Two communities on the northern Pacific coast of Colombia were selected for the study. The town of El Valle was designated for treatment with *R. culicivorax*, while the town of Nuquí served as an untreated control area; the mosquito breeding habitats and climate in both towns are similar, as are the population densities and ethnic characteristics of both communities. Most of the inhabitants are indigenous to the region. Both towns are situated in a malaria transmission zone of high endemicity.

El Valle. The town is located on the coast (6.05N, 77.24W), 20 km south of Bahia Solano (Fig. 1). El Valle has no hospital, and medical care is provided by a small health centre attended a few hours every 2 weeks by a doctor from Bahia Solano. Malaria surveillance is carried out by auxiliary personnel from Servicio Erradicación de la Malaria (SEM) during bimonthly visits and by local health workers trained in the examination of thick blood smears. Antimalaria drugs are available irregularly, and houses in the town are sprayed with DDT every 6–10 months.

All probable *Anopheles* larval habitats in the vicinity of the town were detected by direct inspection from the road leading to Bahia Solano and also along the El Valle river up to 7 km east of the town; inaccessible habitats were detected from an aircraft or helicopter. A total of 21 bodies of water were identified, ranging in surface area from 25 m^2 to 5000 m^2. Three of the breeding sites, located 3–4 km from the centre of the town, and accessible from the road to Bahia Solano, were selected for periodic evaluation over 2 years.

Nuquí. Nuquí, the untreated town that served as a control in the study, is located 60 km south of El Valle (5.44N, 77.16W). Twelve mosquito larval habitats, ranging in area from 10 m^2 to 60 m^2, were identified in the urban area and the population of *Anopheles* larvae in three of them was monitored intermittently over the 2-year study period. The town has a small hospital attended by a permanent doctor,

[g] CALIFORNIA DEPARTMENT OF AGRICULTURE. Certification letter for Mermithidae, 1983.

graduate nurse, and auxiliary personnel. Malaria surveillance in the town is carried out by SEM personnel and by health workers with the assistance of the hospital staff.

Meteorological conditions

Climatic evaluation has been carried out in the area since 1939 from a station in Bahia Solano. From 1939 to 1983 the mean temperature fluctuated from a minimum of 20.0 °C to a maximum of 33.3 °C. The minimum individual temperature during this period was 16.4 °C (July 1973), while the extreme maximum was 40.0 °C (March 1966). The relative humidity fluctuated from 80% to 97% (mean, 90%) and the annual rainfall from 2.026 to 8.663 mm (mean 5.134 mm). No month without rain was recorded from 1939 to 1983, and the monthly rainfall ranged from a minimum of 93 mm to a maximum of 993 mm.

Entomological evaluation

The basic entomological information collected by SEM since 1982 was complemented by an evaluation of the study area conducted jointly with the National Institute of Health in Colombia. Adult *Anopheles* mosquitos were captured indoors using human baits during three 6-hour periods repeated four times—twice prior to seeding with *R. culicivorax* and twice following seeding.

The larval population in the three ponds selected in each community was evaluated twice before introduction of *R. culicivorax*. After the parasite had been seeded in all the ponds surrounding El Valle, the larval population in each of the selected ponds was evaluated 10 times using standard "dipping" (sampling) methods. A sample was taken every 50 cm along the edge of each pond with a total of up to 100 samples per pond. For ponds whose perimeter was less than 50 m, the results were converted by proportion to 100 samples. Differential counts of *Culex* and *Anopheles* larvae were also made.

Seeding with Romanomermis culicivorax

For the ponds that were accessible by road and those on both sides of the El Valle river the seeding was performed directly by hand, in an even distribution pattern over the water surface. Other ponds were seeded from the air. One-half of the *R. culicivorax* eggs (22×10^6) and adults (110 000) was seeded in the first week of April 1983, and the remainder was distributed one month later to all the habitats. The seeding density was approximately 3000 eggs per m^2 of water surface. The following procedures were used to evaluate the establishment and recycling of *R. culicivorax*.

—Direct microscopic observation in the field and dissection of all larvae collected by the dipping method.

—Use of floating "sentinel boxes" containing first and second instar larvae of *Culex pipiens*. The boxes were placed periodically in three of the ponds for 24 hours and then sent by air to Medellín, where the larvae were dissected within 6 hours of receipt. Laboratory-reared *Anopheles* larvae were not used as sentinels in order to avoid introducing a new strain of *A. albimanus* into the study area. *C. pipiens* is a good alternative host for *R. culicivorax* and is normally present in large quantities in the test area.

