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The Journal of Vector Ecology is an international journal published by the Society for Vector Ecology. It is concerned with all aspects of the biology, ecology, and control of arthropod vectors and the interrelationships between the vectors and the disease agents they transmit. The journal publishes original research articles and research notes, as well as comprehensive reviews of vector biology based on presentations at Society meetings. All papers are reviewed by at least two referees who are qualified scientists and who recommend their suitability for publication. Acceptance of manuscripts is based on their scientific merit and is the final decision of the editor, but these decisions may be appealed to the editorial board.

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Copepod Predation on *Anopheles quadrimaculatus* Larvae in Rice Fields

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ABSTRACT: Cyclopoid copepods and mosquito larvae were surveyed in southwestern Louisiana rice fields. Almost every rice field had a natural population of *Mesocyclops ruttneri*, *Acanthocyclops vernalis*, or *Macrocyclops albidus*. Judging from the abundance of pupae, 29% of the fields were responsible for virtually all *Anopheles quadrimaculatus* production, apparently because larval mortality suppressed production in the other fields. *Mesocyclops ruttneri* had the strongest negative association of naturally occurring copepod populations with *An. quadrimaculatus* larvae, though a few fields with *M. ruttneri* had substantial *Anopheles* production. *Macrocyclops albidus*, *M. ruttneri*, *Mesocyclops edax*, and *Mesocyclops longisetus* were introduced to experimental rice field plots. It took two months for the introduced copepods to build up their numbers; *Anopheles* larvae then disappeared from all treated plots while larvae continued to be present in the adjacent control field. Copepods were observed to kill the following number of first instar *An. quadrimaculatus* larvae in the laboratory: *Mesocyclops ruttneri* (36 larvae/day), *Macrocyclops albidus* (23 larvae/day), *Mesocyclops longisetus* (24 larvae/day), and *Acanthocyclops vernalis* (15 larvae/day). It is concluded that introducing select species of copepods and encouraging their populations offer possibilities for contributing to *Anopheles* control in rice fields.

**Keyword Index**: Copepod, mosquito larvae, mosquito control, biological control, *Anopheles*, rice field, malaria.

INTRODUCTION

Some of the larger species of cyclopoid copepods, as predators of first and second-instar mosquito larvae, are now in operational use to eliminate *Aedes* larvae from container habitats such as tires, water storage tanks, and wells (Marten et al. 1994a, Nam et al. 1998). Copepods may also offer possibilities for *Anopheles* control. *Mesocyclops longisetus* (Thiébaut) and *Mesocyclops aspericornis* (Daday) are known to kill large numbers of *Anopheles* larvae in the laboratory (Marten et al. 1989, Brown et al. 1991). Marten et al. (1989) observed *Anopheles albimanus* Wiedeman larvae to be scarce in ponds and other aquatic habitats in Colombia where *M. longisetus* was present.

Rice fields are a major breeding habitat for *Anopheles quadrimaculatus* Say in southeastern United States. We surveyed natural populations of cyclopoid copepods in rice fields of southwestern Louisiana and assessed the impact of natural and introduced populations of copepods on *Anopheles* larvae in the fields.

MATERIALS AND METHODS

**Laboratory Predation Tests**

Laboratory colonies of four species of larvivorous copepods—*Acanthocyclops vernalis* (Fischer), *Macrocyclops albidus* (Jurine), *Mesocyclops longisetus*, and *Mesocyclops ruttneri* Kiefer—were established from collections at a canal in New Orleans, Louisiana. *Megacyclops latipes* (Lowndes) were collected from a roadside drainage ditch in Slidell, Louisiana. Culture methods followed Marten et al. (1997), a system based on wheat seed, *Chilomonas*, and *Paramaecium caudatum*. A laboratory colony of *An. quadrimaculatus* was established from eggs provided by the USDA Medical and Veterinary Entomology Research Laboratory in Gainesville, Florida.

The capacity of each copepod species to kill *An. quadrimaculatus* larvae was assessed by placing single adult female copepods in tissue culture plate wells (35 mm diameter, 18 mm deep) with 50 newly hatched first instar *An. quadrimaculatus* larvae from the laboratory
colony. The number of surviving larvae was counted after 24 hours at a temperature of 24°-26°C.

Field Survey

Thirty-two rice fields in Jefferson Davis Parish, Louisiana, were sampled for mosquito larvae and copepods in late September 1991, about a month after the fields were flooded for the second rice crop of the year. Twenty-four liters of water (approximately 100 dips) were dipped from each field with a standard dipper for mosquito larvae and passed through a net (0.2 mm mesh) to capture mosquito larvae and copepods. All copepods and mosquito larvae were preserved in alcohol for subsequent identification and counting. Copepods were identified to species, and mosquito larvae were identified to genus. All Anopheles larvae appeared to be An. quadrimaculatus, though there may have been some Anopheles crucians Wiedemann that passed unnoticed. Mosquito pupae were held for identification as emerging adults.

Field Experiment

Four adjacent experimental plots were established in a rice field approximately 15 km NW of Jennings, Louisiana. The plots were 10 m on each side and were constructed by placing four parallel levees across one end of a rice field that was fallow the previous year. Approximately 500 adult female Acanthocyclops vernalis, 500 Macrocylops albidus, 500 Mesocyclops longisetus, 500 Mesocyclops ruttenr, and 500 Mesocyclops edax (Forbes), were introduced to each plot in late April 1990, about two weeks after the field was flooded for the first rice crop. The irrigation water was pumped from underground. The introduced copepods came from the same laboratory cultures as copepods in the laboratory predation tests. (M. edax was originally collected from a New Orleans canal.) Each plot was sampled for copepods and mosquito larvae in June and again in late July. The sampling procedure was as described for the field survey, except 40 liters of water were dipped from each plot. Samples from the same field outside the treatment plots served as controls.

The field was harvested in early August, and it was not flooded to produce a second rice crop that year. To monitor for the presence of introduced copepods when the field no longer contained water, samples of moist soil were taken from depressions in the treatment plots in October (two months after the field was drained for harvest). The soil was placed in a bucket of water, and the water was strained through a net several hours later to collect copepods. The following February, copepods were collected from puddles in the treatment plots.

RESULTS

Laboratory Predation Tests

All tested copepod species killed substantial numbers of first-instar An. quadrimaculatus larvae. They usually ate all of the larva except its head capsule, but sometimes they ate only part of a larva. Mesocyclops ruttenr killed the most larvae, and Acanthocyclops vernalis killed the least (TABLE 1).

Field Survey

Almost all surveyed fields contained natural populations of Mesocyclops ruttenr or Acanthocyclops vernalis. Fifty-eight percent of the fields contained Mesocyclops ruttenr, and 38% contained Acanthocyclops vernalis. Only 9% of the fields contained both

<table>
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<th>TABLE 1. Mortality of first instar Anopheles quadrimaculatus larvae due to copepod predation in the laboratory.</th>
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<td>Copepod Species</td>
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</tr>
<tr>
<td>Acanthocyclops vernalis</td>
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<tr>
<td>Macrocylops albidus</td>
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<tr>
<td>Mesocyclops longisetus</td>
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<tr>
<td>Mesocyclops ruttenr</td>
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<td>Megacyclops viridis</td>
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<td>Controls (no cyclopoid)</td>
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¹Average number (± SE) of larvae dead after 24 hours. Fifty larvae were provided to one copepod in each replicate.
species. *Macrocyclops albidus* was found in 6% of the fields.

The following cyclopid copepods, which are not large enough to be significant predators of mosquito larvae, were also encountered: *Mesocyclops reidi* Petkovski, *Tropocyclops extensus* (Kiefer), *Microcyclops rubellus* (Liljestroberg), *Eucyclops aqilis* (Koch), *Eucyclops elegans* (Herrick), *Paracyclops chiltoni* (Thomson), *Paracyclops poppei* (Rehberg), *Thermocyclops inversus* Kiefer, and *Thermocyclops tenuis* (Marsh).

Samples from 81% of the fields in the survey contained *Anopheles* larvae. Average numbers of *Anopheles* larvae declined as they progressed from the first to fourth instar (Fig. 1). The higher instars were concentrated in relatively few fields; 31% of the fields contained 77% of III/IV instar larvae. *Anopheles* pupae were found in 27% of the fields, the number varying from 0.01 to 0.04 pupae/dip. The fields with pupae were the ones that had the largest numbers of III/IV instar larvae.

There was a conspicuous negative association between *Anopheles* and *Mesocyclops ruttneri*. While *Anopheles* larvae were clearly present (>10 larvae) in all fields without *M. ruttneri*, no *Anopheles* larvae were found in 35% of fields that contained *M. ruttneri* (Fig. 2). The number of second-instar *Anopheles* larvae was substantially lower in fields that contained *M. ruttneri* compared to fields with only *Acanthocyclops vernalis* or no larvivorous copepods (Fig. 1). The difference was highly significant with a nonparametric Mann-Whitney U-test (*P* = 0.003, *U* = 190, *n*₁ = 20, *n*₂ = 12, Rohlf and Sokal 1995, p. 129). First and third-instar *Anopheles* larvae were also less numerous in fields with *M. ruttneri*, but the differences were not so great (*P* = .10, *U* = 152 for first instars; *P* = .08, *U* = 158 for third instars). Third/fourth-instar *Anopheles* larvae were found in 75% of the fields without *M. ruttneri*, while third/fourth instar larvae were found in only 22% of the fields that contained *M. ruttneri*. *Anopheles* pupae were found in 42% of the fields without *M. ruttneri*, while pupae were found in only 20% of the fields that contained *M. ruttneri* (Fig. 2).

*Uranotaenia* larvae were in 94% of the fields, but most of the *Uranotaenia* larvae were concentrated in relatively few fields. Twenty-nine percent of the fields had many more first-instar larvae than the other fields; 9% of the fields had 66% of the third/fourth instars. The fields with large numbers of third/fourth-instar *Uranotaenia* larvae were not the same fields that contained larger numbers of third/fourth-instar *Anopheles* larvae. Like *Anopheles*, average numbers of *Uranotaenia* larvae declined as they progressed from the first to fourth instar (Fig. 1). Association of larval numbers with the presence or absence of *M. ruttneri* was not statistically significant (Mann-Whitney *U* = 131, *n*₁ = 20, *n*₂ = 12 for first instars; *U* = 144 for second instars; *U* = 135 for third instars).

Samples from 42% of the fields contained *Culex* larvae. *Culex* larvae were not numerous enough for detailed analysis, averaging 0.008 first-instar larvae/dip, 0.006 second-instar larvae/dip, 0.003 third-instar larvae/dip, and no fourth instars. Fish were seldom collected in the dipping samples.

![Anopheles](image1.png) ![Uranotaenia](image2.png)

**Figure 1.** Average number (±SE) of mosquito larvae/dip in the September field survey. Based on 100 dips/field in 32 Louisiana rice fields. Most fields without *Mesocyclops ruttneri* contained *Acanthocyclops vernalis*.
Field Experiment

No mosquito larvae or copepods were observed in the treatment plots or control field when copepods were introduced to the treatment plots at the end of April. About half of the other newly flooded fields in the district had conspicuous populations of *Acanthocyclops vernalis* at this time.

There were a few copepods, too small to prey on mosquito larvae, in the treatment plots and control field when they were sampled in June. There were small numbers of *Anopheles* larvae (0.10 larvae/dip) in the treatment plots and control field at this time.

Every treatment plot contained adults of all five introduced copepod species when sampled in early July. *Acanthocyclops vernalis* and *Mesocyclops longisetus* were most numerous; the combined abundance of all copepod species exceeded five copepods/dip. No mosquito larvae or pupae were observed in any of the treatment plots. *Acanthocyclops vernalis* was common in the control field when inspected at the same time. No other larvivorous copepod species were observed in the control field. Second to fourth instar *Anopheles* larvae had a combined abundance of 0.07 larvae/dip in the control field.

When soil samples were taken from the drained treatment plots in October and immersed in water, adult *Acanthocyclops vernalis, Mesocyclops ruttneri, Mesocyclops longisetus*, and a small number of *Macrocylops albidus* and *Mesocyclops edax* were swimming in the water within hours. Only *A. vernalis* and *M. albidus* were recovered from puddles in the treatment plots the following February.

**DISCUSSION**

**Copepods**

It was no surprise to find *Macrocylops albidus* in some of the rice fields. *Macrocylops albidus* is the most common large cyclopoid in Louisiana. It is virtually ubiquitous in drainage ditches that have at least some water throughout the year; it is less common in isolated temporary water. *Macrocylops albidus* can survive in moist soil, but it lacks the ability of some cyclopoids such as *Acanthocyclops vernalis* to survive in drier soil for months or more. It is no surprise that *A. vernalis* is common in rice fields because *A. vernalis* is common in

![Figure 2. Percentage of fields with *Anopheles* larvae or pupae in the September field survey.](image-url)
other temporary, shallow-water habitats throughout Louisiana. *Acanthocyclops vernalis* is probably frequently introduced to rice fields in irrigation water, because it is also common in canals. *Macrocylops albidas* and *A. vernalis* should be able to survive the winter in rice fields that have pockets of moist soil because both species tolerate temperatures down to 0°C as long as the water does not freeze (Marten et al. 1994a).

One of the most striking results of the field survey was the complementary distribution of *Acanthocyclops vernalis* and *Mesocyclops ruttneri*; it was unusual to find both species in the same field. It is surprising that *M. ruttneri* is so common in rice fields because we never found this species in other shallow, temporary water in Louisiana. *Mesocyclops longisetus* is an exotic species from Southeast Asia that lives primarily in permanent, deeper water in Louisiana. *Mesocyclops ruttneri* may be abundant in rice fields because it is abundant in irrigation canals.

It is no surprise that *Mesocyclops edax* and *Mesocyclops longisetus* do not occur naturally in Louisiana rice fields. *Mesocyclops edax* is a temperate species for which Louisiana is the southern extreme of its geographic range, and *M. longisetus* is a neotropical species not found north of Louisiana. Neither species is common in Louisiana, occurring naturally only in permanent (relatively deep) water, such as canals or large ponds, and not conspicuously abundant even there. *Mesocyclops edax* is probably associated with deeper water because of its swimming habit. Unlike most other cyclopoid copepods, which spend considerable time clinging to submerged vegetation or resting on the bottom, *M. edax* is always in motion, swimming in the water column. *Mesocyclops longisetus* is restricted to deeper water because it is killed by temperatures below 3°C (Marten et al. 1994a). Although *M. longisetus* is common in shallow water in the tropics, it survives the more severe Louisiana winter cold spells only where the water is deep enough to buffer temperature extremes.

If *M. longisetus* and *M. edax* do not occur naturally in rice fields, why did they do so well when introduced to the experimental plots? It seems the factors that prevent *M. longisetus* and *M. edax* from occupying rice fields over the long term are not operating during the summer. It also appears that neither of these two species is common enough in irrigation water to stock the fields when they are flooded.

Cyclopoid copepods have a generation time of about three weeks at late-spring temperatures, multiplying their numbers about a hundred-fold with each generation. Since it took the copepods about two months to build up their populations after introduction to the experimental plots in April, the same kind of lag should be common for natural populations as well. Because many of the fields in the study area are rotated between rice, soybeans, and fallow, there can be long periods without water, making it difficult for copepods to survive from one rice crop to the next. Moreover, we cannot expect copepods to be introduced with irrigation water pumped from underground. While *Acanthocyclops* is already present in some fields when they are first flooded in April, if they had rice the previous year, the copepod populations in most rice fields probably have to start from small numbers dispersing from nearby fields or introduced with irrigation water from canals. Several months can pass from the time copepods are introduced until they reach sufficient numbers to impact mosquito larvae.

**Anopheles Larvae**

If *Anopheles* oviposition was more or less continuous during the weeks preceding the field survey, the survival of each larval instar at the time of the survey can be inferred from the decline in average numbers of the instars. It appears reasonable to interpret the field survey data this way because light trap records from the survey area showed no large fluctuations in abundance of adult *Anopheles* during the weeks preceding the survey. Using the ratio of pupae to first instars, overall larval survival in the surveyed fields was <1%. It is a common observation that the survival of *Anopheles* larvae in rice fields is <5% (Roger and Bhuiyan 1990).

We can ask whether the bulk of *Anopheles* production comes from a large number of rice fields (all of which produce small numbers of mosquitoes) or from a small percentage of fields that produce many more mosquitoes than the other fields. If we consider the number of pupae to reflect the production of adult mosquitoes, a small percentage of fields in the field survey was responsible for the bulk of *Anopheles* production because pupae were concentrated in a minority of the fields.

**Copepod Predation**

Low survival of *Anopheles* larvae is generally attributed to predators such as fish, odonate nymphs, aquatic bugs, and aquatic beetles (Roger and Bhuiyan 1990). The field survey in this study did not include enough fields without larvivorous copepods to compare larval numbers in fields having *Mesocyclops ruttneri* or *Acanthocyclops vernalis* with numbers in fields that were entirely without copepod predation. However, the abundance of *A. vernalis* or *M. ruttneri* in the fields, combined with the large number of *Anopheles* larvae that these copepods killed in the laboratory, suggests
that cyclopoid copepods are also significant predators.

The lower number of Anopheles larvae in fields with Mesocyclops runtneri compared to fields with Acanthocyclops vernalis (Fig. 1) suggests that M. runtneri is a more effective predator than A. vernalis. The substantial reduction in second-instar larva in fields with M. runtneri would be expected after heavy mortality during the first and second instars. While M. runtneri is probably responsible, the presence of M. runtneri could also reflect a complex of other ecological factors detrimental to early instar survival. Even if M. runtneri is responsible, the first/second instar larval mortality in fields with M. runtneri is not consistently strong enough for M. runtneri to be of use for Anopheles control in the same way that copepods are now used for Aedes control.

The impact of copepods on Anopheles production in the experimental plots was quite different from what we observed with natural copepod populations in the field survey. Once the introduced copepods built up their numbers, they reduced larval populations and the production of adult mosquitoes virtually to zero. We do not know which of the introduced copepod species was responsible, or whether a combination of species was important. We can speculate that M. longisetus was responsible because M. longisetus is already known to reduce Anopheles larvae drastically in other situations (Marten et al. 1989). Mesocyclops edax may also have made a major contribution. While other cyclooids are sedentary much of the time, the continual activity of M. edax in the water column should put it in frequent contact with Anopheles larvae hanging at the surface.

Implications for Anopheles Control

High larval mortality due to predation seems to be responsible, at least in part, for the very low Anopheles production in most rice fields. While the roles of the various naturally occurring predators are not completely clear, it seems likely that Anopheles production can be reduced by using cultivation practices that encourage predator populations in as many fields as possible. Pesticides that kill natural predators should be avoided. To the extent that agronomic considerations allow, the temporal and spatial arrangement of rice fields, alternate crops, fallows, and irrigation water should encourage the survival of predators from one rice crop to the next and facilitate rapid invasion by predators when fields are flooded.

One way to encourage predators is to maintain ponds in the fields while they are dry. Ponds can serve as reservoirs from which copepods and other aquatic predators spread over the fields when they are flooded. Ponds could be stocked not only with predator species that are naturally common in rice fields, but also with predators, such as Mesocyclops longisetus and Mesocyclops edax, which do not occur naturally but which thrive during the summer when introduced. The fact that these two species do not occur naturally should not be an obstacle to their use. Mesocyclops longisetus has proved one of the most effective copepod species for Aedes control, even though natural populations are seldom found in containers (Marten et al. 1994a, 1994b).

Acknowledgments

We thank Edgar Bordes, Michael Carroll, and others on the staff of the New Orleans Mosquito Control Board for numerous contributions to this study. The staff of Jefferson Davis Mosquito Control District facilitated our work in that parish. John Compton provided the rice fields for the field experiment. Janet Reid (Smithsonian Institution) provided species identifications for copepods collected in the field survey.

REFERENCES CITED


Natural Control of *Culex quinquefasciatus* Larvae in Residential Ditches by the Copepod *Macrocyclops albidus*

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ABSTRACT: Natural populations of three larvivorous copepod species live in residential roadside ditches in Louisiana: *Macrocyclops albidus*, *Acanthocyclops vernalis*, and *Megacyclops latipes*. *Macrocyclops* is most common and killed an average of 27 first-instar *Culex quinquefasciatus* larvae/copepod/day in the laboratory. Although severe pollution from septic tank effluent in some parts of the ditches creates havens for *Cx. quinquefasciatus* production by excluding predatory copepods and fish (*Gambusia affinis*), *Macrocyclops* and the fish substantially reduce *Cx. quinquefasciatus* larval survival when present where pollution is not so severe. At natural abundance, *Macrocyclops* reduced the survival of *Cx. quinquefasciatus* larvae (during their first four days) to 2.6%, compared with 46% survival in controls without *Macrocyclops*. During one year of field observation, *Macrocyclops* was common in the spring but disappeared during the summer when fish (which prey on copepods) appeared in many ditches, reduced water flows led to more severe pollution, and water temperatures in very shallow water were sometimes higher than *Macrocyclops* could survive. *Macrocyclops* reappeared in many ditches during autumn and winter, when water temperatures and pollution declined and fish disappeared. Introduction of *Macrocyclops* to ditches in October accelerated its reappearance during autumn and winter and reduced the number of sites with *Cx. quinquefasciatus* larvae to one-quarter the number in control ditches. The most effective way to control *Cx. quinquefasciatus* is to eliminate pollution so predators like fish and copepods can live throughout the ditches, but timely introduction of fish and copepods could also contribute to control. More experience will be necessary to ascertain whether copepod introductions are cost effective.

**Keyword Index:** Copepod, mosquito larvae, mosquito control, biological control, *Culex*, predation.

INTRODUCTION

Roadside drainage ditches in residential areas can be a major breeding habitat for *Culex quinquefasciatus* Say, particularly where effluent from septic tanks empties into the ditches. The pollution generates an abundant supply of bacterial food for *Cx. quinquefasciatus* larvae, but impacts of the pollution on predators of the larvae may also influence mosquito production.

Cyclopoid copepods are known to provide effective natural control of *Aedes* larvae in water storage containers and rain-fed tires (Marten et al. 1994, Nam et al. 1998). However, the relation of cyclopoids to mosquito larvae in other aquatic habitats has only begun to be explored (Marten et al. 1989, Marten et al. 1994). Louisiana is known to have at least 34 species of cyclopoids that might live where mosquitoes breed (Reid and Marten 1995). We investigated the ecological interaction of natural cyclopoid populations with *Cx. quinquefasciatus* larvae in residential ditches polluted by septic tank effluent.

**MATERIALS AND METHODS**

**Field Survey**

The approximately 100 km of roadside ditches in Slidell, a small city in Louisiana, were sampled for cyclopoid copepods at random locations throughout the...
year—a total of 300 samples, each sample consisting of 25 dips with a conventional dipper for mosquito larvae. *Macrocylops albidus* (Jurine) collected from ditches were established in laboratory culture to supply animals for the predation experiments and *Macrocylops* field introductions described below. Culture methods followed Marten et al. (1997), a system based on wheat seed, *Chilomonas*, and *Paramaecium caudatum*, similar to the copepod production system described by Suárez et al. (1992).

**Laboratory Predation Experiments**

The capacity of *Macrocylops albidus*, *Acanthocyclops vernalis* (Fischer), and *Megacyclops latipes* (Lowndes) to kill *C. quinquefasciatus* larvae was evaluated in the laboratory. Single adult female copepods were placed in tissue culture plate wells (35 mm diameter, 18 mm deep) with ditch water. Fifty newly hatched *C. quinquefasciatus* larvae were placed in each well with a small quantity of yeast to ensure food for the larvae. The number of surviving larvae was counted after 24 hours at a temperature of 24°-26°C. Control wells contained larvae and yeast but no copepods.

**Field Predation Experiments**

In September 1990, eight cylindrical sheet-metal enclosures (90 cm diameter, 60 cm high) were placed in residential roadside ditches by imbedding the bottom 15 cm of each cylinder in the mud. The water in the enclosures was 10-15 cm deep and contained typical ditch vegetation. Moderate organic pollution was apparent, and natural food for *C. quinquefasciatus* larvae was abundant. *C. quinquefasciatus* larvae were common at the sites.

*Macrocylops albidus* was introduced to all of the enclosures, which were covered with mosquito netting to prevent mosquito oviposition. Six weeks later, when the *Macrocylops* populations had increased to several adult copepods per dip, all enclosures were checked with an aquarium net to ensure that they contained no fish or mosquito larvae. Ten *C. quinquefasciatus* egg rafts (approximately 2,000 larvae/m²) were placed in four of the enclosures, and 20 egg rafts (4,000 larvae/m²) were placed in each of the other four enclosures. The number of larvae that hatched into each enclosure was estimated from an average of 130 larvae per egg raft observed to hatch in the laboratory. Four days after eclosion, when the larvae had grown too large for copepods to kill, all surviving larvae were removed from each enclosure with a net and counted.

The same experiment was repeated two weeks later in all eight enclosures. At the same time, ten egg rafts and twenty egg rafts, respectively, were placed in two additional enclosures, which contained no copepods or fish and served as controls.

**Bioassays for Pollution**

Approximately 150 water samples were collected from different ditch locations to assess toxicity for copepods and fish. The degree of pollution of each water sample was judged subjectively on the basis of physical appearance and smell. *Macrocylops albidus* was collected from ditches, and ten adults were placed in 300 ml of each water sample for one week of observation in the laboratory. *Gambusia affinis* (Baird and Girard) from the ditches were observed for one week in one-liter water samples from ten moderately to severely polluted ditches. Five *Gambusia* were placed in each sample.

**Field Sites**

Two residential areas with roadside ditches were selected for a year of observation. The first site consisted of four adjacent dead-end streets on which the houses were nearly continuous. The second site consisted of four adjacent streets with small woodlots between many of the houses. Pollution due to septic tank effluent was conspicuously greater in the ditches at the first site.

*Macrocylops* were introduced to two streets at each site to explore the possibility of increasing their presence in the ditches. *Macrocylops* were introduced to 300 m of ditch on both sides of two streets at the first site in late April 1991. Two other streets served as controls to which *Macrocylops* was not introduced. *Macrocylops* were introduced to 500 m of ditch on both sides of two streets at the second site in early May 1991, and they were introduced again to the same two streets in mid-October. Two adjacent streets at the second site served as controls. *Macrocylops* were applied to both sites using a two-gallon backpack sprayer (SP Systems Survivor) at a rate of 10 adult females per meter of ditch.

*Macrocylops, Gambusia*, and mosquito larvae were monitored on a biweekly basis at both sites from April 1991 to April 1992. Each side of the four streets at the first site had seven sampling stations at 25 m intervals. Each side of the streets at the second site had ten sampling stations at 30 m intervals during April-June 1991, three sampling stations at 150 m intervals from July to early September, and the same ten sampling stations at 30 m intervals from late September 1991 to April 1992.

The inspection procedure was to make five dips within 5 m of each station, recording the number of *Macrocylops* and mosquito larvae in the dips. Dips were directed toward spots in the ditch (e.g., vegetation
or conspicuous pollution) that were judged subjectively to be most suitable for *Cx. quinquefasciatus* larvae. The presence or absence of *Gambusia* at the station was assessed visually.

RESULTS

Field Survey

Natural populations of three copepod species large enough to prey on mosquito larvae were found in the ditches. They were *Macrocylops albidus*, *Acanthocyclops vernalis*, and *Megacyclops latipes*. *Acanthocyclops* and *Megacyclops* appeared in scattered populations, primarily during winter and spring. *Megacyclops* was particularly associated with sites that had pine needles in the ditch.

*Macrocylops albidus* was the most common larvivorous copepod in the ditches. It was particularly abundant in the spring but disappeared from many ditches during the summer. *Macrocylops* started to reappear in the ditches by October or November, spreading through many of them as autumn and winter progressed. Regardless of the season, *Macrocylops* was seldom observed in the visibly polluted water that was typically within 5 m of septic tank outlets, even in ditches where this copepod was common further from the outlets. Large numbers of *Macrocylops* were observed only at sites without *Gambusia*.

Laboratory Predation Experiments

All three copepod species killed substantial numbers of *Cx. quinquefasciatus* larvae in the laboratory (TABLE 1). *Macrocylops albidus* killed the most larvae, and *Acanthocyclops vernalis* killed the least. *Macrocylops* and *Acanthocyclops* killed fewer *Cx. quinquefasciatus* larvae than Marten (1990) reported for *Aedes albopictus* (Skuse). The difference can be explained by our observation that these copepods made numerous aborted attacks on *Cx. quinquefasciatus* larvae before actually seizing and killing one. In contrast, nearly all attacks on *Aedes* larvae were carried through to completion. As cyclopoid copepods are known to avoid preying on animals much larger than themselves (Roche 1990), it may be that the prominent bristles on *Cx. quinquefasciatus* larvae intimidate the copepods by giving them the impression the larvae are larger than they really are.

Field Predation Experiments

At the time *Cx. quinquefasciatus* egg rafts were placed in the enclosures containing *Macrocylops*, the number of *Macrocylops* varied from one to five adult dip, corresponding to several hundred to several thousand adults in each enclosure. While 46% of the *Cx. quinquefasciatus* larvae were still alive in control enclosures four days after hatching, only 2.6% of the larvae were still alive in enclosures that contained *Macrocylops* (TABLE 2). The number of mosquitoes that actually emerged was not counted. It is conceivable that the difference in adult mosquito production between enclosures with and without *Macrocylops* would not be so great because of density-dependent survival after the fourth day. Nonetheless, differences in four-day-old larvae should reflect differences in mosquito production

<table>
<thead>
<tr>
<th>TABLE 1. Predation by three species of cyclopoid copepods on <em>Culex quinquefasciatus</em> and <em>Aedes albopictus</em> larvae in the laboratory.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copepod Species</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td><em>Macrocylops albidus</em></td>
</tr>
<tr>
<td><em>Megacyclops latipes</em></td>
</tr>
<tr>
<td><em>Acanthocyclops vernalis</em></td>
</tr>
<tr>
<td>Controls (no copepod)</td>
</tr>
<tr>
<td><strong>Culex quinquefasciatus</strong></td>
</tr>
<tr>
<td><strong>Aedes albopictus</strong></td>
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<tr>
<td><strong>Aedes albopictus</strong></td>
</tr>
<tr>
<td><strong>Aedes albopictus</strong></td>
</tr>
</tbody>
</table>

<sup>1</sup>Average ± SE. Fifty mosquito larvae available to each copepod for 24 hours at 24-26°C. Number of replicates shown in parentheses.

<sup>2</sup>Source: Marten (1990).
because third/fourth instar larval survival in this food-rich habitat is generally high in the absence of predators.

**Bioassays for Pollution**

The survival of *Macrocyclops* in water samples from the ditches was associated strongly with pollution from septic tanks. Assessment of survival was unambiguous because all the *Macrocyclops* in a particular sample either died within 1-3 days or were all still alive after a week. *Macrocyclops* died in 93% (N=43) of the samples of visibly polluted water, which always came from within 5 meters of septic outlets. *Macrocyclops* was killed in 52% (N=46) of the less polluted water samples (cleaner in appearance). *Gambusia* survived in 6 of the 10 moderately to severely polluted water samples in which it was held for one week. In every case, all the fish survived or they all died. With one exception, *Macrocyclops* survived or died in the same samples as *Gambusia*, the exception being a sample in which *Macrocyclops* died and *Gambusia* survived. *Gambusia* was sometimes (though not often) observed at sampling stations where the water killed *Macrocyclops* when tested in the laboratory.

Pollution from septic tank effluent was particularly visible on one of the streets at the first field site. Water from every station on this street killed *Macrocyclops* in laboratory bioassays during summer, autumn, and winter. Only in the spring, when the water was flushed by heavy rainfall, were there stations with water that was not lethal. Water from 40% of the stations on this street killed *Macrocyclops* when field observations began in April, and water from 50% of the stations killed *Macrocyclops* the following March.

The other three streets at the first field site had a patchwork of polluted and unpolluted water. *Macrocyclops* died in water from 27% of the stations on those streets in April. Pollution was visibly greater during summer, autumn, and winter, when the percentage of stations with water that killed *Macrocyclops* fluctuated between 65% and 75%.

There was no conspicuous pollution at the second site when field observations began in April. *Macrocyclops* and *Gambusia* survived in all water samples taken from the second site at that time. Water from 15% of the stations—always the same stations and the only ones with conspicuous pollution—consistently killed *Macrocyclops* during summer, autumn, and winter.

**First Field Site**

*Macrocyclops* and fish were never observed on the most heavily polluted street at the first field site (except a small number of *Macrocyclops* at one end of the street in April). Sixty-two percent of the sampling stations on that street had >50 *Cx. quinquefasciatus* larvae/dip throughout the year. The other stations on that street also had larvae through almost all of the year (except August), though in lower numbers. The following results are based on the other three streets at the first site, where pollution from septic tanks did not completely exclude *Macrocyclops* and *Gambusia*.

