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SEASONALITY, PREVALENCE AND PATHOGENICITY OF THE GREGARINE *ASCOGREGARINA TAIWANENSIS* (APICOMPLEXA: LECUDINIDAE) IN MOSQUITOES FROM FLORIDA

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ABSTRACT. *Aedes albopictus* larvae collected in Gainesville, FL, were infected with the gregarine *Ascogregarina taiwanensis*. Natural prevalence varied from 68 to 100%. Eight mosquito species were tested in the laboratory for susceptibility to *A. taiwanensis* isolated from field-collected *Ae. albopictus*. *Aedes aegypti, Aedes albopictus,* and *Aedes taeniorhynchus* became 100% infected in the larval stage, whereas *Aedes triseriatus* was less susceptible; *Culex quinquefasciatus, Culex nigripalpus, Culex territans,* and *Anopheles quadrimaculatus* were not susceptible. Viable *A. taiwanensis* oocysts from adults were recovered from *Ae. taeniorhynchus* (30%) and *Ae. albopictus* (100%); no oocysts were produced in the other exposed hosts. Mortality induced by *A. taiwanensis* infection was low in all mosquitoes except *Ae. taeniorhynchus*. We conclude that *A. taiwanensis* has little short-term impact on the mortality of the 3 most common container-inhabiting mosquito species in Florida; however, the long-term impact on overall host population regulation has yet to be determined.

INTRODUCTION

Ascogregarina taiwanensis (Lien and Levine) was formally described from *Aedes albopictus* (Skuse) collected in Taiwan (Lien and Levine 1980). This gregarine parasite, according to Munstermann and Wesson (1990), had been reported from *Ae. albopictus* in India (Ray 1933), China (Feng, 1933), and Malaysia (Else and Dangsupa 1974) as *Ascogregarina culicis* (Ross) whose type host is *Aedes aegypti* (Linn.). *Ascogregarina taiwanensis* was reported from infected *Ae. albopictus* in Illinois, Missouri, and Florida (Munstermann and Wesson 1990).

This study presents information on the seasonality and natural prevalence of *A. taiwanensis* in a field population of *Ae. albopictus* from Florida. Additional information is presented on the host specificity and pathogenicity of this gregarine for both its natural and other mosquito hosts.

MATERIALS AND METHODS

Sampling area: An experimental tire site was established on the grounds of the Medical and Veterinary Entomology Research Laboratory (MAVERL), Gainesville, FL. Ten 4th-instar *Ae. albopictus* were taken weekly from shaded tires and sun-exposed tires from October 1992 to September 1993. Each larva was dissected to determine the presence and abundance of gamonts. Larvae of other mosquito species inhabiting the tires were also examined for gregarine infections.

Source of mosquitoes and gregarine oocysts for experimental infection: Oocysts of *A. taiwanensis* were obtained from naturally infected *Ae. albopictus* larvae reared to the adult stage. Both sexes of adults were macerated in well water with a tissue homogenizer and filtered to eliminate most of the host tissue; oocysts were counted using a hemocytometer. For routine maintenance, groups of 100 1st instars of *Ae. albopictus* were exposed to 1×10^3 oocysts/ml. Adults that emerged from these exposures were homogenized with a blender. The homogenate was filtered and stored in water at 4°C as a source of oocysts for cross-infectivity and pathogenicity tests.

Larvae of *Ae. aegypti, Ae. albopictus, Aedes taeniorhynchus* (Wied.), *Aedes triseriatus* (Say), *Anopheles quadrimaculatus* Say, and *Culex quinquefasciatus* Say were obtained from the colonies maintained at MAVERL. Larvae of *Culex nigripalpus* Theobald and *Culex territans* Walker were obtained from field collections as egg rafts or 1st-instar larvae; their taxonomic identification was confirmed as 4th-instar larvae.

Pathogenicity and susceptibility tests: Pathogenicity and susceptibility tests with *A. taiwanensis* were conducted on the 8 culicid species mentioned above. A 1-ml aliquot of the stock

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Table 1. Seasonal infection rates and number of gamonts in a natural population of *Aedes albopictus* larvae infected with *Ascogregarina taiwanensis*.

