PRELIMINARY OBSERVATIONS ON THE INCIDENCE AND BIOLOGY OF A MERMITHID NEMATODE OF *AEDES SOLLICITANS* (WALKER) IN LOUISIANA[1]

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At least 16 species of North American mosquitoes, including *Aedes sollicitans* (Walker), have been reported parasitized by mermithid nematodes (Jenkins 1964). The first report of nematode parasitism in *Aedes sollicitans* came from New Jersey (Smith 1903), and the species was subsequently described as *Agamomermis culicis* by Stiles (1903) from two preadult worms collected by Smith. Smith later (1904) reported finding large numbers of *Agamomermis culicis* parasitizing adult females of *Aedes sollicitans*. He observed that ovarian development did not take place in the parasitized specimens and was certain the parasite was a material check to the multiplication of the mosquito. He made no attempt to locate the parasite in either the larval or pupal stage or to investigate any part of its life cycle. A review of the literature reveals no other published observations of nematode parasitism in *Aedes sollicitans*.

Welch (1960), in comparing the juvenile type of *Agamomermis culicis* with specimens collected by Stabler (1952) from adult *Aedes vexans* (Meigen), found them to be the same. He also concluded that a mermithid reported by Johnson (1903) in larvae of an *Anopheles* species from New Jersey was probably *Agamomermis culicis*. [*Agamomermis* is a collective genus (Stiles 1903) for species of mermithid nematodes based only on immature specimens.]

Parasitized *Aedes sollicitans* were found during the summers of 1965 and 1966 while we were investigating oviposition of *Aedes sollicitans* in southwestern Louisiana. The present study was begun when we observed that parasitized females failed to produce eggs and were usually killed when the nematode escaped.

INCIDENCE OF PARASITISM. Sixteen locations in southwestern Louisiana were surveyed for the incidence of *Agamomermis culicis* in adult female *Aedes sollicitans* from mid-August through December 1966. The results are summarized in Table 1. Seventeen percent of the 2,362 specimens collected were infected with 1–23 parasitic juveniles, and parasitism varied from 0 to 91 percent in the individual populations of biting *Aedes sollicitans*.

During the latter part of the survey, attempts were made to locate specific breeding areas that were producing the infections. Larvae of *Aedes sollicitans* (usually in the fourth instar) were therefore collected from various localities and reared to adults in the laboratory. Then these adults were examined for the presence of nematodes (Table 2). Six of the 19 samples were positive (1–31 parasites per adult with an average of 11.9 per male and 10.8 per female), with parasitism ranging from 4 to 88 percent. The number would undoubtedly have been higher had collections been made during mid-summer. The highest rate of infection (88 percent) of reared *Aedes sollicitans* was found in specimens collected in mid-October. The most heavily infected specimen (an adult female) contained 54 juvenile nematodes and was collected in the grass after emergence of the brood.

The distribution of *Aedes sollicitans* infected with nematodes is not known, but they are found across Louisiana to Mississippi. Also, the incidence of parasitism was lower in southeastern Louisiana since only 2 of 757 female *Aedes sollicitans* supplied by the Louisiana Mosquito Abatement Districts contained nematodes. No

[1] In cooperation with McNeese State College, Lake Charles, Louisiana.

TABLE 1.—Summary of a 6-month survey of adult female *Aedes sollicitans* for nematodes in southwestern Louisiana.

Month	Number of collections	Number females sampled	Number of females infected	Percent infection
August	12	684	237	34.7
September	23	773	117	15.1
October	19	440	67	15.2
November	14	287	63	21.9
December	9	178	18	10.1
Total	77	2362	402	17.0

parasitism was observed after the first prolonged cold weather (5 days below 35° F.) during mid-December.

LIFE CYCLE. The eggs of *Agamomermis culicis* are almost spherical and measure about 53 μ. They are transparent and free of the gelatinous coverings found on the eggs of some other mermithids (Welch, 1960; Phelps and DeFoliart, 1964). Eggs begin to develop almost immediately; the first cleavages occur within 24 to 48 hours, and the first movement of the embryos was observed between 9 and 14 days at ambient temperature after oviposition. The egg appears to have little or no resistance to desiccation.