Forty percent of the stations on those three streets had natural *Macrocyclops* populations when observations began in April (Fig. 1). Only one station had fish in April. Most stations with *Macrocyclops* had no *Cx. quinquefasciatus* larvae; the average number of larvae at stations with *Macrocyclops* was 1.9 larvae/dip (SE = 0.5, N = 16). Most stations without *Macrocyclops* had large numbers of *Cx. quinquefasciatus* larvae in April. The average for all stations without *Macrocyclops* was 30.8 larvae/dip (SE = 8.0, N = 22).

*Gambusia* spread through all three streets during May and June, occupying 35% of the sampling stations by early July (Fig. 1). *Macrocyclops* and *Cx. quinquefasciatus* larvae disappeared from each station as soon as *Gambusia* appeared. During July and August, *Gambusia* disappeared from some of the stations it had just invaded and then expanded briefly on one of the streets in early September. *Gambusia* remained at a smaller number of stations on all three streets through autumn and winter (Fig. 1).

Introduction of *Macrocyclops* at the end of April did not increase the subsequent distribution or abundance.

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**TABLE 2. Survival of Culex quinquefasciatus larvae in field predation experiments.**

<table>
<thead>
<tr>
<th>Number Introduced</th>
<th>Number of Surviving Larvae</th>
<th>Number of Surviving Larvae</th>
<th>Number of Surviving Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Macrocyclops</em></td>
<td><em>Macrocyclops</em></td>
<td><em>Macrocyclops</em></td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>1300</td>
<td>30 ± 14</td>
<td>614</td>
<td>30 ± 14</td>
</tr>
<tr>
<td>(0-99)</td>
<td></td>
<td></td>
<td>(0-99)</td>
</tr>
<tr>
<td>2600</td>
<td>74 ± 41</td>
<td>1,208</td>
<td>74 ± 41</td>
</tr>
<tr>
<td>(0-290)</td>
<td></td>
<td></td>
<td>(0-290)</td>
</tr>
</tbody>
</table>

1 Number of larvae estimated to have hatched from egg rafts placed in the enclosures.

2 Average number of larvae (±SE) in the 0.64 m² enclosures four days after introducing egg rafts. Range shown in parentheses.

3 Eight replicates for 1300 larvae introduced; seven replicates for 2600 larvae introduced.

4 Controls (one replicate for 1300 larvae and one replicate for 2600 larvae).
Figure 1. *Macrocyclops albidus*, *Gambusia affinis*, and *Culex quinquefasciatus* larvae in the ditches of three streets at the first site from April 1991 to April 1992. There was a patchwork of severe septic tank pollution on all three streets. The vertical arrow at the end of April indicates the introduction of *Macrocyclops* to two of the streets. Because most stations at this site had *Cx. quinquefasciatus* larvae almost all year, seasonal changes in larval abundance are best seen as average larvae/dip.

of *Macrocyclops* on the treated streets. *Macrocyclops* disappeared from all stations on both treatment and control streets by July (Fig. 1). A few *Macrocyclops* were observed during November to February. They reappeared in large numbers on one of the streets starting in March, occupying 36% of the stations on that street when observations terminated at the beginning of April.

Stations that had large numbers of *Cx. quinquefasciatus* larvae when observations started in April continued to have large numbers during summer, autumn, and winter if *Gambusia* was not present. The larval population at these stations dropped to zero in August when polluted parts of the ditches dried up. At the less polluted stations, which had *Macrocyclops* and
few *Cx. quinquefasciatus* larvae when observations began in April, there were no larvae during the summer, when *Gambusia* occupied nearly all these stations. The less polluted stations had small numbers of larvae on an intermittent basis during autumn and winter.

**Second Field Site**

There was a conspicuous negative association between the presence of *Gambusia* and the presence of *Cx. quinquefasciatus* larvae at the second site. Over the entire year of observation, *Cx. quinquefasciatus* larvae were present in 12.4% of the inspections at stations with neither *Gambusia* nor *Macrocyclops* (SE = 2.0%, N = 275, range at positive stations: 1-100 larvae/dip). In contrast, *Cx. quinquefasciatus* larvae were observed in only 1.5% of the inspections at stations where *Gambusia* was present (SE = 0.5%, N = 472, number of larvae at positive stations almost always <1 larva/dip). *Gambusia* was also associated negatively with *Macrocyclops*. While *Macrocyclops* was abundant enough to be detected by five dips during 37% of the inspections at stations without *Gambusia* (SE = 2.9%, N = 279), *Macrocyclops* was detected during only 18% of the inspections at stations where *Gambusia* was present (SE = 3.5%, N = 120).

*Gambusia* populations were low on all streets in the spring, but *Gambusia* spread through all the ditches during May and was at 71% of the stations by June. The fish continued at most of the same stations through the summer, being absent primarily from parts of the ditches that were heavily polluted or dry most of the time. *Gambusia* disappeared from most of the stations during September-October, even though they still had water. *Gambusia* repopulated the same stations by December and repeated the same sequence of disappearing and repopulating two more times during the period from January to April (Fig. 2).

There were natural *Macrocyclops* populations at 18% of the stations before *Macrocyclops* was introduced to two of the streets in early May. *Macrocyclops* populations were not increased by the introduction. *Macrocyclops* disappeared from all stations on both treatment and control streets by early June and none were seen again until September. *Macrocyclops* was observed at about 5% of the stations on all streets immediately before its introduction to two of the streets in October.

There were few *Cx. quinquefasciatus* larvae in the ditches at the second site when field observations began in April, and they were virtually absent from the ditches throughout the summer (always <1 larva/dip). *Cx. quinquefasciatus* larvae increased dramatically during September as *Gambusia* declined, and by early October they had large numbers (10-85 larvae/dip) at 7% of the sampling stations. They were present in smaller numbers at an additional 17% of the stations.

The abundance of *Macrocyclops* and *Cx. quinquefasciatus* larvae was distinctly different on treated and control streets after *Macrocyclops* introduction in October (Fig. 2). On control streets, the number of stations with *Macrocyclops* was about the same from October to February, and the same was true for *Cx. quinquefasciatus* larvae (1-25 larvae/dip) after a decline from October to November. The number of control stations with *Cx. quinquefasciatus* larvae did not decline again until *Macrocyclops* spread naturally through the ditches on one of the control streets during March.

The number of stations with *Macrocyclops* on treatment streets increased dramatically within a few weeks after *Macrocyclops* introduction (Fig. 2). From November to March *Macrocyclops* was recorded at three times as many stations on treatment streets compared to control streets. The difference between treatment and control streets during November-April was significant at *P* = .008 (*T* = 0, *N* = 7), using Wilcoxon's rank sum test for matched pairs (Rohlf and Sokal 1995, p. 135). During this period 32%-50% of the stations on the treatment streets had enough *Macrocyclops* to be detected by the inspection procedure of five dips per station. More intensive sampling in January revealed that 80-90% of the remaining stations also had *Macrocyclops*, but in numbers too small to be detected by five dips. Most stations with very low *Macrocyclops* populations had fish.

The treatment streets had twice as many stations positive for *Cx. quinquefasciatus* larvae compared to control streets during the first month after *Macrocyclops* introduction (Fig. 2). However, stations on the treatment streets that had large numbers of *Cx. quinquefasciatus* larvae during September-October experienced a dramatic drop in larval numbers as soon as *Macrocyclops* reached significant numbers in November. Many of those stations continued to have a small number of larvae until December. The remaining larvae, too large for *Macrocyclops* to kill, probably stayed in the ditches because of slow development at cool autumn/winter temperatures. By January there were no larvae on the treatment streets, even when *Gambusia* was absent from most stations (Fig. 2). Small numbers of larvae appeared at a few stations with *Macrocyclops* during March and April.

The impact of *Macrocyclops*, separate from *Gambusia*, can be evaluated by comparing *Cx. quinquefasciatus* at stations having only *Macrocyclops* (but no *Gambusia*) with stations that had neither predator. Using information from all four streets during November-
Figure 2. Populations of *Gambusia affinis*, *Macrocyclops albidus*, and *Culex quinquefasciatus* at the second site after introduction of *Macrocyclops* to two of the streets in October. Pollution on both treated and control streets was moderate and scattered. Because changes in larval numbers at a few highly polluted stations could dominate average larvae/dip, seasonal changes and the impact of *Macrocyclops* introduction on *Cx. quinquefasciatus* larvae at the more numerous less-polluted stations are best seen in terms of the number of stations positive for larvae.
April, 21.2% of the stations with neither *Macrocyclops* nor *Gambusia* had *Cx. quinquefasciatus* larvae (SE = 2.6%, N = 250). On the same streets during the same period, 5.8% of the stations with *Macrocyclops* (but not *Gambusia*) had *Cx. quinquefasciatus* larvae (SE = 2.0%, N = 137).

DISCUSSION

It is apparent from the field observations that *Cx. quinquefasciatus, Macrocyclops albidus,* and *Gambusia affinis* occupy the ditches in a dynamic patchwork that is shaped by seasonal weather fluctuations, ditch hydrology, pollution, and predator-prey relations. Predation by *Gambusia* is a dominant factor. *Cx. quinquefasciatus* larvae are virtually eliminated wherever and whenever *Gambusia* is present, and *Gambusia* suppresses (but does not eliminate) *Macrocyclops*. We consistently observed that if *Macrocyclops* was present on a street, it rapidly increased its numbers at a particular station when *Gambusia* disappeared.

*Macrocyclops* is also a significant predator, though the impact of *Macrocyclops* predation on *Cx. quinquefasciatus* larvae is not as absolute as *Gambusia*. The impact of *Gambusia* and *Macrocyclops* on *Cx. quinquefasciatus* production is limited by expansion and contraction of their populations through the ditches during the year. *Gambusia* is particularly limited in ditches that dry out frequently, and it disappears from many ditches during the winter. Both *Gambusia* and *Macrocyclops* are excluded from severely polluted water. Organic pollution is not responsible for excluding *Macrocyclops* because *Macrocyclops* thrives in the heavy organic pollution of the New Orleans Mosquito and Termite Control Board's mass production system for copepods, which is based on rotting wheat seed (Marten et al. 1997). Further evidence that organic pollution is not responsible came from the survival of *Macrocyclops* in some of the ditch water samples that appeared (and smelled) severely organically polluted. The fact that *Macrocyclops* was sometimes killed by ditch water that lacked noticeable organic pollution suggests that household chemicals are responsible.

Pollution from septic tanks is particularly important because it creates refuges where *Cx. quinquefasciatus* larvae are protected from predation by *Gambusia* or *Macrocyclops*. Although the complementary distribution of *Cx. quinquefasciatus* larvae vis-à-vis *Gambusia* and *Macrocyclops* is probably due in part to selection of organically polluted water by *Cx. quinquefasciatus* for oviposition, existence of the complementary distribution where water was not so severely polluted suggests it was also due to predation.

Natural factors—pollution, fish, and temperature—appear to limit the distribution of *Macrocyclops* during the summer. *Macrocyclops* is suppressed in some parts of the ditches by the expansion of *Gambusia* during early summer. Severe pollution from septic tank effluent is more extensive in the summer because of less water flow. Most ditch water is only a few inches deep during the summer and readily heats up during the day. *Macrocyclops* is killed by temperatures exceeding 37°C (Marten et al. 1994) and was observed to retreat to cooler water in shaded areas and culverts during the hottest times of the summer.

Dispersal seems to be the main factor limiting the distribution of *Macrocyclops* during autumn and winter. Pollution and water temperatures declined sufficiently by October for *Macrocyclops* to be able to live in many parts of the ditches, but natural restocking of the ditches took about six months, perhaps in part because polluted segments (and possibly *Gambusia*) obstructed copepod movement through the ditches. In contrast, *Macrocyclops* was abundant in nearly all suitable parts of the ditches within a few weeks after introduction in October.

Implications for Mosquito Control

What are the implications of this study for operational control of *Cx. quinquefasciatus*? First and foremost is the significance of pollution. Not only does pollution create an abundant supply of bacterial food for *Cx. quinquefasciatus* larvae, it also excludes natural predators. The most obvious and effective way to reduce *Cx. quinquefasciatus* production is to eliminate pollution so predators can provide natural control. Further study could verify whether household chemicals are in fact responsible for excluding copepods from severely polluted parts of the ditches, and if so, which specific chemicals.

Unfortunately, ditch pollution is a fact of life in many places. While the heaviest *Cx. quinquefasciatus* production comes from severely polluted sites where only a larvicide can eliminate production, production from the many kilometers of less polluted segments can also be substantial in the aggregate. It is in these places that appropriately timed introduction of fish and copepods—October for *Macrocyclops* in Louisiana—could reduce the need for larviciding by facilitating natural control. Copepods can help to fill the gap when and where fish are absent. In ditches treated with a larvicide, copepods can help to kill larvae missed by the larvicide.

The lag in the decline of *Cx. quinquefasciatus* larvae observed after introducing *Macrocyclops* in October (Fig. 2) could be eliminated by applying BTI
simultaneously and following up with a single BTI application once the copepod population has built up its numbers (early November). Because BTI kills mosquito larvae of all sizes, it can eliminate larvae that are too large for copepods to kill, and the copepods will prey on new larvae after that. BTI has no detrimental effect on copepods (Marten et al. 1993).

The practical utility of *Macrocylops* introductions remains to be seen. It is clear that *Macrocylops* does not eliminate *Cx. quinquefasciatus* larvae with the same completeness that it eliminates *Aedes* larvae in containers. At best, copepods could fill a specific and limited role in a comprehensive package of integrated *Cx. quinquefasciatus* control. Though millions of copepods can be produced at reasonably low cost (Marten et al. 1997), only further experience will show whether the benefits from introductions are sufficient to justify the cost and effort.

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A Mark-Release-Recapture Study on the Spatial Distribution of Host-Seeking Anophelines in Northern Thailand

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ABSTRACT: A mark-release-recapture experiment was conducted at Mae Taeng, Chiangmai, Thailand, in November 1990 to examine the movement of released host-seeking mosquitoes in a heterogeneous environment. A total of 1,848 mosquitoes of nine anopheline species was field-collected, marked with fluorescent dye, and released. Adult collections were made for four nights after the release at five collection sites surrounding the release site. Three different attractants, dry ice (5 kg/site/night), human, and cow bait were used to collect a large number and a wide variety of adult mosquitoes. The recapture rate of released mosquitoes differed among species, ranging from 0 to 7.5%. The species composition was significantly different among collection sites and collection methods, and samples from dry ice collection showed intermediate species composition between those from human and cow bait collections. The spatial distribution of released mosquitoes was not significantly different from that of unmarked mosquitoes. Different behavioral responses to heterogeneous environments by different species of host-seeking mosquitoes was suggested as the underlying mechanism of species-specific spatial distribution of mosquitoes in the study area.

Keyword Index: Mark-release-recapture, spatial distribution, anophelines, mosquitoes, Thailand.

INTRODUCTION

There is often a spatial variation among sites for the number of host-seeking mosquitoes collected (Service 1993, Smith et al. 1995, Hii et al. 1997, Takagi et al. 1995), and depending on the size of the study area, within- and between-habitat variations can be distinguished. Takagi et al. (1995) observed four different types of frequency distributions of anopheline mosquitoes in northern Thailand. The topography, land use, and vegetation types of their study area differed among adult collection sites; and the observed difference in frequency distribution was considered to be due to spatial variation between habitats. Spatial variations within a single village and small-scale spatial variation were examples of within-habitat variation.

The present study was conducted to better understand the within-habitat spatial variation of host-seeking anophelines at Mae Taeng, northern Thailand, where the between-habitat variation was previously examined by Takagi et al. (1995). Several factors relate to the within-habitat spatial variation, such as behavior of host-seeking mosquitoes, location of breeding habitats, and the distribution of animal hosts. Because of the small size of the study area, this study focused on the behavior of host-seeking mosquitoes as the main factor responsible for the within-habitat spatial variation. A mark-release-recapture experiment was conducted to compare the spatial distribution of released mosquitoes with that of unmarked mosquitoes. If the behavior of host-seeking mosquitoes was the major factor responsible and the released mosquitoes showed a normal host-seeking behavior, the spatial distribution of recaptured mosquitoes should be the same as that of unmarked mosquitoes.

The numbers and the species composition can
differ greatly depending on the attractant used for adult collections (Service 1993). In this study, a combination of three different attractants (human bait, cow bait, and dry ice) was used to collect a large number and a wide variety of mosquitoes. The proportion of each species to the total number collected was compared among samples from different attractants and collection sites, and the host preference of the species was determined.

MATERIALS AND METHODS

The study was carried out during November 1990 at Mae Taeng district, approximately 50 km north of Chiangmai, Thailand. The environmental condition of the village was described in Takagi et al. (1995). Our study area was a small flat area around 1 km² surrounded by hills and corresponded to “station 6” of Takagi et al. (1995).

Mosquito Collection and Mark-Release

Mosquitoes used for the mark-release-recapture experiment were collected using human bait (3 persons) and four cow baited nets at four collection sites for successive three nights. Except for the Anopheles hyrcanus group, the mosquitoes were identified to species and kept in cages with a cotton pad soaked with a 5% sugar solution under humid conditions until the mark-release day. All the collected mosquitoes were marked with a 0.5% water solution of Rhodamine B 5 hr. before release. To minimize any disturbance of normal mosquito activity, the marked mosquitoes were transferred to a large mosquito net (1.5 x 2 x 2m) at the release site at 17:00 hr. and kept under semi-natural conditions for 1.5 hr. before being released 30 min. before sunset.

Recaptures were made at five collection sites surrounding the release site on the night of release and on three subsequent nights. We chose the collection sites with clearly different surrounding environments so that the observed differences in number and species composition of collected mosquitoes were largely ascribed to the environmental differences. The environmental conditions of the five collection sites differed as follows. Site 1 (HL) was located on a small hill covered with grasses and shrubs, without trees and human dwelling, 250 m from the release site. Site 2 (PG) was located at the base of a small hill, an isolated house and a pigsty present, and many fruit trees were planted around the house, 250 m from the release site. Site 3 (PP) was located at the foot of a small hill on which banana, papaya, and some large fruit trees were planted. One house was present, and the distance from the release site was 100 m. Site 4 (RE) was located at the opposite side of site 1 (HL), many fruit trees and shrubs were planted, and distance from the release site was 100 m. Site 5 (RV) was located near the stream, which was the main breeding site of An. minimus in this area. A secondary forest with dense vegetation was present behind this site and there were no houses present. The distance from the release site was 200 m.

In the human bait collection, three persons sat together and biting mosquitoes were aspirated off the hosts for 50 min./hr. from 19:00 to 24:00 hr. In the cow bait collection, one cow was tethered in a double net trap that had a small net (4 x 4 x 2 m) inside of a large net (6 x 6 x 2 m), the latter having the lower edges pulled up and fixed at 50 cm above the ground. At 30-min. intervals from 19:00 to 24:00 hr., a collector aspirated mosquitoes found between the two nets for 10 min. One mosquito net (1.5 x 2 x 2m) was constructed on the ground and 5 kg of dry ice was placed inside the net at 1 m above the ground. Mosquitoes attracted to dry ice were collected similarly as for the cow baited collection. The distance between the attractants was 10–15 m. All collected mosquitoes were identified and counted by species. Mosquitoes were placed on filter paper, rinsed with 80% alcohol, and examined for color under UV light to check for the presence of dye.

Data Analysis

The total number of collected mosquitoes was calculated for each species by collection site and method, and variation in species composition among 15 samples (5 sites x 3 methods) was analyzed by a Chi-square test. Five samples from the same collection method were used for the comparison of species composition among collection sites. The differences in species composition among three collection methods were analyzed using the three samples from the same collection site. To examine the dissimilarity of the species composition among the 15 samples, the proportion of each species to the total number was calculated for each sample, and cluster analysis was conducted. Using Euclidean distance as a dissimilarity measure (Everitt 1993), six different algorithms (Single, Average, Complete, Ward, Centroid, and Median) were tried, and the first four of them showed the same result of clustering. Therefore, the result from Ward minimum variance method was shown in this paper as a representative.

The spatial distribution of recaptured mosquitoes was compared with that of unmarked mosquitoes. Because of the small number of recaptured mosquitoes, samples from three collection methods were pooled and used in the following analysis. Released mosquitoes were distributed according to the spatial distribution of unmarked mosquitoes, and the calculated number of released mosquitoes at each collection site was multiplied.
by the recapture rate of each species to get the expected number of recaptures at each collection site for each species. The difference between the expected and the observed number was tested by the Chi-square test. Except for the dominant species, the total number of recapture for each species was not large enough to carry out the chi-square test, therefore, all species were pooled in the analysis.

The statistical analysis was performed using the SYSTAT statistical software package (Wilkinson 1996).

RESULTS AND DISCUSSION

A total of 11,745 anopheline mosquitoes of 11 species was collected during the study (TABLE 1). The species compositions of the samples were significantly different among collection sites and among collection methods (TABLE 2). Based on the dissimilarity in species composition among 15 samples, a cluster tree was calculated in Figure 1 using the Ward minimum variance method. Three clusters were distinguished. The first cluster was composed of three samples of human bait collection at HL, PP, and RE. The second cluster included four samples: one dry ice sample at HL and three samples from cow bait collection at HL, PP, and RV. The remaining eight samples were included in the third cluster. The location of three clusters in the cluster tree indicated that the species composition of dry ice collection was intermediate between human and cow bait collections. Because CO₂ is a common attractant for host-seeking mosquitoes (Gillies 1980, Service 1993), the species composition of the dry ice collections overlapped with that of human bait collections as well as cow bait collections. The proportion of six major species was calculated for each sample and shown in Figure 2. The samples were arranged according to the order shown in Figure 1. Figure 2 suggested a difference in the host preference among the six major species. The proportion of An. minimus was higher in human bait collections than in cow bait collections showing a high preference toward humans. In contrast, An. hyrcanus gr., An. annularis, and An. nivipes showed preferences toward cows. As the malaria vector in our study area, Takagi et al. (1995) reported the following four species, An. minimus, An. aconitus, An. maculatus and An. annularis. The high preference toward humans and the relatively high density of An. minimus shown in Figure 2 and TABLE 1 suggested that this species was the most important malaria vector in our study area. Although An. annularis showed low preference toward humans, this species was more important than An. aconitus and An. maculatus because of the high density in our study area.

Different spatial distributions were observed among mosquito species in TABLE 1. For example, approximately 46% of An. hyrcanus gr. and 51% of An. barbirostris were collected at HL, whereas, the proportions of An. aconitus ranged between 17 and 22% among the five collection sites. These differences in spatial distributions of host-seeking mosquitoes resulted in significant differences in species compositions among the collection sites (TABLE 2).

A total of 1,848 mosquitoes of nine anopheline species was marked and released. The recapture rate differed among species ranging from 0 to 7.5% with an overall recapture rate of 4.1%. A mark-release-recapture study using unfed An. minimus by Nutsathapana et al. (1986) in a neighboring area of this study reported a recapture rate of 0.7 to 3%.

The spatial distribution of recaptured mosquitoes was compared with that of unmarked mosquitoes to explain the species-specific spatial distribution of host-seeking mosquitoes. Various factors can affect the spatial distribution of host-seeking mosquitoes, such as the locations of breeding sites, resting sites, and host animals, and the topographical and vegetational heterogeneity of the environment. If marked and unmarked mosquitoes showed the same spatial distributions, it may suggest that some of the factors which can affect the host-seeking behavior of mosquitoes are more important to explain the spatial distribution of host-seeking mosquitoes in our study area because the spatial distribution of marked mosquitoes was determined mainly through the movement of the released mosquitoes.

Using the spatial distribution of unmarked mosquitoes and the recapture rate of each species, the expected number of recaptured mosquitoes at each collection site was calculated and shown in TABLE 4. The differences between the expected and observed numbers of recaptured mosquitoes were not significant.

Taking into account the small size, the absence of topographic barriers in our study area, and the flight ability of anopheline mosquitoes (Service 1993, 1997, Nagpal and Sharma 1995), host-seeking mosquitoes may search the whole study area for their host animals. When the host-seeking mosquitoes are exposed to a heterogeneous environment, their movement may differ due to the different behavioral responses to the environment among mosquito species. Therefore, one of the underlying mechanisms of the different spatial distribution of host-seeking mosquitoes observed in this study was considered to be the behavioral response of host-seeking mosquitoes to heterogeneous environments (Billingmayer 1971, Muirhead-Thomson 1982).

The environmental conditions of our collection
Figure 1. Cluster analysis of the dissimilarity of species composition among 15 samples collected by three different methods at five different sites at Mae Taeng, Chiangmai, Thailand. HL, PP, RE, RV, and PG are five collections sites and HB, DI, CW refer to human bait, dry ice, and cow bait collections, respectively.

Figure 2. Compositions of six major anopheline mosquitoes in 15 samples collected at Mae Taeng, Chiangmai, Thailand. The samples were arranged according to the order shown in the result of cluster analysis. niv: *An. nivipes*, ann: *An. annularis*, hyr: *An. hyrcanus* gr., bar: *An. barbirostris*, aco: *An. aconitus*, min: *An. minimus*. 
TABLE 1. Total numbers of adult anopheline collected by three different methods for four nights at five collection sites in November 1990, Mae Taeng, Chiangmai, Thailand.

<table>
<thead>
<tr>
<th>Species</th>
<th>Site 1 (HL)</th>
<th>Site 2 (PG)</th>
<th>Site 3 (PP)</th>
<th>Site 4 (RE)</th>
<th>Site 5 (RV)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO_2</td>
<td>Cow</td>
<td>H.B.</td>
<td>Tl</td>
<td>CO_2</td>
<td>Cow</td>
</tr>
<tr>
<td>An. aconitus</td>
<td>145</td>
<td>429</td>
<td>17</td>
<td>591</td>
<td>326</td>
<td>351</td>
</tr>
<tr>
<td>An. hyrcanus gr.</td>
<td>129</td>
<td>801</td>
<td>16</td>
<td>946</td>
<td>99</td>
<td>104</td>
</tr>
<tr>
<td>An. annularis</td>
<td>90</td>
<td>332</td>
<td>8</td>
<td>430</td>
<td>167</td>
<td>135</td>
</tr>
<tr>
<td>An. minimus</td>
<td>58</td>
<td>267</td>
<td>52</td>
<td>377</td>
<td>188</td>
<td>122</td>
</tr>
<tr>
<td>An. nivipes</td>
<td>44</td>
<td>191</td>
<td>7</td>
<td>242</td>
<td>58</td>
<td>62</td>
</tr>
<tr>
<td>An. barbifronsis</td>
<td>47</td>
<td>577</td>
<td>21</td>
<td>645</td>
<td>20</td>
<td>65</td>
</tr>
<tr>
<td>An. vagus</td>
<td>1</td>
<td>49</td>
<td>0</td>
<td>50</td>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>An. kochi</td>
<td>9</td>
<td>39</td>
<td>3</td>
<td>51</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>An. tessellatus</td>
<td>8</td>
<td>41</td>
<td>2</td>
<td>51</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>An. maculatus</td>
<td>6</td>
<td>20</td>
<td>3</td>
<td>29</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>An. splendidus</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>538</td>
<td>2,752</td>
<td>129</td>
<td>3,419</td>
<td>883</td>
<td>888</td>
</tr>
</tbody>
</table>

1) Dry ice collection
2) Cow bait collection
3) Human bait collection


**TABLE 2.** Results of comparisons of species composition among collection sites and among collection methods.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>( \chi^2 )</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among Collection Sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry ice collection</td>
<td>306.67</td>
<td>40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cow bait collection</td>
<td>1725.26</td>
<td>40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Human bait collections</td>
<td>153.91</td>
<td>40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Among Collection Methods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 1</td>
<td>221.81</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Site 2</td>
<td>82.70</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Site 3</td>
<td>397.65</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Site 4</td>
<td>66.32</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Site 5</td>
<td>226.38</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**TABLE 3.** Number of released and recaptured mosquitoes and the recapture rate.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number Released</th>
<th>Number Recaptured</th>
<th>Recapture Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>An. annularis</td>
<td>667</td>
<td>31</td>
<td>0.046</td>
</tr>
<tr>
<td>An. aconitus</td>
<td>318</td>
<td>11</td>
<td>0.035</td>
</tr>
<tr>
<td>An. hyrcanus gr.</td>
<td>232</td>
<td>11</td>
<td>0.047</td>
</tr>
<tr>
<td>An. nivipes</td>
<td>207</td>
<td>10</td>
<td>0.048</td>
</tr>
<tr>
<td>An. minimus</td>
<td>197</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>An. barbirostris</td>
<td>143</td>
<td>7</td>
<td>0.049</td>
</tr>
<tr>
<td>An. vagus</td>
<td>40</td>
<td>3</td>
<td>0.075</td>
</tr>
<tr>
<td>An. kochi</td>
<td>27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>An. maculatus</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1848</strong></td>
<td><strong>75</strong></td>
<td><strong>0.041</strong></td>
</tr>
</tbody>
</table>

**TABLE 4.** Observed and expected number of recaptured females at five collection sites.

<table>
<thead>
<tr>
<th>Collection Site</th>
<th>An. annularis</th>
<th>Other species</th>
<th>All species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expected</td>
<td>Observed</td>
<td>Expected</td>
</tr>
<tr>
<td>HL</td>
<td>7.41</td>
<td>8</td>
<td>13.61</td>
</tr>
<tr>
<td>PG</td>
<td>5.30</td>
<td>6</td>
<td>6.01</td>
</tr>
<tr>
<td>PP</td>
<td>4.39</td>
<td>5</td>
<td>7.37</td>
</tr>
<tr>
<td>RE</td>
<td>3.69</td>
<td>2</td>
<td>5.61</td>
</tr>
<tr>
<td>RV</td>
<td>10.21</td>
<td>10</td>
<td>11.40</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>31</strong></td>
<td><strong>31</strong></td>
<td><strong>44</strong></td>
</tr>
</tbody>
</table>

\[ \chi^2 \] = 0.999  \quad 3.903  \quad 1.308

\[ p \] = 0.910  \quad 0.419  \quad 0.860
sites differed in many aspects, such as vegetation, topography, locations of breeding sites, and blood sources, etc. Analytical studies on the relationship between these environmental factors and mosquito density are needed to understand and predict the spatial distribution of mosquitoes in a heterogeneous environment.

REFERENCES CITED


224 pp.


Winter Ectoparasites of Mammals in the Northeastern Piedmont Area of Georgia

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ABSTRACT: Twenty-three species of ectoparasites were recovered from 10 species of mammals (89 individuals) in Mistletoe State Park, Columbia County, Georgia, during three winter months of 1998. The collection consisted of 9 species of fleas, 6 mites, 4 sucking lice, 3 ticks, and 1 mammal-associated beetle. Some rarely-collected arthropods were recorded including the ctenophthalmid fleas, Doratopsylla blarinae C. Fox and Epitedia cavernicola Traub, the laelapid mite, Echinonyssus blarinae (Herrin) (first records from Georgia for these three ectoparasites), and the leptinid beetle, Leptinus orientamericanus Peck (second record for Georgia). Varying degrees of host-specificity were exhibited by the 23 species of ectoparasites. Because relatively few mammal ectoparasite surveys are completed during the winter months, some seemingly rare species may, in reality, be more common at this time of year.

Keyword Index: Ectoparasites, mammals, Piedmont Georgia, new state records.

INTRODUCTION

Most ectoparasite surveys of mammals in North America have documented host-parasite associations during the warmer months of the year. The relatively small number of winter ectoparasite surveys in this region have, however, typically shown that different ectoparasite species or different population densities of the same species characterize winter collections (e.g., Morlan 1952, Reisen et al. 1976).

In southern Georgia, where summers are typically hot, some ectoparasites including fleas are infrequently recovered from wild mammals during this season (Morlan 1952, Pung et al. 1994, Durden 1995). For this reason, we decided to survey ectoparasites of mammals during the winter months in Georgia in conjunction with a live-trapping program related to Lyme disease field studies. We hypothesized that certain ectoparasites, especially fleas, would be well represented in our samples and that we might recover some infrequently collected species of ectoparasites.

MATERIALS AND METHODS

Animal sampling was conducted at Mistletoe State Park which is located in Columbia County in the northeastern Piedmont area of Georgia (Fig. 1). The park is characterized mainly by deciduous forest and is heavily used as a recreational facility.

All animals were live-captured in January, November, or December, 1998. Sherman (H.B. Sherman Traps, Inc. Tallahassee, Florida) and Tomahawk (Tomahawk Live Trap Co., Tomahawk, Wisconsin) traps baited with a mixture of rolled oatmeal and birdseed were set in the afternoon at various sites within the park and checked early the next morning. Captured animals were brought back to the laboratory for sampling.

