Prevalence of parasitism and adult survival time of *Aedes albifasciatus* (Diptera: Culicidae) parasitized by *Strelkovimermis spiculatus* (Nematoda: Mermithidae)

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ABSTRACT: We described the carryover of *Strelkovimermis spiculatus* (Poinar and Camino) (Nematoda: Mermithidae) from mosquito larvae, the primary site of maturation, to adults. We analyzed the survival time of male and female *Aedes albifasciatus* (Macquart) (Diptera: Culicidae) parasitized by *S. spiculatus*, the time of emergence of nematodes from adult mosquitoes, and the state of parasitism in the same mosquito cohorts during the immature stages. Mosquito larvae with single and multiple parasitism (up to 11 parasites) were observed. The mortality of mosquito larvae and adults was produced in all cases where at least one mermithid emerged. The mortality of *S. spiculatus* showed an increasing trend in mosquito larvae with larger numbers of nematodes and was higher in larvae parasitized by eight or more nematodes. Maximum survival of parasitized adult females of *Ae. albifasciatus* was 38 days, while non-parasitized adult males and females survived 39 and 41 days, respectively. *Strelkovimermis spiculatus* mortality was observed in *Ae. albifasciatus* larvae with single or multiple parasitisms. The spread of mermithid parasitism in adult mosquito populations is discussed. ***Journal of Vector Ecology* 40 (2): 393-397. 2015.**

*Keyword Index***:** Floodwater mosquito, Aedini, parasite, mortality, Argentina.

INTRODUCTION

Aedes albifasciatus (Macquart) is a neotropical mosquito that ranges in distribution from southern Brazil and Bolivia to Tierra del Fuego, Argentina (Bachmann and Bejarano 1960, Prosen et al. 1960, Foratini 1965). Its larvae occupy temporary puddles formed by rainwater, without aquatic vegetation, and the eggs withstand prolonged periods of desiccation and hatch when the puddles are flooded again. Larval development is completed in at least six days (Fontanarrosa et al. 2000, Campos and Sy 2003) and adult females can live up to about 50 days (Ludueña Almeida and Gorla 1995). *Aedes albifasciatus* cohorts emerge simultaneously and blood feed on humans and livestock, causing significant economic losses (Ludueña Almeida and Gorla 1995). This species was identified as a vector of western equine encephalitis in Argentina (Avilés et al. 1992).

Aedes albifasciatus populations are regulated by different natural enemies, the most common being *Strelkovimermis spiculatus* (Poinar and Camino) (Nematoda: Mermithidae), discovered in Argentina (Poinar and Camino 1986). This mermithid can cause over 80% mortality in cohorts of *Ae. albifasciatus* larvae (Maciá et al. 1995, Micieli and García 1999). Natural infections of *Ae. albifasciatus* by *S. spiculatus* in the temperate region of Argentina occur with varying prevalence and intensity throughout the year, with epizootics during all seasons except summer (Maciá et al. 1995, Micieli and García 1999, Campos and Sy 2003, Micieli et al. 2012). The dynamics of epizootics and the factors involved are little known and the results obtained to date are sometimes controversial.

Mermithid penetration always occurs in the larval stage of the mosquito, but mermithid maturation can occur in either the larval or adult stage depending on the nematode species (Platzer 1981). For example, *Aedes sollicitans* (Walker) populations have been recorded with a high prevalence of parasitism in adults, caused by *Perutilimermis culicis* (Stiles) (= *Agamomermis culicis*), whose maturation is completed in the adult mosquito (Petersen et al. 1967). In contrast, few records are known of adult mosquitoes parasitized by mermithids whose maturation occurs primarily in the larva. As an example, we can cite the infrequent infections in adult *Aedes sierrensis* (Ludlow) by the mermithid *Octomyomermis troglodytis* Poinar and Sanders (Washburn et al. 1986), or the occasional finding of adult *Culex dolosus* Lynch Arribálzaga parasitized by *S. spiculatus* (García et al. 1994).