In the laboratory, mature eggs of *Agamomermis culicis* hatch when they are flooded with water, some almost immediately and others apparently over several days (Fig. 1). Newly hatched preparasitic juveniles remain passive for a half-hour or more before they become active and free swimming. This free living stage is somewhat truncated anteriorly and tapers uniformly to the posterior end; the approximate length and width are 0.709 and 0.012 mm., respectively. No directional motion

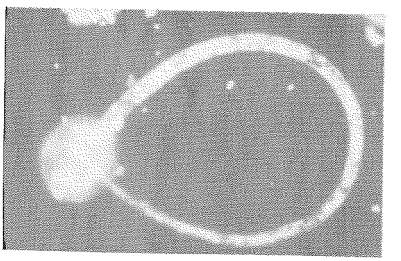

FIG. 1.—Hatching preparasitic juvenile of *Agamomermis culicis*.

is apparent in the undulating activities of the parasitic juvenile, even when it approaches first instar mosquito larvae. Contact seems to be made by accidental collision between the two organisms. On several occasions, these pre-parasitic juveniles were observed to penetrate the abdominal cuticle and enter the haemocoele of mosquito larvae, and sometimes such penetration and ingress required less than 2 minutes (Fig. 2). However, we are not certain this is the principal mode of entry because, when first instar mosquito larvae were exposed to the parasite for a short time, the majority of

TABLE 2.—Nematode parasitism in *Aedes sollicitans* collected as larvae and reared to adults in southwestern Louisiana.

Month of collection	Number of areas sampled	Number sampled		Number positive for nematodes		Percentage parasitism
		Male	Female	Male	Female	
October	10	118	116	34	30	27.3
November	4	57	58	0	1	0.9
December	5	60	64	0	0	0

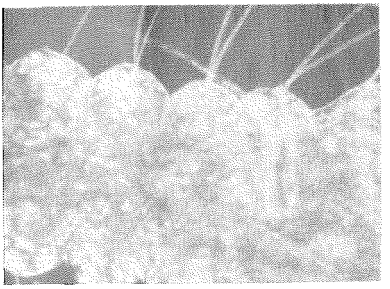

FIG. 2.—Preparasitic juvenile of *Agamomermis culicis* entering haemocoele of first instar *Aedes sollicitans*.

the parasites was found within the head capsule, perhaps because the mouth brushes of the mosquito tend to draw the parasites into the oral cavity. Ingesting infective eggs apparently does not cause parasitism since many mature eggs were ingested and passed through the body of the mosquito with no observed hatching.

The degree of parasitism in the laboratory is governed primarily by the concentration of nematodes and mosquito larvae. First instar larvae are apparently unable to tolerate more than 8 or 9 nematodes, but much larger numbers of nematodes per mosquito seem to occur if the rate of parasitism is lower and continues throughout larval development of the mosquito. After the pre-parasitic juvenile enters an early instar mosquito larva, it can be seen moving about, but unlike most mermithid species that parasitize North American mosquitoes, *Agamomermis culicis* grows little in the preadult stages of *Aedes sollicitans*. The approximate length and width of the sickle-shaped parasitic juveniles of *Agamomermis culicis* obtained from late fourth instar larvae were 1.18 and 0.09 mm., respectively.

The parasites occur throughout the haemocoele of the mosquito larva but are most often found in the head capsule or among the malpighian tubules. Growth of the parasite is rapid following emergence of the adult *Aedes sollicitans;* it may grow as long as 30 mm. in about 7–9 days. Parasitic juveniles in the adult mosquitoes have the characteristic shape of the adult nematode, and a caudal appendage is present in this stage that persists into the post parasitic juvenile stage. This appendage makes it easy to separate juveniles and adults after emergence of the parasites from the adult mosquito.

Nematodes are usually found among the malpighian tubules in newly emerged adult *Aedes sollicitans*. While we have not determined just when the nematodes migrate to the abdomen, we believe the movement takes place in the pupal stage. *Agamomermis culicis* remains in the abdomen of the adult mosquito unless levels of infection are high in which case some move into the thorax after 2 or 3 days. This movement of the nematode into the thorax is probably caused by competition for food and/or space. Also, a nematode was twice found completely filling the head capsule of heavily parasitized female *Aedes sollicitans*. Migration from the abdomen usually results in death of the host.