In the laboratory, animals were removed from the traps and anesthetized with a 1:10 mixture of xylazine (Mobay, Animal Health Division, Shawnee, Kansas): ketamine hydrochloride (Avenco Co. Inc., Fort Dodge, Iowa) injected intramuscularly. Ectoparasites were recovered from these animals by combing back the fur and then collecting them with fine jeweler's forceps. Ticks recovered were kept alive in humidified chambers for an ongoing Lyme disease study. Other ectoparasites were stored in appropriately labeled vials filled with 70% ethanol for subsequent species identification. All the host animals were kept alive and housed in an animal facility for the Lyme disease project. All procedures dealing with animals were approved by the Institutional Animal Care and Use Committee and the Institutional Biosafety Committee. The approvals and the State Collecting Permits are on file in J. H. Oliver's office at
Georgia Southern University.

Ectoparasites collected were identified to species and stage. Live ticks were identified using a binocular microscope. Some alcohol-stored, uncleared fleas, sucking lice, and mesostigmatid mites were also identified using a binocular microscope. Other fleas and sucking lice were cleared in 10% potassium hydroxide and slide-mounted in Canada balsam, whereas, mite specimens were cleared in lactophenol and mounted in Hoyer's medium before they were identified using phase-contrast microscopy. All specimens are deposited in the collection at the Institute of Arthropodology and Parasitology, Georgia Southern University.

RESULTS

A total of 89 mammals representing 10 mammal species was captured at Mistletoe State Park during the study period. These consisted of 50 field mice, Peromyscus spp.; 17 cotton rats, Sigmodon hispidus Say and Ord; 6 eastern wood rats, Neotoma floridana (Ord); 5 southern short-tailed shrews, Blarina carolinensis (Bachman); 4 golden mice, Ochrotomys nuttalli (Harlan); 3 southern flying squirrels, Glaucomys volans (L.); 2 eastern gray squirrels, Sciurus carolinensis Gmelin; 1 pine vole, Microtus pinetorum (Le Conte); and 1 Virginia opossum, Didelphis virginiana Kerr. Populations of Peromyscus gossypinus (Le Conte) and Peromyscus leucopus (Rafinesque) overlap within the study area and it was difficult to accurately identify some of these mice to species. However, we definitively identified 15 P. gossypinus and 11 P. leucopus among those captured; the remaining 24 mice, some of which were immatures, could not be identified to species.

Twenty-three ectoparasite species were recovered from the 89 mammals captured during the study (TABLE 1). These consisted of 3 species of ticks, 6 mites, 9 fleas, 4 sucking lice, and 1 beetle. TABLE 1 includes host-parasite association data and infestation parameters for the 23 species of ectoparasites.

DISCUSSION

As we had hypothesized, a relatively rich community of 23 ectoparasite species (TABLE 1) was collected from a modest number (89) of mammalian hosts during the winter months (January, November, and December) at one site in the northeastern Piedmont area of Georgia. Although we have not surveyed rodent ectoparasites at this precise location during the summer, other studies have shown that relatively depauperate ectoparasite faunas are found on rodents in Georgia during the warmer months (Morlan 1952, Pung et al. 1994, Durden 1995).
TABLE 1. Winter ectoparasites recovered from 89 mammals at Mistletoe State Park, Columbia County, Georgia, 1998.

<table>
<thead>
<tr>
<th>Host Species</th>
<th>Total Collected</th>
<th>Ectoparasites*</th>
</tr>
</thead>
</table>
| *Peromyscus* spp.  | 50              | Tick  
|                    |                 | *Dermacentor variabilis* (32%, 1.0±2.4, 10N, 42L)  
|                    |                 | Fleas  
|                    |                 | *Peromyscopsylla scotti* (50%, 1.2±1.8, 6M, 54F)  
|                    |                 | *Stenoponia americana* (22%, 0.5±1.4, 12M, 12F)  
|                    |                 | *Epitedia wenmanni* (14%, 0.1±0.4, 5M, 5F)  
|                    |                 | *Peromyscopsylla hesperomys* (4%, 0.1±0.3, 3F)  
|                    |                 | Sucking louse  
|                    |                 | *Hoplopleura hesperomydis* (2%, 0.1±0.4, 1M, 1F, 1N)  
|                    |                 | Mites  
|                    |                 | *Androlaelaps fahrenholzi* (2%, 0.04±0.28, 2F)  
|                    |                 | *Ornithonyssus bacoti* (2%, 0.02±0.14, 1F)  
| Sigmodon hispidus  | 17              | Tick  
|                    |                 | *Dermacentor variabilis* (12%, 0.5±1.7, 3N, 5L)  
|                    |                 | Fleas  
|                    |                 | *Polygenus gwyni* (59%, 1.5±2.3, 6M, 20F)  
|                    |                 | *Peromyscopsylla scotti* (6%, 0.1±0.2, 1F)  
|                    |                 | *Ctenophthalmus pseudagyrtes* (6%, 0.1±0.2, 1M)  
|                    |                 | Sucking louse  
|                    |                 | *Hoplopleura hirsuta* (18%, 0.2±0.6, 1M, 2F, 1N)  
|                    |                 | Mites  
|                    |                 | *Androlaelaps fahrenholzi* (35%, 1.4±2.5, 22F, 1N)  
|                    |                 | *Prolistrophorus bakeri* (18%, 64.7±161.8, ca. 1100)  
|                    |                 | *Radfordia sigmodontis* (6%, 0.1±0.2, 1F)  
| Neotoma floridana  | 6               | Fleas  
|                    |                 | *Epitedia cavernicola* (17%, 0.2±0.4, 1F)  
|                    |                 | *Polygenus gwyni* (17%, 0.2±0.4, 1F)  
| Blarina carolinensis| 5               | Flea  
|                    |                 | *Doratopsylla blarinae* (20%, 0.2±0.5, 1M)  
|                    |                 | Mite  
|                    |                 | *Echinonyssus blarinae* (20%, 0.4±0.9, 2F)  
|                    |                 | Beetle  
|                    |                 | *Leptinus orientamericanus* (20%, 0.2±0.5, 1M)  
| Ochrotomys nuttalli| 4               | Tick  
|                    |                 | *Dermacentor variabilis* (25%, 0.3±0.5, 1L)  
|                    |                 | Fleas  
|                    |                 | *Peromyscopsylla scotti* (25%, 0.5±1.0, 1M, 1F)  
|                    |                 | *Epitedia wenmanni* (25%, 0.3±0.5, 1F)  
| Glaucomys volans    | 3               | Flea  
|                    |                 | *Orchopeas howardi* (33%, 9.0±7.9, 15M, 12F)  

Table 1 continued on next page.
Table 1 continued

<table>
<thead>
<tr>
<th>Host Species</th>
<th>Total Collected</th>
<th>Ectoparasites*</th>
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</thead>
<tbody>
<tr>
<td><em>Sciurus carolinensis</em>*</td>
<td>2</td>
<td>Tick</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Amblyomma maculatum</em> (50%, 1L)</td>
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<td></td>
<td></td>
<td>Flea</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Orchopeas howardi</em> (100%, 1M, 5F)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sucking lice</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Hoplopleura sciuricola</em> (50%, 9M, 31F, 10N)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Neohaematopinus sciuri</em> (50%, 1M, 4F, 3N)</td>
</tr>
<tr>
<td><em>Microtus pinetorum</em>*</td>
<td>1</td>
<td>Flea</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Ctenophthalmus pseudagyrites</em> (8M, 8F)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mites</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Androlaelaps fahrenholzi</em> (4F, 1N)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Haemogamasus longitarsus</em> (1F)</td>
</tr>
<tr>
<td><em>Didelphis virginiana</em>*</td>
<td>1</td>
<td>Tick</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Ixodes scapularis</em> (2F)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses represent prevalence (% infested). Mean±SD per host, and total number collected by stage, respectively; M=Male(s), F=Female(s), N=Nymph(s), L=Larva(e).

**For hosts with two or less captures, means are omitted; for hosts with one capture, prevalences are also omitted.

Fleas were especially well represented (9 species), and as also hypothesized, some infrequently encountered species were recovered. These include the ctenophthalmid fleas, *Doratopsylla blarinae* C. Fox and *Epitedia cavernicola* Traub, both of which are recorded in Georgia for the first time. *Doratopsylla blarinae* typically parasitizes *Blarina* spp. short-tailed shrews, and *E. cavernicola* is a nest flea associated with eastern woodrats (Durden and Kollars 1997). Both of these fleas appear to be more common during the cooler winter months (Benton 1980).

Other fleas collected include several species that mainly parasitize *Peromyscus* spp. mice and occasionally golden mice, as they did in this survey, i.e., the ctenophthalmids *Epitedia wenmanni* (Rothschild) and *Stenoponisa americana* (Baker), and the leptopsyllids *Peromyscopsylla hesperomys* (Baker) and *Peromyscopsylla scotti* I. Fox (Durden and Kollars 1997). The rhopalopsyllid flea *Polygenus gwnyi* (C. Fox) was principally collected from its main host, the cotton rat (Durden et al. 1999) but also from an eastern woodrat. The ctenophthalmid flea *Ctenophthalmus pseudagyrites* Baker parasitizes many species of insectivores and small rodents (Durden and Kollars 1997) and was recovered from a pine vole and a cotton rat in our survey. As expected, the squirrel flea *Orchopeas howardi* (Baker) was recovered from both the eastern gray squirrel and the southern flying squirrel.

Mites were the next most frequently encountered ectoparasites with respect to the number of species recovered (Table 1). The commonly collected laelapid, *Androlaelaps fahrenholzi* (Berlese), was recovered from *Peromyscus* spp., *S. hispidus*, and *M. pinetorum*, reflecting the wide host range of this mite (Whitaker and Wilson 1974). Other laelapids collected were *Haemogamasus longitarsus* (Banks) from a pine vole and *Echinonyssus blarinae* (Herrin) (1st record from Georgia for this species) from a southern short-tailed shrew. The former mite species has been recovered from several species of small mammals whereas, the latter appears to be host specific to *Blarina* spp. (Whitaker and Wilson 1974, Whitaker et al. 1994). Other mites recorded were the tropical rat mite, *Ornithonyssus bacoti* (Hirst), from *Peromyscus* and both the listrophorid, *Prolistrophorus bakeri* (Radford), and the myobiid, *Radfordia sigmodontis* Radford, from cotton rats. The tropical rat mite parasitizes many species of mammals but the last two listed mite species are relatively host specific to *S. hispidus* (Whitaker and Wilson 1974).

Four species of sucking lice (Anoplura) were collected during this survey and all are host specific to varying degrees (Durden and Musser 1994). *Hoplopleura hesperomysis* (Osborn) mainly parasitizes *Peromyscus* spp., *Hoplopleura hirsuta* Ferris parasitizes...
S. hispoidus and some related Sigmodon spp., the Holarctic Neohaematopinus scutari Jancke parasitizes S. carolinensis in North America and Sciurus vulgaris L. in Europe, and Hoplopleura scutariola Ferris parasiizes several species of New World tree squirrels (Kim et al. 1986, Durden and Musser 1994). These host associations were also noted in this survey (Table 1).

We recovered three species of ticks: adults of the blacklegged tick, Ixodes scapularis Say from a Virginia opossum, a larval Gulf Coast tick, Amblyomma maculatum Koch from a gray squirrel, and immature stages of the American dog tick, Dermacentor variabilis (Say) from Peromyscus spp., S. hispoidus, and O. nuttali (Table 1). The winter phenomena of these stages of I. scapularis and D. variabilis in Georgia have been reported previously (Durden and Oliver 1999). Significantly, I. scapularis is a vector of the agent of Lyme borreliosis, whereas, D. variabilis is a vector of the agent of Rocky Mountain spotted fever, two important zoonoses in North America (Oliver 1996a, b, Felz and Durden 1998).

One non-parasitic mammal/mammal nest associated beetle, the lephtid Leptinus orientamericanus Peck, was collected from a B. carolinensis specimen during this survey. Although this appears to be only the second record of this beetle in Georgia, it is widespread in the eastern United States (Peck 1982) and probably is not rare in Georgia. It also has been recovered from B. carolinensis at a nearby location in South Carolina (Whitaker et al. 1994).

Overall, we recovered a relatively rich fauna of ectoparasite species from just 89 individual mammals during three winter months at one site in the Piedmont region of Georgia. Because some of the ectoparasites recovered have rarely or never been recorded in Georgia previously, we suggest that a slightly different, and infrequently sampled, ectoparasite fauna can be associated with mammals during the winter in temperate climates.

Acknowledgments

We thank Craig Banks and Andrew Kinsey for their assistance in field sampling. This study was supported in part by grant R 37Al-24899 from the National Institute of Allergy and Infectious Diseases.

REFERENCES CITED


Use of Water Drums by Humans and *Aedes aegypti* in Trinidad

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ABSTRACT: This report documents the results of a countrywide survey by county for *Aedes aegypti* (L.) mosquitoes found breeding in drums in Trinidad, West Indies. The island-wide mean number of positive drums was 8.1 ± 2.9 per county and the mean number of positive drums per house was 3.0 ± 0.7 and ranged from 1.6 in St. George East to 4.9 in St. Andrew/St. David. The Breteau index varied from 6.2 in St. Andrew/St. David to 52.5 in Victoria East, with the median figure of 29.5. The most common location of drums was under the eaves of houses (71.4%) in eight out of nine counties surveyed, and a high proportion (82.3%) was positive for *Ae. aegypti* at this location. Water stored in drums was used primarily for washing clothing (57.5%), with 6.6% for drinking, 12.9% for bathing, and 23.0% for miscellaneous purposes. An average of 68.4% of all drums at the nine sites were uncovered and were located under the eaves of houses, with 76.2% being filled under the eaves by rainfall. Tanks were the main potable water storage container found in all nine counties, with a mean number of 1.4 ± 0.6 per county. In our survey, all water drums were treated as if they were for potable use, but this had virtually no correspondence with the actual findings in each county per household. We conclude that vector control strategies based on insecticide treatment and source reduction need to be revised in light of the current findings.

**Keyword Index:** *Aedes aegypti*, dengue fever, breeding sites, water drums, vector control, Trinidad.

INTRODUCTION

Within the Caribbean and Latin American region *Aedes aegypti* (L.) is the primary vector of urban yellow fever and dengue fever, including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Christophers 1960). Over the last 20 years, *Ae. aegypti* eradication and control programs have been conducted throughout the Caribbean region (PAHO 1994), but in spite of these efforts, DHF has emerged as a serious public health problem (Pinheiro 1989). Dengue is now endemic throughout the Caribbean with the exception of the Cayman Islands, where the vector *Ae. aegypti* is absent (Nathan and Knudsen 1991, PAHO 1994).

Although the breeding habitats of *Ae. aegypti* in the Caribbean are well known and include both artificial and natural containers like water drums, rock holes, tree holes, tires, buckets, tin cans, flower pots, animal watering pans, and cisterns, (Nathan 1993, Chadee et al. 1998), few control programs have targeted these containers on a systematic basis. In Trinidad and Tobago the breeding habitats of *Ae. aegypti* include buckets, small cans, tubs, water drums, and tires with drums representing over 70% of the breeding sites in Trinidad (Chadee 1992) and over 35% in Tobago (Chadee 1990). Water drums are used to store water for drinking, washing, bathing, and other household needs. Therefore, in theory, control of this vector in water drums could be attained by the provision of an adequate water supply, eliminating two-thirds of the disease vectors, and possibly reducing the incidence of dengue cases (Focks and Chadee 1997). However, in Trinidad large sections of the human population live in rapidly expanding urban areas with inadequate water supplies due to rapid population growth and poor urban planning (Halstead 1988, Tonn 1988). Consequently, water collection and storage in drums is a common practice especially where water supply is unreliable or where traditional water storage habits persist (Knudsen 1983, Nathan and Knudsen 1991, Focks and Chadee 1997).

During 1993-1996, the Insect Vector Control Division, Ministry of Health, Trinidad and Tobago, embarked on a pilot community-based program (Rosenbaum et al. 1995) to control *Ae. aegypti*
mosquitoes through health education, psychosocial approaches, community participation, chemical treatment, and source reduction strategies. Considerable experience was gained from this project in the development of strategies to reduce the incidence of *Ae. aegypti* in water drums in Trinidad. This study was conducted to understand the functional domestic role of water drums because programs aimed at removal and destruction of these sites may be unacceptable to householders.

**MATERIALS AND METHODS**

**Study Site and Date of Survey**

Trinidad is divided into eight counties (Fig. 1) but due to variable county size and unequal population distribution, some counties have been administratively merged for vector control purposes (St. Andrew and St. David into one and Nariva and Mayaro into another), while others have been subdivided (County St. George into St. George West, St. George Central, and St. George East and County Victoria into Victoria East and Victoria West). Using available data at the Insect Vector Control Division, a site with drums in each of nine areas was randomly selected for determination of number of drums, physical location of drums within the premises, and drums that were positive for *Ae. aegypti* immatures.

Trinidad experiences a wet season from May to November and a dry season from December to April. Our survey was conducted during the wettest portion of the year between September and October 1998.

**Survey Methodology**

All natural and artificial containers at more than 100 houses and compounds in each of the nine sites were inspected using the PAHO guidelines by senior staff attached to the Insect Vector Control Division, Ministry of Health, Trinidad (PAHO 1968). All indoor and outdoor containers, including natural habitats, were inspected to determine whether they were positive or negative. The total number of drums per premises, whether positive or negative, location of the drums on the premises, whether drums were covered or uncovered, the drum filling methodology, and use of water in each drum was recorded at each house.

All immatures in wet containers were collected using ladles and pipettes, placed into vials, labeled, and taken to IVCD laboratory where they were allowed to emerge for adult identification. The Breteau indices were calculated for each county using the number of *Ae. aegypti* infested containers per 100 houses. In addition, both house (percentage of houses infested) and container indices (percentage of containers infested) were calculated (PAHO 1968).

**RESULTS**

**Species Identification**

All immatures collected from water drums at the nine collection sites emerged and were identified as *Ae. aegypti*. No other associated mosquito species were identified during this study.

**Infestation Levels**

**TABLE 1** shows the number of houses surveyed in each county (site), the number of drums positive, the mean number of drums per house, the range, and the Breteau indices (number of *Ae. aegypti* infested containers per 100 houses). Between 109 and 130 houses were inspected per county. The number of drums per house ranged from 0 to 24. The mean number of drums containing *Ae. aegypti* immatures at the time of the inspection ranged from 1.6 to 4.9 while the Breteau index varied from 6.2 in St. Andrew/St. David to 52.5 in Victoria East, with the median figure of 29.5. The number of houses that were positive varied from 6 in St. George West to 28 in Victoria West while the number of positive drums ranged from 7 in St. George East to 57 in Victoria East.

**Island-Wide Distribution of Drums Among Households**

The drum locations were placed into five categories (Fig. 2). Low density locations included roadsides or along streets, under houses, and at indoor sites (0.3-12.6%). More common were drums located in the yard (9.1 to 41.4%) and the most frequent location of water drums (>50%) were under the eaves of houses in eight out of nine counties inspected (71.4%). The island-wide average density of *Ae. aegypti* positive drums was 8.1 ± 2.9 per county. The average number of positive drums per house was 3.0 ± 0.7 and ranged from 1.6 in St. George East to 4.9 in St. Andrew/St. David.

**Locations of Positive Drums**

Of the 255 drums found breeding *Ae. aegypti* immatures, 210 or 82.3% were found under the eaves of houses in all counties except St. George East where the distribution of drums in the yard and under eaves of houses were similar (Fig 2). In St. George East, however, more positive drums were found in the yard and under houses (70%) than under the eaves (29%).

**Use of Water in Drums**

The various functional uses of water in drums were classified into four categories (Fig. 1). Water in drums was used for drinking on an average of 6.6% of the times with the lowest incidence occurring in county St. Patrick.
Figure 1. Countrywide percentages of water stored in drums for drinking, washing, bathing, and for miscellaneous purposes in accordance with Insect Vector Control Division administrative divisions of the island of Trinidad.
(2.8%) and the highest in County St. George West (11.0%). While water in drums was used for bathing purposes on an average of 12.9% with the lowest incidence occurring in county St. George East (2.1%) and the highest in two counties St. George West (21.7%) and St. Andrew/St. David (22.4%). In addition, water in drums used for various or miscellaneous (e.g., watering plants) purposes were reported on an average of 23.0% in the various counties but with the lowest incidence in County St. George East (14.6%) and the highest in County Caroni (41.2%). However, the most common (G=25.6; df 3; P>0.01) use of water in drums was for washing clothing with an average of 56.8% in the various counties.

TABLE 3 shows the use of water in drums located indoors at all nine areas surveyed in Trinidad.

Of the 41 drums located indoors, 18 (43.9%) were used for drinking while the majority (56.1%) were used for bathing, washing (26.8%), and miscellaneous use (14.6%).

Use of Water Tanks

Tanks with a holding capacity in excess of 2,000 liters served as the main potable water storage container at all nine sites. The mean number of water tanks found at all sites was 1.4 ± 0.6, but with <5% of tanks found in county St. George West (4.4%), 5.1 to 10.0% of tanks found in counties St. George East (8.8%), St. Andrew/St. David (8.8%), Caroni (5.6%), and Victoria East (9.6%), 10.1 to 20.0% of tanks found in St. George Central (13.4%), Victoria West (12.1%), and St. Patrick (15.9%), and >21.0% of tanks found in Nariva/Mayaro (21.3%).

Island-Wide Water Drum Management

Uncovered water drums accounted for over 90% of the Ae. aegypti breeding recorded during our inspections. On average, 68.4% of all drums at the nine sites were uncovered, with the lowest incidence found in county St. George East (51.2%) and highest at two sites, county St. Patrick (88.2%) and county St. Andrew/St. David (89.8%). In contrast, an average of 31.6% of the water drums were covered, with the lowest incidence found in St. Andrew/St. David (10.2%) and St. Patrick (11.8%) and the highest incidence of covered drums at three sites, County St. George West (46.9%), St. George Central (48.8%), and St. George East (46.6%). Most drums were positioned under the eaves of houses (Fig. 2). On average 76.2% of drums were filled under eaves while in the rest of the sites drums were filled by rainfall (TABLE 2).

DISCUSSION

This study confirms that water drums are a major breeding focus of Ae. aegypti in Trinidad with 81.2% of the breeding sites coming from these containers. These results also suggest that from 1976 to the present, Ae. aegypti has not changed its oviposition preferences for water drums even though these containers are periodically recharged with water by man or by rainfall and are frequently treated with insecticides (Knudsen 1983, Chadee 1984, Chadee 1992).

The present study demonstrated that drums were most frequently located under the eaves of houses (Fig. 2; TABLE 2), a position which allows frequent overflowing during the rainy season, diluting the
Figure 2. Location of drums within premises in every Insect Vector Control Division administrative divisions of Trinidad. IN/HOUSE refers to drums located inside houses, U/HOUSE refers to drums located under houses built on stilts, U/EAVES refers to drums located under the eaves of the houses built on stilts and those at ground level. YARD refers to any other location within the premises not protected from the elements, and RD/SIDE refers to drums located along the roadside or street and generally found in front of houses.
insecticide concentrations when applied. Therefore, *Ae. aegypti* immatures occupying these drums may be exposed to sublethal concentrations of insecticides, leading to insecticide resistance, as reported by Vaughan et al. 1998 in Trinidad and by Rawlins (1998) in the Caribbean region. These findings are consistent with that reported by Chadee (1984) who demonstrated that drums treated with 1% temephos insecticide effectively controlled or killed *Ae. aegypti* immatures for only six days when the water in drums was used and refilled on a daily basis.

Our survey indicated that in Trinidad water stored in drums was not used for drinking (<10%) but rather for washing purposes (Fig. 2). Nathan and Knudsen (1991) also showed that within the Caribbean region drums provided the means of storing potable water in communities with inadequate pipe-borne water supply, while Barrera et al. (1993) reported in Venezuela that communities with “reasonably” adequate water supplies still stored water in drums as a precaution and that areas with and without an adequate water supply both had comparable *Ae. aegypti* house and container indices.

The present data suggest that water storage practices cannot be analyzed purely from the standpoint of regularity of tap-borne water supplies but rather in accordance with the functional use of the stored water. The fact that most residents at all nine sites inspected had both water tanks and drums suggests that water storage practice may have changed within recent years with tanks providing storage of potable water and drums providing storage of water for domestic use like washing and other miscellaneous purposes. This change in

<table>
<thead>
<tr>
<th>County</th>
<th>Total No. of Positive Drums</th>
<th>Drums Positive Under Eaves</th>
<th>Uncovered Drums Positive Under Eaves</th>
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<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
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<td>41</td>
</tr>
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<td><strong>TOTAL</strong></td>
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<th>County</th>
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<td>6</td>
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<td>0</td>
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<td>Caroni</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>3</td>
<td>15</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Nariva/Mararo</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>18</td>
<td>6</td>
<td>11</td>
<td>6</td>
<td>41</td>
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</table>
container utility may well explain the storage of water in drums in spite of an adequate supply of potable water in certain communities in Trinidad (Focks and Chadee 1997) and in Venezuela (Barrera et al. 1993).

Focks and Chadee (1997) also demonstrated that counties in close proximity to the capital city of Port of Spain, Trinidad, generally have a more reliable water supply and better environmental sanitation with fewer foci per hectare and fewer *Ae. aegypti* mosquitoes. During the present study similar results were obtained with fewer drums found in St. George East (191) and in St. George Central (303) than that found in St. Andrew/St. David (548) and Nariva/Mayaro (378). However, the number of drums with *Ae. aegypti* immatures were similar with 13.5% of drums positive in St. George Central and 13.2% positive in Nariva/Mayaro. This indicates that the residents of these counties practice little or no water management/mosquito prevention, resulting in the daily production of large numbers of *Ae. aegypti* mosquitoes.

The results of the house inspections showed only 41 (1.3%) drums were located indoors (TABLE 3). Similar results were reported by Focks and Chadee (1997) who suggested that due to their small numbers and lack of appreciable *Ae. aegypti*, these indoor containers are inconsequential and can be best controlled by educating householders rather than by centralized control measures, which are difficult to execute because of the high number of "closed houses" (Chadee 1988) encountered during house inspection and treatment programs in Trinidad.

Based on the results of the present study, the *Ae. aegypti* control strategies currently being used need to be revised. For example, in 1997, 987 bags or 24,675 kgs of 1% temephos were used in stored water treatment, especially drums; but during this study only 6.6% of drums were used for storing potable water. Thus, approximately 70 bags or 1727.3 kgs should have been used for potable water treatment in drums (23 grams/drum) and alternative insecticides or treatment strategies used for domestic water stored in drums and other containers.

Temephos is one of the few insecticides recommended by the World Health Organization for treatment of potable water at 1 ppm and should be used appropriately in light of insecticide resistance reported in the Caribbean region (Vaughan et al. 1998, Rawlins 1998).

To prevent the proliferation of *Ae. aegypti* breeding in water drums, a number of options should be made available, with residents choosing the most adequate method to suit their needs. For example, the provision of house connections from a central water supply, a long-term objective, would not solve the water storage problem unless it is a reliable service, as demonstrated in Venezuela where communities with reliable and unreliable water supplies had similar *Aedes* indices. Alternatively, the physical exclusion of mosquitoes by providing protective covers for drums is another option, but this measure would be effective only if the covers are easy to use (PAHO 1994). It is noteworthy that the introduction of larvivorous fish such as *Poecilia reticulata* (L.) or other biological control agents such as predatory copepods, the "la untadita" method of combining detergent and bleach to destroy *Ae. aegypti* eggs (Sherman et al. 1998), or the use of appropriate insecticides are other available options. However, the enforcement of existing legislation should be the last alternative if householders support and compliance are not gained using the above approaches.

Acknowledgments

We wish to thank Dr. C. C. Tilluckdharry for his support and Messrs. C. Dabreau, K. Maharaj, W. Mohammed, and other senior Public Health Inspectors and Health Control Officers attached to Insect Vector Control Division, Ministry of Health, Republic of Trinidad and Tobago, for assistance in the field. We also thank Deryck Ayow for statistical assistance. This research was supported by a Forgaty International Collaboration Award R03 TW00800.

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Susceptibility of Geographically Distinct *Aedes aegypti* L. from Florida to *Dirofilaria immitis* (Leidy) Infection

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ABSTRACT: In the present study, five geographically distinct populations of *Aedes aegypti* L. from various regions of Florida were compared for their susceptibilities to *Dirofilaria immitis* (Leidy) infection. The populations were from Gainesville (North Central), Jacksonville (North Eastern), Naples (South Western), Sarasota (West Central), and Vero Beach (East Central). Mosquitoes of all the populations had fewer parasites in their Malpighian tubules 6 and 13 days after blood feeding compared to the number of microfilariae initially ingested. There was no significant difference in the mean number of the total parasites per female 6 and 13 days after taking an infectious blood meal. In comparison, significantly larger numbers of the microfilariae completed development in the laboratory-selected Vero Beach susceptible strain. Females excreted microfilariae within 72 hr. of engorgement on an infected dog and the Gainesville, Jacksonville, Naples, and Sarasota populations had 20-23% females that lacked ovarian development six days after blood feeding. Out of these, in 16-22% of the females from the Jacksonville, Naples, and Sarasota populations, parasite development was arrested mostly as moribund prelarvae and only few developing larvae were observed. All females of the Gainesville population that did not develop eggs (20%) also lacked parasites. In the Jacksonville population, 23% of the females that matured eggs had no infection. Eight percent of the females from the Sarasota population exhibited a similar response. Low survivorship was observed at 6 and 13 days after imbibing an infectious blood meal. Females showed melanization of microfilariae in the Malpighian tubules, on the midgut wall, and in the hemocoel. Cellular melanization response was observed in the hemocoel, whereas, a humoral melanization response was observed in the Malpighian tubules. The Vero Beach susceptible strain showed maximum vector efficiency and lowest survival at 13 days post-blood meal compared to the Jacksonville and the Gainesville populations.

**Keyword Index:** Culicidae, *Aedes aegypti*, *Dirofilaria immitis*, transmission, susceptibility.

INTRODUCTION

*Aedes aegypti* L., a container breeding species, is widely distributed in the USA. It is a domestic pest mosquito that utilizes varied habitats, such as discarded tires, empty food cans, and drums for larval development. Hendrix et al. (1986) showed that under laboratory condition, infected *Ae. aegypti* females successfully transmitted *Dirofilaria immitis* third stage infective larvae to dogs. There is an overlap between the geographical infection range of *D. immitis* in dogs and domestic breeding habitats of *Ae. aegypti* in Florida. The present study was initiated to determine the role of various geographical populations of *Ae. aegypti* from...
Florida in the transmission of *D. immitis* to dogs. It is known that geographically distinct populations of *Ae. aegypti* vary in their susceptibilities to *D. immitis* infection (Roubaud 1937, Buxton and Mullen 1981). *Aedes aegypti* populations from various geographical areas of the world exhibit a range of immune responses to *D. immitis* microfilariae after imbibing an infectious blood meal (Roubaud 1937). Physiological responses, including complete developmental arrest, melanization, cellular encapsulation of microfilariae, and normal development to infective third stage larvae have been well documented in literature (Kartman 1953, Buxton and Mullen 1981). In *Ae. aegypti*, susceptibility to *D. immitis* infection is an inheritable character controlled by the gene *f* (filarial susceptibility *D. immitis*) (Sulaiman and Townson 1980). Microfilariae of *D. immitis* are capable of successfully migrating in the Malpighian tubules of both refractory and susceptible strains of *Ae. aegypti*, but development of *D. immitis* is arrested as moribund prelarvae in the former. In certain infected mosquitoes, development of microfilariae is not arrested completely, and both prelarvae and infective third-stage larvae can be observed simultaneously (McGreevy et al. 1974, Nayar and Sauerman 1975).

In this paper, five geographically distinct populations of *Ae. aegypti* from Florida and a laboratory selected susceptible strain from Vero Beach were fed on a dog with circulating microfilariae of *D. immitis*. Their susceptibilities and vector efficiencies were then determined. Further, the immune responses exhibited by *Ae. aegypti* to *D. immitis* infection and the effect of *D. immitis* infection on mosquito survival and vector efficiency is discussed.

**MATERIALS AND METHODS**

**Mosquito Collection and Maintenance**

**Strains:** Eggs of various strains were collected using *Ae. aegypti* oviposition traps by mosquito control personnel from the following Florida locations: 1) Gainesville (North Central), Jacksonville (North Eastern), Naples (South Western), Sarasota (West Central), and Vero Beach (East Central) (Fig. 1). A laboratory selected susceptible strain from Vero Beach (Mahmood and Nayar 1989) was used as control for determining the normal course of nematode development in susceptible mosquitoes.

**Mosquito Rearing:** Eggs from each strain were hatched in water containing brewer’s yeast and mosquitoes were colonized using previously described methods (Nayar and Sauerman 1970). Colonized adults were kept in an insectary maintained at 25 ± 2°C, 16:8 (L:D) photoperiod, and 80 ± 10% relative humidity. Larvae (*F*₂ and *F*₃ generations) were reared in white enamel pans containing tap water and were fed brewer’s yeast. After emergence, adults were kept in cages constructed from 3.8 liter ice cream cartons and were provided with 10% sucrose solution as a carbohydrate source, in 100 ml conical flasks with cotton wicks. The sucrose solution was replaced twice each week. Two hundred females of each population aged 4-6 days were used in the following experiments to determine their susceptibility and immune mechanisms to *D. immitis* infection.