In South America, the first record of *S. spiculatus* parasitizing adult mosquitoes in nature was reported by García et al. (1994), who isolated a nematode from a female *Culex dolosus* collected by a CDC trap. Subsequently, Campos and Sy (2003), in a study on the mortality of immature stages in cohorts of *Ae. albifasciatus* parasitized by *S. spiculatus,* found parasitized male and female pupae with single and multiple mermithid parasitism.

For the remaining seven species of mosquitoes that are parasitized by *S. spiculatus* in nature, *Culex chidesteri* Dyar, *Culex maxi* Dyar, *Culex mollis* Dyar & Knab, *Cx. pipiens*, *Aedes crinifer* (Theobald), *Psorophora ciliata* (F), and *Psorophora cyanescens* (Coquillett) (Achinelly and Micieli 2013), parasitism has not been reported in the adult mosquito.

One of the most important aspects of mermithid parasitism in adult mosquitoes is the potential for the mosquito to disperse the mermithid to other water bodies, potentially facilitating colonization of new environments. Essential for this achievement are the mosquito flight and survival capacity when parasitized, and the emergence time delay of the mermithids. It is known that the females of *Ae. sollicitans* parasitized by *P. culicis* exhibit normal activity and seek blood meals. Females that survive the release of the nematodes can take up to two blood meals and lay eggs (Petersen et al. 1967). Conversely, it appears that female *Aedes stimulans* (Walker) parasitized by mermithids die shortly after emergence of the parasite (Gaugler et al. 1984). These aspects of mermithid biology are unknown for the parasitism of *S. spiculatus* in *Ae. albifasciatus*.

When searching for larvae of *Ae. albifasciatus* parasitized by *S. spiculatus*, in order to establish a nematode colony in the laboratory, eight pools containing *Ae. albifasciatus* larvae were sampled and an infrequent event of parasitism in the adult mosquitoes was observed in one of them. Here we report the estimation of the survival time of *Ae. albifasciatus* males and females parasitized by *S. spiculatus,* the state of parasitism in the cohort of the immature stages from which the studied adult mosquitoes were obtained, and *S. spiculatus* mortality in *Ae. albifasciatus* larvae.

MATERIALS AND METHODS

The sampling site where parasitism by *S. spiculatus* occurred was in Ensenada (34° 49' 20.6" S, 57° 58' 6" W), Buenos Aires province, Argentina. It is a shallow puddle of rainwater, formed in a ground depression covered by grass and exposed to direct sunlight. Sampling took place on 3 June 2014 at 10:00 h when larvae were in stage IV, using a 350 ml dipper to collect the water from the pond, and a fine 1 mm mesh sieve to filter the water and retain the larvae. The sample was transferred to the laboratory in a 10 liter pail containing water from the puddle. Two subsamples of *Ae. albifasciatus* larvae were separated from the original samples. The first sample assessed the prevalence of *S. spiculatus* in immature stages, and the second to estimate the prevalence of parasitism in adult mosquitoes.

A subsample of 240 randomly selected mosquito larvae were reared individually in 1.5 ml cultivation cells with dechlorinated water. Individuals were maintained under these conditions until death or until their pupal stage. For each mosquito larva, the numbers of mermithids that emerged or died within each mosquito were quantified. The remaining larvae were raised in 52 x 36 x 7 cm plastic trays containing water from the larval habitat. All predators were removed and no food was added. Emerged mermithids were removed daily. All mosquitoes reaching the pupa stage (n=785) were sexed and transferred to plastic trays 15 x 11 x 5 cm, which were placed in 27 x 23 x 27 cm cages. After adult mosquito emergence, a sub-sample of 68 males and 111 females was isolated to estimate the survival time of parasitized and non-parasitized adults. Each mosquito was placed individually in a 29 x 59 mm plastic vial covered with a tulle cap. A cloth was placed at the base of the vial, covered with wet absorbent paper to maintain moisture. Adult mosquitoes were kept at room temperature and fed a 10% sugar solution through embedded cotton, renewed every other day. The date of mosquito death and the emergence time of the mermithid were recorded. Each adult mosquito was dissected with entomological forceps under a stereoscopic microscope (magnification: 6.7x) to quantify the presence of remained mermithids. The remaining 606 adult mosquitoes, 279 females and 327 males, were frozen and then dissected to estimate parasitism caused by *S. spiculatus*.

