FIELD APPLICATION OF *ROMANOMERMIS CULICIVORAX* (MERMITHIDAE: NEMATODA) TO CONTROL ANOPHELINE LARVAE IN SOUTHERN IRAN

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ABSTRACT. The efficacy of the parasitic nematode *Romanomermis culicivorax* in controlling anopheline larvae, including malaria vectors, was studied in Fars and Baluchistan provinces. Twenty sites were treated once with the preparasitic stage of *R. culicivorax* in 1984 with 3,000, 5,000 or 10,000 preparasites per m^2 surface area, depending on larval density. The average parasitism of anopheline larvae ranged from 56 to 69% based on 24 hr posttreatment dissections. No correlation was found between the level of parasitism and the density of mosquito larvae present in a site. About 61% parasitism was obtained when different rates of preparasites were released in 14 larval breeding sites in 1985. No apparent difference was observed in the rate of parasitism in 10 sites receiving one treatment compared with four sites receiving two treatments, with a seven day interval between each treatment. *Romanomermis culicivorax* was established in the release sites but caused only minor reductions in anopheline larval populations. The nematode would be of limited use in antimalaria campaigns in southern Iran.

INTRODUCTION

Malaria is still the most important health problem in Iran and its neighboring countries. Annual prevalence rates average 30-40 thousand and most cases occur in the south and southeastern parts of the country. *Anopheles stephensi* Liston, the primary vector of malaria in southern Iran, is resistant to DDT, dieldrin and malathion (Mofidi and Samimi 1960, Manouchehri et al. 1976) and *An. culicifacies* Giles, the major vector of malaria in southeastern Iran, is resistant to DDT (Zaini and Manouchehri 1973) and probably malathion. Propoxur has been used for the control of these vectors since 1978, and it is expected mosquito resistance will occur in the near future.

Because of the emergence and spread of insecticide resistance in malaria vectors in Iran, the high cost of new types of insecticides and concern for environmental pollution, vector control can no longer solely depend on the use of chemicals. Alternative methods of control have to be developed.

Romanomermis culicivorax Ross and Smith is an obligatory endoparasitic nematode, the larvae of which complete their development inside mosquito larvae. This nematode has been extensively studied for the past 19 years. It is relatively easy to mass produce (Petersen and Willis 1972, Petersen et al. 1978a), presents no hazard to mammals or other non-target organisms, and its environmental limitations are well documented (World Health Organization 1980a, 1980b). At least 17 mosquito species are known to be naturally infected and over 87 species have been experimentally infected by this nematode (Petersen and Chapman 1979). Based on small scale field experiments it has been shown that it may have the potential for biological control of mosquitoes in specific habitats (Finney 1981, Platzer 1981, Poinar 1979, Westerdahl et al. 1982). The largest successful field trial using the preparasitic stage was carried out against *An. albimanus* Wiedemann in a volcanic lake in El Salvador and resulted in a 17-fold reduction of *Anopheles* populations (Petersen et al. 1978b). However, more large scale studies, especially based on periodic inundative release of parasites in warmer climates, need to be conducted.

This study was conducted during 1984–86 to determine the efficacy of *R. culicivorax* in controlling anopheline mosquitoes, including malaria vectors, in southern Iran and to study the nematodes' performance and especially the possibility of recycling in the warm climates of that area.

MATERIALS AND METHODS

The mass culture of *R. culicivorax*, supplied by the late E. I. Hazard, Director of the Gulf Coast Mosquito Research Laboratory, Lake Charles, LA, USA, was achieved at the rearing facilities of the School of Public Health, Teheran University of Medical Sciences 8–10 weeks in advance of tests to permit maturation of the nematode cultures.

The basic mass rearing procedure required the exposure of groups of 200 first instar *Culex pipiens* Linn. to preparasites of *R. culicivorax* at a 4:1 parasite-host ratio (causing more than 90% parasitism and an average of 2.5 nematodes per parasitized larvae) in 37 x 22 x 4 cm rearing

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trays. Seven days later, host larvae were removed from trays, concentrated in groups of about 2,000, washed and placed in nematode collecting containers. After emergence, nematodes were pooled, washed and placed in cylindrical plastic containers (18.5 cm diam x 14 cm) containing clean sterile sand (1.5 cm) covered to a depth of 4 cm with chlorine-free water. The water was removed after 10 days and the moist cultures were stored for an additional 5–7 weeks before use. The cultures were shipped in the plastic containers in which they were established. A 2-cm thick styrofoam pad was placed over the sand in each container to immobilize the culture. Shipping containers were made of styrofoam and each held six cultures. Packaging material was placed around culture containers to prevent their sideways movement. Shipping containers were transported to the nearest airport and then transported to the study site by car (not exceeding 150 km).