**Infection of Mosquitoes with *D. immitis***

Two hundred, 4-6-day-old mated females from the *F*₂ and *F*₃ generations of the above populations were allowed at 9:00 A.M. to blood feed partially to completely on the hind legs of a dog with *D. immitis* infection. Adults were briefly anesthetized using chloroform and females with one-half blood meal were separated and allowed to refed to repletion on a tethered chicken (Edman et al. 1975). This procedure was adopted to avoid excessive mortality of the blood-fed females due to high level of microfilaremia of the dog’s blood. Immediately after blood feeding, 10 females of each population with one-half blood meals were dissected and the numbers of total microfilariae ingested by them were counted under 100X magnification of a dissection microscope.

**Effect on Mosquito Survivorship**

Females were allowed to blood feed as above and 50 infected females of each population were maintained in separate 3.8 liter ice cream carton cages with 10% sucrose solution available. Dead females from each cage were removed daily and their numbers recorded. Simultaneously, 50 females of each strain were blood fed on chicken, and their survivorship was also determined at the above time intervals to serve as controls. Survivorship of the infected females of each population was determined 6 and 13 days after the initial blood meal and was adjusted for mortality in the controls.

**Susceptibility to *D. immitis* Infection**

Ten to 18 females of each population, that took a one-half blood meal on an infected dog as mentioned above were dissected in Hank’s solution (Hanks and Wallace 1949) just after blood feeding, and the total number of microfilariae ingested per female was determined. The remainder was maintained in 3.8 liter ice cream carton cages and provided with 10% sucrose solution that was changed twice a week. Fifty females of each strain that were fed by the previously mentioned
procedures were dissected 6 and 13 days post-blood meal. The time intervals 6 and 13 days post-blood feeding were selected because maximum mortality of mosquitoes was observed at the time when microfilariae enter in the Malpighian tubules, or when the infective third-stage larvae exit the Malpighian tubules (Kershaw et al. 1953).

Females that were dissected six days post-blood meal were checked for the presence of prelarvae, arrested presausage, sausage, or developing L1 and melanized stages in the Malpighian tubules, gut wall, and hemocoel. The number and stages of parasites were recorded in each region of the body of mosquitoes, and egg maturation was also recorded. Ovaries from mosquitoes without mature eggs were placed in a drop of distilled water and air-dried. The females were classified as either nulliparous or parous by checking their ovarian tracheation (Detinova 1962).

The rest of the females (50/population) were dissected 13 days post-blood meal and Malpighian tubules, abdomen, thorax, head, and proboscis were examined. The Malpighian tubes were examined for the presence of prelarvae, presausage, sausage, L1, L2, L3, and any melanized stages (Taylor 1960). The thorax, head, and proboscis were examined for the presence of L1 infective larvae, and the number of parasites was recorded. The abdomens, midgut walls, and hemocoels of the mosquitoes were searched for the presence of melanized prelarvae. Differences in vector potential and vector efficiency of the above Ae. aegypti populations were estimated using the formulas of Wharton (1960), Ramachandran (1970), and Sabry (1991). The infectivity index (Number of mosquitoes with L3 larvae/number of mosquitoes surviving through the incubation period)
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(Brito et al. 1998) was calculated for each population and the laboratory selected Vero Beach strain.

**Excretion of Microfilariae**

Just after taking an infectious blood meal, 10-16 fully blood-fed females were immobilized by pasting their wings and thoraxes to glass slides using adhesive tape. The glass slides were kept in separate covered petri dishes and were maintained in a humid environment by placing pieces of wet paper towel inside the petri dishes. Excreta of each female was washed with Hank’s solution, collected using a pipette, and placed on glass slides that were examined under a compound microscope. The number of total excreted microfilariae per female was counted at 24 hr. intervals for three days and the percent mortality of females was recorded at the same time.

**Statistical Analysis**

Numbers of total ingested microfilariae at 0 hr. after blood feeding were compared to the total number of parasites (moribund prelarvae, presausage, sausage, melanized prelarvae, developing $L_1$, $L_2$, and $L_3$ infective larvae/mosquito) present at 6 and 13 days post-blood feeding by one way ANOVA ($P < 0.001$) (SPSS analysis package 1998). If significant differences were observed, the mean total number of parasites/female were then compared by Duncan’s test for within population differences and the differences at various times after mosquito infection.

**RESULTS**

A comparison of test populations of *Ae. aegypti* in their ability to sustain different levels of *D. immitis* infection at various times after blood feeding is presented in TABLE 1. Females of all the populations ingested significantly more microfilariae just after blood feeding than the total number of parasites retrieved from their bodies 6 and 13 days after imbibing an infectious blood meal ($P < 0.001$). Females of the Gainesville population had 8.6 and 11.5 times more microfilariae just after blood feeding compared to 6 and 13 days post-blood meal, respectively ($F = 42.06; df = 2, 115; P < 0.001$). After 13 days a non-significant decrease (1.2 times less parasites than at 6 days) was observed in this population. Similar trends were observed in the other populations except for the Vero Beach (Wild) and Vero Beach (Susceptible) females which had 18.7 and 2.1 times more microfilariae, respectively, at 6 hr. than at six days after blood feeding. Comparison of the number of microfilariae ingested by different populations showed that Vero Beach (wild) females ingested significantly more microfilariae and there was no difference in the number of total parasites found in all test populations at 6 and 13 days post-blood feeding.

The Vero Beach susceptible females had significantly more parasites at 6 ($F = 26.751; df = 5, 292; P < 0.001$) and 13 days ($F = 6.26; df = 5, 294; P < 0.001$) post-blood feeding than females of the other test populations.

**TABLE 1.** A comparison of mean total number of *Dirofilaria immitis* larvae found in different *Aedes aegypti* populations at 0 hr., 6 days, and 13 days post-blood feeding.

<table>
<thead>
<tr>
<th>Population</th>
<th>Microfilariae/female at 0 hr. Mean ± SD (n)*</th>
<th>Immature/female at 6 days Mean ± SD (n)*</th>
<th>Immature/female at 13 days Mean ± SD (n)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gainesville**</td>
<td>47.3 ± 45.4 (18) b&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5.5 ± 6.2 (50) b&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.1 ± 4.1 (50) c&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Jacksonville**</td>
<td>33.4 ± 35.4 (17) b&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5.1 ± 7.4 (50) b&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.8 ± 5.8 (50) c&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Naples**</td>
<td>66.6 ± 61.4 (17) b&lt;sup&gt;1&lt;/sup&gt;</td>
<td>8.3 ± 8.1 (50) b&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5.4 ± 5.0 (50) b&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sarasota**</td>
<td>59.5 ± 63.7 (17) b&lt;sup&gt;1&lt;/sup&gt;</td>
<td>6.3 ± 6.1 (50) b&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.6 ± 5.0 (50) c&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vero Beach (Wild)**</td>
<td>112.1 ± 77.8 (10) a&lt;sup&gt;1&lt;/sup&gt;</td>
<td>6.0 ± 6.2 (50) b&lt;sup&gt;2&lt;/sup&gt;</td>
<td>6.6 ± 6.8 (46) b&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vero Beach (Sus)**</td>
<td>61.6 ± 48.7 (15) b&lt;sup&gt;1&lt;/sup&gt;</td>
<td>29.0 ± 27.0 (50) a&lt;sup&gt;2&lt;/sup&gt;</td>
<td>8.9 ± 7.3 (50) a&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Mean ± SD (number of females) followed by same letters in columns and numbers in rows were not significantly different when compared by Duncan’s test ($P < 0.001$).

**All strains had moribund prelarvae, and few moribund presausages, melanized prelarvae, and few developing $L_1$, $L_2$, and $L_3$ stages.

***Vero Beach (susceptible) strain had all normal developing $L_1$ larvae six days after blood feeding and $L_1$, $L_2$, and $L_3$ larvae 13 days post-blood feeding.
populations. The Vero Beach susceptible strain was 99% susceptible, and 92% of the females had infective larvae (L_3) in their proboscis, ranging from 1-14 with a mean of 4.7 larvae per female after 13 days. In comparison, 2.5 and 2.6% of the Gainesville and the Jacksonville females had 1 and 4 L_3 larvae in their proboscis. Growth of filaria was slower in other populations and 4.4, 2.6, and 8.9% of females from the Naples, Sarasota, and Vero Beach (Wild) population had developing L_3 in their Malpighian tubules 13 days after the ingestion of an infective blood meal. The majority of the females were refractory in all the wild populations and moribund prelarvae and/or moribund sausage stages were encountered in the Malpighian tubules 6 and 13 days after an infectious blood meal compared to normal development in the Vero Beach susceptible strain (Figs. 2, 3, and 4).

The females were classified by different loads of D. immitis parasite infection found in various parts of their bodies at different times after blood feeding (TABLE 2). Females from all strains had variable numbers of parasites just after blood feeding, but a number of females from all wild populations except the Naples females lacked parasites after six days (TABLE 2). No uninfected females were present in the Vero Beach (sus) strain, but the percentage of females with 1-10 and 11-20 parasites increased after 6 and 13 days due to high mortality of females with more than 20 parasites in their bodies. After 13 days, most of the females of wild populations had 0-20 parasites (TABLE 2).

The percentages of females that excreted microfilariae at different times after blood feeding from the Gainesville, Jacksonville, Naples, and Sarasota populations are presented in TABLE 3. The Jacksonville and the Sarasota populations survived for 72 hr. and the Gainesville and the Naples females survived up to 48 hr. The maximum numbers of microfilariae were excreted 24 hr. after blood feeding by the Jacksonville females, whereas, during the three days of observation period the maximum percentage of the Sarasota females excreted microfilariae.

The excretion of microfilariae after blood feeding was also supported by the presence of 6-23% females without egg development in the different populations (TABLE 4). Such females showed nulliparous ovaries with tight ovarian skeins (Detinova 1962) and 16-22% of such females had 1-29 D. immitis larvae in their Malpighian tubules. Some females had no prelarvae in their Malpighian tubules but the melanized prelarvae were present either on their midgut walls or in their hemocoels. The Jacksonville and the Sarasota populations showed 22.9 and 8% females with fifth stage eggs, respectively, but no parasites were observed in

<p>|TABLE 2. Variations in the number of Dirofilaria immitis parasites found in the different populations of Aedes aegypti at 0 hr., 6 days, 13 days after blood feeding on an infected dog. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|Range of Mean Total Parasites Per Female |</p>
<table>
<thead>
<tr>
<th>Gainesville</th>
<th>Jacksonville</th>
<th>Naples</th>
<th>Sarasota</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>28</td>
<td>60</td>
<td>81</td>
</tr>
<tr>
<td>11-20</td>
<td>19</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>21-30</td>
<td>5</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>31-40</td>
<td>28</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>41-70</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>71-100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt;100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 2. Distal end of the Malpighian tubule of *Aedes aegypti* (Gainesville) showing complete cellular destruction (arrow) by arrested prelarvae (AP) and melanized prelarvae (MP). Bar equals 100 µm.

Figure 3. A moribund sausage (MS) from the Malpighian tubule of *Aedes aegypti* (Jacksonville strain). Bar = 100 µm.
TABLE 3. Comparisons of the excretion of microfilariae (mf) of *Dirofilaria immitis* by different populations of *Aedes aegypti* females at various times after blood feeding.

<table>
<thead>
<tr>
<th>Population</th>
<th>Total Females Observed</th>
<th>Hr. after Blood Feeding</th>
<th>Percent Excreted</th>
<th>Percent Mortality</th>
<th>Range of Excreted mf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gainesville</td>
<td>10</td>
<td>24</td>
<td>30.0</td>
<td>16.7</td>
<td>2-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>0.0</td>
<td>66.7</td>
<td>0</td>
</tr>
<tr>
<td>Jacksonville</td>
<td>9</td>
<td>24</td>
<td>12.5</td>
<td>11.1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>28.6</td>
<td>22.2</td>
<td>4-40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72</td>
<td>57.1</td>
<td>0.0</td>
<td>1-8</td>
</tr>
<tr>
<td>Naples</td>
<td>16</td>
<td>24</td>
<td>37.5</td>
<td>0.0</td>
<td>1-4</td>
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<td></td>
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<td>48</td>
<td>71.4</td>
<td>56.3</td>
<td>1-8</td>
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<tr>
<td>Sarasota</td>
<td>10</td>
<td>24</td>
<td>20.0</td>
<td>0.0</td>
<td>1-2</td>
</tr>
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<td></td>
<td></td>
<td>48</td>
<td>30.0</td>
<td>0.0</td>
<td>3-17</td>
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<td></td>
<td></td>
<td>72</td>
<td>62.5</td>
<td>20.0</td>
<td>1-10</td>
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</tbody>
</table>

*No data available about the Vero Beach populations.

TABLE 4. Variations in egg maturation by the different populations of *Aedes aegypti* after blood feeding on a *Dirofilaria immitis* infected dog.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total Females</th>
<th>Egg Development (%)</th>
<th>Parasite development (%)</th>
<th>Total parasites*</th>
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<tr>
<td></td>
<td></td>
<td>No</td>
<td>Yes</td>
<td>Mean</td>
</tr>
<tr>
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<tr>
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<td></td>
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<td>80.0</td>
</tr>
<tr>
<td>Jacksonville</td>
<td>48</td>
<td>22.9</td>
<td></td>
<td>77.1</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>1-31</td>
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<tr>
<td>Vero Beach (wild)</td>
<td>50</td>
<td>6.0</td>
<td></td>
<td>94.0</td>
</tr>
<tr>
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<td></td>
<td>6.0</td>
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<td></td>
<td>94.0</td>
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<td>6.0</td>
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<td>94.0</td>
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<td>6.9</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-100</td>
</tr>
<tr>
<td>Vero Beach (sus)</td>
<td>50</td>
<td>100.0</td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>

*Total parasites include moribund prelarvae, presausage, sausage, L₁, and melanized microfilariae.
TABLE 5. Differences in the survivorship of the various populations of Aedes aegypti after imbibing Dirofilaria immitis from an infected dog.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Survivorship 6-days post Infection*</th>
<th>Survivorship 13-days Post Infection*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gainesville</td>
<td>.38</td>
<td>.46</td>
</tr>
<tr>
<td>Jacksonville</td>
<td>.69</td>
<td>ND**</td>
</tr>
<tr>
<td>Naples</td>
<td>.53</td>
<td>.45</td>
</tr>
<tr>
<td>Sarasota</td>
<td>.60</td>
<td>.46</td>
</tr>
<tr>
<td>Vero Beach (wild)</td>
<td>.45</td>
<td>.42</td>
</tr>
<tr>
<td>Vero Beach (Sus)</td>
<td>.45</td>
<td>.31</td>
</tr>
</tbody>
</table>

* Total alive/Total blood-fed females
** ND = Not determined

their bodies. Both of these strains lived longer and more than half of the females excreted large numbers of microfilariae (TABLE 3). This observation was also supported by their high survivorship compared to other strains after six days (TABLE 5). Increased mortality of mosquitoes with a large number of filaria, resulted in lower survivorship of females after 13 days except in the Gainesville population where mortality after 6 and 13 days was noted on two separate cages due to mortality caused by temperature change. The Vero Beach susceptible females, that harbored a large number of developing parasites died at the time of the exit of nematodes from the Malpighian tubules. Thus, resulting in lower survivorship (31%) and the fewer developing larvae in this strain (6.2 times less at 13 days as compared to 2 times less at 6 days) (TABLE 1). It also explains the lack of females within the 41-130 developing larvae category in the susceptible strain, whereas, at six days post infection, 35% females supported such developing larvae (TABLE 2).

Parameters describing the vector potential and the vector efficiency of different populations of *Ae. aegypti* from Florida are presented in TABLE 6. The Naples, Sarasota, and Vero Beach (wild) females were incapable of transmitting *D. immitis* after 13 days, and the Gainesville and the Sarasota populations had a zero infectivity index. Although, Jacksonville females had high intensity of infection and such a female would be capable of transmitting immature L₃ that would develop into female and male adults in a dog. The chances of more than one infected female with L₃ biting the same dog would be remote due to its low vector efficiency.

All strains of *Ae. aegypti* exhibited melanization of prelarvae to a variable degree and the maximum percentage of females with melanized parasites were present in the Naples and Sarasota populations at 6 and 13 days after imbibing an infective blood meal (TABLE 7). The range of the total melanized prelarvae did not exceed from 1-2 per female. Prelarvae that melanized

TABLE 6. Various parameters depicting differences in ability of the various strains of Aedes aegypti to transmit Dirofilaria immitis to dogs in Florida.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Infectivity Index = A*</th>
<th>Survival Rate at 13 Days = B**</th>
<th>Intensity of Infection = C***</th>
<th>Number of L₂/Number of Blood fed Mosquitoes****</th>
<th>Vector Efficiency*****</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gainesville</td>
<td>0.08</td>
<td>.46</td>
<td>1.25</td>
<td>0.05</td>
<td>0.21</td>
</tr>
<tr>
<td>Jacksonville</td>
<td>ND******</td>
<td>ND</td>
<td>12.00</td>
<td>ND</td>
<td>0.72</td>
</tr>
<tr>
<td>Naples</td>
<td>0.00</td>
<td>.45</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Sarasota</td>
<td>0.00</td>
<td>.46</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Vero Beach (Wild)</td>
<td>0.00</td>
<td>.45</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Vero Beach (Sus)</td>
<td>0.99</td>
<td>.31</td>
<td>8.43</td>
<td>2.45</td>
<td>13.41</td>
</tr>
</tbody>
</table>

* Number of mosquitoes with L₂ larvae/number of mosquitoes surviving through the incubation period.
** Number of mosquitoes surviving through incubation period (13 days)/number of blood-fed mosquitoes.
*** Number of L₂ larvae/number of mosquitoes with L₃ larvae.
**** A x B x C.
***** (L₂ per mosquito/number of microfilariae ingested per mosquito) x 100.
****** ND = Not determined.
Figure 4. Distal end of the Malpighian tubule of *Aedes aegypti* (Vero Beach susceptible strain) showing normal development of *Dirofilaria immitis* larvae (arrow). Bar = 100 µm.

Figure 5. Melanized prelarva (MP) in the hemocoel of *Aedes aegypti* (Jacksonville) showing a cellular capsule (CC). Bar = 100 µm.
inside the Malpighian tubules did not show cellular encapsulation, a response exhibited against melanized prelarvae that were present on the outer midgut wall and inside the hemocoel (Fig. 5).

DISCUSSION

Observations on the survival of infected females were similar to those reported earlier (Kershaw and Duke 1954) with D. immitis infection resulting in increased mortality of mosquitoes 6 and 13 days after blood feeding. Intake of large numbers of microfilariae has a direct impact on the survival of susceptible mosquitoes (Paige and Craig 1975, Samarwickerma and Laurence 1978, Mahmood and Nayar 1989). Aedes aegypti from all populations excreted microfilariae during the 72 hr. observation period after ingesting an infectious blood meal and some females were capable of expelling all the blood meal. Crans (1973) observed 20% uninfected Anopheles gambiae females when fed on a carrier with Wuchereria bancrofti infection and persevered in 0.5 ml of 3% acetic acid within 30 min. after feeding. He suggested that mosquito infection was dependent on their mode of blood feeding, i.e., mosquitoes ingested more parasites when they fed through a capillary than on blood from a lacerated capillary. In the wild populations of Ae. aegypti from Florida the excretion of microfilariae, expulsion of blood meal, and/or uptake of blood from a lacerated capillary might have resulted in Ae. aegypti females with no parasites. Presence of parasites in nulliparous females lacking ovarian development also supported this observation. In the Vero Beach susceptible strain, no females were found with undeveloped ovaries six days after blood feeding. Apparently, excretion of infected blood meal was a physiological barrier to infection in the refractory females.

The wild type females harbored a significantly smaller number of larvae six days after blood feeding, suggesting that most of the females that had more than 40 parasites either died or excreted their parasites during this time. The Jacksonville females had the highest vector efficiency (0.72); but it would not act as an efficient vector because if we assume 50% survival after 13 days, its infectivity index would be only .02 (Brito et al. 1998).

In this study, developmental arrest of microfilariae was observed at the presausage and sausage stages compared to the earlier reports by Buxton and Mullen (1981), who suggested that all developmental arrest occurred at the prelarvae stage. In the wild type Florida strains, arrested presausage and sausage stages were also found in the Malpighian tubules of females 13 days after imbibing an infectious blood meal. Fewer (8.9%) females in the wild type Vero Beach strain had developing larvae compared to 21% females with developing larvae that were observed by Buxton and Mullen (1981) in the same strain.

A higher percentage of females with normal developing larvae from all populations had a mixed infection and also showed arrested prelarvae suggesting better survival of such females. In the homozygous
refractory females the proximal and distal cells of the Malpighian tubules are completely destroyed by the persistent motion of moribund prelarvae. Whereas, in homozygous susceptible females many females die due to the damage caused by the developing first stage larvae to the proximal and distal cells of the Malpighian tubules (Nayar and Sauerman 1975). Townson (1971) observed that Ae. aegypti refractory to Brugia pahangi infection survived better with a genotype F/f or F/f* compared to the susceptible individuals with genotype f/f*.

Melanization response exhibited against D. immitis microfilariae and prelarvae was similar to the previously described immune responses (Buxton and Mullen 1981). Melanization in the Malpighian tubules was humoral (Bradley and Nayar 1985) compared to the cellular response observed in the hemocoel and was similar to the immune response observed by Christensen et al. (1984) to intrathoracically inoculated microfilariae of D. immitis. The basic modes of refractoriness in the present strains were arrest of microfilariae as moribund prelarvae or as arrested presasuges and the excretion of the microfilariae. Therefore, the populations of Ae. aegypti tested in this study have been found to be ineffective vectors of D. immitis in Florida.

REFERENCES CITED


A Probability Model of Vector Behavior: Effects of DDT Repellency, Irritancy, and Toxicity in Malaria Control\(^1\)

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ABSTRACT: A probability model of how DDT residues may function within a malaria control program is described. A step-wise organization of endophagic behaviors culminates in a vector acquiring a human blood meal inside the house. Different vector behaviors are described, epidemiologically defined, temporally sequenced, and quantified with field data. Components of vector behavior and the repellent, irritant, and toxic actions of insecticide residues are then assembled into a probability model. The sequence of host-seeking behaviors is used to partition the total impact of sprayed walls according to the three chemical actions. Quantitatively, the combined effect of repellency and irritancy exert the dominant actions of DDT residues in reducing man-vector contact inside of houses. These relationships are demonstrated with published and unpublished data for two separate populations of Anopheles darlingi, for Anopheles gambiae and Anopheles funestus in Tanzania, and Anopheles punctulatus in New Guinea.

**Keyword Index:** Probability model, malaria control, vector behavior, DDT, Anopheles.

INTRODUCTION

Efficient vectors of human malaria typically move to a house, enter, and bite indoors. These activities are interrupted by intervals of resting. The acts of entering and exiting a house involve finding and entering openings in a physical barrier (the house wall), so they are distinct from movement or flight from one location to another. House entering and indoor biting (endophagic) behaviors are epidemiologically important because they influence the likelihood that a mosquito will bite a human and imbibe an infectious blood meal or transmit malaria.

Endophagic behaviors are equally important in malaria control to the extent that an insecticide may prevent vectors from entering a sprayed house or stimulate vectors to exit a house before they bite.

Practically every important vector of malaria exhibits insecticide avoidance behavior (Elliott and de Zuluet 1975 and Lockwood et al. 1984). Yet the epidemiological significance of these chemically-induced behaviors is controversial. In this paper we describe a model for defining the importance of repellent and irritant actions of insecticide residues. The term excito-repellent will be used to describe all chemically-

\(^1\)Disclaimer: The views expressed are those of the authors and do not reflect the official policy or position of the USUHS, the Department of Defense, or the United States Government.
induced behaviors, including both repellent and irritant actions (Roberts and Andre 1994). A repellent is a chemical that causes insects to make oriented movements of avoidance without tarsal contact with the chemical. An irritant is a chemical that causes insects to make oriented movements of avoidance after tarsal contact.

The sequential nature of mosquito host-seeking behaviors is used to partition the separate contributions of repellency, irritancy/repellency, and toxicity that comprise the overall impact of DDT-sprayed houses. A series of mathematical formulas are described that explicitly isolate and quantify these actions. The formulas are used with field data from experimental hut studies on four important malaria vectors to show that repellency is the primary action of DDT residues in preventing human-vector contact.


MATERIALS AND METHODS

Components of Indoor Host-Seeking Behaviors of Malaria Vectors

The primary scenarios of host-seeking activities that occur within the peridomestic environment and inside houses are listed in Figures 1 and 2. All 10 scenarios might be represented within natural host-seeking populations of a vector species. The sequence of directed movements at sunset and early evening, followed by variable levels of indoor biting activity through the remainder of the night have been documented for An. darlingi populations in Brazil (Roberts et al. 1987), Colombia (Elliott 1972), and Suriname8. Although the patterns of biting activity were not identical, the sequence of activities was essentially the same. Figures employed in Figures 1 and 2 and in the probability model are:

\[ c = \text{control house (unsprayed)}, \]
\[ t = \text{treated house (sprayed)}, \]
\[ a = \text{average number of mosquitoes during a post-spray interval (can indicate numbers entering, exiting, or biting indoors)}, \]
\[ a' = \text{average number of mosquitoes during a pre-spray interval (can indicate numbers entering, exiting, or biting indoors)}, \]
\[ h = \text{index of reduced numbers entering, biting indoors, or exiting a treated house}, \]
\[ r = \text{resting for a prolonged period (2-3 days)}, \]
\[ r' = \text{not resting for a prolonged period (temporary period of minutes to hours)}, \]
\[ r_s = \text{resting at site through oogenesis (time required for egg development)}, \]
\[ o = \text{outside of house (outdoors)}, \]
\[ i = \text{inside of house (indoors)}, \]
\[ f = \text{fed (blood engorged)}, \]
\[ u = \text{unfed (not blood engorged)}, \]
\[ b = \text{biting}, \]
\[ b' = \text{not biting}, \]
\[ s = \text{surviving for 24 hours after exiting}, \]
\[ s' = \text{not surviving (death) 24 hours after exiting the house, and} \]
\[ m = \text{movement from one site to another}. \]

Endophily characterizes a mosquito that rests indoors until oogenesis is completed \( (r_s) \), and then departs to lay eggs. Exophilic mosquitoes rest outdoors during oogenesis \( (r_s) \). Endophagy characterizes indoor biting \( (b_s) \), and exophagy characterizes biting out of doors \( (b_l) \).

A malaria-susceptible mosquito that moves into the peridomestic environment, enters a house \( (m_j) \), bites a human indoors \( (b_i) \), and subsequently departs \( (m_j) \) for an outdoor resting site \( (r_u) \) is epidemiologically important. In the context of human malaria, a mosquito

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Figure 1. Patterns of outdoor host-seeking behaviors of Anopheles mosquitoes, illustrating the primary schemes of exophagic behavior, with or without house entering. Symbols are indicative of: \( r \) = resting for prolonged period (1-3 days); \( r' \) = not resting for prolonged period; \( r_e \) = resting at site through oogenesis (time required for egg development); \( u \) = unfed (unengorged); \( o \) = outdoors; \( b \) = biting; \( m \) = moving; \( i \) = indoors; \( f \) = fed (engorged).
Six host-seeking scenarios:

- $r'_{uo} \rightarrow m_{iu} \rightarrow b_i \rightarrow r_{ei}$
- $r_{uo} \rightarrow m_{iu} \rightarrow b_i \rightarrow m_{of} \rightarrow r_{eo}$
- $r_{uo} \rightarrow m_{iu} \rightarrow b_i \rightarrow r_{fi} \rightarrow m_{of} \rightarrow r_{eo}$
- $r_{uo} \rightarrow m_{iu} \rightarrow r_{ui} \rightarrow b_i \rightarrow r_{ei}$
- $r_{uo} \rightarrow m_{iu} \rightarrow r_{ui} \rightarrow b_i \rightarrow m_{of} \rightarrow r_{eo}$
- $r_{uo} \rightarrow m_{iu} \rightarrow r_{ui} \rightarrow b_i \rightarrow r_{fi} \rightarrow m_{of} \rightarrow r_{eo}$

**Epidemiological Descriptors**

- $b_i, r_{eo} =$ endophagic and exophilic
- $b_i, r_{ei} =$ endophagic and endophilic

Figure 2. Patterns of indoor host-seeking behaviors of *Anopheles* mosquitoes, illustrating the primary schemes of endophagic behavior. Symbols are indicative of: $r'$ = not resting for prolonged period; $r_i$ = resting at site through oogenesis (time required for egg development); $u$ = unfed (unengorged); $o$ = outdoors; $b$ = biting; $m$ = moving; $i$ = indoors; $f$ = fed (engorged).
that bites some nonhuman host outdoors \((b_s)\), then rests indoors through oogenesis \((r_s)\) is not epidemiologically important. The former is exhibiting endophagic \((b_s)\) and exophagic \((r_s)\) behaviors, and the latter is exhibiting exophagic \((b_s)\) and endophagous \((r_s)\) behaviors. Behavioral characterizations of vectors should reflect the mix and relative frequency of host-seeking scenarios (Fig. 1 and 2) expressed by vector populations under field conditions.

**Data Sources**

A behavioral study on *An. darlingi* populations was used to develop basic probability statistics (Roberts et al. 1987, Roberts and Alecrim 1991). The study was conducted along the Ituxi River in Amazonas, Brazil in 1979-1981 and was used here because the primary author has the complete data set. Comparative data were also extracted from studies conducted on *An. darlingi* in Suriname\(^8\), *An. gambiae* and *An. funestus* in Tanzania (Smith and Webley 1969), and *An. punctulatus* in Irian Jaya (West New Guinea)\(^9\).

*Anopheles darlingi* females captured in entrance and exit traps were examined for physiological condition, e.g., using Detinova's (1962) descriptions of Sella's stages of blood digestions and egg development. Based on observations by Roberts et al. (1983), specimens described as being in Sella's stages 4 through 7 probably fed 24 hours or more before they were captured. We will refer to these specimens as a group of “old” fed specimens. Specimens marked as “recent” or “late” fed or in Sella's stages 2 and 3 will be grouped as having fed the night they were captured.

**Methods of Calculating Probabilities**

Data from biting collections and entrance and exit trap collections were compiled for the two houses for several days before one of the houses was sprayed with DDT. Discrete probabilities \((p)\) were developed by dividing the average number captured during an interval of time by total number collected for the whole night. As an example, consider that, over several nights of collecting, an average of 80, 50, 20, 10, 5, and 4 mosquitoes/night were captured in entrance traps during six two-hour intervals from 6:00 P.M. to 6:00 A.M. The sum of average values is 169 and the estimated discrete probability for the first two-hour interval is 80/169, or 0.47. The estimated discrete probability for the second hour was 50/169, or 0.296. These values suggest that the probabilities for mosquitoes to enter the house from 6:00 to 8:00 P.M. is 0.47 and from 8:00-10:00 P.M. is 0.296. Alternatively, cumulative probabilities \((cump)\) would be 80/169, or 0.47 for the first interval; (80+50)/169, or 0.769 for the second interval; and so forth until the estimated cump for the last interval (4:00 to 6:00 A.M.) would equal 1.0.

The entrance and exit traps were identical in dimensions and design and were employed in equal number simultaneously in identical sampling intervals. The traps functioned as unbiased and equal estimators of population movements into or out of the house. Consequently, we adjusted patterns of entering unsprayed houses by subtracting the average number of unfed females that exited by window traps \(m_e\) from the average number of unfed females that entered \(m_i\) through window traps for each time interval during the night. This “adjusted” value corrected for background movement of unfed females into and out of the house that seemed to vary according to the numbers of unfed females that entered during a collection interval. In our opinion, the adjustment provided a more refined estimate of numbers of unfed females that both entered the house, remained longer indoors and possibly took a blood meal indoors \(m_e, b\). These adjusted values were then converted to cump values.

The cump values were used to estimate times for 50% or 75% of the population to enter, bite and exit unsprayed houses. Smoothening splines were used to plot the cump data points (with cump values on the y-axis; time on the x-axis; cump values plotted by the midpoint of each time interval). Estimates of time for 50% or 75% of mosquitoes to enter, bite, or exit were obtained by drawing lines from the 0.5 and 0.75 values on the y-axis. A line was dropped to the x-axis from points intersecting the graphical plot of cump values. Estimates of time were read from the x-axis.