—Pre-parasitic *R. culicivorax* larvae taken from the surface of the ponds were placed in water samples containing *Anopheles* larvae and dissected after 72 hours' exposure.

—Examination of mud from the bottom of the ponds for adult *R. culicivorax*.

[h] Samples of *R. culicivorax* eggs and adults were provided by the Sutter-Yuba Mosquito Abatement District, Yuba City, CA, USA.

Malaria epidemiological evaluation

The prevalence of malaria among the schoolchildren in El Valle and Nuquí was determined five times over the 2-year study period, and an epidemiological evaluation of the disease was carried out prior to seeding with *R. culicivorax*. A thick film of blood was obtained from all children attending schools on the day of the evaluation, and films of the smears were examined by experienced technicians. Quality control checks of the evaluation procedure were performed by the Antioquia State Public Health Laboratory on a 10% random sample of positive and negative smears.

RESULTS

Entomological evaluation

A. albimanus was the only *Anopheles* species captured in the urban areas of both communities. However, specimens of *A. pseudopunctipennis* and *A. punctimacula* were identified 6 km to the east of El Valle. Also, *A. (Kertszia) neivai* larvae were found in bromelids in trees along the El Valle river; however, no adult of this species was captured in the urban areas of El Valle.

Anopheles mosquitos were captured using human bait over four 24-hour periods. The number of females caught in the two evaluations prior to seeding with *R. culicivorax* (February and April 1983) was 74.5 in El Valle and 67.5 in Nuquí. After seeding, two further evaluations (in June and August 1983)

Table 1. Mean number of *Culex* and *Anopheles* larvae in the three designated ponds in El Valle and Nuquí

	Mean number of larvae[a]			
	El Valle		Nuquí	
	Culex	*Anopheles*	*Culex*	*Anopheles*
1983				
March	295	99	230	145
April	305	126	285	98
June	208	72	210	155
July	165	52	265	107
August	149	12	168	86
September	122	27	185	91
1984				
January	185	11	–	–
June	145	2	–	–
December	187	0	223	127
1985				
April	152	6	–	–
July	178	15	–	–
August	139	12	292	223
1986				
January	189	15	–	–

[a] In each case values are the average number in the three ponds.

indicated that the mean number captured had decreased to 17.5 in El Valle but had remained relatively high in Nuquí (53). In El Valle there was a 90% reduction in the number of adult females caught in June 1983 (32) and August 1983 (3).

A reduction of more than 50% in the total larval population at breeding sites in El Valle was recorded. The sharpest decline was observed for *Anopheles* larvae, whose number was reduced by 90% by January 1984 (Table 1). In contrast, in Nuquí there was no sustained decrease in the total population of *Anopheles* or in the relative proportion of larvae of *Anopheles* spp. to those of *Culex* spp.

Recycling of R. culicivorax

Three ponds at El Valle were studied periodically to determine whether *R. culicivorax* had established itself and was recycling. From June 1983 to August 1985 such evaluations by direct microscopy and dissection of the larvae collected were performed. In pond 1, the nematode was detected in nine of the eleven evaluations, while in ponds 2 and 3 it was identified five and three times, respectively (Table 2). Altogether 2% to 6% of the *Anopheles* larvae examined were infected.

Sentinel boxes were also used on six occasions to monitor the ponds. The nematode was detected four times in ponds 1 and 3, and once in pond 2, while pre-parasitic *R. culicivorax* larvae were detected three times in pond 1 and twice in ponds 2 and 3.

Adult *R. culicivorax* were also found in mud from pond 1, but not in mud from the other ponds.

Epidemiological results

The prevalence of malaria among schoolchildren in both communities is shown in Table 3. The study in April 1983 was carried out before seeding with *R. culicivorax*. In El Valle the prevalence of the disease among the school population declined progressively from 21.7% in April 1983 to 0.81% in April 1985. In contrast, in Nuquí the prevalence among schoolchildren fluctuated from 9.7% to 12.2% between April 1983 and August 1984; by April 1985 it had decreased to 4.8%. A more recent epidemiological evaluation, carried out in January 1986, indicated that the prevalence of malaria had subse-

Table 2. Results of the evaluation of recycling of *R. culicivorax* in the three designated ponds in El Valle

	Method of evaluation[a]											
	Pond 1				Pond 2				Pond 3			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
1983												
June	+	–	–	–	–	–	–	–	+	–	–	–
July	+	–	+	–	+	–	–	–	+	–	+	–
August	+	+	+	–	+	–	+	–	–	+	–	
September	+	–	–		+	–	–		–	+	–	
1984												
January	+	+	–	+	–	–	–	–	–	+	+	
June	–	+	+		–	+	+		–	+	–	
December	+	–	–	–	–	–	–	–	+	–	–	–
1985												
April	+	+	–		–	–	–		–	–	–	
June	+	–		–	+	–		–	–	–		–
August	+	–		–	+	–		–	–	–		–
1986												
January	–	–		–	–	–	–		–	–	–	

[a] I = Direct observation; II = sentinel boxes; III = detection of pre-parasitic immature nematodes; IV = examination of mud for adult nematodes.

