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Larval mosquito fauna (Family Culicidae) of Sta. Ignacia, Tarlac, Province, Philippines

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ABSTRACT
Surveillance of the presence of larval mosquitoes was performed in the suburban municipality of Sta. Ignacia, Tarlac Province, Philippines, with approximately 94.0% of the land area still devoted to agriculture. Five districts were covered, namely: Macageuing, San Francisco, San Vicente, Sta. Ines Centro, and Sta. Ines East. The standard dipping method was used to collect mosquito larvae from rice fields, ponds, creeks, streams and used tires and larvae were identified at the genus level. Culex sp. was the most abundant with a calculated mean breeding index of 22 larvae per dip. Anopheles sp. and Aedes sp. were likewise identified with mean breeding indices of five and two larvae per dip, respectively. In two samplings, Culex sp. had the highest mean larval breeding index (MLBI) of 9 larvae/dip in Sta. Ines Centro (West); while Sta. Vicente registered higher MLBI of Anopheles sp. and Aedes sp. relative to sites in other districts. Considering the endemicity of malaria, lymphatic filariasis and dengue in the country, present findings are valuable in the formulation of control measures specifically targeting presently identified breeding sites that revealed considerable mosquito population. To arrive at a comprehensive picture, we highly recommend a validation of current data in similar sampling sites in the other districts of Sta. Ignacia, concurrent with a species-specific surveillance of culicidae mosquitoes.

Key words: mosquito larval surveillance; breeding index; Sta. Ignacia; Tarlac Province; Philippines

INTRODUCTION
In tropical countries like the Philippines where many viral and parasitic infections are endemic, mosquito surveillance is an integral part in the prevention and control of vector-borne diseases and in the evaluation of pest control strategies. Agroecosystems, rice fields, coastal areas and artificial water-holding containers provide suitable breeding places of adult mosquitoes (Lichtenberg and Getz, 1985; Mutero et al., 2000; Sunahara et al., 2002; Caglar et al., 2003; Muturi et al., 2006; Blaustein and Chase, 2007). Mosquito vectors rely on vertebrate blood meals for egg development partly utilized for yolk-protein and renewed lipid synthesis and storage synthesis, and on sugars from plants essential for flight and energy reserves (Scott et al., 1997; Ziegler and Ibrahim, 2001; Briegel, 2003).

In the Philippines, mosquitoes (family Culicidae) are widely distributed (Delfinado, 1966; Basio, 1971). In Northern Luzon, Palawan and Mindanao, Anopheles spp., Aedeomyia spp., Aedes spp., and Culex spp. commonly inhabit paddy fields, shaded pools, streams and artificial water holding-containers (Miyagi et al., 1985). Several Culex spp. and Aedes spp. transmit viral pathogens (Rudnick et al., 1962; Doherty et al., 1963; Chow et al., 1998; Kay et al., 2002); while Anopheles spp. and Aedes spp. are important vectors of malaria and lymphatic filaria (Ishii et al., 1983; Belizario, 1993; Lansang et al., 1997; Espino and Manderson, 2000; Bell et al., 2001 and 2005). In view of the importance of data on mosquito fauna in the development and execution of appropriate programs for the prevention and control of mosquito-borne diseases like malaria, dengue and lymphatic filariasis, all of which are endemic in the country, the present surveillance
was carried out in Sta. Ignacia, Tarlac Province, Philippines, a suburban municipality with approximately 94.0% of the land area still devoted to agriculture.

MATERIALS AND METHODS

Study Area

Sta. Ignacia is a suburban municipality of Tarlac Province divided into 24 districts (National Statistical Coordination Board, 2007). It covers a 14,607-hectares land area, of which only approximately 6.5% are used for residential, commercial and industrial purposes. Its 2007 projected population is 44,742 with a population density of three persons per hectare. Currently, there are 8,054 households (average household size: 6 persons). The mosquito surveillance study covered three rural (Macagueing, San Vicente and Sta. Ines East) and two urban (San Francisco and Sta. Ines West) districts (Fig. 1).