Since entrance and exit trap collections were continued for 24-hour periods, values for \(p\) and cump for entering and escaping houses were arrayed for both 12 and 24-hour intervals. However, emphasis was placed on defining activities during the 12 hours of darkness. Biting collections were conducted from 6:00 P.M. to 6:00 A.M. The series of cump values for house-entering covered the same 6:00 P.M. to 6:00 A.M. interval of time; but we used a 30-min. offset because entrance and exit trapping started at 6:30 P.M.

The impact of DDT on numbers entering the treated house \((t)\) was estimated by dividing the average number entering a sprayed house \((a)\) for each time interval/night with the average number that entered before the house

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was sprayed \((a', h)\). For the control house, the formula \(a'/a\) was used to measure the natural change in numbers entering houses from the pre-spray to post-spray intervals of time. The two calculations were combined to adjust for natural difference between the treated and control houses under pre-spray conditions and for natural change in mosquito populations from the pre- to post-spray time intervals. Thus, the formula for estimating proportions entering the sprayed house is \((a'/a)\), or \((a'x a'/x a)\). We converted this to an index of reduced numbers entering the sprayed house with the formula:

\[
\text{Index of Reduced Numbers} = 1 - \frac{a}{x a}/\frac{a'}{x a'} = h. \quad \text{(Formula 1)}
\]

This formula was also used with numbers exiting the experimental houses.

The Probability Model

In general, probabilities employed in this model are conditional simply because a mosquito cannot enter a house unless it has first moved near the house, cannot bite indoors unless it has first moved into the house, and cannot escape engorged until it has first taken a blood meal inside the house. Reduced house entering (see formula 3) was estimated with entrance trap data from the Ituxi River study. Alternatively, investigators, using indirect measures, in the Suriname, Tanzania, and New Guinea studies developed estimates of reduced house entering. Measures of reduced indoor biting (see formula 5) were obtained from biting collections conducted simultaneously in the treated and control houses, and by proportions of females captured in exit traps that were blood engorged. Reduced survival (see formula 8) was estimated by holding females captured in exit traps from both treated and control houses for 24 hours and recording numbers that died at end of the holding period.

All parameters of this model were standardized with data from the control house. To standardize data, we assume that the maximum possible number of females will enter and bite indoors and that the smallest possible numbers of specimens will subsequently die. As a method of standardization, we assume that \(p(m_{ur}) = 1\), and \(p(b_{ur}) = 1\). We also assume that \(p(s_{r}) = 1\), whether the mosquito remains indoors throughout oogenesis or departs the house after biting. Finally, we assume that

\[
p(m_{ur}) = p(b_{ur}) = p(m_{pr}) = p(s_{r}) = 1,
\]

and that \(p(s_{r}) = 1 - p(s_{r}) = 0\).

To examine the effect of a repellency action that prevents mosquitoes from entering the sprayed house, let \(m_{ur}\) be the event that the unfed mosquito enters the sprayed house \((m_{ur}'\) for not entering), then the probability of entering will be denoted by \(p(m_{ur})\). We assume the probability that an unfed mosquito will enter the control house is \(p(m_{ur}) = 1\). It follows that the probability of reduced entry for the sprayed house is

\[
p(m_{ur}) = p(m_{ur}) = 1 - p(m_{ur}). \quad \text{(Formula 2)}
\]

Thus, the percent reduction in numbers entering the sprayed house is

\[
(1 - p(m_{ur})) \times 100. \quad \text{(Formula 3)}
\]

Discrete and cumulative probabilities for entering the unsprayed house can be used with \(h\) values to estimate discrete and cumulative probabilities for entering the sprayed house (remember, \(h\) is a measure of reduced entering, biting, or exiting for the sprayed house). Using the earlier example, the probability of entering the control house \(p(m_{ur})\) during the 6:00 to 8:00 P.M. is 0.47. Let’s suppose that the index of reduced numbers, \(h\), entering the sprayed house from 6:00 to 8:00 P.M. is 0.95. As a consequence, the index of entering the house is 0.05, or 1 - \(h\). The discrete probability for entering the sprayed house from 6:00 to 8:00 P.M. is \(p(m_{ur})(1 - h)\), or 0.47 * 0.05 = 0.0235. Estimates of discrete probabilities for all time intervals during the night can be summed to give a standardized, total probability \((cump)\) for unfed females to move into the sprayed house, that is, \(p(m_{ur})\).

Impact of irritant/non contact repellent actions on mosquitoes after they enter the house is defined by numbers biting indoors. Let \(b_{ur}\) be the event that a mosquito will bite in the sprayed house \((b_{ur}'\) for not biting), then the probability that the mosquito will bite in the sprayed house is denoted by \(p(b_{ur})\), or \(p(b_{ur}) = p(m_{ur})\). We denote the probability of the mosquito entering and biting in the control house as \(p(b_{ur}) = p(m_{ur}) = 1\). Given these relationships, the probability of reduced biting in the sprayed house is

\[
\frac{p(b_{ur}m_{ur})}{p(m_{ur})} - [p(b_{ur}m_{ur}) * p(m_{ur})] = 1 - [p(b_{ur}m_{ur}) * p(m_{ur})]. \quad \text{(Formula 4)}
\]

Consequently, the percent reduction in numbers that enter the sprayed house and subsequently bite indoors can be defined as

\[
(1 - p(b_{ur}m_{ur}) * p(m_{ur})) \times 100. \quad \text{(Formula 5)}
\]

To define the reduction in numbers escaping after feeding indoors, let \(m_{er}\) be the event that the mosquito
will escape from the sprayed house \((m'_{\text{ef}}\) for not escaping). The probability a mosquito entered, took a blood meal indoors, and escaped from the sprayed house is \(p(m_{\text{ef}}, b, m_{\text{ui}}) = p(m_{\text{ef}} | m_{\text{ui}}, b) \cdot p(b) \cdot m_{\text{ui}} \). Alternatively, the probability that the mosquito will escape from the control house can be denoted by \(p(m_{\text{ef}}, b, m_{\text{ui}}) = 1\). The probability of entering, biting indoors, and escaping the sprayed house is

\[
p(m_{\text{ef}}, b, m_{\text{ui}}) = \frac{1 - p(m_{\text{ef}} | m_{\text{ui}}, b) \cdot p(b) \cdot m_{\text{ui}}}{p(m_{\text{ui}})}. \quad \text{(Formula 6)}
\]

If mosquitoes die indoors, then formula 6 can be used to capture this element of insecticide-vector interaction. Alternatively, numbers dead inside the house can be included in formulas 7 and 8. In the Ituxi River study, too few females entered and fed in the sprayed house and no indoor deaths were observed. Additionally, engorged females that were marked and released indoors rapidly escaped and none were found dead as a result of DDT exposure. As a consequence, the probability of moving out of doors from the treated house was treated as unity (i.e., \(p(m_{\text{ef}}, b, m_{\text{ui}}) = 1\)).

Let \(p(s, m_{\text{ef}}, b, m_{\text{ui}})\) be the event that the mosquito will survive for 24 hours after entering, feeding, and escaping from the sprayed house \(p(s, m_{\text{ef}}, b, m_{\text{ui}})\) for not surviving), then the probability is

\[
p(s, m_{\text{ef}}, b, m_{\text{ui}}) = [p(s | m_{\text{ui}}, b) \cdot p(b) \cdot m_{\text{ui}}] \cdot p(m_{\text{ef}} | m_{\text{ui}}, b) \cdot p(b) \cdot m_{\text{ui}} \cdot p(m_{\text{ui}}). \quad \text{(Formula 7)}
\]

We assume that all mosquitoes entering the control house will survive and this probability is denoted by \(p(s, m_{\text{ef}}, b, m_{\text{ui}}) = 1\). Using these relationships, the probability of reduced survival (which is equal to probability of increased mortality) after entering and escaping the sprayed house can be obtained by

\[
p(s, m_{\text{ef}}, b, m_{\text{ui}}) = [p(s | m_{\text{ui}}, b) \cdot p(b) \cdot m_{\text{ui}}] \cdot p(m_{\text{ef}} | m_{\text{ui}}, b) \cdot p(b) \cdot m_{\text{ui}} \cdot p(m_{\text{ui}}).
\]

The percent reduction in probabilities of 24-hour survival of specimens that have taken a blood meal inside the treated house is defined as

\[
\frac{1 - (p(s, m_{\text{ef}}, b, m_{\text{ui}}) \cdot p(m_{\text{ef}} | m_{\text{ui}}, b) \cdot p(b) \cdot m_{\text{ui}}) \cdot p(m_{\text{ui}})) \cdot 100. \quad \text{(Formula 8)}
\]

Overall epidemiological impact of DDT residues is described by proportional reductions in house entering, indoor biting, and subsequent survival. Parameters that compose this total effect are quantified with formulas 3, 5, and 8. The singular impact of toxicity, \(p(s, b)\), in relation to the overall impact of repellent and irritant actions of DDT can be defined as

\[
\frac{p(b, m_{\text{ui}})}{p(b, m_{\text{ui}}) - [p(s, b, m_{\text{ui}}) \cdot p(b, m_{\text{ui}}) \cdot p(m_{\text{ui}})]} = \frac{100}{1 - p(s, b, m_{\text{ui}}) \cdot p(b, m_{\text{ui}}) \cdot p(m_{\text{ui}})}.
\]

To calculate percent reduction due to mortality,

\[
p(b, m_{\text{ui}}) \cdot p(m_{\text{ui}}) \cdot p(s, b, m_{\text{ui}}) \cdot 100. \quad \text{(Formula 9)}
\]

We used this probability model to quantify the actions of DDT residues on four important vectors of malaria, with emphasis on the use of formulas 1, 3, 5, 8, and 9. In the Ituxi River study, the numbers of females entering, biting, and escaping the sprayed house were small, e.g., in a single night after spraying DDT, 386 females were captured in entrance and exit traps in the control house and only six females were captured in an equal number of trap collections in the sprayed house. For this reason, no standard statistics, e.g., means or standard deviations, are used to describe parameters of the probability model. Regardless, the magnitude of differences between treatment and control are amply defined by probability values, and the probability values are based on actual numbers captured.

As stated above, the numbers of specimens entering the treated house were so small that no direct estimates of biting rates and mortality were possible. Consequently, we used Rozendaal's estimate of reduced biting in the sprayed house. For mortality, we used Rozendaal's estimate that 95% of all escaping An. darlingi females died after entering the sprayed house. We also performed comparative calculations using an estimated mortality of 22%, which was the mortality rate reported by Smith and Webley (1969) for An. gambiae mosquitoes.

RESULTS

Pre-Spray Observations in Experimental Houses

Data in TABLES 1 and 2 show that An. darlingi females of varying physiological conditions routinely entered and exited houses. Unfed females entered the house in the early evening, whereas, blood-fed females entered later at night. The "old" fed females also entered the house late at night (data not shown).

Data in TABLE 2 show that females of varying physiological conditions exit houses. Only during the sunrise period did the numbers of fed females represent the majority of exiting specimens.

Of fed specimens exiting the house, roughly 3%
TABLE 1. Summary statistics for 151 entrance trap and 208 exit trap collections of *Anopheles darlingi* females in two experimental, unsprayed houses along the Ituxi River, Amazonas, Brazil. Collections were conducted for two-hour intervals throughout the night and, for selected sampling regimes, two- to six-hour sampling periods throughout daylight hours. Numbers represent the average number collected per trap per collection by physiological condition of the specimen.

<table>
<thead>
<tr>
<th>Time intervals</th>
<th>Entrance Trap Collections</th>
<th>Exit Trap Collections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfed</td>
<td>Recent* fed</td>
</tr>
<tr>
<td>6:30-8:30 P.M.</td>
<td>15</td>
<td>0.1</td>
</tr>
<tr>
<td>8:30-10:30 P.M.</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>10:30 P.M.-00:30 A.M.</td>
<td>13</td>
<td>0.1</td>
</tr>
<tr>
<td>00:30-2:30 A.M.</td>
<td>8.6</td>
<td>0</td>
</tr>
<tr>
<td>2:30-4:30 A.M.</td>
<td>5.8</td>
<td>0.1</td>
</tr>
<tr>
<td>4:30-6:30 A.M.</td>
<td>7.1</td>
<td>0.1</td>
</tr>
<tr>
<td>6:30-8:30 A.M.</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>8:30 A.M.-12:30 P.M.</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>12:30-6:30 P.M.</td>
<td>1.8</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Blood engorged female with red blood visible in the abdomen.

**Blood engorged female with black blood visible in the abdomen; but without the level of oogenesis as apparent in a Sella stage 2 female (Detinova 1962).

TABLE 2. Discrete (p) and cumulative probabilities (cump) of unfed and blood-fed *Anopheles darlingi* females entering or exiting two experimental houses along the Ituxi River, Amazonas, Brazil. Probabilities derived from collections for two-hour intervals throughout the night and, for selected sampling regimes, two- to six-hour sampling periods through daylight hours. Specimens that fed during the night of capture (specified as exiting fed) are represented by specimens that were recently fed*, late fed**, or were in Sella's stages*** 2 or 3 (numbers in parentheses are cump).

<table>
<thead>
<tr>
<th>Time Intervals</th>
<th>Probabilities**** of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Entering Unfed</td>
</tr>
<tr>
<td>6:30-8:30 P.M.</td>
<td>0.22(0.22)</td>
</tr>
<tr>
<td>8:30-10:30 P.M.</td>
<td>0.22(0.44)</td>
</tr>
<tr>
<td>10:30 P.M.-00:30 A.M.</td>
<td>0.20(0.64)</td>
</tr>
<tr>
<td>00:30-2:30 A.M.</td>
<td>0.12(0.76)</td>
</tr>
<tr>
<td>2:30-4:30 A.M.</td>
<td>0.09(0.85)</td>
</tr>
<tr>
<td>4:30-6:30 A.M.</td>
<td>0.11(0.96)</td>
</tr>
<tr>
<td>6:30-8:30 A.M.</td>
<td>0.01(0.97)</td>
</tr>
<tr>
<td>8:30 A.M.-12:30 P.M.</td>
<td>0.00(0.97)</td>
</tr>
<tr>
<td>12:30-6:30 P.M.</td>
<td>0.03(1.00)</td>
</tr>
</tbody>
</table>

* Blood engorged female with red blood visible in the abdomen.

** Blood engorged female with black blood visible in the abdomen; but without the level of oogenesis as apparent in a Sella stage 2 female.

*** Stages of blood digestion and egg development, see Detinova (1962).

**** Probability = Average number collected by time interval/Total number collected over 24 hours.
had fed more than 24 hours before exiting. The "old" fed specimens also had a peak exit time at sunrise; 33% exited from 4:30 to 6:30 A.M. and another peak in exiting occurred during the afternoon. Proportionally, the highest number of specimens that fed during the night of collecting exited during late morning, with peak numbers exiting from 4:30-6:30 A.M.

Two compilations of probability (p) values and cumulative probabilities (cump) of house-escaping and house-entering activities were developed. The first set of statistics was for a 24-hour period (TABLE 3). The second set of statistics was developed for direct comparison with the 6:00 P.M. to 6:00 A.M. observations on paired indoor/outdoor landing collections. Graphical representations (not shown) of cump values (from TABLE 3) showed that 50% of all females that would enter the house over 24 hours, entered within the first 3.6 hours, and 75% entered within the first 6.8 hours. Of all unfed females that exited the house during a full 24-hour period, 50% had exited within the first 5.6 hours and 75% had exited within the first 9.5 hours. Of all females that fed during the night in which they were collected, 50% exited within the first 9.8 hours and 75% within the first 12 hours of a 24-hour period.

Graphical representations (not shown) of cump values for indoor biting were also used to show that highest indoor landing rates occurred early in the evening, with declining rates through the remainder of the night. Time adjusted cump values (from 6:00 P.M. to 6:00 A.M.) were also calculated for house entering as defined by entrance trap collection data. However, numbers that entered the house were further "adjusted" (as described in materials and methods) to give a more realistic estimate of numbers of unfed females that both entered the house and took a blood meal indoors. Using this adjustment, the cump for times of house entry were calculated and analyzed graphically (not shown). The cump values showed that 50% of unfed females that would subsequently bite indoors had entered the house within the first 2.67 hours, compared to an average of 3.64 hours for 50% of the females to bite. For 75% of the females to enter and bite, 4.37 and 6.8 hours were required, respectively. Based on these statistics, the average female that fed indoors must have rested in the house at least one hour before taking a blood meal.

**Behavior in a DDT-Sprayed House**

Summary statistics from systematic collections in each of the two houses after one of the two houses was sprayed were arrayed (not shown) according to the data format presented in TABLES 1 and 2.

Data in TABLE 4 show that the early evening surge of house entering by unfed females did not occur in the sprayed house (h=1.0 or h*100 =100% reduction). DDT residues also eliminated the peak exiting activity near sunrise.

As described in materials and methods for formula 3, the estimates of h were multiplied by the discrete p values in TABLE 3 to obtain adjusted probabilities for entering and exiting the sprayed house. The cumulative adjusted probabilities are presented in TABLE 4. Overall, 95% fewer unfed *An. darlingi* females entered the sprayed house compared to the unsprayed house. Cumulative adjusted probability for blood-engorged females to exit the sprayed house was only 0.038.

Summary probability values for *An. darlingi* (Ituxi River populations), along with comparable data for other vector populations*, (Roberts and Alercím 1991, Smith and Webley 1969) are presented in TABLE 5. Normally, the impact of insecticide on biting can be determined by comparing the relative mix of fed and unfed specimens in control versus sprayed houses. Unfortunately, so few specimens were collected in the sprayed house in the Ituxi River study that we could not quantify the reduction in biting by populations that entered the houses. As an alternative, we used Rozendaal's estimate of 0.56 as an estimated probability for biting in the sprayed house, so p(b*0|m_a)=0.56.

Likewise, mortality determinations were not possible because too few specimens escaped from the sprayed house. For comparison, we used two different estimates of mortality. First, we used Rozendaal's (Rozendaal 1990) estimate of 95% mortality, equating to a p(s*) = 0.95 or p(s) = 0.05; and we also used the estimate of 22% mortality from the study with *An. gambiae* (Smith and Webley 1969), equating to p(s*) = 0.22 or p(s) = 0.78.

When formula 8 is used with *An. darlingi* (Ituxi River-Brazil) data in TABLE 5, we see that DDT residues reduced joint probabilities of house entering, biting indoors, exiting, and subsequently surviving (using 95% mortality) by 99.81%, that is (1-0.0019)*100. This probability is reduced by 97% (versus 99.81%) when a mortality of 22% is substituted into the equation. Using formula 8, the joint probabilities for entering, biting, and surviving were reduced in the sprayed house by 98.1% for *An. darlingi* (Suriname), by 72% for *An. gambiae*, by 88% for *An. funestus*, and by 66% for *An. punctulatus*.

Reduced house entry as a result of repellency, calculated on the basis of (1-p(m_a))/p*100, showed that repellency actions were responsible for 95, 32, 60, 63, and 50% of the overall effect of DDT sprayed walls against *An. darlingi* (Ituxi River), *An. darlingi* (Suriname), *An. gambiae*, *An. funestus*, and *An. punctulatus* populations, respectively. Finally, formula
TABLE 3. Indexes (h values)* of reduced numbers of unfed and blood-fed**
_Anopheles darlingi_ females that entered or exited a DDT-sprayed house along the Ituxi River, Amazonas, Brazil. Estimates are presented for two-hour intervals.

<table>
<thead>
<tr>
<th>Time Intervals</th>
<th>Indexes (h values) of Reduced Numbers of Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Entering Unfed</td>
</tr>
<tr>
<td>6:30-8:30 P.M.</td>
<td>1.00</td>
</tr>
<tr>
<td>8:30-10:30 P.M.</td>
<td>0.95</td>
</tr>
<tr>
<td>10:30 p.m.-00:30 A.M.</td>
<td>0.95</td>
</tr>
<tr>
<td>00:30-2:30 A.M.</td>
<td>0.83</td>
</tr>
<tr>
<td>2:30-4:30 A.M.</td>
<td>1.00</td>
</tr>
<tr>
<td>4:30-6:30 A.M.</td>
<td>0.97</td>
</tr>
<tr>
<td>6:30-8:30 A.M.</td>
<td>0.74</td>
</tr>
</tbody>
</table>

*Index of reduced numbers = \(1-f(a, \alpha) \times (a', \alpha') = h\), with \(t\) representing the sprayed house, \(c\) the control house and \(a\) representing the average number entering a sprayed house and \(a'\) representing the average number that entered before the house was sprayed.

**Specimens classified as recent fed (red blood visible in the abdomen), late fed (black blood in abdomen), or in Sella's stage 2 or 3 (stages of blood digestion or egg development, see Detinova (1962)).

TABLE 4. Estimates of probabilities (p) and cumulative probabilities (cump) of _Anopheles darlingi_ females entering and exiting a DDT-sprayed house. These probabilities are estimated from (a) the discrete, normal probability (p) of a behavioral event occurring at a specified time in an unsprayed house (see TABLE 2 for relevant values), and (b) the index of numbers (1-h) entering the sprayed house (see TABLE 3 for estimates of h). So, \((p \times (1-h))\) equates to the probability that the behavioral event will occur in the sprayed house during a specific interval of time. The value for \((p \times (1-h))\) represents a discrete probability, the _cump_ is obtained by summing discrete probabilities over the whole night (values in parentheses). Data compiled from studies of unfed and blood-fed _An. darlingi_ females that entered or exited sprayed and unsprayed experimental houses along the Ituxi River, Amazonas, Brazil.

<table>
<thead>
<tr>
<th>Time Intervals</th>
<th>Discrete and Cumulative Probabilities, p and (cump)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Entering Unfed</td>
</tr>
<tr>
<td>6:30-8:30 P.M.</td>
<td>0.0(0.0)</td>
</tr>
<tr>
<td>8:30-10:30 P.M.</td>
<td>0.011(0.01)</td>
</tr>
<tr>
<td>10:30 P.M.-00:30 A.M.</td>
<td>0.01(0.02)</td>
</tr>
<tr>
<td>00:30-2:30 A.M.</td>
<td>0.02(0.04)</td>
</tr>
<tr>
<td>2:30-4:30 A.M.</td>
<td>0.0(0.04)</td>
</tr>
<tr>
<td>4:30-6:30 A.M.</td>
<td>0.003(0.04)</td>
</tr>
<tr>
<td>6:30-8:30 A.M.</td>
<td>0.0026(0.05)</td>
</tr>
</tbody>
</table>
TABLE 5. Probabilities for vector populations to enter a DDT-sprayed house, \( p(m_{ui}) \); bite indoors after entering, \( p(b_u | m_{ui}) \); survive 24 hours after exiting the house with a full blood meal, \( p(s_p | b_u, m_{ui}) \); and joint probability of entering, biting, escaping, and surviving, \( p(s_{edr}, m_{ef}, b_u, m_{ui}) \).

<table>
<thead>
<tr>
<th>Study populations</th>
<th>Enter House</th>
<th>Bite Indoors</th>
<th>Survive 24-hours</th>
<th>Survive after Entering, Biting, and Escaping</th>
<th>Data Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anopheles darlingi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unpublished data and</td>
</tr>
<tr>
<td>(Ituxi River study)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>References (Roberts et al.</td>
</tr>
<tr>
<td>95 % mortality:</td>
<td>0.05</td>
<td>0.56*</td>
<td>0.05*</td>
<td>0.0019</td>
<td>1987, Roberts and Alecrim</td>
</tr>
<tr>
<td>22 % mortality:</td>
<td>0.05</td>
<td>0.56*</td>
<td>0.78**</td>
<td>0.03</td>
<td>1991 and Rozendaal⁸</td>
</tr>
<tr>
<td>Anopheles darlingi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Suriname)</td>
<td>0.68⁴</td>
<td>0.56⁴</td>
<td>0.05⁴</td>
<td>0.02</td>
<td>Rozendaal⁸</td>
</tr>
<tr>
<td>Anopheles gambiae</td>
<td>0.4⁴</td>
<td>0.9⁵</td>
<td>0.78⁴</td>
<td>0.28</td>
<td>Smith &amp; Webley 1969</td>
</tr>
<tr>
<td>Anopheles funestus</td>
<td>0.37⁴</td>
<td>0.82⁵</td>
<td>0.41⁴</td>
<td>0.12</td>
<td>Smith &amp; Webley 1969</td>
</tr>
<tr>
<td>Anopheles punctulatus</td>
<td>0.5⁴</td>
<td>0.85⁵</td>
<td>0.76⁴</td>
<td>0.32</td>
<td>Sloof⁹</td>
</tr>
</tbody>
</table>

*Numbers of females captured exiting house were not sufficient for estimating reduction in biting due to irritant/non contact repellency. Estimate of 0.56 was taken from Rozendaal’s study⁸.

**Value extracted from estimates of mortality from Smith and Webley (1969) and Sloof⁹.

⁴Values extracted directly from published study.

⁵Value derived by comparing ratio of numbers fed and unfed in sprayed and unsprayed houses.

9 was used to show that toxic actions of DDT residues accounted for <10% of the total impact of DDT residues for An. darlingi (Ituxi River), An. gambiae (Tanzania), and An. punctulatus (Irian Jaya) populations. Toxicity accounted for 36.2% of the total effect of DDT on An. darlingi populations in Suriname and for 18.3% of total effect on An. funestus populations in Tanzania. However, it is important to note that repellency and irritancy together, \( [1 - p(b_u | m_{ui}) * p(m_{ui})] \times 100 \), accounted for 61.9% of the overall DDT impact on An. darlingi populations in Suriname.

DISCUSSION

The probability model described in this report isolates and quantifies the repellent, irritant/repellent, and toxic actions of DDT residues on vector behavior. This model challenges the idea that residual spraying exerts control over malaria by killing mosquitoes and reducing vector population longevity. This model also challenges conventional approaches to collecting and interpreting malaria control data.

The critical importance of endophagic behavior is defined by the fact that mosquitoes have options of multiple hosts outdoors; but are generally limited to a human host once they enter a house. Field data show that repellent actions of DDT may prevent mosquitoes from entering and biting inside of houses. As a consequence, a truly endophagic vector may exhibit predominantly exophagic behavior when a house is sprayed with DDT.

Studies of vector ecology and malaria epidemiology are commonly conducted in areas covered by routine house spray programs. Under such conditions, vector studies based on indoor or paired indoor/outdoor collections in DDT-sprayed houses may show that the vectors are exophagic. Epidemiological studies of malaria in populations covered by house spray programs may reveal age-sex distributions of cases indicative of outdoor transmission. Such results might be interpreted as indicating that residual spraying is not useful because vectors bite outside and malaria cases are acquired out of doors. This is a reasonable interpretation if DDT only functions by killing vectors and reducing vector
population longevity. However, in both types of studies, the indicators of exophagic behavior may result from repellent and/or repellent/irritant actions of DDT residues. In other words, out of doors biting and disease transmission can be chemically induced. As a consequence, the results would belie the true endophagic nature of the vector and the true potential for indoor transmission. It is critically important to reexamine claims of exophagic behaviors because if exophagy and out of doors transmission are chemically induced, then elimination of residual spraying will allow a return of natural endophagic behavior, malaria transmission will move indoors and become more efficient, and malaria rates will increase.

In the Ituxi River study, repellent action reduced the probabilities that An. darlingi females would enter the house by 95%. In other words, the repellent effect was the dominant action of DDT residues. The females that still entered the sprayed house were often stimulated to leave without feeding. Rozendaal8 showed that for females that entered the house, biting was 44% less than for a comparable population in the control house. We propose that reduced biting and rapid exit were due primarily to irritant, and secondarily, to repellent actions of DDT.

Roberts and Alecrim (1991) determined that biting in the sprayed house along the Ituxi River was reduced by 96.4% (this effect includes reduced house entry by An. darlingi females). Similar levels of reduced endophagic behavior were documented for the sprayed house two months later, and there was significant suppression of endophagic behaviors one year after the house had been sprayed. The toxic (killing) properties of DDT residues produced an additional, albeit small, overall impact.

The Ituxi River study is just one of many studies against just one of many vectors that show dominant excito-repellent effects of DDT residues. For An. darlingi, excito-repellency tests by Charlwood and Paraluppi (1978), Roberts et al. (1984), and Rozendaal8, and experimental hut studies by Roberts and Alecrim (1991) and Rozendaal8 have all shown powerful excito-repellent responses to DDT residues. Even earlier investigations suggested a similar phenomenon, e.g., de Bustamente et al. (1952) and Elliott (1972).

A high frequency of strong behavioral responses of malaria vectors to DDT residues was suggested by statistics extracted from field studies on populations of An. gambiae and An. funestus in Tanzania (Smith and Webley 1969) and An. punctulatus in Irian Jaya (West New Guinea)7. In each case, when formulas 3, 5, 8, and 9 were used with probability data, the joint action of repellency and irritancy were the dominant functions of DDT residues. Rozendaal’s study in Suriname8 showed that the probability of An. darlingi females entering, biting indoors and surviving was 0.02 in the sprayed house. However, the joint action of repellent and irritant actions accounted for 62% of the overall DDT impact.

We propose that reported changes in mosquito host-seeking activities after houses are sprayed with DDT are primarily a result of the vector’s natural behavioral responses to DDT. Repellent actions that reduce numbers of females entering houses explain the findings of larger numbers biting outdoors relative to numbers biting inside sprayed houses. This is a change in relative proportions only, and may not result in an increase in absolute numbers biting outside.

Contact irritancy, and secondarily, non-contact repellency, can account for a change in time of peak indoor biting activity. As shown for An. darlingi populations, some malaria vectors enter houses in the early evening. However vectors will not remain indoors in the presence of contact irritant and non-contact repellent actions of DDT. Therefore, early evening biting may still occur; but biting later at night will be eliminated. In the Ituxi River study, one year after the experimental house was sprayed with DDT, biting occurred in the sprayed house only in the early evening, while biting in the control house continued throughout the night (Roberts and Alecrim 1991). Similar behavioral responses have been documented for An. albimanus and An. vestitipennis in Belize7 (Grieco et al. 2000).

The primary goal for spraying houses is to reduce the indoor transmission of malaria. Presumably, outdoor transmission is less affected by this control measure. Given these relationships, a better quantification of indoor versus outdoor behavior patterns is needed. In particular, it is important to systematically sample populations as they enter and exit houses. Beyond this, the probability model could be expanded to include probabilities for movement of vector populations from sylvatic to peridomestic environments, and ensuing probabilities for biting humans, versus other vertebrates, outdoors. Through this process, we could begin to better understand the dynamics of malaria transmission on a more quantitative basis and more accurately predict the outcome of a house spray program.

The probability model described in this report may not be descriptive of behavioral responses of all vector species in all environmental settings. However, the model and underlying concepts can be used to evaluate the frequent reports of changes in peak biting times and the relationships of indoor to outdoor biting in the presence of insecticide residues. Indeed, it provides a more realistic perspective on the individual contributions of repellent, irritant, and toxic actions of DDT residues.
and other insecticides having similar properties, e.g., pyrethroids. The model also establishes a basis for understanding how DDT-sprayed houses might exert control over malaria transmission even when vector populations are physiologically resistant to DDT, as exemplified by malaria control operations in India and Mexico (Roberts and Andre 1994).

The probability model was first developed in 1996-1997. Since that time, additional studies have, to varying degrees, confirmed with other vector species that repellency and irritancy are dominant functions of DDT residues, e.g., An. pseudopunctipennis, An. albimanus, and An. vestitipennis. The recent study by Casas et al. (1998) showed that excito-repellency produced satisfactory reductions of indoor biting of An. pseudopunctipennis females when two 0.8-m-wide horizontal swaths of DDT were applied to interior walls. The most recent study by Grieco et al. (2000) demonstrated that repellent actions of DDT residues almost completely eliminated house entering by An. vestitipennis females in southern Belize. These results, in combination with many previous studies that showed similar DDT actions, provide a solid basis for suggesting that future evaluations of insecticides for malaria control should be designed to account for actions of repellency and irritancy, and not just toxicity alone. These results also suggest that studies of vector ecology and malaria epidemiology should be designed to account for chemically-induced versus natural exophagy of important vector species.

Acknowledgments

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A Comparison Study of House Entering and Exiting Behavior of *Anopheles vestitipennis* (Diptera: Culicidae) Using Experimental Huts Sprayed with DDT or Deltamethrin in the Southern District of Toledo, Belize, C. A.¹

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ABSTRACT: An investigation of the house entering and exiting behavior of *Anopheles vestitipennis* Dyar and Knab was undertaken in the Toledo District of Belize, Central America, between March and December of 1998. Three untreated experimental huts were either fitted with exit or entrance interception traps or used as a control for human landing collections. Human landing collections showed that *An. vestitipennis* exhibited a high level of biting activity shortly after sunset and continued biting at high levels throughout the night. Under unsprayed conditions, the use of exit and entrance interception traps demonstrated that doors, windows, and eaves were the primary mode of entry; whereas, cracks in the walls served a secondary role. The peak entrance time for *An. vestitipennis* occurred between 6:45 P.M. and 9:45 P.M. and a peak exit time occurred between 11:45 P.M. and 4:45 A.M. Additional trials were conducted after spraying one of the huts with DDT and another with deltamethrin. The excito-repellent properties of deltamethrin did not affect entrance times but did result in a peak exiting behavior that was five hours earlier than under pre-spray conditions. Deltamethrin also exhibited a repellency effect, showing 66% fewer *An. vestitipennis* entering the hut two weeks post-spray. DDT had an even more powerful repellency effect resulting in a 97% post-spray reduction of *An. vestitipennis* females entering the hut up to two weeks post-spray. The control hut showed only a 37% reduction in *An. vestitipennis* as compared to pre-spray conditions.