Month	Sun		Shade	
	No. gamonts[1]	% prevalence[2]	No. gamonts[1]	% prevalence[2]
October 1992	9.4 ± 13.1	80	17.6 ± 14.9	95
November 1992	15.3 ± 13.4	85	14.7 ± 17.0	95
December 1992	20.2 ± 26.5	80	21.3 ± 22.1	95
January 1993	32.5 ± 41.0	95	64.7 ± 80.1	100
February 1993	27.1 ± 27.5	95	29.2 ± 25.9	85
March 1993	23.3 ± 31.4	85	16.7 ± 25.8	85
April 1993	14.3 ± 20.5	68	17.4 ± 30.3	80
May 1993	15.5 ± 13.9	90	19.9 ± 16.3	100
June 1993	13.3 ± 13.6	90	14.5 ± 11.0	100
July 1993	9.7 ± 9.9	88	17.2 ± 16.3	100
August 1993	13.0 ± 9.7	100	16.4 ± 16.8	95
September 1993	16.2 ± 21.2	85	15.1 ± 14.1	85

[1] Mean ± SD.
[2] n = 10 4th instars per week from shaded tires and 10 from sun-exposed tires.

solution (1×10^4 oocysts/ml) of *A. taiwanensis* was added to 9 ml of well water for a final test concentration of 1×10^3 oocysts/ml. Twenty-five 1st instars of each species were exposed for 24 h and then transferred to a plastic cup with 100 ml of water. Exposed and control larvae were held at room temperature (26–27°C) and fed as needed. Mortality was recorded daily through adult emergence. A portion (usually 25%) of the exposed 4th instars, pupae (25%), and adults (100%) were dissected to detect gregarine infection and count gamonts. Some *Ae. taeniorhynchus* pupae and adults were dissected after death. All the other species dissected were living specimens. The gamonts present in 4th instars were measured and quantified for each infected host. Tests were repeated 4 times for the *Aedes* and *Anopheles* species and twice for the 2 *Culex* species. Each test consisted of 3 replicates (4 replicates for *Ae. taeniorhynchus*). Controls for each species were maintained under the same conditions without the addition of oocysts.

RESULTS AND DISCUSSION

Gregarine identification: We determined that the gregarine isolated from *Ae. albopictus* is a strain of *A. taiwanensis* based primarily on the results of susceptibility experiments reported below. In addition, morphological characteristics of this Florida strain resembled the original description of *A. taiwanensis* from Taiwan (Lien and Levine 1980) and the strain from *Ae. albopictus* found in Illinois (Munstermann and Wesson 1990). Oocyst dimensions were similar between the original description (10×5 μm) and the Florida strain ($\bar{X} = 9.3 \times 4.6$ μm; 8.3–9.9 × 4.3–4.9 μm). However, the gamonts of *A. taiwanensis* from the original description (Lien and Levine 1980) were larger ($\bar{X}$ = 234 [193–274] × 32 μm) than the Florida strain (53.3 [45-69] × 26.5 [23-30] μm) (Fig. 1).

Monthly prevalence of Ascogregarina *in natural populations of* Aedes albopictus: The natural prevalence of *A. taiwanensis* infections in *Ae. albopictus* varied from 68 to 100% during the collection period (Table 1). The number of gamonts in infected 4th instars from sun-exposed tires ranged from 1 to 153 ($\bar{X}$ = 18.8) and from 1 to 298 ($\bar{X}$ = 23.4) for infected larvae from shaded tires. Higher numbers of gamonts per larva occurred during the winter months of December to March (Table 1). The lower number of gamonts in larvae during the summer might be explained by lower infection rates due to reduced viability of oocysts exposed to high summer temperatures (June–August) which averaged 22.4°C (range 20.9–24.2°C) in the shade and 31.2°C (range 24.5–34.4°C) in the sun. McGray et al. (1970) demonstrated a reduction in the viability of *A. culicis* oocysts held at high temperatures in the laboratory.

Experimental cross-infection: Trophozoites and gamonts were present in all *Ae. albopictus* larvae exposed to oocysts of *A. taiwanensis* (Table 2). Gamonts fused to form gametocysts during pupation and were present in both pupae and adults. Sporulation of these gametocysts resulted in the production of oocysts in all *Ae. albopictus* adults.

All *Ae. aegypti* larvae exposed to *A. taiwanensis* oocysts harbored gamonts in their guts but

Table 2. Development of *Ascogregarina taiwanensis* in different mosquito species.