Data analysis

The prevalence of parasitism between immature and adult mosquitoes and between males and females, and the prevalence of dead mermithids between larvae with single parasitism and larvae with multiple parasitism were compared with the Fisher Exact test. The number of mermithids per mosquito was compared among larvae, adult females, and adult males with the Kruskal-Wallis test and post-hoc comparisons of mean ranks. The total number of nematodes (N_{ti}) and the sum of dead nematodes (N_{di}) from larvae with parasites (number of parasites i = 1-11) was calculated, and from these data the proportion of dead nematodes was calculated in each i parasitism category (P_{di}). N_{ti} and N_{di} corresponded to categories of nine or more nematodes grouped to ensure the representation of data from more than one mosquito in each category. The increasing linear trend in the proportions obtained was evaluated with a chi-square test for independent proportions quantitatively ordered (Fleiss et al. 2003).

Survival curves of adult *Ae. albifasciatus* were constructed from 178 individuals obtained from 4th instar larvae collected in the field. After emergence of the imago, the number of adult mosquitoes who died each day was recorded. The survival curve was constructed with the proportion of survivors at age x (l_x), where l_x was calculated as N_x / N_0; being N_x the total number of individuals at x age, and N_0, the initial number of individuals (Rabinovich 1978). The survival time of adult mosquitoes was compared between sexes in non-parasitized individuals, and between parasitized and non-parasitized females with the Mann-Whitney *U*-test.

RESULTS

Parasites in larvae and adults of *Aedes albifasciatus*

The only mosquito species collected in the puddle where parasitism by *S. spiculatus* occurred was *Ae. albifasciatus*. Of the 240 mosquito larvae examined, a total of 223 (93%) were parasitized by *S. spiculatus*, 690 mermithids were obtained, 623 individuals emerged from larvae, and 67 died within the larvae. Approximately 24 h after the sample of larvae was extracted from the puddle, pupation started and in the following days 785 adult mosquitoes were obtained, among which 105 individuals (13.4%) were infected. The prevalence of parasitism in adult mosquitoes was significantly lower than in immature stages (Fisher Exact test, p<0.001), while no significant difference was detected between females (15.1%,

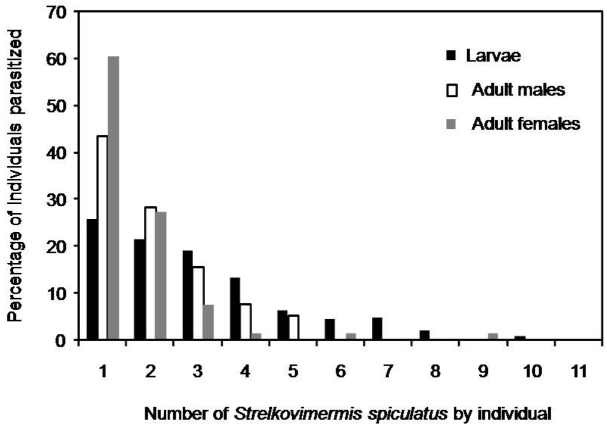

Figure 1. Percentage of *Aedes albifasciatus* larvae and adults (males and females) parasitized with different numbers of *Strelkovimermis spiculatus*.

n = 66/438) and males (11.2%, n = 39/347) (Fisher Exact test, p=0.13).