Hatching of preparasites was induced by flooding mature cultures 14–20 hours prior to each treatment and the number of preparasitic nematodes per unit volume of water was determined by volumetric dilutions as described by Petersen and Willis (1972). Final dilutions were made at the site of the treatment by using water from the test site.

Three locations were selected as treatment sites in southern Iran: (1) Anarestan (ANR), Kazeroun, Fars Province (latitude 29°37′ N and longitude 51°38′ E) with permanent, shallow, standing bodies of water, with emergent and floating vegetation; (2) Pir Sabz (PSB), Kazeroun, Fars Province (close to site 1), with permanent, very slow running bodies of water with floating vegetation; and (3) Abtar (ABT), Iranshahr, Baluchistan Province (latitude 60°33′ N and longitude 25°27′ E) with permanent, very slow running water with floating and emergent vegetation. All treatment sites were exposed to sunlight and contained water with relatively low salinity (maximum conductivity reading of 1,160 microhms/cm). The following anophelines were present: in site-ANR: *An. dthali* Patton and *An. superpictus* Grassi; in site-PSB: *An. dthali, An. superpictus, An. sergentii* (Theobald) and *An. turkhudi* Liston; and in site-ABT: *An. turkhudi, An. dthali, An. culicifacies.*

In the first attempt (September–October 1984), preparasitic nematodes were released only once in the mosquito breeding sites of the three named locations (six sites in PSB; six sites in ANR; and eight sites in ABT). Percent parasitism of anopheline larvae, based on 24 hour posttreatment dissections, was recorded. In the second attempt (July and August, 1985), 14 mosquito breeding sites of 4 m^2 surface area each, were selected in PSB (different from those used in previous releases). Ten sites were treated with preparasitic *R. culicivorax* larvae once (six sites on July 25 and four on August 1) and the other four twice, with a 7 day interval between treatments. The rate of parasitism, recycling and overwintering was observed. All nematode applications were made in the late afternoon, by pouring them carefully all over the water surface. Chemical analyses of water samples from each site were performed to determine quantitatively the chemical nature of the water.

RESULTS AND DISCUSSION

The level of parasitism, based on 24 hour posttreatment dissections of anopheline larvae, fluctuated greatly between sites and treatments (Tables 1–3). Also no correlation was noticed between the levels of parasitism and the density of mosquito larvae present in a site. The average parasitism of anopheline larvae in the six test sites of PSB, six sites of ANR and eight sites of ABT was 61.7, 69.2 and 56.3, respectively. First and second instars were relatively more susceptible to infection by *R. culicivorax* than third and fourth. Percent parasitism of first and second instars in PSB, ANR and ABT were 63.3,

Table 1. Parasitism in larvae of *Anopheles* spp.* 24 hours after preparasitic *Romanomermis culicivorax* were released, Pir Sabz (PSB), Fars Province, Iran (September 1984). Forty larvae were dissected from each site.

		Larval density per dip by instar								
		Anopheline		Culicines			No. preparasites released per m^2 surface area	Percentage parasitism of anophelines by instar		
Site no.	Surface area (m^2)	1–2	3–4	1–2	3–4	Total		1–2	3–4	Total
PSB-2	1	3.2	1	0	0	4.2	3,000	55	30	42.5
PSB-3	1	2.1	1.2	0	0	3.3	3,000	60	70	65
PSB-4	1	1.1	0.9	0	0	2	3,000	30	70	50
PSB-13	1	4.8	6.5	0.1	0.1	11.5	5,000	90	65	77.5
PSB-14	1	6.3	6	0	0	12.3	5,000	70	55	62.5
PSB-15	1	4.3	7.1	0	0.4	11.8	5,000	75	70	72.5

* *An. dthali, An. superpictus* and *An. sergentii.*

Table 2. Parasitism in larvae of *Anopheles* spp.* 24 hours after preparasitic *Romanomermis culicivorax* were released, Anarestan (ANR), Fars Province, Iran (September 1984). Forty larvae were dissected from each site.