Host	Percent larvae[1] with		Percent pupae[1] with		Percent adults[1] with	
	Trophozoites	Gamonts	Gamonts	Gametocysts	Gametocysts	Oocysts
Aedes aegypti	100	100	0	0	0	0
Aedes albopictus	100	100	100	100	100	100
Aedes taeniorhynchus	100	100	100	65	95	30
Aedes triseriatus	15	12	0	0	0	0
Anopheles quadrimaculatus	18	0	0	0	0	0
Culex nigripalpus	0	0	0	0	0	0
Culex territans	0	0	0	0	0	0
Culex quinquefasciatus	0	0	0	0	0	0

[1] $n = 25$.

few were found in 24-h-old pupae; none were present in older pupae or adults (Table 2). Gamonts, however, were often observed within a bolus in the posterior midgut of dissected pupae. To assure that stages had not been overlooked, *Ae. aegypti* adults were individually examined ($n = 350$) or ground ($n = 675$) and examined for the presence of oocysts. Oocysts were not detected in any of these samples. In addition, a suspension of the ground adults was fed to 1st instars of *Ae. aegypti* and *Ae. albopictus* to verify the absence of oocysts. None of these exposed larvae were infected.

In used tires located in Levy County, FL, both *Ae. aegypti* and *Ae. albopictus* larvae were infected with *A. taiwanensis*. Examination of the gregarine from 4th instars of these mosquito species revealed no differences in the number, size, or shape of the gamonts. But oocysts were produced only in adults of *Ae. albopictus* and not in the adults of *Ae. aegypti*. This confirmed our laboratory observations that *A. taiwanensis* does not complete its life cycle in *Ae. aegypti*.

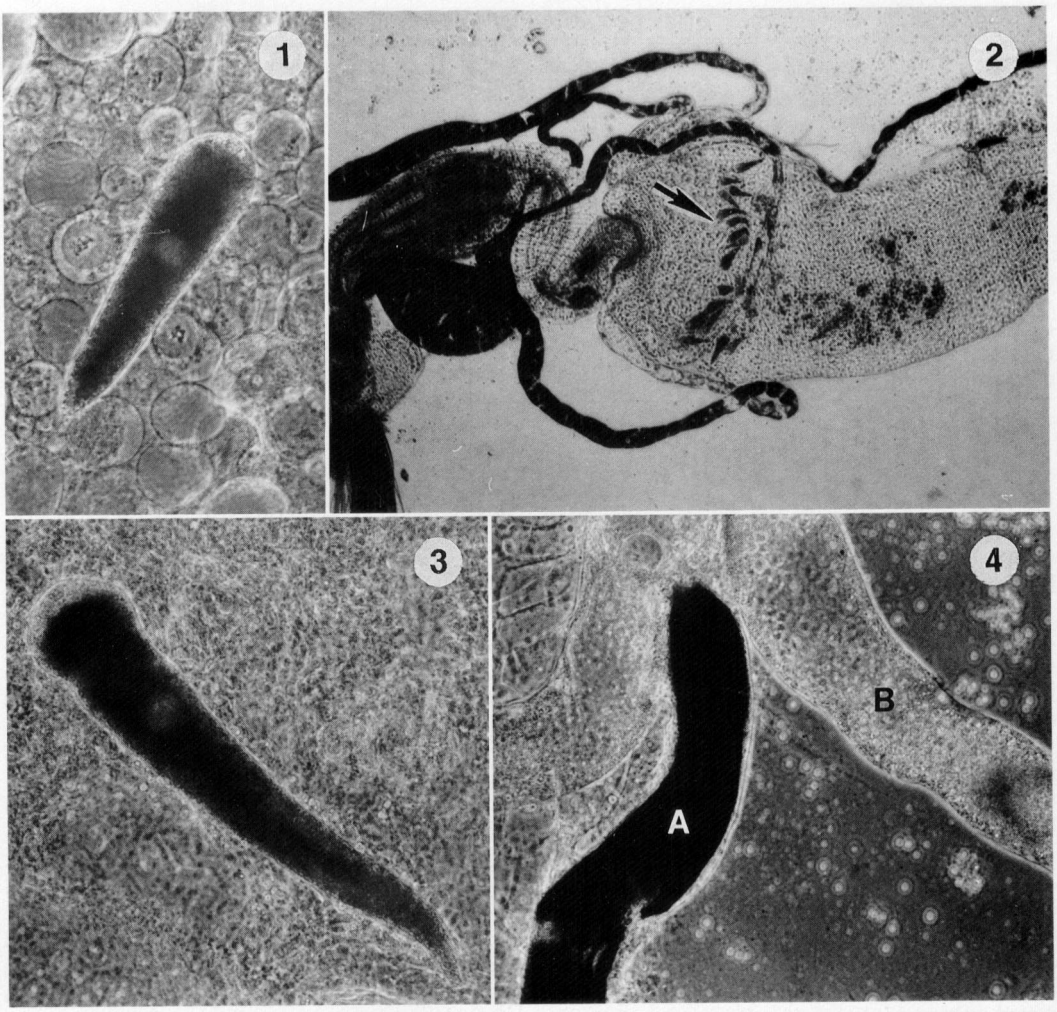
Lien and Levine (1980) reported a 100% infection rate in *Ae. albopictus* larvae exposed to *A. taiwanensis* from Taiwan and a 48% infection rate in *Ae. aegypti* larvae. Oocysts were not produced in *Ae. aegypti* adults. Munstermann and Wesson (1990) reported large numbers of trophozoites and 100% larval infection in 3 strains of *Ae. albopictus* exposed to *A. taiwanensis*. They also exposed 2 strains of *Ae. aegypti* to *A. taiwanensis* and obtained relatively low infection rates in larvae (56.3 and 12%) and observed an abnormal pattern of infection including oversized trophozoites in larvae and frequently melanized gamonts in the Malpighian tubes of pupae. In addition, they reported the production of a few viable *A. taiwanensis* oocysts from *Ae. aegypti* capable of infecting larvae of *Ae. albopictus*. This differs from the findings in our report and those of Lien and Levine (1980).