Single and multiple parasitism was observed in larvae as well as in male and female adults (Figure 1). Twenty-six percent of the larvae were parasitized by one mermithid, while in adults single parasitism was 44% and 61% in males and females, respectively (Figure 1). In all cases when at least one mermithid emerged, this caused the death of the mosquito larvae or adult. Of the 17 non-parasitized mosquito larvae, two reached the adult stage and the remaining 15 died as stage IV larva. The number of mermithids per mosquito showed significant differences among the analyzed groups (H=41.37; df=2; n=328; p<0.001). Post-hoc tests showed a higher number of mermithids per mosquito in larvae than in male adults (p < 0.01) or in female adults (p < 0.001), while no significant differences were detected between male and female adults (p = 0.34).

For *Ae. albifasciatus* males, 12.8% of *S. spiculatus* stayed in the thorax (Figure 2), 76.9% in the abdomen, and 10.3% occupied simultaneously both tagma. In females, 7.6% of the nematodes were located in the thorax, 87.6% in the abdomen, and 4.8% in both simultaneously. The maximum number of mermithids observed in the abdomen of males was five, and nine in females, whereas in the thorax up to four mermithids were observed in mosquitoes of both sexes. In the male individuals with mermithids in both tagmas, one mermithid was observed in the thorax and one to three in the abdomen. In the only female observed, two mermithids were found in the thorax and one in the abdomen.

Survival of *Ae. albifasciatus* adults

The range of survival time of adult males and females of *Ae. albifasciatus* non-parasitized was 4-39 days and 1-41 days, respectively. The mean (±SD, n) was 12.22 (± 6.42, 67) and 18.60 (±9.14, 99) days, this difference being significant (*U*=4217, n=67, 99; p<0.001). On the other hand, the range in females parasitized by *S. spiculatus* was 4 to 38 days with a mean equal to 16.83 (±10.13, 12) days (Figure 3). *Strelkovimermis spiculatus* parasitism in adult females of *Ae. albifasciatus* did not significantly affect their survival

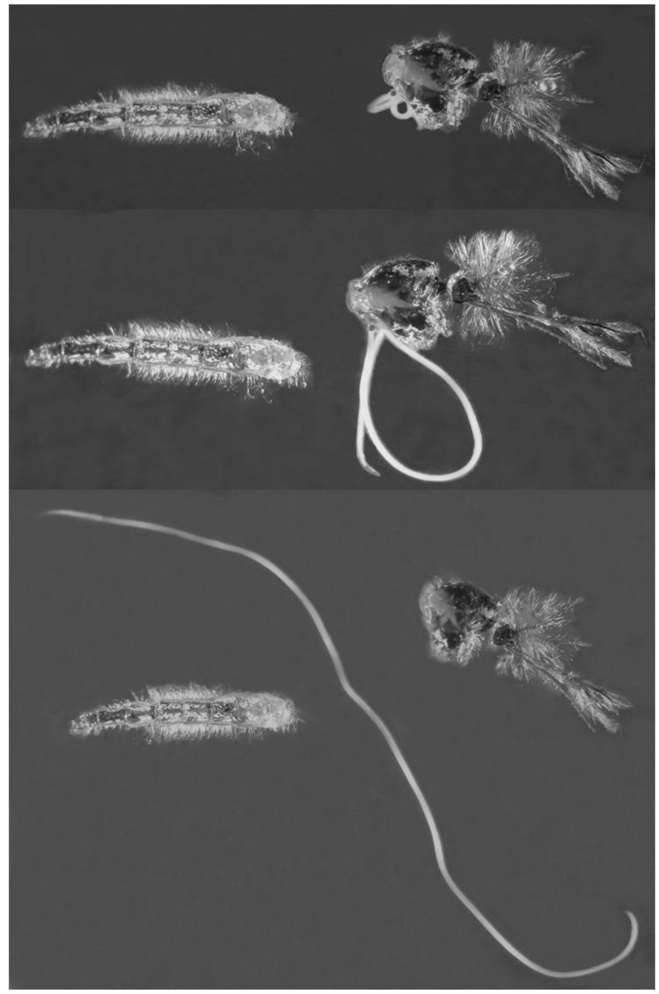

Figure 2. Male of *Aedes albifasciatus* with one *Strelkovimermis spiculatus* located in the thorax.