*Keyword Index:* *Anopheles*, behavior, DDT, deltamethrin, Belize.

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INTRODUCTION

The resurgence of malaria in tropical regions of Central and South America has created a renewed urgency for information pertaining to *Anopheles* vectors (PAHO 1994). This is evident in Belize and many other countries of the Americas where the prevalence of malaria has increased. This increase is associated with a reduced emphasis on vector control measures, primarily house spraying with residual insecticides (Roberts et al. 1997).

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¹Disclaimer: The views expressed are those of the authors and do not reflect the official policy or position of the USUHS, the Department of Defense, or the United States Government.
(Kennedy 1947, Muirhead-Thomson 1960, Elliott and de Zulueta 1975). The term repellency is used to describe oriented movements of avoidance made by the insect in response to a chemical stimulant without having made tarsal contact with the chemical (Roberts et al. 2000). Irritancy refers to oriented movements made by the insect away from a chemical stimulus only after making tarsal contact with the chemical (Roberts et al. 2000). Excito-repellency implies a series of oriented avoidance behaviors made with or without tarsal contact to a chemical stimulus (Roberts and Andre 1994).

A long-standing belief was that the only true action of an effective malaria campaign was the reduction of the vector population. This belief is based on Macdonald's early mathematical models (1950, 1957), which emphasize the relationship between decreased vector survival and reduced rates of malaria transmission. These models pointed out that any effect that decreases the probability of a lethal contact with the insecticide would negatively affect the reduction in malaria transmission. This notion led to the assumption that behavioral avoidance of residual insecticides would, in essence, protect the vectors and, therefore, prevent overall reductions in the vector populations (Muirhead-Thomson 1950). This assessment, however, overlooks the possibility that prevention of indoor biting and resting behaviors might be an effective approach to reducing malaria transmission. Others have also stressed the relationship between altered behavioral patterns associated with insecticide interaction, and to their implications for control of vector-borne disease (Cullen and De Zulueta 1962, Hamon et al. 1970; Elliott 1972, and Gillies 1988). Indeed, present understanding of disease transmission indicates that the disruption of the host-vector interaction may be more important than an actual reduction in mosquito populations.

Research efforts in Belize have focused on four anopheline species, which have been incriminated in the transmission of human malaria including: Anopheles albimanus Weidemann; An. darlingi Root; An. pseudopunctipennis Theobald; and An. vestitipennis Dyar and Knab. Both An. darlingi and An. pseudopunctipennis have shown the ability to transmit malaria in areas of Central America (Padilla et al. 1992) while Anopheles albimanus is widely believed to be the primary vector in this region (Ramsey et al. 1994). Although this species readily feeds on humans, recent studies in Belize have indicated that it exhibits a weak endophagic behavior (Bangs 1999, Roberts et al., unpub. data).

Anopheles vestitipennis is found throughout the coastal regions of Mexico, Central America, regions of northern South America, Cuba, Dominican Republic, and Puerto Rico (Loyola et al. 1991, Mekuria et al. 1991, Padilla et al. 1992, Marqueti et al. 1992), and until recently, its role as an important vector of human malaria has not been well established. In fact, Boyd (1949) reported that An. vestitipennis is of little importance in the transmission of malaria. Loyola et al. (1991), however, observed this species to be both endophilic and endophagic, and found native populations of An. vestitipennis in Chiapas, Mexico, positive for Plasmodium vivax. In a malaria vector survey conducted in Belize, Kumm and Ram (1941) found malaria sporozoites in the salivary glands of An. vestitipennis and An. darlingi, but not in An. albimanus. More recently, natural P. falciparum infections in An. vestitipennis collected from Belize have been identified by sporozoite ELISA (Achee et al., unpub. data). This data indicates that An. vestitipennis had a higher minimum field infection rate (0.282%) than either An. albimanus (0.162%) or An. darlingi (0.271%). Evidence for the role of An. vestitipennis as a vector of malaria in Belize has been mounting from thorough documentation of human-vector contact (Roberts et al. 1993 and Bangs 1999), relatively high natural infection rates (Achee et al., unpub. data), and confirmation of malaria sporozoites in naturally infected salivary glands (Kumm and Ram 1941). Taken together, these observations incriminate An. vestitipennis as one of three important malaria vectors in Belize.

MATERIALS AND METHODS

Study Area

In the early part of 1998, an experimental hut study was undertaken to determine the house entering and exiting behavior of An. vestitipennis in the Toledo District, located in southern Belize (Fig. 1). The study was conducted in the village of Rancho (N 16° 09.954, W 88° 50.529), which was composed of 166 houses with a total population of approximately 824 people based on survey statistics from 1998. The village was located about four miles from the Gulf of Honduras near the town of Punta Gorda. Study site selection was based on the Belize Ministry of Health's classification of Rancho as one of the five most malarious areas within the Toledo District and preliminary surveys which detected large numbers of An. vestitipennis larvae and adults.

The rainy season in the southern portion of Belize begins in late April and continues through December. The average yearly rainfall in the south is generally greater than 160 inches. The coolest temperatures in the region occur from November to December with an average temperature of 24°C while the warmest temperatures occur May through September (average
Figure 1. Map of the Toledo District in Southern Belize. The location of the experimental hut site is indicated by the star.
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temperature of 27°C). The prevailing winds blow off of the Gulf of Honduras from the Southeast from January through August and shift out of the Northwest beginning in September.

Three identical huts were constructed for experimental purposes and for a series of 15-hour collections. Huts were modeled after the basic design of a Mayan house that is common in southern Belize. The walls of the hut measured 7.8 m long x 4.2 m wide x 2.1 m high and consisted of plank wood walls, dirt floor, and a thatch roof constructed from Cahune palm. The apex of the angled roof measured 4.5 m from the floor of the hut. A walkway running lengthwise down the center of each hut was constructed 3 m above the floor to enable examination of the thatch during collections. Each hut had three windows and a north-facing door. In addition, each hut was equipped with a small auxiliary door (0.5 m wide x 1 m high) through which collectors could enter or exit the huts without removing the traps. This door remained shut and locked except when collectors were rotated. All three huts were constructed to accommodate window and door interception traps. The huts were situated in a triangle with the most northern house (Hut A) positioned 15 m from the other two structures and Hut B and Hut C positioned 30 m from each other. Two additional huts, that housed the collectors and their families, were located about 40 to 50 m from the experimental hut site.

**Interception Traps**

With minor modifications, window and door interception traps were constructed according to the design of Muirhead-Thomson (1950). The window traps were 0.6 m x 0.6 m x 0.6 m boxes and were constructed with lengths of 1.3 cm PVC pipe. The door traps were constructed in a similar fashion and measured 1.8 m x 0.9 m x 0.9 m. Both trap types were covered by green polyester insect netting (BioQuip Products Inc.) and were sewn into place. The front of the traps were left open to form a five-sided box. Sleeve material was attached over a hole in the rear of the trap to facilitate the removal of resting mosquitoes. Initial efforts were made using a funnel in the front of the trap to act as a one way baffle. After a number of trials, this apparatus was removed as an obstacle to mosquito passage. Additionally, a 3 m x 0.3 m x 0.3 m eaves trap and a 3 m x 1.8 m x 0.8 m wall trap were constructed to aid in determining the mode of entry into the huts. These traps were constructed in a similar fashion to that used for the window and door traps.

**Collection Method**

Simultaneous collections were conducted using all three huts. During a single night’s collection (4:30 P.M. to 7:30 A.M.), one hut was affixed with exit traps while another was affixed with entrance traps. The third hut was maintained as a control hut at which an indoor/ outdoor human landing collection was conducted. The type of collection conducted at each hut was rotated on subsequent nights in order to minimize slight variations between the huts. A host presence was established in each hut prior to the collection by placing collectors in the huts one hour before the beginning of the sampling period. Two collectors were placed in the center of each trap hut to act as bait during the collection.

Human landing collections at the control hut were conducted for 30-minutes each hour. Indoor collectors were positioned in the center of the hut while outdoor collectors were positioned on the side of the hut at least 3 m from any openings. Collections consisted of collectors aspirating mosquitoes from their exposed, lower legs for a 30-minute time period. Indoor collectors switched with outdoor collectors halfway through each 30-minute collection period. All collectors rotated their positions between huts throughout the night to eliminate the possibility of collector bias. Post-spray collections were conducted with traps being placed in the same position (i.e., both with exit traps or both with entrance traps) on the two sprayed huts. Post-spray human landing collections were conducted at the control hut in the same manner as described above.

Resting mosquitoes were collected from the interior aspects of the traps using a mouth aspirator for a period of 30 minutes every hour. Collected mosquitoes, from both human landing and interception traps, were placed in separate cardboard cages and labeled with the date, time, and hut location for each collection period. Each hour represents the 30-minute sampling period, which occurred beginning at the top of that hour. Mosquitoes were killed with chloroform vapor, and species were identified the following morning.

**Insecticide Application**

On November 27, 1998, after the initial pre-spray trials had been completed, trained personnel of the Belize Malaria Control Program sprayed two of the huts with insecticide following established protocol. The interior walls, lower 1 m of thatch, and outside eaves of Hut C were sprayed with DDT wettable powder (75% wdp) that had been suspended in clean water to make a 4% (technical grade) formulation. Spraying was conducted using a Hudson X-Pert compression sprayer equipped with an 8002E Teejet nozzle in order to obtain a fan application to all hut surfaces. Hut B was sprayed in an identical manner as that applied to Hut C except...
using a 5% deltamethrin wettable powder. Four ounces of wettable powder were suspended in 4 gallons of clean water. The application rate was 2 grams per square meter. Both huts were allowed to dry completely (for no less than 12 hours) before a collection was performed.

RESULTS

Pre-Spray Collections

A total of 26 pre-spray human landing collections were conducted from February to December 1998 resulted in the collection of the following anopheline species placed in order of abundance: An. vestitiennis (23,087), An. punctimacula (437), An. albimanus (158), An. apicimacula (17), An. gabaldoni (10), An. darlingi (3) and An. neivai Howard, Dyar & Knab (1). Analysis of five preliminary all-night human landing collections performed at all three huts simultaneously indicated the mean number of anopheline mosquitoes collected at the three huts were not significantly different (F value = .03; p>0.05).

Anopheles vestitiennis showed a constant high level of landing/biting activity beginning shortly after sunset and continuing until approximately one hour prior to sunrise (6:00 A.M.) (Fig. 2). An examination of the indoor/outdoor biting populations showed no difference between the number of An. vestitiennis collected inside the huts (11,220) compared to outside the hut (11,867), for an O:I ratio of 1:0.9.

The nightly biting activity of the other anophelines found at the study area conforms to what is already found in the literature (Hobbs et al. 1986, Taylor 1966, Rachou et al. 1965 and Muirhead-Thomson and Mercier 1952). Outdoor:indoor ratios, however, were calculated for the other anopheline species collected during this study to document the behavior of these species for the southern portion of Belize, Central America. The density of outdoor and indoor biting populations of An. albimanus exhibited a marked difference with 91% (144) of the population being collected outside and only 9% (14) of the population being collected inside. The O:I ratio for An. albimanus was 1:0.1. Anopheles punctimacula also exhibited a greater propensity for biting outdoors with 71% (309) of the biting population being collected outdoors and only 29% (128) being collected indoors. These data translate into an O:I ratio of 1:0.4. The remaining four species, An. apicimacula, An. gabaldoni, An. darlingi, and An. neivai were all collected in outdoor collections.

Mode of Entry

Studies on mode of entry into the hut employed the simultaneous use of three window traps, one door trap, one wall trap, and one eaves trap. Overall, intercept traps were effective collecting devices for An. vestitiennis mosquitoes. The traps were, however, more effective as exit traps (3,929) than as entrance traps (2,807).

The pre-spray trap collections of An. vestitiennis were conducted over 21 trap nights between August and November of 1998 and consisted of multiple collections each night. The collection totals were averaged by time period and graphed against the midpoint for that time interval. Peak entering occurred between 6:45 P.M. and 9:45 P.M. (Fig. 4). The vast majority of the entering population was collected in the first half of the night with 91% of the An. vestitiennis being collected before midnight. Peak exiting began at approximately 11:45 P.M. and continued until 4:45 A.M. (Fig. 5). Exiting An. vestitiennis were primarily collected during the latter half of the night. Only 31% of all exiting An. vestitiennis were collected prior to midnight while 69% were collected between midnight and 6:45 A.M.

Only three anophelines collected from all the entrance traps were blood fed (0.1%). Two of these were An. punctimacula and only one was An. vestitiennis. For this reason, the gonotrophic status of the entering mosquitoes collected was classified as unfed. The door and window openings clearly proved to be the preferred mode of entry with 1,044 anopheline mosquitoes (35.3%) being collected from the window traps and 1,760 (59.5%) being collected from the door trap (Fig. 3). The eaves and walls appeared to contribute very little to the overall buildup of entering anopheline mosquitoes, with 4.4% (130) entering through the eaves and 0.8% (24) entering through cracks in the wall. These traps only sampled 1/8 of total wall or eaves space in the house (3 m of 24 m of wall or eaves space). Assuming random movement into the house, the number of mosquitoes entering through other areas, such as the eaves or walls should be proportional to that obtained from the trapped portion. For this reason, collections from the eaves and wall were adjusted to reflect the true size of these entry points. After adjustment, the doors and windows remained as primary modes of entry (43.6% and 25.9%, respectively). The eaves also showed to be a primary mode of entry after adjustment (25.7%). Only the walls continued to contribute very little to the overall indoor population of anopheline mosquitoes (4.8%).

Entry/Exit Times (Post-Spray)

All night post-spray collections (5 entrance and 5 exit) were conducted between November and December of 1998. The pattern of An. vestitiennis biting activity was similar to pre-spray patterns of activity. Biting
Figure 2. Average number of *Anopheles vestitipennis* collected from human baited collections during 26 collection nights at Rancho, Toledo District, Belize, from March to November of 1998. Each collection was conducted for 15 hours by two collectors collecting for 30 minutes each hour throughout the night. Data is plotted against the midpoint of each 30-minute sample period.

Figure 3. Percentage of total *Anopheles vestitipennis* populations collected from entrance interception traps during 21 nights of collections. Window and door traps represent collection from entire portal region while the eaves and wall trap represent only 1/8 of the total surface area for that mode of entry.
Figure 4. Comparison of the average number of *Anopheles vestitipennis* collected under pre- and post-spray conditions from entrance interception traps conducted during 21 and 5 collection nights, respectively, at Rancho, Toledo District, Belize, from August to December of 1998. Each collection was conducted for 15 hours by two collectors collecting for 30 minutes each hour throughout the night. Data is plotted against the midpoint of each 30-minute sample period. During a single night's collection, both sprayed huts were affixed with entrance traps, and a human baited collection was conducted at the control hut.

Figure 5. Comparison of the average number of *Anopheles vestitipennis* collected under pre- and post-spray conditions from exit interception traps conducted during 21 and 5 collection nights, respectively, at Rancho, Toledo District, Belize, from August to November of 1998. Each collection was conducted for 15 hours by two collectors collecting for 30 minutes each hour throughout the night. Data is plotted against the midpoint of each 30-minute sample period. During a single night's collection, both sprayed huts were affixed with exit traps and a human baited collection was conducted at the control hut.
began shortly after sunset (6:45 P.M.) and continued at a high level throughout the remainder of the night in the control hut (Fig. 5). The O:1 ratio was calculated to be 1:0.8. Numbers of *An. vestitipennis* collected in the unsprayed hut, compared to pre-spray collections, showed a 37.4% reduction.

Both the DDT and the deltamethrin sprayed huts exhibited a reduction in population levels from pre-spray trials. Numbers of *An. vestitipennis* collected in pre-spray traps compared to the numbers trapped post-spray documented a 66% reduction in the deltamethrin hut and a 97% reduction in the DDT hut. Trap data for the DDT hut showed that two of the nights with the largest collections occurred with winds of greater than 5 mph and bouts of heavy rain.

The numbers of *An. vestitipennis* that entered the DDT hut post-spray were very low and only small peaks of entrance/exit activity occurred in the early hours after sunset (5:45 P.M. to 7:45 P.M.) (Figs. 4 and 5). The deltamethrin hut, however, continued to have substantial numbers of *An. vestitipennis* enter the house after spraying. In the deltamethrin-sprayed hut, the peak entrance time generally followed the same pattern as that seen in the pre-spray trials. A peak occurred between 6:45 P.M. and 9:45 P.M. (Figs. 4, 5, and 7). The exit times, however, demonstrated a dramatic shift to earlier in the evening. In addition visual examination of the unsprayed portions of the thatch during the collection indicated that there were no mosquitoes resting on these surfaces during the course of the night. After spraying, the peak in exit activity occurred between 8:45 P.M. and 11:45 P.M. Almost all mosquitoes had exited the deltamethrin-sprayed hut by midnight.

An ANCOVA analysis was performed on the entrance activity to examine the affect population density had on trap collection totals (Fig. 8). The analysis compared both pre- and post-spray entrance trap collection totals to the population density on the same night based on the human-landing collection from the control hut. As population densities increased, the collection from the entrance traps also increased for both pre-spray huts and for the post-spray deltamethrin hut. The slope of the DDT hut regression line, however, was not significantly different from zero (> 0.05) and, therefore, represented no increase in entrance activity with increased population density.

**DISCUSSION**

Experimental hut studies provide critical information on the behavioral activities of vector populations in malarious areas. The present study demonstrates the differences in the biting activities of three anopheline species commonly found in the southern regions of Belize. *Anopheles albimanus* showed a much stronger exophagic behavior than either *An. vestitipennis* or *An. punctimacula*. *Anopheles punctimacula*, however, showed a greater tendency towards exophagy than *An. vestitipennis*, with only 29% of the biting females collected indoors.

At the Rancho study site, *Anopheles vestitipennis* was collected in the greatest abundance and, therefore, exhibited the strongest trends. Continuous high levels of biting activity occurred throughout the night both indoors and outdoors. Activity began shortly after sunset and continued until just prior to sunrise. In addition, this species demonstrated the strongest endophagic behavior with a calculated O:1 ratio of 1:0.9, clearly documenting this species' strong tendency to enter a house and feed.

Data pertaining to the mode of entry into the huts show that the door, window, and eaves all contribute to the overall indoor population of *Anopheles vestitipennis*. The door, windows, and eaves present very large portals of entry. A host-seeking mosquito would find little or no obstacle to house entry when presented with one of these openings. The gaps between the slates of the wall, on the other hand, are very narrow (ranging from 1 to 2 cm wide). Those few mosquitoes entering through the wall may be host-seeking females that accidentally find one of these cracks in the process of trying to locate a window or door. *Anopheles vestitipennis*, for example, were observed to land on the exterior wall surface prior to house entry. After a short outdoor resting period, the mosquito would again take flight, bouncing along the wall in either a horizontal or vertical pattern. It would continue this movement until it reached either a window, door, or eaves opening. It is possible that during this behavior a proportion of mosquitoes would locate a suitable gap in the wall. This may account for the small number of anophelines that were collected from the wall trap.

Entrance trap collections from unsprayed huts showed that the majority of *An. vestitipennis* entering the house were collected during the first six hours after sunset. Of those mosquitoes entering the hut, 91% were collected prior to midnight. While peak entry occurred shortly after sunset, the biting activity continued at a high level throughout the night. This implied that a large proportion of the host seeking female population rested within the interior of the hut prior to obtaining a blood meal. Under identical conditions, exiting of *An. vestitipennis* females occurred during the last six hours before sunrise. Those *An. vestitipennis*, which entered the unsprayed hut, peaked in their exiting behavior at three to four hours before sunrise, at which time they
Figure 6. A comparison of entrance and exit times for *Anopheles vestitiennis* post-spray with deltamethrin. Entrance and exit collections were conducted on different nights. Average number of mosquitoes collected per 30-minute sample period are plotted against the midpoint of that sample period.

Figure 7. Average number of *Anopheles vestitiennis* collected from human baited collections conducted post-spray during 10 night collections at Rancho, Toledo District, Belize, from November to December of 1998. Each collection was conducted for 15 hours by two collectors collecting for 30 minutes each hour throughout the night. Data is plotted against the midpoint of each 30-minute sample period.
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Figure 8. Ancova analysis showing the relationship between increasing population density and increasing entrance trap collection for *Anopheles vestitipennis*. An increase in the number of mosquitoes collected from the traps is positively correlated with all treatments except the DDT treated hut where the slope of the line is not significantly different from zero (P>0.05).

returned to the outdoor environment in search of suitable resting sites.

After deltamethrin application, time of peak exiting for *An. vestitipennis* populations was shifted to earlier in the night, by approximately five hours. Examination of the thatch during the collection indicated that there were no mosquitoes resting on the unsprayed portions of the thatched roof. This shift, in conjunction with very little deviation from pre-spray entrance behavior, indicated that *An. vestitipennis* was sufficiently irritated or repelled by deltamethrin to leave shortly after entering the hut. Although this effect was observed, there still remained a two-hour period between peak time of entering and peak time of exiting in which biting occurred, allowing for the possibility of malaria transmission to also occur. In addition, it should be noted that a common practice during insecticide application is the removal of personal items from inside the hut prior to spraying the walls, beams, and ceiling. When returned to the hut, these items provide a number of suitable, untreated resting sites. These unsprayed surfaces may decrease the overall irritancy effect, as well as, allow mosquitoes to avoid contact with the chemical, and thereby avoid uptake of a lethal dose of insecticide.

After spraying with DDT, there was a reduction of 97% in the total population of *An. vestitipennis* coming to the treated hut. This equates to the collection of an average of 4.9 mosquitoes per night in the DDT hut. This is compared to a 66% reduction (or an average nightly collection of 55.4 mosquitoes/night) in the deltamethrin hut and a 37% reduction (or an average nightly collection of 465.9 mosquitoes/night) in the control hut. This illustrates a strong repellency effect due to the DDT, and a lower but, perhaps important level of deltamethrin repellency. A reduction in the number of mosquitoes actually coming into the hut indicates a clear reduction in the vector/host interaction and a potential break in the transmission cycle.

Pre- and post-spray variations in *An. vestitipennis* collected from the entrance traps were not due to natural changes in population density as determined by the ANCOVA analysis. Both pre-spray huts demonstrated increased trap collections in association with increasing population densities. This same positive linear trend was also seen after deltamethrin application. The DDT treated hut, however, showed no increase in the number of *An. vestitipennis* collected from entrance traps as the mosquito population levels increased. This means that the number of *An. vestitipennis* entering a hut will increase in both untreated and deltamethrin treated huts as mosquito densities increase but will be repelled from entering a DDT treated hut regardless of vector...
population levels.

In conclusion, An. vestitipennis in southern Belize exhibited a strong endophagic behavior. This species readily fed indoors and was a persistent biter throughout the night. The deltamethrin hut showed a definite irritancy effect. Although the chemical did not alter the pattern of house entering activity, it did alter the time of exiting. DDT on the other hand proved to have a very powerful repellency effect. Under normal reduced population levels (37.4% in control hut), deltamethrin only exhibited a 66% reduction, while DDT showed a reduction of 97%. This effect goes contrary to earlier observations made by Roberts et al. (1993) that An. vestitipennis was undeterred by DDT residues. These early observations were based on 45 minute indoor/outdoor human landing collections conducted in houses that where last sprayed with DDT months prior to the actual collection activity. The present study sampled anopheline populations throughout the night and was conducted for two weeks after the initial spray. Differences in the house entering behavior of An. vestitipennis documented by these two studies are clearly associated with the duration of the residual effect of DDT. Due to the short time frame of the post-spray observations in the present study, it is clear that additional studies are required to examine the residual effects of both DDT and deltamethrin in order to determine their long-term effectiveness.

The repellency effect documented in the DDT-sprayed house essentially excluded human-vector contact within that house. Reduced levels of mosquitoes entering and biting will strongly reduce the potential for malaria transmission. While the irritancy effect of deltamethrin reduced the potential window of opportunity for transmission, it did not preclude transmission from occurring within the house during those few hours in which An. vestitipennis females were in contact with humans.

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Insecticide Resistance Status, Esterase Activity, and Electromorphs from Mosquito Populations of *Culex quinquefasciatus* Say (Diptera: Culicidae), in Houston (Harris County), Texas

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ABSTRACT: *Culex quinquefasciatus* Say is a vector of St. Louis encephalitis (SLE) in Texas. This disease is endemic and prevalent in the Houston area. Disease prevention through mosquito control is mainly targeted against adults by application of a resmethrin-piperonyl butoxide formulation (Scourge®). Immature mosquitoes were collected from eight areas in Harris County during 1998. The susceptibility status of these populations to Scourge®, malathion, and resmethrin, the latter alone or with an esterase inhibitor as a synergist, was determined using a bottle assay with females. The population structure was investigated by electrophoretic analysis of esterases and their activity. Individual females were also analyzed for esterase activity by plate assay and for isoenzyme pattern by native PAGE. Bioassays indicated high levels of resistance to malathion in all areas. In addition, the effectiveness of Scourge® in mosquitoes from area 51 deteriorated throughout the season. A localized, distinctive esterase pattern and activity level was observed in mosquitoes from different areas. Overall, the frequency of esterases Esta2 (A2)/ Estβ2 (B2) was higher than that of Estβ1 (B1). Altogether, these results indicate the onset of a fragile situation for mosquito control that should be further analyzed to effectively maintain the SLE prevention program for Harris County.

**Keyword Index:** Malathion resistance, resmethrin resistance, carboxylesterases, cross resistance, Scourge®

INTRODUCTION

There were 237 confirmed human cases of St. Louis encephalitis (SLE), with 24 human fatalities, in Harris County, TX from 1975 to 1998 (Texas Dept. of Health, http://www.tdh.state.tx.us, under Epigram). During 1998 there were four cases of SLE reported in Harris County. The onset of all four cases was within a two-week period starting on August 19; no deaths occurred (Rawlings and Marengo 1999). There is no vaccine for the disease and the primary means of prevention is surveillance of the SLE arbovirus (flavivirus) in local mosquito populations and in feral birds that are captured and screened for antibodies to SLE virus, and control of the mosquito vector. Calculations predict that global warming will increase the transmission of SLE, not only in subtropical, but also in now temperate regions of the U. S. (Gratz 1999, Reeves et al. 1994).

The Harris County Mosquito Control Division operational area is divided into 268 sectors serving about 3,000,000 people. Current practices of adult mosquito control in Harris County include the application of resmethrin, a pyrethroid, formulated with the synergist piperonyl butoxide (PBO) (Scourge®) as Ultra Low Volume (ULV) space spray against *Culex quinquefasciatus*, and ULV space spray malathion, an organophosphate, to control other pest mosquito species. These ULV applications of both insecticides are by truck. Control activity is largely confined to residential neighborhoods and most intense spraying occurs within the limits of the interstate 610 freeway (I-610) loop in Houston, where SLE is more prevalent.
We conducted this study in 1998 with collections of *Cx. quinquefasciatus* mosquitoes from Harris County to better understand the local distribution of resistance to the above adulticides, the frequency of resistance, and their associated esterase levels and electromorphs. It is known that worldwide overproduction of esterases in *Culex* spp. is a major mechanism of resistance to organophosphates and a secondary mechanism of resistance to carbamates (Hemingway and Karunaratne 1998). The general mechanism of esterase overproduction is the amplification of the esterase genes at the Est-2 (esterase B or β) and Est-3 (esterase A or α) loci (Raymond et al. 1998). These two esterase genes are on the same chromosome, at less than one percent recombination (Rooker et al. 1996). Overproduction of all β esterases studied so far, e.g. Estβ1 (Mouchès et al. 1990), Estβ2 (Vaughan and Hemingway 1995), Estβ4 (Poirié et al. 1992), Estβ5 (Severini et al. 1997) and Estβ6, is due to gene amplification (Rooker et al. 1996). The mechanism for overproduction of Estα2 is also gene amplification (Vaughan and Hemingway 1995). The *Culex* amplified esterases Estα2 and Estβ2 were first reported in the U. S. by Raymond et al. (1987) and the genes have been cloned. The genes are together ‘head to head’ in the amplicon and this explains their linkage disequilibrium (Vaughan et al. 1997). There are conflicting reports on the origin of the amplicon, some indicating that it has arisen once and spread worldwide (Raymond et al. 1991), others indicating independent amplification events (Hemingway et al. 1990). All amplified esterases in different species of *Culex* mosquitoes act by sequestration; they bind the insecticides at a faster rate than those esterases that are not amplified (Cuany et al. 1993, Ketterman et al. 1992, Hemingway and Karunaratne 1998). Slow turnover of the insecticide may occur subsequently (Ketterman et al. 1992). Although gene amplification of esterases is common, a genetic regulatory mechanism was reported for overproduced Estα1 (Rooker et al. 1996, Guillemaud et al. 1997). For current *Culex* esterase nomenclature and a review see (Vaughan and Hemingway 1995, Hemingway and Karunaratne 1998).

In Harris County, applications of malathion to control *Cx. quinquefasciatus* started in 1965 and ceased in 1993, but currently continue against other species of mosquitoes. Consequently, malathion use has substantially decreased since 1993. Most insecticide applications against *Cx. quinquefasciatus* are now scheduled on a need-only basis using a resmethrin-piperonyl butoxide formulation (Scourge®), believed to be highly effective. Since the cessation of malathion use against *Cx. quinquefasciatus*, a comprehensive analysis of esterases in these populations has not been undertaken.

There are, however, references in the literature indicating the presence of esterases Estα2 and Estβ2 and Estβ1 in *Cx. quinquefasciatus* from Houston (Beyssat-Arnaouty et al. 1989, Raymond and Pasteur 1996). We determined the susceptibility status and the esterase activity and pattern in individual mosquitoes from various areas. The localized nature of resistance development and consequences for mosquito management and SLE prevention are discussed.

**MATERIALS AND METHODS**

**Mosquito Collections**

Larvae and pupae of *Culex quinquefasciatus* Say were field collected within eight of the areas serviced by the Harris County Mosquito Control Division, from July to November, 1998. The first week of the calendar year 1998 was considered week 0. Immature mosquitoes were reared in the laboratory until adult emergence (F₁ generation). Females, 2-5 days old were used in bioassays, for quantitative analysis of esterase activity, and pattern of esterase activity by native PAGE. Mosquito collections for quantitative esterase analysis and electrophoresis were as follows: area 54 collected in early July (week 27); area 55 collected twice, in early July (week 27) and in late August (week 33); areas 42 and 66 collected in early August (week 31); area 512 in late August (week 33). Mosquitoes from areas 51, 106, and 206 were collected on September 1 (week 35); an additional collection from area 51 was in early November. These adult F₁ mosquitoes were kept frozen at -80°C until analysis; in all cases for less than one month. Maintenance at this temperature does not alter the esterase pattern on PAGE (Callaghan 1993).

**Bioassays**

The susceptible *Cx. quinquefasciatus* Sebring reference strain was obtained from Dr. J. K. Olson (Dept. of Entomology, TAMU) and was originally obtained from the USDA-ARS in Gainesville, Florida. Females were tested for susceptibility to malathion, Scourge®, and resmethrin using a bottle assay with single discriminatory dosages that estimate effectiveness through comparisons of percentage mortality at various times (Brogdon and McAllister 1998a). An esterase inhibitor S, S, S-tributyl-phosphorotriothioate (DEF) (98%) (Chem Service, Inc., West Chester, PA) was used at the sublethal dosage of 90 μg/bottle. Each test consisted of four 250-ml bottles; three coated with insecticide or insecticide plus synergist and one with acetone as a control. The average number of mosquitoes per bottle was 15.1, ranging from 11-19 per bottle in individual tests, depending on availability. The number
of tests per collection was most commonly three, but ranged from 2-6 (TABLE 1). Malathion was tested at 400 µg/bottle and resmethrin at 30 µg/bottle. The bottles treated with Scourge® contained 90 µg/bottle PBO and 30 µg/bottle of resmethrin. Susceptible mosquitoes were used first to establish the time at which 100% of them died, this was approximately 30 min. for malathion and 15 min. for resmethrin and Scourge®. For field collected mosquitoes, the number dead was recorded at these respective exposure times and the percentage alive was calculated. Additional subsequent readings were every 15 min. for a total exposure of 135 min. Resmethrin was technical grade (99%) obtained from Cheminova and Scourge® (18% resmethrin, 54% of piperonyl butoxide, a mono-oxygenase inhibitor) from AgrEvo.