Table 3. Prevalence of malaria among schoolchildren in El Valle and Nuquí

	El Valle		Nuquí	
	No. of children	Positive (%)	No. of children	Positive (%)
1983				
April[a]	254	21.7	390	10.0
August	170	8.8	288	12.2
November	180	6.6	218	11.5
1984				
August	233	4.3	300	9.7
December	192	3.4	286	9.1
April 1985	247	0.81	304	4.8
January 1986	242	7.1	280	6.9

[a] Date when ponds were seeded with *Romanomermis culicivorax* larvae.

quently increased in both communities: in El Valle from 0.85% in August 1985 to 7.1% in January 1986; and in Nuquí from 4.8% to 6.9% over the same period. By January 1986 no further recycling of *R. culicivorax* in the ponds could be detected.

DISCUSSION

Since 1975, recycling of *R. culicivorax* in new habitats has been reported in Louisiana and California (*10*). However, attempts to recycle the nematode failed in Thailand (*3*) and El Salvador (*8*).

It has been suggested that the best use of *R. culicivorax* in the control of vector populations is its periodic deployment for immediate control of *Anopheles* larvae. Our results show the nematode can recycle for at least 2 years in a tropical zone and can effectively reduce the *A. albimanus* population. A coincidental reduction in the transmission of malaria was demonstrated by a decrease in the prevalence of the disease among schoolchildren in El Valle.

The marked decrease found in the population of *Anopheles* relative to that of *Culex* spp. is not unexpected, since *R. culicivorax* prefers *Anopheles* larvae. This probably arises because the latter adopt a horizontal position below the water surface, thereby facilitating their invasion by the pre-parasitic larvae of the nematode, which exhibits negative geotropism. Recent observations in our laboratory (A. E. Montoya & O. Gallo, unpublished results) show that *R. culicivorax* kills the first and second instar larvae of *A. albimanus* and of *A. nunez-tovari* within 28 hours; however, the parasite does not attain a post-parasitic form in these larvae. In contrast, in *C. pipiens* or *Aedes aegypti* larvae, the nematode is able to complete its development, and the invaded larvae die within 5 to 7 days, upon emergence of the post-parasitic form.

Of the four methods used in the field to evaluate the capability of *R. culicivorax* to recycle, direct observation of the larvae captured from the infected ponds was the most satisfactory. The other three methods (sentinel boxes, detection of pre-parasitic larvae, and detection of adults in the mud of ponds) less frequently provided unequivocal results.

The climate in the study area (high relative humidity, maximum temperatures below 35 °C, and frequent rains that prevent the ponds from drying up) is important for the recycling of *R. culicivorax*. For example, studies in four geographical areas of Colombia indicated that dry conditions and high temperatures can kill the nematode after it has recycled for several months.

Evaluation of the adult *Anopheles* population was time-consuming, presented risks for the persons used as bait for the mosquitos, and was expensive, thus preventing use of the procedure throughout the study.

Of note was the impressive decrease in the prevalence of malaria among schoolchildren in El Valle from 21.7% in April 1983 to 0.81% in April 1985. No extra malaria control measures were taken in the study area during the investigation period; however, in both the study and control areas symptomatic children with positive smears received chemotherapy. Furthermore, there was no increase in DDT spraying coverage or in the use of insecticides, and there were no changes in weather conditions.

The decrease in the prevalence of malaria in El Valle is therefore consistent with a reduction in the vector population caused by the recycling of *R. culicivorax* in the *Anopheles* larval habitats. The reason why the nematode ceased to recycle is not clear, but it may have been due to the onset of the dry season or to a new biological equilibrium that reduced its population levels that were too low to detect using the methods we have described. The availability of better medical care could account for the lower initial prevalence of malaria in Nuquí, which was 50% less than that at the beginning of the study in El Valle. However, it is not clear why the prevalence of the disease in Nuquí decreased moderately over the study period.