(parasitized females vs non-parasitized: *U*=613, n=12, 99, *p*=0.58). The only parasitized male survived eight days. Because many of the emerged post parasites died before molt, we could not establish any mermithid sex relationship.

Strelkovimermis spiculatus mortality in _Ae. albifasciatus_ larvae

Strelkovimermis spiculatus mortality was observed in larvae of *Ae. albifasciatus* when single or multiple parasitisms occurred. Most of the dead mermithids came from mosquito larvae with multiple parasitism, except for three cases that corresponded to larvae with single parasites. In 35 larvae with multiple parasitism, after the emergence of at least one parasite, it was found that between one and six individuals were dead inside the body of the mosquito larvae. The prevalence of dead mermithids was significantly lower in larvae with single parasites (5.2%, n = 58) than in larvae with multiple parasitism (17.9%, n = 196) (Fisher Exact test, p<0.02).

The mortality of *S. spiculatus* showed an increasing trend in larvae with greater numbers of mermithids (χ^2=6.29; df = 1; *p*<0.02), with a linear relationship (difference from linearity χ^2 = 2.08; df = 7, p = 0.95). The relationship can be described as $P_{di} = 0.018 + 0.018 * i$. The mortality rate for single parasitism

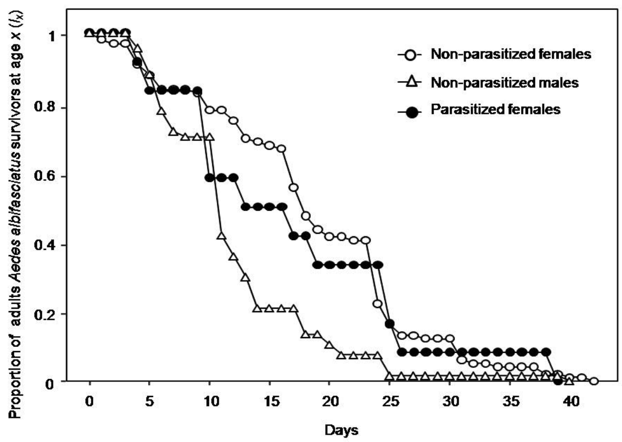

Figure 3. Survival curves of adult *Aedes albifasciatus* non-parasitized and parasitized by *Strelkovimermis spiculatus*.

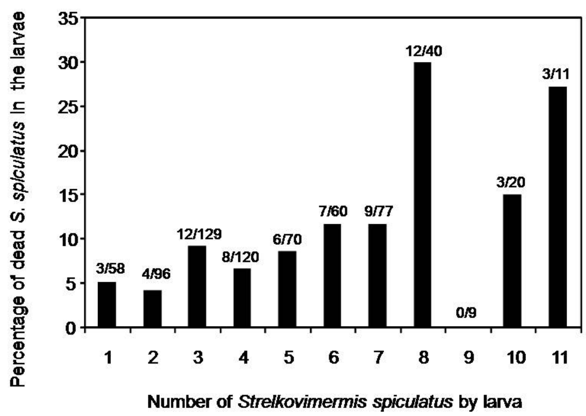

Figure 4. Percentage of dead *Strelkovimermis spiculatus* in *Aedes albifasciatus* larvae parasitized with different numbers of individuals. Number on bars indicates: Number of dead *S. spiculatus* / Total number of *S. spiculatus*.

was 5.2%, while in the multiple parasitism it varied between 4.1 and 30%, increasing considerably in larvae parasitized by eight or more nematodes (Figure 4).