**Esterase Plate Assays**

Carboxylesterase activity was determined from single mosquitoes using a microplate assay as described (Dury et al. 1990). Thirty non-blood fed females were analyzed from each of the eight areas under study, while 15 males and 15 females were analyzed from the *Cx. quinquefasciatus* Sebring susceptible strain. The average esterase specific activity between males and females was not significantly different in the Sebring strain. Briefly, single females were immobilized by cold exposure and homogenized in 100 µl of phosphate buffer on a porcelain plate. Esterase activity in the homogenate was measured by monitoring the production of α-naphthol from α-naphthyl acetate after the addition of Fast Garnet GBC salt (Sigma, St. Louis, MO). The absorbance of this complex was measured at 490 nm with a Bio-Rad (Richmond, CA) plate reader. Similarly, a standard curve was calibrated by measuring the absorbance at 490 nm of various known quantities of α-naphthol after the addition of Fast Garnet. The average absorbance of three determinations per mosquito was used to estimate esterase activity. Esterase activity (U) was expressed as moles of α-naphthyl acetate hydrolyzed/min Protein concentration was estimated with the BCA protein assay kit (Pierce, Rockford, IL) and bovine serum albumin as a standard; absorbance readings were at 595 nm.

Statistical analysis was performed with SigmaStat software (SPSS Inc., Chicago, IL). Pooled specific esterase activity data of individual mosquitoes from all areas, and esterase activity within each area were tested for normality. To compare the activity among all areas the Kruskal-Wallis one way ANOVA on ranks, followed by the multiple comparison Dunn’s test, was performed.

The upper limit of esterase activity in the susceptible Sebring strain was calculated as the mean specific esterase activity (0.053 U/mg) plus three standard deviations (3 x SD= 0.039). From normal distribution theory results that P(x) > 0.09 U/mg = 0.135%. This specific activity of 0.09 U/mg was used as the discriminatory value between susceptible and resistant populations, with a probability of 0.135% that a value higher than this corresponds to the susceptible population.

**Native PAGE**

Ten to fourteen females from each area were run on native 7% polyacrylamide gels followed by esterase activity staining to visualize electromorphs. Running buffer was 0.1 M TBE, pH 8.8, and the gels were pre-run at 200 V and 5°C for 30 min. prior to loading the samples (Karunaratne et al. 1995, Callaghan et al. 1994). Individual mosquitoes were crushed in 100 µl of 0.1 M TBE, pH 8.8. Crude homogenates were drawn up through tissue paper and spun briefly at 15,000 g to remove solids. Twenty µl of the supernatant were mixed with 10 µl of native sample buffer (0.1% bromophenol blue and 30% glycerol), vortexed, and loaded into a well. Gels were run at 4°C and at 100 V until the bands concentrated on top of the resolving gel, and then run at 200 V for 4 hr. Approximately 10-15 µg of protein were loaded per lane, determined with the Pierce Protein Assay kit. Prior to staining, resolved gels were soaked in 0.5-M cold boric acid for 30 min. and rinsed with 0.1-M phosphate buffer, pH 6.5. Soaking each gel at 25°C in 50 ml of 0.1 M phosphate buffer, pH 6.5, plus 10 mg each of α- and β-naphthyl acetate in 5 ml of acetone revealed the esterase bands. The gels were shaken gently in this substrate solution for 15 min., then 100 mg of Fast Garnet GBC in 5 ml phosphate buffer was added to stain the esterase bands, and kept with agitation for 15 to 30 min. (Pasteur et al. 1988). Gels were then fixed in 50% methanol/10% acetic acid for 30 min., followed by rinsing with double distilled water. Gels were dried onto blotting paper using a Bio-Rad Model 350 gel dryer at -80°C for 1 hr. under vacuum, followed by photographing or digital scanning. The number of females analyzed (84) is higher than numbers reported in similar studies (Raymond et al. 1996, Raymond et al. 1987).

**RESULTS**

The Harris County Mosquito Control Division operational areas investigated are shown in Figure 1. Areas 42, 51, 54, 55, and 66 are within the limits of the I-610 Houston loop. Areas 106 and 206 are north of it and area 512 is south.
### TABLE 1. Areas from Harris County, number of insecticide treatments in 1998 and laboratory bioassay results of corresponding *Culex quinquefasciatus* females.

<table>
<thead>
<tr>
<th>Collection Site (Area #)</th>
<th># Treatments Mal.</th>
<th># Treatments Scourge</th>
<th>Week Collected</th>
<th>Week Tested</th>
<th>No. of Tests</th>
<th>Insecticide Tested</th>
<th>L^2 (%)</th>
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<td>51</td>
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<td>22</td>
<td>27</td>
<td>28</td>
<td>3</td>
<td>malathion</td>
<td>79 - 93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27</td>
<td>29</td>
<td>2</td>
<td>resmethrin</td>
<td>0 - 2</td>
</tr>
<tr>
<td></td>
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<td>35</td>
<td>36</td>
<td>3</td>
<td>malathion</td>
<td>98 - 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>38</td>
<td>1</td>
<td>resmethrin + DEF</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>44</td>
<td>45</td>
<td>3</td>
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<td>22 - 42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>Scourge</td>
<td>16 - 20</td>
</tr>
<tr>
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<td></td>
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<td>3</td>
<td>resmethrin + DEF</td>
<td>20 - 24</td>
</tr>
<tr>
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<td>35 - 42</td>
</tr>
<tr>
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<td>0</td>
<td>16</td>
<td>27</td>
<td>28</td>
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<td>98 - 100</td>
</tr>
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<td>3</td>
<td>resmethrin</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>29</td>
<td>3</td>
<td>Scourge</td>
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</tr>
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<td>84 - 98</td>
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<td>33</td>
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<td>96 - 98</td>
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<td>30</td>
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<td>resmethrin</td>
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</tr>
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<td>27</td>
<td>29</td>
<td>3</td>
<td>Scourge</td>
<td>0</td>
</tr>
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<td>106</td>
<td>0</td>
<td>1</td>
<td>26</td>
<td>27</td>
<td>3</td>
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<td>resmethrin</td>
<td>0 - 3</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>27</td>
<td>28</td>
<td>3</td>
<td>Scourge</td>
<td>0</td>
</tr>
<tr>
<td></td>
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<td>resmethrin (CS-99)</td>
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<td>1</td>
<td>26</td>
<td>27</td>
<td>3</td>
<td>malathion</td>
<td>77 - 100</td>
</tr>
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<td></td>
<td></td>
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<td></td>
<td>3</td>
<td>resmethrin</td>
<td>0</td>
</tr>
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<td>Scourge</td>
<td>0</td>
</tr>
<tr>
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<td></td>
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<td>36</td>
<td>3</td>
<td>resmethrin (CS-99)</td>
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<td></td>
<td></td>
<td>2</td>
<td>resmethrin (AGR-90)</td>
<td>0 - 2</td>
</tr>
</tbody>
</table>

1Malathion ULV.

2Percentage of live mosquitoes after all susceptible mosquitoes have died, about 30 min. for malathion or 15 min. for resmethrin.

Insecticide Field Treatments: In Harris County, one treatment with Scourge® or malathion is defined as the one-time area-wide application of ULV material. The application rate for malathion is 0.05 lbs. per acre and that of Scourge® is 0.003 lbs. per acre.
Figure 1. Map of Harris County showing the areas serviced by the Mosquito Control Division. The eight areas studied are darkened.
Bioassay results for the F<sub>0</sub> generation of Cx. quinquefasciatus females are shown in TABLE 1. For malathion, the percentages of living mosquitoes after 30 min. of exposure or after all susceptible Cx. quinquefasciatus Sebring mosquitoes died, were very high in all areas tested, ranging from 77-100%. It is noteworthy that 100% mortality was never achieved with malathion for Cx. quinquefasciatus from all areas tested, not only after 135 min. but in some cases after longer exposures of up to one day (not shown). The potential for decreased susceptibility to resmethrin was seen in some areas. A few mosquitoes (2-7%) from areas 55, 66, 206, and 106 survived the discriminatory resmethrin concentration. In areas 55 and 66, this may be a consequence of the high number of treatments applied. Area 55 received 10 Scourge® (resmethrin/PBO) applications and area 66 received 12 (TABLE 1). Mosquitoes from area 51 showed increased survival to resmethrin exposure throughout the season. In early July (week 27) the percentage of survival to resmethrin in these mosquitoes was 0-2% and this percentage increased to 42% by mid-November (week 44) (TABLE 1). Although DEF, an esterase inhibitor, synergized the activity of resmethrin in mosquitoes from area 51 collected during week 35, no synergism was observed by week 44, allowing 20-42% survival. Similarly, Scourge® (containing an inhibitor of cytochrome P450 or microsomal monoxygenases) was not effective, allowing 20% survival in the same week (TABLE 1).

The esterase specific activity of the susceptible strain and that of the pooled mosquitoes from the eight areas were analyzed and tested for normality. Although the esterase activity data of the Sebring strain were distributed normally around a mean of 0.0573 U/mg (TABLE 2), the esterase activity of pooled mosquitoes from the eight areas was not (Fig 2). This indicates that the frequency distribution of esterase activity is asymmetrical, the majority within 0.1-0.9 U/mg and extending to the right with fewer mosquitoes exhibiting extremely high activity values. The highest activity measured was about 1.75 U/mg. The upper limit of esterase activity for the Sebring (0.09 U/mg) was used as discriminatory for susceptibility. There were 226 measurements from individual field collected mosquitoes with esterase activities higher than this value (Fig. 2). Based on esterase activity, the frequencies for Harris County would predict that about 93.77% of the field population could survive the discriminatory concentration. This percentage was in agreement with the malathion bottle bioassay survival which averaged about 90% for most areas (TABLE 1). The Spearman rank order correlation analysis between the percent live mosquitoes after 30 min. of exposure to malathion (TABLE 1) and the percentage of mosquitoes with >0.09 U/mg specific activity in each area (Fig. 3), resulted in a highly significant (P < 0.005) correlation coefficient of 0.83. This indicates that survival was strongly associated with increase esterase activity and that these two variables tend to increase together.

The individual esterase specific activity from mosquitoes from the eight areas was also tested for normality within each area (Fig. 3). All groups were

**TABLE 2.** Specific activity of carboxylesterases from Culex quinquefasciatus females from Harris County.

<table>
<thead>
<tr>
<th>Strain or Area</th>
<th>Female Total No.</th>
<th>Mean U/mg</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
<th>C. I. of mean</th>
<th>Median U/mg</th>
<th>25% U/mg</th>
<th>75% U/mg</th>
<th>Dunn's Test Result&lt;sup&gt;1&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>66</td>
<td>♀ 30</td>
<td>0.7996</td>
<td>0.396</td>
<td>0.046</td>
<td>0.095</td>
<td>0.841</td>
<td>0.530</td>
<td>0.970</td>
<td>b</td>
</tr>
<tr>
<td>512</td>
<td>♀ 30</td>
<td>0.7019</td>
<td>0.119</td>
<td>0.073</td>
<td>0.149</td>
<td>0.591</td>
<td>0.447</td>
<td>1.014</td>
<td>bc</td>
</tr>
<tr>
<td>42</td>
<td>♀ 30</td>
<td>0.6830</td>
<td>0.303</td>
<td>0.072</td>
<td>0.147</td>
<td>0.71</td>
<td>0.336</td>
<td>0.885</td>
<td>bc</td>
</tr>
<tr>
<td>106</td>
<td>♀ 30</td>
<td>0.5672</td>
<td>0.345</td>
<td>0.033</td>
<td>0.069</td>
<td>0.578</td>
<td>0.456</td>
<td>0.703</td>
<td>bc</td>
</tr>
<tr>
<td>54</td>
<td>♀ 16</td>
<td>0.5476</td>
<td>0.254</td>
<td>0.075</td>
<td>0.161</td>
<td>0.442</td>
<td>0.317</td>
<td>0.782</td>
<td>bc</td>
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<tr>
<td>206</td>
<td>♀ 30</td>
<td>0.538*</td>
<td>0.185</td>
<td>N/A</td>
<td>N/A</td>
<td>0.397</td>
<td>0.186</td>
<td>0.610</td>
<td>c</td>
</tr>
<tr>
<td>55</td>
<td>♀ 30</td>
<td>0.466*</td>
<td>0.598</td>
<td>N/A</td>
<td>N/A</td>
<td>0.335</td>
<td>0.242</td>
<td>0.618</td>
<td>cd</td>
</tr>
<tr>
<td>51&lt;sup&gt;2&lt;/sup&gt;</td>
<td>♀ 30</td>
<td>0.1996</td>
<td>0.400</td>
<td>0.021</td>
<td>0.044</td>
<td>0.178</td>
<td>0.109</td>
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<tr>
<td>Sebring</td>
<td>♀♂ 8</td>
<td>0.0537</td>
<td>0.013</td>
<td>0.003</td>
<td>0.007</td>
<td>0.054</td>
<td>0.044</td>
<td>0.059</td>
<td>a</td>
</tr>
</tbody>
</table>

*Not a normal distribution; N/A = not applicable; Sebring = susceptible laboratory strain, all others are Harris County area identification numbers.

<sup>1</sup>Rows with the same letters indicate that the means for esterase activity (U/mg) are not significantly different between areas compared. 51<sup>2</sup> = Early season data.
normally distributed except esterase activities of mosquitoes collected from areas 206 and 55 (TABLE 2). The subsequent analysis of variance detected highly significant differences in specific esterase activity among areas. The Dunn's test indicated that the mean esterase activity in all areas, except that of area 51, differed significantly from that of the susceptible Sebring strain (TABLE 2 and Fig. 2). Esterase activity in Area 66, the highest, was significantly different from those of areas 206, 55, and 51, but not significantly different from those of areas 512, 42, 106, and 54 (TABLE 2). The activity in these last four areas was not different from activity in areas 206 and 55, but differed significantly from area 51. Activity from area 55 was also not different from 51. Activity in area 51 early in the season was not significantly different than the Sebring susceptible strain using this test. Because these two sets of data (Sebring and area 51) are distributed normally, they were further compared with a t-test, and a highly statistically significant difference in esterase activity between the two was detected (P = <0.00002). In area 51, the average activity was 3.7-fold that of the susceptible strain. This average level (0.2 U/mg) exceeds the discriminatory value of 0.09 U/mg and was sufficient to produce a high frequency of malathion resistant mosquitoes (Fig. 3). In area 51, about 77% of the mosquitoes had esterase activity above the discriminatory value during week 35. This was in agreement with the observed survival of 79-93% in early bioassays with malathion on week 27 (TABLE 1). Activity measured from mosquitoes collected in area 51 in early November (Week 44) was 0.69 U/mg in average, with upper values of 1.33 U/mg. The increase in the average esterase value is consistent with the observed increase in malathion survival during week 35 (98-100% survival) and possibly with the observed resistance to resmethrin (7-20% survival) that was synergized by DEF (0% survival) on that week (TABLE 1).

Additional topical bioassays with F0 females during week 45 revealed a median lethal dose (LD95) of 11.3 ng resmethrin/female for the Sebring strain, and a LD95 of 95 ng resmethrin/female of area 51, with an estimated resistance ratio of 8.4. The LD95 of resmethrin

![Figure 2. Frequency distribution of specific esterase activity from Cx. quinquefasciatus susceptible (Sebring) and field collected mosquitoes from all areas studied in Harris County, 1998. Note that the frequency distribution of the field-collected mosquitoes is asymmetric.](image-url)
Figure 3. Frequency distribution of specific esterase activity of female mosquitoes, identified by area.
Figure 3. Continued.
was 36.5 ng/female for the Sebring strain and >1 mg/female from area 51; the resistance ratio was estimated to be >27. For Scourge® the LD₉₅ of the Sebring strain was 4.7 ng/female and for area 51 females was 13 ng/female, with a resistance ratio of 2.76. The LD₉₅ for Scourge® was 10.3 ng/female of the Sebring strain and 62 ng/female of area 51, with a resistance ratio of 6.

The mean esterase activity of the field populations from areas 66, 512, 42, 106, and 54 was 11-15-fold higher than that of the susceptible strain (TABLE 2). Activities in areas 206 and 55 were statistically significantly below these, and about 10-fold over the mean of the susceptible strain. The mean activity in area 51 was about 3.7 times that of the susceptible strain (TABLE 2). All areas with high esterase activity, 66, 512, 42, and 54, exhibited 84-100% survivorship to malathion. These localities also received more than 10 Scourge® treatments during the year. Note that on week 27, mosquitoes from the areas with the lowest esterase activity (206, 55, and 51) also showed the highest percent kill (% dead after all susceptible mosquitoes had died) in the first three malathion bottle assays, of 23%, 11%, and 21%, respectively.

Native PAGE patterns were similar to those obtained by several researchers worldwide (Ketterman et al. 1992, DeSilva et al. 1997, Gopalan et al. 1997). A localized distribution of esterase phenotypes, consistent with the known focal nature of mosquito resistance development was observed (Brogdon and McAllister 1998b, Silvestrini et al. 1998). The Est2Estβ2 phenotype is the most frequent in the areas tested, with noticeable overproduction of these enzymes specifically in areas 106, 206, 512, 55, and 66 (Fig. 4). The estimated frequency of this phenotype for the pooled data is about 68.7%, followed by esterase Est2Estβ1Estβ2 and Estβ1 phenotypes, each with about 11% of the mosquitoes exhibiting these phenotypes. About 7% of the mosquitoes did not appear to have amplified esterases. In at least 20% of the mosquitoes analyzed from all area populations in Harris County, we observed a weak, fast migrating band that developed blue in color in the presence of α-naphthyl acetate, and thus, possibly represents a novel esterase α (Fig. 4).

Based on the esterase electromorphs on native PAGE we defined the apparent phenotype of mosquitoes from the different areas to determine if the presence of distinct phenotypes could be associated with level of esterase activity, resistance to malathion or resmethrin or specific locations (TABLE 3). Two areas with a low level of esterase activity, 206 and 55 (TABLE 2), were also characterized by no phenotypic variability, composed only of the Est2Estβ2 phenotype (TABLE 3). All the other areas, especially those with the highest esterase activity had the Est2Estβ1Estβ2 phenotype at low frequency, and a variable percentage of individuals apparently only carrying β1 (Fig. 4, areas 42 and 512). Area 51, in which resistance to malathion and survival to Scourge® and resmethrin changed throughout the season, had all three phenotypes and the highest percentage of the Est2Estβ1Estβ2 phenotype among all areas.

With respect to location, the two areas with phenotypes Est2 Estβ2, areas 106 and 206, are north of the I-610, and close to one another. The two areas with the highest frequency of Estβ1 phenotypes, areas 51 and 42, are adjacent to each other within the loop. All areas within the I-610 loop, except 55, exhibited the three phenotypes. Area 512, south of I-610 is similar to the two northern areas where the Est2Estβ2 phenotype prevails.

**DISCUSSION**

Local information on resistance levels is crucial for the effective control of mosquitoes, especially during SLE virus outbreaks. Females emerged from field

---

**TABLE 3.** Apparent phenotypic frequency by area defined as a percentage of the total number of mosquitoes (84) from Harris County individually analyzed by PAGE in 1998.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>A 66 (%)</th>
<th>A 512 (%)</th>
<th>A 42 (%)</th>
<th>A 106 (%)</th>
<th>A 54 (%)</th>
<th>A 206 (%)</th>
<th>A 55 (%)</th>
<th>A 51 (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>1.19</td>
<td>0</td>
<td>3.57</td>
<td>0</td>
<td>1.19</td>
<td>0</td>
<td>0</td>
<td>5.59</td>
<td>11.54</td>
</tr>
<tr>
<td>A2 B2</td>
<td>8.33</td>
<td>10.71</td>
<td>4.76</td>
<td>11.9</td>
<td>5.95</td>
<td>11.9</td>
<td>9.52</td>
<td>5.59</td>
<td>68.7</td>
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<tr>
<td>A2B1B2</td>
<td>2.38</td>
<td>1.19</td>
<td>1.19</td>
<td>0</td>
<td>2.38</td>
<td>0</td>
<td>0</td>
<td>4.76</td>
<td>11.9</td>
</tr>
<tr>
<td>No Detect.</td>
<td>0</td>
<td>0</td>
<td>2.38</td>
<td>0</td>
<td>2.38</td>
<td>0</td>
<td>2.38</td>
<td>0</td>
<td>7.14</td>
</tr>
</tbody>
</table>
Figure 4. Native PAGE from individual *Cx. quinquefasciatus* females stained for carboxylesterase activity, showing the diverse phenotypes in five areas studied. One fifth of a single female homogenate was loaded per lane. The letter S indicates that the sample is a mixture of the homogenates of mosquitoes shown in the same gel, used as an internal standard, equivalent to 1/5 of a female (loaded 1/50 of each female). SBR indicates the same equivalent from a mixture of susceptible Sebring females showing no obvious esterase activity. In area 512, the symbol * indicates a female with the Est2Estß1Estß2 phenotype. Vertical arrows in the bottom of the gels indicate the females in which the putative fast-running esterase A is obvious, also indicated on the side by an arrow with a question mark. Co. = County.
collected immature mosquitoes were analyzed using three different methods: bioassay, esterase activity, and esterase pattern. Bioassays indicated a broadly distributed resistance to malathion in the Houston area, defined by a high frequency of resistant mosquitoes as well as a high level of resistance in individual mosquitoes. In Harris County these high resistance levels caused the termination of malathion use for control of Cx. quinquefasciatus in 1993. Currently, malathion applications in Harris County target only nuisance mosquitoes; Cx. quinquefasciatus is not considered a biting nuisance.

The evolution of resistance to resmethrin should be closely monitored considering that the rate of Scourge® at 0.003 lb/acre is in the middle range of the manufacturer’s recommended rates for mosquito control. High frequency of mosquito survivorship from area 51 to resmethrin and Scourge® may indicate the presence of an additional specific mechanism responsible for this increased survival. The effectiveness of resmethrin in this area decreased during 17 weeks, with mortality declining from an initial 98-100% to about 60-80%. Scourge® allowed 16-20% survivorship during week 44. Survivorship (17%) to resmethrin was also detected in area 414 in one test (not shown), unfortunately no additional mosquitoes were available from this area for further testing. Further research is needed to elucidate the mechanism(s) involved in survival to resmethrin and Scourge®. While areas 206 and 106 only received one treatment with Scourge®, very low and uniform levels of tolerance to resmethrin detected in areas 66, 55, 106, and 206 could be maintained as a consequence of the residential use of pyrethroids and pyrethrin. It would be of interest to quantify the residential use of these products in Houston. Interestingly, high levels of resistance resulting from residential use of pyrethroid coils and sprays in cities in Burkina Faso was observed in the absence of an organized control campaign with pyrethroids against Cx. quinquefasciatus. No correlation with agricultural use was found (Chandre et al. 1998).

The presence of a potential new esterase Esta, as revealed by a bluish band when the substrate α-naphthyl acetate is used, was observed in mosquitoes from Harris County. A similar band was sporadically observed in a 1989 survey in Harris County (Wirth, unpublished, personal communication) and more recently in a survey in 1994 (Nawrocki, unpublished). It would be interesting to determine if this esterase is associated with the development of resistance to resmethrin in mosquitoes.

We confirmed the broad distribution of highly active esterases in Cx. quinquefasciatus populations in Houston (Harris County, TX). Highly active esterases were present in all populations sampled, although the specific activity varied among sites. The highest activity, of about 1.75 U/mg (areas 42 and 512, Fig. 4), is similar to values reported from resistant mosquitoes of various species, such as those from a fenitrothion and permethrin resistant Anopheles albimanus strain from Guatemala (Brogdon et al. 1999).

The observation of mosquitoes with amplified esterases Estβ1, Esta2Estβ2 or both is consistent with prior observations on the Houston mosquitoes from 1989 (M. Wirth, personal communication) and 1994 (S. Nawrocki, unpublished). The association observed between high levels of resistance and esterase activity in areas 42 and 512 and a higher frequency of Estβ1 is similar to the report by Raymond et al. (1987) who found that under high organophosphate selection pressure the frequency of Estβ1 increases over those of the other two phenotypes. Knowledge of the distribution of mosquitoes with the Estβ1 phenotype is important because high levels of amplification of the Estβ1 gene appear to confer higher levels of resistance to organophosphates in mosquitoes than a high number of Esta2Estβ2 amplicons (Raymond et al. 1987, Ferrari and Georgiou 1990, Wirth et al. 1990). In another study of Cx. quinquefasciatus from Santiago de Cuba, however, Estβ1 appeared more frequently associated with resistance to pyrethroids (Rodriguez et al. 1997). This is a similar to our observation of survival to resmethrin in area 51, where Estβ1 was frequent. Other possible mechanisms of malathion resistance in Harris County, such as insensitive acetyleholinesterase, malathion carboxylesterase, or monooxygenases were not analyzed in this study but are currently under investigation.

Our results on esterase phenotypic frequencies differ from unpublished data from 1994 (S. Nawrocki and K. Flatt) in which 104 mosquitoes were analyzed by PAGE. At that time, about 20% were found carrying Estβ1, 40% Esta2Estβ2, and 40% Esta2Estβ1Estβ2 (not shown). Mosquitoes without amplified esterases (susceptibles) were not found. This apparent decrease in the frequency of amplified Estβ1 observed in the last five years is consistent with the decrease in malathion use by the Harris County Mosquito Control Division since 1993, both, in the number of areas treated with malathion and in the number of applications in the remaining malathion treated areas. It appears that in Harris County, as in many other areas worldwide, the Esta2Estβ2 phenotype is being favorably selected. In Italy, the amplified Esta2Estβ2 were detected in an OP-treated area but apparently disappeared when OP treatment was suspended (Severini et al. 1993). In Cx. quinquefasciatus from Cuba treated with organophosphates, the apparent selective advantage of the
Esta2/Estβ2 amplicon over the more rare estβ1 could not be explained by differences in their bimolecular rate constants for interaction with OP insecticides, since both are similar (Small et al. 1998). Further, the advantage of the Esta2/Estβ2 amplicon cannot be explained by the fact that it carries two esterases, instead of one as does the estβ1 amplicon, because one strain of Cx. quinquefasciatus (COL strain) with elevated Estβ1 esterases which also carries an elevated Estβ3, does appear to have a selective advantage (DeSilva et al. 1997).

The results from native PAGE support the assertion that the detection of the Esta2/Estβ2 haplotype by restriction map length polymorphism cannot be used to indicate the level of amplification or any particular degree of resistance, even when the restriction maps of the amplicons are almost identical in populations of diverse origin, including Texas (Pasteur and Raymond 1996, Callaghan et al. 1998a). A 13-fold variation in enzyme levels can be observed depending on the numbers of copies present (Callaghan et al. 1998a). In our study, the levels of activity for the areas with apparent higher frequency of Esta2Estβ2 have about ten-fold higher enzymatic activity compared to control mosquitoes (TABLE 2). Significant variation among single mosquitoes with Esta2Estβ2 esterases was observed by native PAGE, especially in areas 106, 206, 55, 42, and 54 (Fig. 4). Mosquitoes from area 51 showed an apparently higher frequency of Estβ1 and Esta2Estβ1Estβ2 than mosquitoes in all other areas. Mosquitoes from area 51 also exhibited resistance to malathion and resmethrin. It appears that esterases are partially involved in this resistance because DEF synergized resmethrin's activity in mosquitoes collected on week 35 (TABLE 1). This resistance could be attributable to esterase Estβ1 because of its high frequency in area 51, or maybe to the putatively novel Esta. An additional resistance mechanism to resmethrin, possibly sodium channel insensitivity or kdr-like, could be present because during week 44, neither DEF nor PBO (Scourge®) synergized the activity of resmethrin in this population. It is possible that the phenotypes in this area can be rapidly changed after migration and subsequent insecticide application. It is also possible that monooxygenases are responsible for resmethrin resistance during week 44. Although PBO did not appear to act as a synergist of resmethrin (week 44), the remaining survivorship could be due to a remaining independent esterase activity, not necessarily from the widespread amplified esterases Esta2/Estβ2 and Estβ1. Experiments with simultaneous application of both, DEF and a monooxygenase inhibitor other than PBO is needed to help elucidate the mechanism involved (Bergé et al. 1998). Despite this phenotypic diversity in area 51, the staining intensity of the electromorphs and quantitative esterase activity suggests that the number of copies of the esterase genes may be variable but not excessively high. The mosquito collection site in area 51 is located in a low-income neighborhood. There are no obvious physical barriers to adult mosquito migration and, although it is believed that the residential use of pesticides is sporadic and possibly low, this area is the site of illegal public dumping of trash and various chemicals and contaminants, which may also select for the B1 electromorph. The presence of susceptible mosquitoes and those with low esterase activity early in the season may also be explained by the fact that this area had not received any insecticide treatments in 1997. Therefore, 22 Scourge® treatments in 1998 may have exerted enough selection pressure to select for target site insensitivity, kdr-like mechanism or a glutathione-S-transferase mechanism. These hypotheses need to be tested for Harris County, considering that the kdr-like mechanism has been detected in Cx. quinquefasciatus from neighboring Louisiana as well (Brogedon and McAllister 1998b). In addition, cross-resistance to lambda cyhalothrin, another pyrethroid, was obtained after only six generations of selection of a malathion-resistant Cx. quinquefasciatus strain in Cuba, which had an overproduced Estβ and insensitive acetylcholinesterase (Bisset et al. 1997). The specific mechanism was not elucidated but it did not confer resistance to other pyrethroids such as deltamethrin and cypermethrin (Bisset et al. 1997). This indicates that specific resistance mechanisms to particular pyrethroids can be rapidly selected in Cx. quinquefasciatus and must be analyzed locally. In 1998, area 66 was treated preventively with Scourge® on 12 occasions due to the detection and isolation of SLE arbovirus from mosquito surveillance. These mosquitoes showed the highest esterase activity and the highest variance among the areas with a similar mean. Interestingly, mosquitoes in this area were collected in a vegetated drainage ditch close to the treated street and probably received environmental pollutants from multiple sources. Variability in esterase activity has been suggested as a better measure of environmental contamination than the mean activity (Callaghan et al. 1998b). Our data on insecticide resistance, electromorphs, and esterase activity suggest strong local selection effects. This suggestion is in agreement with the analysis of SLE arbovirus data in that certain strains are associated with certain areas (L. Chandler, UTMB, personal communication). It is possible that esterases are locally selected depending on insecticide use and that despite gene flow, fitness differences between insects with
different aldozymes play an important role in maintaining a localized distribution of phenotypes (Karunaratne and Hemingway 1996, Silvestri et al. 1998). Targeted malathion use against Cx. quinquefasciatus for 28 years selected for amplified esterases present at high frequency and these are possibly being maintained by a nontarget effect of malathion applications against other mosquito species, by home and garden insecticide use and by environmental contaminants in breeding sites. Our data provide a record for the most populated city in the subtropical U.S. and are of significance for future comparisons of local evolution of insecticide resistance and associated esterases.

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Isolations of Enteric Pathogens from Synanthropic Flies
Trapped in Downtown Kuala Lumpur

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Abstract: Four species of synanthropic flies were trapped in downtown Kuala Lumpur: Chrysomya megacephala, Chrysomya rufifacies, Musca domestica, and Musca sorbens. Burkholderia pseudomallei, the organism causing melioidosis, was the dominant bacteria isolated from Chrysomya megacephala. Klebsiella oxytoca, commonly associated with nosocomial infections, was commonly isolated from Chrysomya megacephala, Musca domestica, and Musca sorbens. Aeromonas hydrophila, the bacteria causing gastroenteritis, was predominantly isolated from Chrysomya megacephala and also from Musca domestica and Musca sorbens. A total of 18 bacterial species was isolated from the synanthropic flies trapped. Burkholderia pseudomallei had been reported for the first time.

Keyword Index: Synanthropic flies, Burkholderia pseudomallei, Klebsiella oxytoca, melioidosis, gastroenteritis.

INTRODUCTION

In Malaysia, the house fly, Musca domestica L., the face fly, Musca sorbens Wiedemann, and blow flies, such as Chrysomya megacephala (Fabricius), are commonly found foraging on or breeding in garbage and other filth. As they also visit clean food, they are important mechanical transmitters of pathogens causing human diseases (Reid 1953, Greenberg 1973). The dangers of house flies and blow flies as carriers of diseases are increased by their feeding habits. They feed on feces, then land on human food, and regurgitate for pre-digestion before ingestion. They may mechanically transfer disease organisms, such as protozoal cysts, human helminth parasites, bacteria, and enteroviruses, to food that is later consumed by humans. In Malaysia, Sulaiman et al. (1988a) found that adults of C. megacephala carried eggs of Ascaris lumbricoides, Trichuris trichiura, and hookworm on the external body surface and in the gut lumen. In an urban slum of Kuala Lumpur, filariform larvae of the hookworm, Necator americanus, were found in the gut of the face-fly, M. sorbens Wiedemann and C. megacephala. Sulaiman et al. (1988b) also isolated enteropathogenic bacteria from the body surface and gut contents of adults of M. domestica and C. megacephala trapped at a refuse dump and a poultry farm in Malaysia.