Larval Mosquito Sampling and Identification

Mosquito surveillance consisted of three collections period in June, 2007, which were scheduled one-week apart. The specific sites surveyed in the rural districts were stagnant creeks to streams (Macagueing), rice fields and streams (San Vicente) and rice fields and old/unused tires (Sta. Ines East), while the sites surveyed in the urban districts of San Francisco and Sta. Ines Centro (West) were rice fields and shallow ponds, respectively (Fig. 2). Mosquito larvae were collected using the standard dipping method (O’Malley, 1989). Sampling sites were first observed for the presence of larvae which were collected using a white plastic dipper, then larvae were placed in hot water and preserved in 80% ethyl alcohol. Larvae were identified at the genus level based on established morphological characteristics (Pratt, 1993; Dagnall Teaching Laboratory, 1998). Larvae of *Anopheles* sp. were distinguished based on the absence of a breathing tube at the 8th abdominal segment and presence of ventral palmate hairs; and the presence of a breathing tube with more than one pair of hair tufts for *Culex* sp. contrasted with the presence of single pair of hair tufts in *Aedes* sp. (Fig. 3).

Figure 1. Five districts covered by mosquito larval survey, Sta. Ignacia, Tarlac Province, 2007.
Figure 2. Sample sites from the five districts covered in the study: (A) Macagueing creek, (B) San Francisco rice field, (C) San Vicente rice field, (D) Sta. Ines East rice field, and (E) Sta. Ines Centro pond, Sta. Ignacia, Tarlac, 2007.
Figure 3. Larval mosquitoes. A and B. Anopheles sp. Absence of a breathing tube (BT) (arrow) and presence of ventral palmate hairs. C and D. Culex sp. E. Aedes sp. Note presence of BT (arrow) with more than one pair of hair tufts in Culex sp., and presence of single pair of hair tufts only in Aedes sp.

Data Analysis

The first larval collections for the five districts were pooled to serve as baseline data for the municipality. In the second and third sampling, the number of larvae per genus per district was recorded, and the data were compared across the five districts. To determine the total density of mosquito larvae per genus in the municipality and for each of the five districts, the breeding index (Belkin, 1954) was used calculated as: \( BI = \frac{TLP}{ND \times BP} \), where BI = breeding index; TLP = total number of larvae collected; ND = number of dips; and BP = number of sampling stations/ breeding places.

RESULTS AND DISCUSSION

A total of 226 mosquito larvae were collected from 36 sampling stations (Table 1). Of the total 137 larvae collected during the second and third sampling, 56 (40.9%) were from Sta. Ines Centro (West), followed by 48 (35.1%) from San Vicente (Table 2).

The larvae identified were predominantly Culex sp. (176: ~78%), followed by Anopheles sp. (39: ~17.0%) and Aedes sp. (11: ~5.0 %). Variation in the larval breeding indices between sampling periods revealed significantly higher density of Culex sp. (Fig. 4). The mean larval breeding index (MLBI) was computed as follows: Culex sp. (22 ± 5.7 larvae/dip); Anopheles sp. (5 ± 3.0 larvae/ dip); and Aedes sp. (2 ±
Larval mosquito fauna of Sta. Ignacia

2.1 larvae/dip). *Culex* sp. registered the highest MLBI of 9 larvae/dip in Sta. Ines Centro (West), followed by higher MLBI of *Anopheles* sp. and *Aedes* sp. in Sta, Vicente, and an overall low larval densities, including the absence of *Anopheles* sp. and *Aedes* sp. in Macagueing and Sta. Ines East district, respectively (Fig. 5).

Present data clearly indicate the study sites as natural breeding sites of *Aedes* sp., *Anopheles* sp. and *Culex* sp., with the highest mosquito larval collection from Sta. Ines Centro (West). The sampling sites at Sta Ines Centro (West) are situated in close proximity to piggery and poultry farms and shallow ponds rich in flora and other organic

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<tr>
<th>Sampling period</th>
<th># sampling stations</th>
<th># dips</th>
<th>Total # larvae</th>
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<tr>
<td>First*</td>
<td>15</td>
<td>42</td>
<td>89*</td>
</tr>
<tr>
<td>Second</td>
<td>11</td>
<td>28</td>
<td>71</td>
</tr>
<tr>
<td>Third</td>
<td>10</td>
<td>30</td>
<td>66</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>36</strong></td>
<td><strong>100</strong></td>
<td><strong>226</strong></td>
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- larvae collected were pooled from five districts

<table>
<thead>
<tr>
<th>District</th>
<th># of Larvae Collected (%)</th>
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<tr>
<td>Macagueing</td>
<td>16 (11.68)</td>
</tr>
<tr>
<td>San Francisco</td>
<td>12 ( 8.76)</td>
</tr>
<tr>
<td>San Vicente</td>
<td>48 (35.04)</td>
</tr>
<tr>
<td>Sta. Ines Centro</td>
<td>56 (40.87)</td>
</tr>
<tr>
<td>Sta. Ines East</td>
<td>5 ( 3.65)</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td><strong>137</strong></td>
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Table 1. Larval mosquito surveillance data (June 9, 16 and 23, 2007) in Sta. Ignacia, Tarlac Province Philippines.