DISCUSSION

Micieli and García (1999) and Micieli et al. (2012) showed that multiple parasitism by *S. spiculatus* in *Ae. albifasciatus* larvae was common in nature, but the incidence of more than three mermithids in a single larva was sporadic. Micieli and García (1999) studied six cohorts of *Ae. albifasciatus* in natural conditions, and found that in one of them the mean was 5.5 nematodes per larvae, finding an exceptional case of a larva parasitized by 19 individuals of *S. spiculatus*. In a cohort with lower intensity of parasitism (mean = 1.8 parasites per larva), the extreme number of parasites per larva was seven. Another report of high intensity parasitism caused by *S. spiculatus* under natural conditions was for floodwater mosquito larvae *Ps. cyanescens*, where up to eight nematodes parasitizing larvae (Achinelly and Micieli 2013) were observed. In this study, multiple parasitism occurred in 74% of the parasitized larvae of *Ae. albifasciatus*, with a range of 2-11 parasites per larva. A trend for higher mortality of *S. spiculatus* was observed with increased numbers of mermithids per larva. Similar observations on mortality were conducted by Camino (1988) for a laboratory experiment with larvae of *Cx. pipiens*, where a high mortality of *S. spiculatus* was detected when the number of individuals per larva was increased to ten. Multiple parasitism seems to favor the proliferation of *S. spiculatus* when the number of mermithids per mosquito larvae is low, which may regulate wild populations of mermithids in a density dependent manner.

In the present study it was observed that *S. spiculatus* parasitism in larvae does not always result in the death of the mosquito and under certain circumstances parasitized individuals can develop to the adult stage. However, the lower number of mermithids in adults than in larvae suggests that a differential mortality occurs in larvae with different numbers of parasites, thus enhancing the survivorship to adulthood of mosquitoes with lower parasitism levels. It is not clear whether the presence of mermithids in adults responds to a delay in the development time of mermithids to favor dispersal, or it is a consequence of the infection of later instar larvae. The latter hypothesis may be supported by the findings of Camino and Reboredo (1994), who demonstrated in a laboratory trial that the presence of mermithids in *Culex pipiens* adults was infrequent and occurred only when the infection was initiated in stage four larvae. In a previous study, Campos and Sy (2003) found an adult male *Ae. albifasciatus* parasitized by seven *S. spiculatus*. These findings suggest that *Ae. albifasciatus* can survive to the adult stage even with a high infection. A surprising result of this study was a prolonged survival of infested adult mosquitoes. A female with a single parasite survived 38 days, another one containing three parasites survived 19 days, while the only male obtained survived eight days. Until now, it was known that adult mosquitoes parasitized by *S. spiculatus* could not survive for more than four days (Camino and Reboredo 1994), but our results show that the mean survival time is 16 days, similar to that of healthy individuals.

Campos and Sy (2003) found no significant differences in weight and size of parasitized and non-parasitized adults of *Ae. albifasciatus*, suggesting that there would be no impediment for parasitized mosquitoes to move. While the flying ability of parasitized individuals was not directly assessed in this study, it was observed that both females and males of *Ae. albifasciatus* flew without evidencing differences from healthy individuals. This fact, coupled with the survival time and the ability to support more than one nematode in the body of the adult mosquito, could constitute the factors that allow *Ae. albifasciatus* to disperse *S. spiculatus* to other pools. An unknown issue is whether the infected females of *Ae. albifasciatus* can effectively blood feed and subsequently oviposit, as was observed in *Ae. sollicitans* parasitized by *P. culicis* (Petersen et al. 1967). These behaviors favor the dispersion because the female mosquitoes must travel to find a blood source, and oviposition site. Otherwise, as with *Ae. stimulans*, parasitized females whose ovaries do not develop fail to oviposit and die soon after the mermithid emergence (Gaugler et al. 1984), the possibility of moving to other breeding sites being limited.

In short, parasitism in adult *Ae. albifasciatus* could contribute to *S. spiculatus* in the following ways: If the mosquito does not move from the pond of origin, nematode emergence help increase the local population. In the event of displacement of the parasitized mosquitoes, they could contribute to the spreading of *S. spiculatus* and to the colonization of puddles where they were not present, or they could facilitate genetic drift to puddles where a nematode population exists. Further studies are necessary in order to interpret with higher precision the role of *Ae. albifasciatus* in the dispersion of *S. spiculatus.*

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