All larvae of *Ae. taeniorhynchus* were infected when exposed to oocysts of *A. taiwanensis* (Table 2). Gamonts and gametocysts were observed in pupae, and mature oocysts were observed in the Malpighian tubules of male and female adults. There were some notable differences between infections in *Ae. taeniorhynchus* and *Ae. albopictus*. In infected *Ae. taeniorhynchus*, gamonts in larvae were larger in size and number (Table 3). Gregarine stages in pupae were often melanized and

Table 3. Number and size of gamonts of *Ascogregarina taiwanensis* in mosquito larvae exposed to 1×10^3 oocysts/ml.

Host	No. gamonts/larvae[1] (mean ± SD)	Gamont size (μm)[2] (mean ± SD)
Aedes aegypti	27.9 ± 15.2	66.4 ± 9.3 × 31.0 ± 3.0
Aedes albopictus	22.2 ± 16.9	53.3 ± 7.1 × 26.5 ± 1.9
Aedes albopictus[3]	—	234 × 32 (193–274 × 32)
Aedes taeniorhynchus	79.3 ± 62.0	131.6 ± 16.4 × 32.9 ± 6.6
Aedes triseriatus	0.6 ± 1.2	57.5 ± 8.2 × 28.1 ± 2.3

[1] $n = 25$ larvae.
[2] $n = 25$ gamonts.
[3] Ref. Lien and Levine (1980).



Figs. 1–4. Gamonts of *Ascogregarina taiwanensis*. 1. Gamont in *Aedes albopictus* larva, ×300; 2. Group of gamonts (arrow) in the posterior midgut of *Aedes taeniorhynchus* larva, ×30; 3. Gamont in *Aedes taeniorhynchus* larva, ×300; 4. Malpighian tubules (×200) of infected *Aedes taeniorhynchus* adult. A. Melanized tubule; B. Normal tubule.

resulted in death. Adults that died during emergence were also found to have melanized gamonts, gametocysts, and oocysts. Some infected adults developed oocysts in their Malpighian tubules. These oocysts produced infections in larvae of *Ae. aegypti, Ae. albopictus,* and *Ae. taeniorhynchus*. Gamonts in mature, 4th instars of *Ae. taeniorhynchus* were located uniquely in a row across the posterior midgut; individual gamonts were usually oriented parallel to the longitudinal axis of the gut (Fig. 2).

The number and size of gamonts of *A. taiwanensis* were similar in larvae of *Ae. albopictus* and *Ae. aegypti* at a dose of 1×10^3 oocysts/ml (Table 3). In *Ae. taeniorhynchus* however, gamonts were 3 times more numerous (Table 3) and 2.5 times larger (Fig. 3) than those in *Ae. albopictus* and *Ae. aegypti* when exposed at the same dose.

Melanization of *A. taiwanensis* did not occur in larvae, pupae, or adults of infected *Ae. albopictus* or *Ae. aegypti*. Likewise, melanization did not occur in larvae of *Ae. taeniorhynchus* but intense melanization of gamonts and gametocysts due to host reaction was observed in the guts and Malpighian tubules of infected pupae and adults (Fig. 4). Most infected pupae died. Oocysts were present in a few dead pupae and in all adults of *Ae. taeniorhynchus* that died during eclosion. Examination of surviving adults from infected larvae revealed that 90% had less than 5 melanized gamonts and only one or 2

Table 4. Mortality in 4 mosquito species infected as first instars to 1 × 10^3 oocyst/ml of *Ascogregarina taiwanensis*.