CONCLUSIONS

The results of the study indicate that *R. culicivorax* became established and recycled for 27 months in

Anopheles larval habitats of the Pacific coast of northern Colombia. An acute decrease in the larval population of *A. albimanus* relative to that of *C. pipiens* was observed in the test area, indicating the preference and greater pathogenic effect of *R. culicivorax* for *A. albimanus*. A reduction in the prevalence of malaria among schoolchildren in El Valle coincided with deployment of *R. culicivorax* and was probably due to the recycling of the nematode in the ponds of the town.

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RÉSUMÉ

DIMINUTION DE LA PRÉVALENCE DU PALUDISME APRÈS INTRODUCTION DE *ROMANOMERMIS CULICIVORAX* (MERMITHIDÉS: NÉMATODES) DANS DES GÎTES LARVAIRES D'ANOPHÈLES EN COLOMBIE

La reprise du paludisme dans le monde constitue un problème de santé publique préoccupant et de nouvelles méthodes de lutte contre les vecteurs de la maladie se révèlent nécessaires. Dans le cadre de la lutte biologique contre les vecteurs, on a récemment envisagé de recourir à des agents microbiologiques pathogènes pour les larves d'anophèles. La présente étude évalue les résultats de la lutte biologique contre *Anopheles albimanus* au moyen de nématodes *Romanomermis culicivorax*, dans deux villes, El Valle et Nuquí, situées sur la partie septentrionale de la côte pacifique de Colombie. El Valle (6,05° N, 77,24° O) ne dispose d'aucun hôpital, mais un petit centre de santé, où un médecin assure une permanence de quelques heures tous les quinze jours, offre des soins à la communauté.

Tous les gîtes hébergeant probablement des larves d'anophèles et qui se trouvent à proximité d'El Valle ont été identifiés par inspection directe depuis la route conduisant à Bahia Solano et le long de la rivière El Valle jusqu'à 7 kilomètres à l'est de la ville. Les gîtes inaccessibles depuis la route ou la rivière ont été repérés par avion ou hélicoptère. On a localisé au total 21 étendues d'eau ayant une superficie de 25 à 5000 m^2. On a choisi trois de ces gîtes larvaires, situés à 3–4 km du centre de la ville et accessibles par la route, pour y procéder à des évaluations échelonnées sur une période de deux ans.

La ville de Nuquí (5,44° N, 77,16° O), qui a servi de ville témoin pour l'étude, se trouve à 60 km au sud d'El Valle. Douze habitats de larves de moustiques, d'une surface comprise entre 10 et 60 m^2, ont été identifiés dans la zone urbaine. La population larvaire dans trois d'entre eux a été évaluée périodiquement pendant les deux ans qu'a duré l'étude. La ville dispose d'un petit hôpital doté d'un médecin permanent, d'un(e) infirmier(ère) diplômé(e) et de personnel auxiliaire.

On a contrôlé à deux reprises la population larvaire dans les trois étangs désignés dans chaque communauté avant de les ensemencer en *R. culicivorax*. En outre, après l'ensemencement en nématodes de tous les étangs qui entourent El Valle, on a évalué 10 fois la population larvaire dans les trois étangs choisis dans cette zone urbaine. Quatre méthodes ont été utilisées pour évaluer la capacité de reproduction de *R. culicivorax*: observation directe des larves capturées dans les étangs, boîtes-sentinelles, détection des larves préparasitaires, et détection des nématodes adultes présents dans la vase recueillie. Parmi ces méthodes, c'est l'observation directe qui a donné les résultats les plus nets.

La prévalence du paludisme parmi les enfants scolarisés des deux communautés a été contrôlée à cinq reprises au cours des deux années et l'on a procédé à une étude épidémiologique du paludisme parmi ces enfants avant l'ensemencement en *R. culicivorax*. On a constaté avec intérêt que la prévalence de la maladie parmi les enfants scolarisés d'El Valle avait chuté de 21,7% à 0,81% pendant les deux années de l'étude. On peut légitimement en conclure que cette diminution, coïncidant avec la réduction de la population vectrice, est due à la reproduction des nématodes dans les gîtes larvaires d'*Anophèles*. On n'a pas élucidé pourquoi *R. culicivorax* avait cessé de se reproduire au bout de deux ans environ.

Si les résultats que nous avons présentés s'appliquent à d'autres régions géographiquement identiques, la lutte biologique à long terme contre les *Anophèles* vecteurs du paludisme au moyen de larves de *R. culicivorax* pourrait se révéler intéressante. Parallèlement aux méthodes classiques de lutte, l'alternative qu'offre cette approche pourrait contribuer à réduire la prévalence du paludisme dans les régions de forte endémicité.

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