Site no.	Surface area (m^2)	Larval density per dip by instar: Anophelines 1–2	Anophelines 3–4	Culicines† 1–2	Culicines† 3–4	Total	No. preparasites released per m^2 surface area	Percentage parasitism of anophelines by instar: 1–2	3–4	Total
ANR-1	1	5.9	2.8	3.8	0.9	13.4	10,000	60	85	72.5
ANR-2	1.5	3.3	2.4	2.6	0.3	8.6	10,000	85	80	82.5
ANR-3	1	4.3	3.4	4	2.9	14.6	10,000	55	75	65
ANR-4	1.5	8.3	3	4.1	0.3	15.7	10,000	60	25	42.5
ANR-5	1	5.7	5.7	2.7	1.5	15.6	10,000	55	80	67.5
ANR-6	1.5	4.4	2.4	2.4	2.1	15.9	10,000	90	80	85

* *An. dthali* and *An. superpictus.*
† *Cx. perexiguus.*

Table 3. Parasitism in larvae of *Anopheles* spp.* 24 hours after preparasitic *Romanomermis culicivorax* were released, Abtar (ABT), Baluchistan, Iran (October 1984). Forty larvae were dissected from each site.

Site no.	Surface area (m^2)	Larval density per dip by instar: Anophelines 1–2	Anophelines 3–4	Culicines 1–2	Culicines 3–4	Total	No. preparasites released per m^2 surface area	Percentage parasitism of anophelines by instar: 1–2	3–4	Total
ABT-1	1	3.2	1.4	0	0	4.6	5,000	80	70	75
ABT-2	1	2.1	2.2	0	0	4.3	5,000	60	60	60
ABT-4	1	1.9	1.2	0	0	3.1	5,000	85	60	72.5
ABT-6	1	1.8	1.6	0	0	3.4	5,000	75	60	67.5
ABT-7	1	2.9	2	0	0	4.9	5,000	65	75	70
ABT-9	1	3.3	1	0	0	4.3	5,000	10	10	10
ABT-10	1	2.7	1.6	0	0	4.3	5,000	40	55	47.5
ABT-11	1	2.5	1.3	0	0	3.8	5,000	50	45	47.5

* *An. turkhudi, An. dthali, An. superpictus* and *An. culicifacies.*

67.5 and 58.1 in contrast to 60, 70.8 and 54.4 in third and fourth instars, respectively.

Parasitism of anopheline larvae in PSB in July and August 1985 averaged 60.7%, with no correlation between levels of parasitism and density of mosquito larvae present within a site (Tables 4 and 5). Again, the degree of parasitism fluctuated greatly between sites and treatments.

Anopheline dissections were made 38 days after the first release and later on to determine the possibility of establishment and overwintering of the nematode in the release sites (Table 6). Parasitism was generally low in anopheline larval populations and no apparent difference was observed in the rate of parasitism in sites receiving one or two applications of *R. culicivorax.* Parasitized larvae were collected from only two out of the 14 release sites in July 1986. It is noteworthy that anopheline larval activity (mainly *An. dthali* and *An. superpictus*) was present in the test site (PSB) until early December 1985, after which no larvae were present until May 1986. The minimum temperature (0°C) during this period (July 1985–July 1986) occurred in January, and the maximum temperature (46°C) occurred in June 1986.

The average rate of parasitism of anopheline larvae, especially in cases where 10,000 and 15,000 parasites were released per m^2, was much lower than expected. Based on other published reports, normal field dosages against anophelines in fresh, non-moving bodies of water have usually ranged from 1,500 to 5,000 preparasites/m^2 with the higher dosages giving 80–90% parasitism (Dr. H. C. Chapman, personal communication). A dosage rate of 10,000 preparasites/m^2 in Anarestan (ANR) with nonmoving bodies of water and relatively high larval densities resulted in an average parasitism rate of 69.2%.

Low rates of parasitism by *R. culicivorax* preparasites in the past have been attributed mainly to water quality, quality of inocula and host tolerance. However, in this study, the treatment sites had reasonably good quality of water, shown by maximum conductivity reading of 1100 microhms/cm; calcium 141 mg/liter; potassium 16.5 mg/liter; sodium 20 mg/liter; bicarbonate 380 mg/liter; sulfate 190 mg/liter and chloride 67 mg/liter for PSB and ANR; and conductivity of 1160 microhms/cm; calcium 65 mg/liter; potassium 5 mg/liter; sodium 125 mg/liter; bicarbonate 259 mg/liter; sulfate 120 mg/

Table 4. Parasitism in larvae of *Anopheles* spp.* 24 hours after preparasitic *Romanomermis culicivorax* were released, Pir Sabz (PSB), Fars, Iran (July 25, 1985). Fifty larvae were dissected from each site.