Table 2. Mosquito larval distribution across the five districts in Sta. Ignacia, Tarlac Province, 2007 (pooled from second and third sampling).

materials, conditions ideal for mosquito breeding and larval development (Miyagi et al., 1985). Larval density of *Anopheles* sp. and *Aedes* sp. was highest in San Vicente, a predominantly rice-producing community along a main road, where vulcanizing shops and the municipal water pump station are located. Earthenware jars and metal and concrete water tanks and discarded automobile tires and food containers used to collect water in close proximity to human habitation, favor the breeding and oviposition of *Aedes* sp. (Kay et al., 2002; Lenhart et al., 2005; Mahilum et al., 2005; Roiz et al., 2007). In Sta. Ines East district had low numbers of *Culex* sp. and *Anopheles* sp. and none of *Aedes* sp. This may be attributed to the place being primarily irrigated rice fields that are regularly sprayed with pesticides and herbicides.
Figure 4. Variation in the breeding indices of mosquito larvae during three samplings pooled from 36 sampling sites in five districts in Sta. Ignacia, Tarlac Province, 2007.

Figure 5. Mean larval breeding indices per district surveyed (2nd and 3rd Sampling) at Sta. Ignacia, Tarlac Province, 2007.

The abundance of Culex sp. in Sta Ignacia possess a major concern in view of the association of particular species in the transmission of avian Sindbis virus (Rudnick et al., 1962; Sammels et al., 1999; Buckley et al., 2003); Japanese B encephalitis infection (Doherty et al., 1963; Ritchie and Rochester, 2001; Vythilingam et al., 2002), and avian malaria and Bancroftian filaria (Cabrera and Valeza, 1978; Valeza and Grove, 1979; Suguri et al., 1985). The occurrence of Anopheles and Aedes needs to be highlighted considering the endemicity in the country of infections like malaria, with Anopheles flavirostris as the principal vector (Saul et al., 1997; Torres et al., 1997; Miguel et al., 1998; Bell et al., 2005); and lymphatic filariasis, particularly Bancroftian filarial transmitted mainly by Aedes poicilus, a night-biting and banana-axil breeding species (Ishii et al., 1983; Go, 1993; Walker et al., 1998; Kron et al., 2000; Ramirez et al., 2004). The classic dengue fever and dengue hemorrhagic fever which are transmitted by Aedes aegypti and Aedes albopictus (Chow et al., 1998) are endemic in the country, as well. In Cebu City, Philippines, serological assay of patients revealed 94.0% positive cases of dengue, of the 489 breeding sites surveyed, 29.4% were infested with Ae. aegypti larvae, with discarded tires registering the highest breeding site at 69.4% (Mahilum et al., 2005).
Daphnia sp., tadpoles and snails were abundant in the collection sites. Copepods are beneficial as food to mosquito larvae and by feeding on mosquito predators/pathogens (Blaustein and Chase, 2007). While tadpoles and mosquito larvae co-exist, they compete for a scarce food resource (Blaustein and Margalit, 1994). Mokany and Shine (2003), observed a mutually negative effect on the growth rate of tadpoles and mosquitoes and survival of mosquitoes even at higher food levels, suggestive of an interaction that goes beyond food competition. In view of the crucial role of mosquitoes as vectors of important human diseases, they had asserted that the global decline in amphibian populations may have more impact on human health than has generally been thought of.

Present preliminary surveillance data are encouraging and may prove valuable in the formulation of workable control measures specifically targeting presently identified breeding sites that revealed considerable mosquito population. To provide a comprehensive picture of the mosquito fauna in Sta. Ignacia, Tarlac Province, we highly recommend a validation of present surveillance data in comparable sampling sites in the other 19 districts of Sta. Ignacia, concurrent with a species-specific surveillance of culicidae mosquitoes.

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