Species	No. tests	% mortality Control	% mortality Treated[1]
Aedes aegypti	4[2]	1.3	9.1
Aedes albopictus	4[2]	2.0	9.5
Aedes taeniorhynchus	4[3]	19.8	76.1
Aedes triseriatus	4[2]	3.3	1.7

[1] Corrected by Abbott's formula.
[2] Each test consisted of 75 larvae exposed and 75 control larvae.
[3] Each test consisted of 100 larvae exposed and 100 control larvae.

gametocysts within their Malpighian tubules; 30% produced infective oocysts and 10% had no gregarine stages (Table 2). *Aedes taeniorhynchus* is the 5th host in which viable oocysts of *A. taiwanensis* can be produced. Viable oocyst production has been reported from *Aedes alcasidi* Huang (Lien and Levine 1980), *Ae. aegypti, Aedes atropalpus* (Coq.), and *Aedes epactius* Dyar and Knab (Munstermann and Wesson 1990).

Aedes triseriatus larvae were only slightly susceptible to *A. taiwanensis,* with infections observed in 15% of the 2nd and 3rd instars. Only 11.7% of the 4th instars had gamonts (Table 2). Gamonts were normal in size and shape but their number was low, ranging from one to 5 per infected larva (Table 3). No parasites were observed in pupae or adults (Table 2).

Only trophozoites were observed in *An. quadrimaculatus* larvae exposed to this parasite; 4th instars, pupae, and adults were free of parasites (Table 2). Intracellular stages and gamonts were not observed when oocysts of *A. taiwanensis* were fed to 1st instars of *Cx. nigripalpus, Cx. territans,* and *Cx. quinquefasciatus* (Table 2).

Mortality induced with A. taiwanensis: Laboratory mortality rates are presented in Table 4 for the 4 species of mosquitoes exposed to *A. taiwanensis* oocysts. Gregarine infection had little impact on its natural host, *Ae. albopictus,* or on the other hosts, *Ae. aegypti* and *Ae. triseriatus.* Munstermann and Wesson (1990) reported that "mortality in *A. taiwanensis*-infected *Ae. aegypti* seemed quite high." We found no difference in the mortality between *Ae. albopictus* and *Ae. aegypti* challenged with this Florida strain of *A. taiwanensis.* It is known, however, that the biological activity of different strains can vary considerably. Sulaiman (1992) reported significant differences in infection and mortality rates among 4 strains of *A. culicis* from Malaysia in its natural host *Ae. aegypti.*

Table 5. Differential mortality rates in stages of *Aedes taeniorhynchus* exposed to *Ascogregarina taiwanensis* oocysts.

Host stage	% mortality[1] Control	% mortality[1] Treated[2]
Larva	18.8	24.6
Pupa	1.3	46.7
Adult	0.0	42.2

[1] $n = 400$.
[2] Corrected by Abbott's formula.

In contrast to the low mortality rates for the 3 species of *Aedes* discussed above, mortality in *Ae. taeniorhynchus* due to *A. taiwanensis* infections was very high (Table 4). Significant differences in the mortality rate between control and exposed *Ae. taeniorhynchus* were found for larvae ($\chi^2 = 35.45$, $P < 0.00001$, df = 1), pupae ($\chi^2 = 161.93$, $P < 0.00001$, df = 1), and adults ($\chi^2 = 121.44$, $P < 0.00001$, df = 1) infected with *A. taiwanensis* (Table 5). Mortality was greatest for infected pupae and emerging adults where an intense melanization reaction against *A. taiwanensis* was observed.

In general, pathogenicity and parasite prevalence are related inversely (Anderson 1979). The high prevalence and low mortality rates found for this strain of *A. taiwanensis* in *Ae. albopictus* suggest a well-adapted, host–parasite relationship. Susceptibility tests also indicate a specificity of this strain for its natural host, *Ae. albopictus,* even though other hosts were susceptible. Based on our study, this Florida strain of *A. taiwanensis* has little short-term impact on the mortality of the 3 most common container-breeding mosquito species in Florida. Other strains of *A. taiwanensis* from different geographic locales may differ in both pathogenicity and specificity for their natural and other hosts.

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