Site no.	Surface area (m^2)	Larval density per dip by instar: Anophelines 1–2	Anophelines 3–4	Culicines† 1–2	Culicines† 3–4	Total	No. preparasites released per m^2 surface area	Percentage parasitism of anophelines by instar: 1–2	3–4	Total
PSB-25	4	2.7	0.4	2.1	1.5	6.7	10,000	90	**	90
PSB-27	4	0.3	0.2	0.7	1.1	2.3	10,000	62	80	71
PSB-29	4	2.7	0.3	4.7	3.6	11.3	10,000	50	28	39
PSB-31	4	2.6	2.3	8.5	8.7	22.1	10,000	50	100	75
PSB-40	4	1.1	0.5	2.7	2.1	6.4	10,000	56	72	64
PSB-22	4	3.6	4.2	19.9	14.4	42.1	15,000	44	44	44
PSB-23	4	4.9	2.7	11.4	11.8	30.8	15,000	50	36	43
PSB-30	4	4.4	4.4	11.1	5.4	25.3	15,000	**	60	60
PSB-34	4	3.5	5.1	4.6	6.3	19.5	15,000	56	88	72
PSB-36	4	6.9	5.3	8	8	28.2	15,000	56	56	56

* *An. dthali, An. superpictus* and *An. turkhudi.*
† *Cx. perexiguus, Cx. theileri* and *Cx. pipiens.*
** No larvae could be found.

Table 5. Parasitism in larvae of *Anopheles* spp.* 24 hours after preparasitic *Romanomermis culicivorax* were released, Pir Sabz (PSB), Fars, Iran (August 1, 1985). Fifty larvae were dissected from each site.

Site no.	Surface area (m^2)	Larval density per dip by instar: Anophelines 1–2	Anophelines 3–4	Culicines† 1–2	Culicines† 3–4	Total	No. preparasites released per m^2 surface area	Percentage parasitism of anophelines by instar: 1–2	3–4	Total
PSB-37	4	1	0.1	2.2	1.2	4.5	2,500	28	82	55
PSB-38	4	0	0	0.7	0.6	1.3	2,500	**	18	18
PSB-26	4	0	0.1	0.5	0.6	1.2	5,000	100	50	75
PSB-33	4	1.3	0.6	4.6	6.9	13.4	5,000	86	94	90
PSB-25††	4	1.6	1.1	0.8	2.2	5.7	10,000	14	74	44
PSB-27††	4	0	0.2	0.2	1.7	2.1	10,000	50	100	75
PSB-29††	4	0	0.3	2.3	3.2	5.8	10,000	**	50	50
PSB-31††	4	0.4	0.1	1.8	5.1	7.4	10,000	68	80	74

* *An. dthali, An. superpictus* and *An. turkhudi.*
† *Cx. perexiguus, Cx. theileri* and *Cx. pipiens.*
** No larvae could be found.
†† Sites previously treated on July 25, 1985.

Table 6. Percent parasitism of anopheline larvae 38 days posttreatment and thereafter, Pir Sabz (PSB), Fars Province, Iran (1985–86).

Date of dissection	No. larvae examined	Percent parasitism of anopheline larvae per site no.*: 22	23	25	26	27	29	30	31	33	34	36	37	38
Sept. 8, 1985	25	4	4	4	0	0	0	8	36	16	8	4	0	4
Sept. 11, 1985	25	8	0	24	0	0	0	12	64	4	12	0	0	8
Sept. 14, 1985	25	8	8	8	0	0	0	8	56	4	4	4	0	4
Sept. 16. 1985	25	8	8	0	0	4	0	8	48	0	0	4	0	8
Sept. 21, 1985	50	4	0	0	0	0	0	0	26	0	6	34	0	24
July 4, 1986	50	0	0	0	0	4	0	0	0	0	0	0	0	8

* All instars included.

liter and chloride 161 mg/liter for ABT, and rates of parasitism of anopheline larvae in such water was compared with larvae reared in tap water in the laboratory with no noticeable difference. The quality of inocula was checked for each release and ruled out as a possible influence on the low rate of parasitism. Future studies on differential susceptibility tests of anopheline species to *R. culicivorax* will be undertaken to show if host tolerance could be a factor in the observed rate of parasitism.

It seems that although *R. culicivorax* may have

a role in biological control of anopheline mosquitoes in southern Iran, effective long term control is not likely to occur from a few artificially created epizootics. Further, the technical procedures of production, storage and transportation of the nematode make it costly to use it for periodic inundative releases for immediate control. Thus *R. culicivorax* seems to be of limited use in antimalaria campaigns in southern Iran, based on our present knowledge.

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