Maturation of the nematode to the postparasitic juvenile stage within previously engorged female mosquitoes requires 5 to 9 days at room temperature (23–26° C). The nematode usually escapes through the integumental membranes just anterior or posterior to the eighth abdominal segment of the mosquito (Fig. 3). Postparasitic juveniles wind themselves into tight masses when they are placed on moist soil and remain in this condition

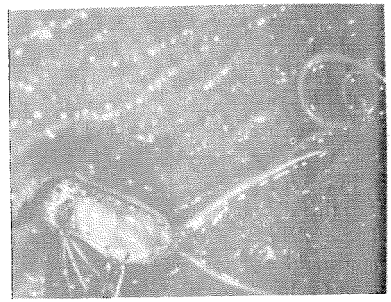

FIG. 3.—Escaping postparasitic juveniles of *Agamomermis culicis* from female *Aedes sollicitans*.

until they molt to the adult stage and copulate. Oviposition occurred from 4 to 21 days (average 10 days) after the nematodes escaped from the host, and many thousand eggs were laid in 2 to 4 days. Both sexes may remain alive for several days after the completion of egg laying. The nematodes apparently do not take in food after maturing to the postparasitic stage, and the adult nematode dies when the food reserves are depleted.

EFFECTS OF THE NEMATODE ON THE HOST. Penetration of the preparasitic nematode into the haemocoele appears to have little effect on the host larva though death results when heavy infections occur, especially in first instar larva. This mortality is probably the result of the movement of the numerous parasites within the body cavity of the host rather than from the wounds inflicted by the entering parasites. First instar *Aedes sollicitans,* when they are heavily parasitized (usually with 10 or more nematodes), die in a few hours. However, they can tolerate much heavier infections and develop normally if the penetrations occur over a long period and in a later instar. The parasite appears to cause no observable damage or reduction in activity to either the larval or pupal stage and can be detected only by dissection. Also, the fat body of the host is not depleted in the late fourth instar larva as is normal with nematodes that terminate their parasitic stages in the larval host.

Retarded emergence (compared with non-parasitized females) was not observed with parasitized *Aedes sollicitans.* When parasitism was not extreme (about 15), adult females exhibited normal activity and readily sought a blood meal. Females infected with as many as 23 nematodes have been collected in the field while they were attempting to take a blood meal.

In the laboratory, infected *Aedes sollicitans* that did not receive a blood meal died in 2–5 days, depending upon the degree of parasitism. Raisins were not a sufficient food source.

The effect of *Agamomermis culicis* upon male *Aedes sollicitans* is not known. Probably the male does not live long enough in nature for the nematode to complete the parasitic stage. We do not know if the male reproductive organs are injured by the nematodes. However, in more than 500 parasitized female *Aedes sollicitans* collected, none had ovarian development beyond Christophers' "Stage III" (Fig. 4). Similar observations were reported by Smith (1904).

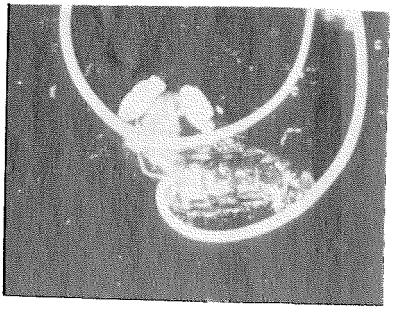

FIG. 4.—Dissected females of *Aedes sollicitans* showing the extent of ovarian development when parasitized by *Agamomermis culicis.*

To better understand the effects of *Agamomermis culicis* on oviposition, 50 engorged females were selected from a population known to be about 60 percent infected. These females were placed in individual shell vials containing moist cotton. Eighteen were eventually found to be free of nematodes, and 16 of the 18 developed normal egg batches. None of the 32 females with nematodes developed eggs, and 21 either died with or shortly after the escape of the nematodes. Of the 11 females surviving the escape, only 4 survived long enough to re-engorge, develop and deposit eggs. Three of the four re-fed twice and laid two egg batches before succumbing. The seven egg batches from these four females contained from 51 to 95 eggs and averaged 69. The 16 uninfected females averaged 148 eggs per oviposition, with a range of 102 to 220 eggs.

The rate of mortality of infected adult mosquitoes is probably high. Also, infected females that lose their nematodes and live are incapable of laying an egg

batch for about 5 days after the emergence of the nematode, which, in itself, greatly reduces the number of eggs laid by a generation of adults because many females will not live long enough to lay eggs. If the once-infected females survive these obstacles, they are apparently capable of laying only about half the normal complement of eggs.

The external characteristics and size of adult mosquitoes were not changed by parasitism. Infected adults could be detected visually only when parasitism was so high that the abdomen was distended.

ALTERNATE HOSTS. Although our observations indicate that *Agamomermis culicis* is not specific for *Aedes sollicitans* in the larval stage, the species is the only one found in which the nematode can reach maturity. More than 400 adult specimens of 10 species (5 genera) collected in the field, in addition to *Aedes sollicitans,* were checked for parasitism by *Agamomermis culicis* and none was positive. Also, on two occasions when reared *Aedes sollicitans* were heavily parasitized (76 percent and 88 percent), *Aedes taeniorhynchus* (Wiedemann), *Aedes vexans,* and *Psorophora confinnis* (Lynch-Arribalzaga) from the same areas were parasite-free.

In addition, preliminary attempts were made to infect mosquito species in the laboratory (Table 3). When first instar larvae were placed in depression slides containing newly hatched preparasitic *Agamomermis culicis,* all mosquito species tested except *Orthopodomyia signifera* exhibited some parasitism. However, given the small samples used, we can only conclude that little or no selection occurs at the onset of parasitism. In the laboratory, only infected larvae of *Aedes sollicitans* survived to the fourth instar.

Later, first instar larvae of *Aedes sollicitans, Aedes vexans, Aedes taeniorhynchus, Aedes sticticus, Aedes triseriatus, Psorophora ferox,* and *P. confinnis* were placed in a common container, exposed to preparasitic *Agamomermis culicis,* and reared to adults. The adults of all seven species were subsequently checked for parasitism. All were negative except *Aedes sollicitans*; this species had a 100 percent infection and averaged 10.1 parasites per individual. Thus, in nature, if early instar larvae of other species become infected, they apparently die and are not collected by the usual sampling techniques or develop host resistance.

EFFECT OF THE HOST ON THE NEMATODE. Welch (1960) reported finding occasional

TABLE 3.—Parasitism after exposure of first instar mosquito larvae to preparasitic juveniles of *Agamomermis culicis* for 16 hours.

Mosquito species	Number of larvae exposed	Percentage of larvae parasitized	Average number of nematodes per parasitized larva
Aedes			
sollicitans	8	100	7.0
sticticus (Meigen)	8	100	3.2
vexans	8	75	1.2
triseriatus (Say)	10	60	1.1
taeniorhynchus	9	44	2.5
Psorophora			
ferox (Humboldt)	10	100	6.3
confinnis	10	100	4.0
Culex			
salinarius Coquillett	10	10	1.0
Culiseta			
inornata (Williston)	10	90	2.3
Orthopodomyia			
signifera (Coquillett)	5*	0	...

* Four larvae were second instar.

first and second instar larvae of *Aedes communis* exhibiting a host resistance to *Hydromermis churchillensis* Welch by encapsulation of the parasite. Encapsulation was not observed in either the aquatic stages or adults of *Aedes sollicitans* parasitized by *Agamomermis culicis*.

CONCLUSIONS. During the last 6 months of 1966, large populations of *Aedes sollicitans* were found parasitized by the mermithid nematode *Agamomermis culicis*. Seventeen percent of the adult females sampled were infected, and parasitism ranged from 0 to 91 percent in individual populations. Parasitism was equal in the two sexes.

Eggs of *Agamomermis culicis* hatched at flooding, and preparasitic nematodes entered by penetrating the integument of the host larva. The nematode matures to the postparasitic stage only in the adult mosquito. At maturation, the postparasitic juvenile escapes, molts to the adult form, copulates, and lays eggs in 14–21 days at room temperature. Eggs mature in 9–14 days.

No lethal effects of the nematodes were observed in fourth instar and pupal stages of *Aedes sollicitans* though such damage is characteristic of many mermithid parasites of mosquitoes. *Agamomermis culicis*, when present, prevents egg development in *Aedes sollicitans* and usually kills the host at emergence. When female *Aedes sollicitans* do survive the emergence, they develop an egg batch, but the number of eggs is about half that of an uninfected female.

Parasitism of other species of mosquitos by *Agamomermis culicis* occurs in the laboratory but appears to be lethal at a very early larval stage.

Agamomermis culicis apparently can and does materially reduce populations of *Aedes sollicitans* and certainly is a biological control agent worthy of further study.

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