This study was undertaken to examine the enteropathogenic flora present on the body surfaces and gut contents of flies caught in downtown Kuala Lumpur.

MATERIALS AND METHODS

Study Sites

At the study site, the Chow Kit area of the downtown of Kuala Lumpur, flies were collected from food stalls, the wet market, and rubbish dumping site.

Collection of Flies

Adult flies were trapped with fly traps consisting of a vertical rectangular cage made of aluminum gauze (35 cm in length, 35 cm wide, and 45 cm high) and strengthened with stainless steel, standing on four legs 10 cm above a transparent plastic sheet (35 cm x 35 cm). The lower end of the trap formed an inverted gauze cone open at the base and the apex so that flies could enter. To attract flies, 100 g of prawns were placed in a Petri dish as bait and placed below the fly trap. Three traps were used, and these were operated from 09.00 to 11.00 hr.
weekly between August 1998 and January 1999. The sampling time was used as standard time throughout the study period. Each trap was placed at the food stall, the wet market, and at a rubbish dumping ground for each sampling period during the dry period.

**Processing of Flies and Isolation of Microbes**

All the trapped flies were killed by deep freezing at -20°C, identified, counted, and pooled into groups according to species. From each field collection, half of the number of each fly species was placed individually in test tubes containing sterile 5 ml peptone water. The other half was used for parasites study. However, no parasites were found. Those in peptone water were then rinsed in sterile distilled water and dissected with sterile dissecting needles. The gut content of each individual fly was placed in test tubes containing sterile 5 ml peptone water. The samples were left at room temperature (28 ± 1°C) from two to five hours before being processed.

A loop full of each sample was streaked on blood agar and Mac Conkey agar plates and incubated at 37°C overnight. The plates were examined and bacteria isolates identified on the basis of a series of biochemical tests, such as triple sugar iron, motility, urease production, and sugar reactions following the methods of Cowan and Steel (1993). Further studies to confirm the identification of the bacterial isolates was conducted using the Microbact 24E Identification System (Medvet).

**RESULTS AND DISCUSSION**

Eighteen species of bacteria were isolated from four fly species. *Musca domestica* had nine bacterial species isolated (TABLE 1). The most common bacteria isolated were *Klebsiella oxytoca*, followed by *Citrobacter freundii*, *Aeromonas hydrophila*, *Enterobacteragglomerans*, *Burkholderiapseudomallei*, and to a lesser extent *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens*. *Musca sorbens* trapped had indicated the presence of twelve bacterial isolates. *Aeromonas hydrophila* was the most common species observed, followed by *Burkholderiapseudomallei*, *Aeromonas sobria*, *Citrobacter freundii*, *Klebsiella pneumoniae*,

**TABLE 1**  Bacteria isolated from fly species at the Chow Kit area of Kuala Lumpur.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th><em>Musca domestica</em></th>
<th><em>Musca sorbens</em></th>
<th><em>Chrysomya megacephala</em></th>
<th><em>Chrysomya rufifacies</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>4</td>
<td>8</td>
<td>20</td>
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<tr>
<td><em>Aeromonas caviae</em></td>
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</tr>
<tr>
<td><em>Aeromonas sobria</em></td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
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</tr>
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<td>0</td>
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</tr>
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<td><em>Klebsiella oxytoca</em></td>
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<td>0</td>
</tr>
<tr>
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<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Klebsiella ozaenae</em></td>
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<td>0</td>
<td>4</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
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<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
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</tr>
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</tr>
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<td><em>Pseudomonas cepacia</em></td>
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<tr>
<td><em>Pseudomonas fluorescens</em></td>
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<td>0</td>
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<tr>
<td><em>Burkholderiapseudomallei</em></td>
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</tr>
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<td><em>Pseudomonas stutzeri</em></td>
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<td><em>Salmonella arizonae</em></td>
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<td>0</td>
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<tr>
<td><em>Vibrio alginolyticus</em></td>
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</table>
Klebsiella oxytoca, Salmonella arizonae, Enterobacter agglomerans, Escherichia coli, Klebsiella ozaenae, Pseudomonas cepacia, and Vibrio alginolyticus. Bacterial isolations from the Chrysomya megacephala indicated that twelve different species were obtained. Burkholderia pseudomallei was the most common bacteria species isolated, followed by Aeromonas hydrophila, Klebsiella oxytoca, Klebsiella pneumoniae, Aeromonas caviae, Citrobacter freundii, Enterobacter agglomerans, Proteus mirabilis, Proteus vulgaris, Pseudomonas cepacia, Pseudomonas stutzeri, and Salmonella arizonae. Bacterial isolations from the Chrysomya rufifacies trapped indicated the presence of five species. The most common were Escherichia coli, Klebsiella ozaenae, and Pseudomonas fluorescens, followed by Proteus vulgaris and Burkholderia pseudomallei.

Investigations on the role of flies as mechanical vectors or carriers of pathogenic microorganisms have been conducted (Greenberg 1973, Sulaiman et al. 1988b). In Japan, Takeuchi et al. (1966) studied the persistence of bacteria in flies and the quantity of bacteria transmitted. They found that the pathogenic bacteria Shigella dysenteriae and Salmonella typhimurium ingested by the larvae died during the pupal stage. Bidawid et al. (1978) found that non-biting flies played the role of transmitting enteric pathogens viz., Salmonella spp. and Shigella spp. in Beirut, Lebanon. In Norway, Rosef and Kapperud (1983) found Musca domestica to be involved as a mechanical vector in the epidemiology of gastroenteritis caused by Campylobacter fetus subsp. jejuni. The results indicated Burkholderia pseudomallei, the organism causing melioidosis, was unexpectedly the most predominant bacteria isolated from flies especially Chrysomya megacephala. This was rather unusual and may be the first report of its isolation. However, the relationship of these flies to the transmission of the disease is questionable. Bacteria causing gastroenteritis, such as Aeromonas hydrophila, were predominantly found in Chrysomya megacephala. Salmonella arizonae were isolated from Chrysomya megacephala and Musca sorbens and Vibrio alginolyticus isolated from Musca sorbens only. Citrobacter strains are often found in the feces of humans and occasionally have been associated with urinary tract infections (Jensen et al. 1997). The presence of Citrobacter freundii in Musca sorbens, Musca domestica, and Chrysomya megacephala adults indicated the important role played by the fly species to transmit pathogenic bacteria. Klebsiella sp. are a fairly common cause of urinary tract infection and occasionally give rise to cases of severe bronchopneumonia, sometimes with chronic destructive lesions and multiple abscess formation in the lungs (Greenwood et al. 1992). Three species of Klebsiella viz. Klebsiella oxytoca, Klebsiella pneumoniae, and Klebsiella ozaenae were isolated from the flies. Klebsiella oxytoca was found in all fly species with the exception of Chrysomya rufifacies.

Organisms of the Enterobacter group occur in soil, dairy products, water, and sewage as well as in the intestine of man and animals. Enterobacter agglomerans were isolated from Musca domestica, Musca sorbens, and Chrysomya megacephala, an indication that the fly species could play a potential role in transmission to man and animals.

Proteus mirabilis is a prominent cause of urinary tract infection in children and in domiciliary practice (Greenwood et al. 1992). Two species of Proteus were isolated from the flies trapped in downtown Kuala Lumpur, viz. Proteus mirabilis and Proteus vulgaris. Certain strains of Escherichia coli are known to exhibit pathogenicity, the major categories are enterotoxigenic, enteropathogenic, enterohemorrhagic, and enteroinvasive (Jensen et al. 1997). In this study two specimens of Escherichia coli from Musca sorbens and four specimens from Chrysomya rufifacies were isolated. Thus, the flies could potentially serve as vectors of pathogenic organisms in Malaysia.

Due to the habits of synanthropic flies feeding on feces, waste matter, regurgitating, and transferring disease organisms to human food, these fly species could play an important role in harboring and transmitting bacterial pathogens to man.

Acknowledgments

We wish to thank Universiti Kebangsaan Malaysia for providing research facilities and Ministry of Science Technology and Environment Malaysia for awarding the research grant IRPA 06-02-0006. We also thank Mr. Abdul Razak Abdul Rahman and John Jeffery for technical support and Mrs. Wirda Hassan for typing the manuscript.

REFERENCES CITED


Field Evaluation of Deltamethrin/S-Bioallethrin/Piperonyl Butoxide and Cyfluthrin against Dengue Vectors in Malaysia

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ABSTRACT: Deltacide® (S-bioallethrin 0.71% w/v, deltamethrin 0.5% w/v, piperonyl butoxide 8.9% w/v excipients to 100% w/v) and Solfac UL 015® (cyfluthrin 1.5% w/v) were evaluated against the sentinel sugar-fed adults and 4th-instar larvae of Aedes aegypti at 17 storey high-rise apartments in Malaysia using ULV applications. The impact of both insecticides on field populations of Ae. aegypti and Ae. albopictus larvae was monitored weekly using bottle containers. Both Deltacide® and Solfac UL 015® showed adulticidal and larvicidal effects. This was the first field trial using Deltacide® against dengue vectors in Malaysia and showed its potential for use in dengue vector control programs.

Keyword Index: Deltacide®, Solfac UL015®, Aedes aegypti, ULV spraying, dengue vector control.

INTRODUCTION

Aedes aegypti (L.) is the primary vector of dengue viruses in Southeast Asia with Aedes albopictus (Skuse) serving as a secondary vector (Russell et al. 1969, Chan et al. 1971, Jumali et al. 1979, Harinasuta et al. 1984). Gratz (1991) reported that properly applied and timed ultralow-volume (ULV) insecticide application could be effective in suppressing dengue vectors at the time of an epidemic. Kilpatrick et al. (1970) conducted a series of field trials of ULV insecticide applications to determine their efficiency for rapid control of Ae. aegypti in Thailand. A rapid reduction in natural mosquito population occurred at an application of 438 ml/ha. Sulaiman et al. (1998) evaluated cyfluthrin and malathion 96TG ULV application on the dengue vectors around high-rise apartment buildings in Malaysia, and although both insecticides showed adulticidal effects, cyfluthrin showed more significant larvicidal effect than malathion 96TG.

The objective of this study was to compare the efficacy of deltamethrin 0.5% w/v/s-bioallethrin 0.71% w/v/piperonyl butoxide 8.9% w/v excipients to 100% w/v (Deltacide®) ULV application provided by AgrEvo H.S. (Malaysia) Sdn. Bhd., while a second block was sprayed with cyfluthrin (Solfac UL015®) ULV application supplied by Bayer (M) Sdn. Bhd. Both insecticides were diluted at a dilution rate of 1:5 parts with diesel. The third block was sprayed with diesel diluent only as the control. All the windows and doors of apartments remained open during ULV spraying. A LECO/Model 1600 cold aerosol generator mounted on a vehicle was used for the spraying.

MATERIALS AND METHODS

Three blocks of 17 storey high-rise apartments in Kuala Lumpur were chosen for this study. Each block was 300-600 m apart. One block received a s-bioallethrin 0.71% w/v deltamethrin 0.5% w/v/piperonyl butoxide 8.9% w/v excipients to 100% w/v (Deltacide®) ULV application provided by AgrEvo H.S. (Malaysia) Sdn. Bhd., while a second block was sprayed with cyfluthrin (Solfac UL015®) ULV application supplied by Bayer (M) Sdn. Bhd. Both insecticides were diluted at a dilution rate of 1:5 parts with diesel. The third block was sprayed with diesel diluent only as the control. All the windows and doors of apartments remained open during ULV spraying. A LECO/Model 1600 cold aerosol generator mounted on a vehicle was used for the spraying.
The sprayer head nozzle was pointed at an angle of 45° to horizontal line and directed to the building. The flow rate of ULV application was 104 ml/min. (3.5 fl oz/min.) and a vehicle velocity selected at 6 km/hr (3.7 mph). The spraying route covered a distance of 260 m around each building block. Each insecticide was sprayed with the ULV equipment four times over a two month period at 1700 hr. Teflon coated slides were placed inside and outside each apartment of the 17 storey building.

Knockdown and mortality of caged four-day old sugar-fed Aedes aegypti were used to evaluate the efficiency of the two insecticides. Cylindrical screened sentinel cages (26 cm long x 18 cm diam.) were filled with 25 sugar-fed female Aedes aegypti and the cages were hung inside a room in each apartment and outside at 1.5 m above the floor. A sugar cube was placed on top of each cage for ad lib feeding. Two apartments in each of the level 1, 3, 5, 7, 9, 11, 13, 15, and 17 of each block of high-rise apartments were chosen at random for the study. Thus, a total of 18 cages was placed outside the apartments and another 18 cages inside the rooms in the building. In addition, 100 ml water-filled bottle containers (5.7 cm high x 6.5 cm diam.) were placed on the floors against the walls inside and outside of each apartment. Both the screened cages and the bottle containers were left at their placement sites 24 hr. after the ULV spray applications. In order to monitor Aedes field populations, bottles containing water were placed inside and outside each apartment six weeks before spraying to serve as breeding sites for wild mosquitoes. Each week the number of larvae in the bottle containers was counted and removed. Data analysis used the least significant different test (Choi 1978).

RESULTS AND DISCUSSION

Both deltamethrin/s-bioallethrin/piperonyl butoxide and cyfluthrin were significantly different from the control in causing adult knockdown one hour after spraying both outside and inside apartments (P<0.0005) (TABLE 1). The latter was slightly more effective than the former in causing adult knockdown (P < 0.01), probably as a result of the low dose applied. There was no significant difference between deltamethrin/s-bioallethrin/piperonyl butoxide and cyfluthrin in causing adult mortality both inside and outside apartments (P > 0.05). There was, however, a significant difference between the two insecticides and the controls both inside and outside (P < 0.0005). These results indicate that deltamethrin/s-bioallethrin/piperonyl butoxide and cyfluthrin were effective in controlling adult populations of Aedes aegypti.

Both deltamethrin/s-bioallethrin/piperonyl butoxide and cyfluthrin were significantly different from the control in causing a low level of larval mortality both inside and outside apartments (P<0.05). Cyfluthrin had a slightly faster action on the larvae than deltamethrin/s-bioallethrin/piperonyl butoxide. However, there was no significant difference between both insecticides in causing larval mortality inside and outside apartments (P > 0.05) (TABLE 1). The results indicate that both deltamethrin/s-bioallethrin/piperonyl butoxide and cyfluthrin were effective in causing adult mortality of

| TABLE 1. Overall knockdown and mortality effects of ULV spraying with Deltacide® and Solfac UL 015° against sentinel Aedes aegypti adults and larvae inside and outside high-rise apartment buildings. |
|---|---|---|---|---|---|---|---|
|   | Adults |   | Larvae |   |   |   |
|   | Mean 1-hr. | Mean 24-hr. |   | Mean 1-hr. | Mean 24-hr. |   |
| Treatment | knockdown (%) | mortality (%) |   | knockdown (%) | mortality (%) |   |
| Deltacide® | outside 58b | inside 57b | outside 95a | inside 95a | outside 4b | inside 3b | outside 9a | inside 7a |
| Solfac UL015° | outside 82a | inside 72a | outside 95a | inside 97a | outside 10a | inside 11a | outside 8a | inside 10a |
| Control | outside 11c | inside 11c | outside 11b | inside 10b | outside 1b | inside 0b | outside 1b | inside 2b |

Means within a column followed by the same letter are not significantly different (P > 0.05; least significant difference)
Ae. aegypti in the field. This study supported previous work on cyfluthrin (Sulaiman et al. 1998), whereby, cyfluthrin had adulticidal effects and a more significant larvicidal effect than malathion 96TG. It is concluded that deltamethrin/s-bioallethrin/piperonyl butoxide (Deltacide®), a new generation insecticide, is effective in controlling dengue vectors in the field. Even at an application dose of one-third to that of cyfluthrin, deltamethrin/s-bioallethrin/piperonyl butoxide was effective in its impact on dengue vectors in the field.

Based on our results of the droplet size, the volume median diameter (VMD) for each floor was uneven, ranging from 30 µm on the 11th floor to 93 µm on the 17th floor (TABLE 2). Perhaps, the wind factor might contribute to the unevenness. The percentage knockdown for Ae. aegypti adults at the lower floor levels was as would be expected, higher compared to the higher floors for both deltamethrin/s-bioallethrin/piperonyl butoxide and cyfluthrin (TABLE 2). Surprisingly high adult mortality also occurred at the higher floor levels. No doub resulting from ideal air movement up the side of the apartments during application. Both insecticides had caused higher larval mortality compared to the control (P<0.05). However, the highest larval mortality by Delta-cide® at 14% and Solfac ULO15® at 22% are still considered low.

Figure 1 shows the larval populations of Ae aegypti and Ae. albopictus in field breeding containers before and after ULV spraying with Deltacide® (deltamethrin/s-bioallethrin/piperonyl butoxide) and Solfac ULO 015®. (cyfluthrin). A reduction of larval population was observed after every spraying by both insecticides while the larval population in the control remained generally higher than the peak larval population in the treated areas. This study showed that both insecticides were effective in suppressing field larval populations of Aedes spp. Similar reductions in the field population, and apparent residual action following ULV applications of malathion have been documented (Pant et al. 1971). In conclusion, this study indicates that ULV formulations of both Deltacide® (deltamethrin/s-bioallethrin/piperonyl butoxide) and Solfac ULO15® (cyfluthrin) can cause high mortality of the dengue/dengue hemorrhagic fever vector Ae. aegypti at all levels of 17 story high-rise apartments. Cyfluthrin has previously been utilized for dengue control in Kuala Lumpur. With deltamethrin/s-bioallethrin/pipronyl butoxide (Deltacide®), another new generation insecticide may now be considered as a suitable product to include in such dengue control operations.

Acknowledgments

We wish to thank the Ministry of Science, Technology and Environment Malaysia for awarding the research grant IRPA 06-02-02-0006 to support this study and Universiti Kebangsaan Malaysia for providing research facilities. We appreciate the support of the technical staff, Department of Biomedical Science and Department of Parasitology and Medical Entomology, Universiti Kebangsaan Malaysia. We are grateful to

<table>
<thead>
<tr>
<th>Floors</th>
<th>Droplet Size (VMD)</th>
<th>Deltacide® Knockdown (%)</th>
<th>Solfac UL015® Knockdown (%)</th>
<th>Control Knockdown (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33µ</td>
<td>50µ</td>
<td>76</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>48µ</td>
<td>40µ</td>
<td>66</td>
<td>96</td>
</tr>
<tr>
<td>5</td>
<td>38µ</td>
<td>35µ</td>
<td>66</td>
<td>93</td>
</tr>
<tr>
<td>7</td>
<td>33µ</td>
<td>55µ</td>
<td>55</td>
<td>88</td>
</tr>
<tr>
<td>9</td>
<td>48µ</td>
<td>50µ</td>
<td>55</td>
<td>70</td>
</tr>
<tr>
<td>11</td>
<td>30µ</td>
<td>53µ</td>
<td>48</td>
<td>69</td>
</tr>
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<td>13</td>
<td>40µ</td>
<td>38µ</td>
<td>55</td>
<td>70</td>
</tr>
<tr>
<td>15</td>
<td>68µ</td>
<td>48µ</td>
<td>45</td>
<td>41</td>
</tr>
<tr>
<td>17</td>
<td>93µ</td>
<td>68µ</td>
<td>53</td>
<td>67</td>
</tr>
</tbody>
</table>
Figure 1. Field population of Aedes spp. (Aedes aegypti and Aedes albopictus) larvae before and after ULV spraying with Deltacide® and Solfac UL015®.

References Cited


The Effects of Flea Egg Consumption on Larval Cat Flea (Siphonaptera: Pulicidae) Development

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ABSTRACT: Cat flea larvae feeding on the feces of adult fleas that were maintained on cats and were provided with frozen flea eggs ad libitum each consumed an average of 21.7 ± 3.9 eggs and developed rapidly with 100% adult emergence. In contrast, 93.4% of larvae held individually and provided with only flea feces did not survive to the adult stage. Developing larvae consumed eggs in the presence of yeast and rearing diet. In a second experiment, larvae provided with flea feces and eggs and maintained at 55% RH consumed 26.9 ± 2.7 eggs per larva, compared to larvae maintained at 75% RH that consumed 20.4 ± 1.9 eggs per larva.

Keyword Index: Cat fleas, eggs, cannibalism.

INTRODUCTION

Adult cat fleas, Ctenocephalides felis (Bouché), spend most of their time on the host and scatter eggs and feces into the host’s environment. In nature, cat flea larvae require adult flea feces as a food source. When cat flea larvae are reared in cohorts on dried blood or adult flea feces, greater than 75% development to the adult stage has been reported (Moser et al. 1991). When larvae are reared individually, development beyond the third instar often does not occur without additional nutrients (Silverman and Appel 1994). In laboratory colonies, larval rearing diets of dried blood are supplemented with yeast and/or ground dog food (Henderson and Foil 1993).

Reitblat and Belokopytova (1974) reported that flea larvae of both Xenopsylla cheopis Rothschild and Ceratophyllus tesquorum Wagner were cannibalistic, and Kern (1991) indicated that cat flea larvae feed upon a variety of materials including pet dander, injured larvae, and eggs. The objective of this study was to determine the effects of egg cannibalism upon larval cat flea development.

MATERIALS AND METHODS

Flea eggs ≤ 24 hr old were collected beneath cats that were infested twice per week with 50 adult cat fleas (Henderson and Foil, 1993). For the collection of larvae, a 20 mm hole was drilled off-center in the bottom of a plastic 60 x 15 mm petri dish, and a 25 mm glass funnel was attached below. Half of the top of the petri dish was painted black. Viable eggs (VE) were placed in the dish beneath the clear portion of the top. A fluorescent lamp was positioned over the collecting device and when newly hatched larvae crawled to the darkened side, they fell into 16 x 100 mm test tubes. The larvae used in all experiments were ≤ 24 hr old.

Individual spirals of feces (CHF) from adult fleas that were maintained on cats were aspirated from the debris in the egg collection trays. Frozen eggs (FE) were held at -15°C for 24 hr to prevent them from hatching. Larval rearing diet consisted of 100 g of ground dog food, 20 g of dried whole beef blood (United States Biochemical Corp., Cleveland, OH), and 15 g of brewer’s yeast.

Experiments were conducted in a Precision Model
incubator at 22.5 ± 2.0°C and constant darkness. Relative humidity (RH) of 75 or 55% was maintained in glass-covered 19 liter aquariums containing saturated salt solutions of sodium chloride or calcium nitrate, respectively (Sweetman, 1933). Experimental units consisted of sterile 16 x 100 mm test tubes containing a single larva. Mortality and developmental stages were recorded every other day. Adults were stimulated to emerge from the cocoons by gently prodding them with a blunted dissecting needle.

**Experiment 1**

Ten FE and ten CHF were added to each of 30 tubes, and ten CHF only were added to 30 additional tubes. Subsequently, five FE were added to those units having fewer than five eggs remaining and five CHF spirals were added to units having fewer than five spirals. CHF only units were held for 50 d. In a second trial, 10 CHF and 15 FE were added to each of 60 test tubes. Larval rearing diet was then added to 30 of these units. The experiment was terminated at 100% cocoon formation (day 14) and the number of consumed eggs was recorded.

**Experiment 2**

Diets for larvae to be held at 75% RH were 10 FE, 5 mg of yeast (USB, Cleveland, OH), CHF, FE+ Yeast, CHF+ FE, CHF+ Yeast, and CHF+ FE+ Yeast (30 units each). Diets for larvae to be held at 55% RH were CHF, CHF+ FE, and CHF+ Yeast (30 of each). All diets were provided ad libitum. The fleas were maintained for 24 d, at which time adults were stimulated to eclose.

**Statistical Analysis**

The Chi-square test was used for comparison of certain treatments. Developmental time data were analyzed by the nonparametric ANOVA Kruskal-Wallis test followed by Dunn's multiple comparisons test. The accepted level of significance for all comparisons was P ≤ 0.05. The LT50 and LT90 values (i.e., the length of time required for the death of 50 and 90% of the larvae, respectively) were calculated by probit analysis.

**RESULTS**

In experiment 1, larvae maintained individually and provided with CHF+ FE consumed an average of 21.7 ± 3.9 eggs; and by day 24, there was 100% adult emergence in this group. Significantly fewer (6.6%) individuals in the CHF only group developed into adults. The mean time until pupation for larvae provided with FE was 10.0 ± 1.6 days. On day 24, 19 live larvae and 2 pupae remained in the CHF group. The LT50 and LT90 values for the CHF only treatment were 26.6 and 48.5 d, respectively. All larvae fed CHF+ FE or CHF+ FE+ rearing diet each consumed 15 eggs and 100% pupation occurred between days 10-14 in both groups.

After 24 d at 75% RH, there were no significant differences in mortality among larvae provided with either FE, Yeast, or FE+ Yeast, or among larvae provided CHF+ FE, CHF+ Yeast, or CHF+ FE+ Yeast, while mortality in the CHF only treatment was significantly different from all others (TABLE 1). Larvae provided with CHF only survived longer than larvae provided

**TABLE 1.** The effects of diet upon the development of cat flea larvae maintained individually for 24 d in treatment groups of 30 each at 75% relative humidity and 22.5 ± 2.0°C.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Percent Mortality</th>
<th>Development Time (d)</th>
<th>No. Pupae</th>
<th>% of No. Eclosed</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE 1</td>
<td>100a</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Yeast 2</td>
<td>100a</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CHF 3</td>
<td>30b</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>FE+Yeast</td>
<td>73a</td>
<td>19.0 ± 2.0a</td>
<td>4b</td>
<td>0b</td>
</tr>
<tr>
<td>CHF+Yeast</td>
<td>0c</td>
<td>12.1 ± 1.8b</td>
<td>30a</td>
<td>93.3a</td>
</tr>
<tr>
<td>CHF+FE</td>
<td>0c</td>
<td>13.4 ± 2.0ab</td>
<td>30a</td>
<td>90.0a</td>
</tr>
<tr>
<td>CHF+FE+Yeast</td>
<td>3c</td>
<td>10.2 ± 1.5c</td>
<td>29a</td>
<td>100.0a</td>
</tr>
</tbody>
</table>

1 Frozen flea eggs.
2 Brewer's yeast.
3 Feces from adult fleas maintained on cats.

Means with the same letters within columns are not statistically different (p > 0.05); N=30.
with FE, yeast, or Yeast + FE (TABLE 2). There were no significant differences among the number of pupae or percent eclosion in groups provided with CHF+ FE, CHF+ Yeast, or CHF+FE+ Yeast; four pupae developed on FE+ Yeast but failed to eclose (TABLE 1). Mean FE consumption was not significantly different between CHF+ FE (20.4 ± 1.9) and CHF+ FE+ Yeast (15.6 ± 3.1). However, developmental time was significantly shorter in the group provided CHF+FE+ Yeast.

After 24 d at 55% relative humidity, there was significantly higher mortality in the groups provided with CHF and CHF+ Yeast than for those provided with CHF+ FE (TABLE 3). The number of pupae was different among all groups as was the number of adults.

Larvae that were provided with CHF+ FE and maintained at 55% RH consumed an average of 26.9 ± 2.7 eggs per larva, which was greater than the number of eggs consumed (20.4 ± 1.9) by larvae held at 75% RH. No mortality occurred in the group provisioned with CHF+ Yeast at 75% RH compared to 43.3% mortality in the group with CHF+ Yeast at 55% RH. There was higher mortality in the group with CHF at 55% RH than the group with CHF at 75% RH. In the CHF+ Yeast treatments, fewer pupae formed in the group held at 55% RH than in the group at 75% RH, but there was no significant difference between pupal formation in the groups provided with CHF+ FE and held at either 55% or 75% RH.

**DISCUSSION**

Although 6.6% adult emergence occurred on a diet of CHF alone, our study demonstrates that the potential for successful and rapid development of fleas can be

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>LT₉₀</th>
<th>LT₉₀</th>
<th>Slope±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE¹</td>
<td>30</td>
<td>2.67</td>
<td>5.03</td>
<td>4.66±0.81</td>
</tr>
<tr>
<td>Yeast²</td>
<td>30</td>
<td>8.67</td>
<td>18.88</td>
<td>3.79±0.85</td>
</tr>
<tr>
<td>Yeast+FE</td>
<td>30</td>
<td>13.48</td>
<td>42.83</td>
<td>2.55±0.67</td>
</tr>
<tr>
<td>CHF³</td>
<td>30</td>
<td>29.31</td>
<td>48.06</td>
<td>5.97±2.98</td>
</tr>
</tbody>
</table>

¹Cat flea eggs previously held at -15°C for 24 hours.
²Brewer’s yeast.
³Feces from adult fleas maintained on cats.

**TABLE 2.** The effects of larval diet on the survival (d) of cat flea larvae held at 75% relative humidity and 22.5±2.0°C.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Percent Mortality</th>
<th>No. of Larvae</th>
<th>No. of Pupae</th>
<th>% Eclosed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHF ⁸</td>
<td>53.3a</td>
<td>12a</td>
<td>3a</td>
<td>0a</td>
</tr>
<tr>
<td>CHF + Yeast⁹</td>
<td>43.3a</td>
<td>3b</td>
<td>14b</td>
<td>93b</td>
</tr>
<tr>
<td>CHF + FE ⁴</td>
<td>6.7b</td>
<td>2c</td>
<td>26c</td>
<td>88c</td>
</tr>
</tbody>
</table>

⁸ Feces from adult fleas maintained on cats.
⁹ Brewer’s yeast.
⁴ Frozen eggs.

Means within columns followed by the same letter are not significantly different (p > 0.05); N=30.
increased by consumption of flea eggs, as well as yeast. Silverman and Appel (1994) found that no development occurred on a diet of adult flea feces only, but reported 13% larval development on dried blood alone. Because egg cannibalism was not reduced by the addition of rearing diet, we suggest that this phenomenon occurs in the presence of adequate food sources.

Egg consumption was higher for larvae held at 55% RH than for those held at 75% RH, but the percentage of adult eclosion was equivalent. Survival of larvae provided with CHF+ Yeast or CHF+FE was equivalent at 75% RH, but survival was higher for larvae with CHF+ FE than those with CHF+ Yeast at 55% RH. These results indicate that eggs may be a potential source of water. However, we did not measure possible microchanges in RH within the units due to the addition of FE.

Larvae provided with only flea eggs, yeast, or flea eggs and yeast did not survive as long as those provided CHF. On a diet of only flea feces, larvae were found to develop from the first to third instar within three days and over 50% of the larvae survived for more than 29 d, which suggests that flea larvae are capable of surviving for extended periods on adult flea feces alone. Our study indicates that if larvae feed on either yeast or eggs during this period, essentially 100% adult development occurs.

The results of this study show that a single cat flea larva is capable of consuming > 20 eggs over a seven day period. Thus, an established larval population could consume enough eggs to affect local populations. Egg cannibalism could be a factor in the cyclic rise and decline reported in natural flea populations (Silverman and Rust 1983).

In conclusion, cat flea larvae living in their natural environment require a form of blood plus additional nutrients for successful development (Bruce 1948, Strenger 1973, Silverman and Appel 1994). Yeast is used as an additive to dried blood or flea feces in laboratory flea colonies, but yeast is not widely available in most of the natural cat flea larval habitats. In natural settings, adult feces and eggs are distributed together relative to the activity of the host animal and our study indicates that these are two sources of nutrition for developing flea larvae. Egg consumption provides nutrients that promote rapid growth and development, which in turn would favor host exploitation. Furthermore, at low relative humidity, egg cannibalism may compensate for water loss.

Acknowledgments

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REFERENCES CITED


A State-by-State Survey of Ticks Recorded From Humans in the United States

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ABSTRACT: Based on tick specimens accessioned into the U.S. National Tick Collection, the species of ticks recorded as ectoparasites of humans are documented and compared for 49 U.S. states. A total of 44 tick species was recorded as parasites of humans, consisting of 11 species of soft ticks (Argasidae) and 33 species of hard ticks (Ixodidae). Four of the hard tick species are not native to the U.S. and were removed from travelers returning from foreign destinations. Therefore, we record 40 of the 84 species of U.S. ticks as ectoparasites of humans. Predictably, in the southern U.S. and Atlantic states, all active stages of the lone star tick, Amblyomma americanum (L.), were common ectoparasites of humans. Also, in the eastern U.S., adults of the American dog tick, Dermacentor variabilis (Say), and all active stages (especially adults) of the blacklegged tick, Ixodes scapularis (Say), were commonly recorded. In certain Rocky Mountain and western states, all active stages of the Rocky Mountain wood tick, Dermacentor andersoni (Stiles), predominated, whereas, in most far western states, adults of the western blacklegged tick, Ixodes pacificus Cooley and Kohls, were relatively common parasites of humans. Parasitism by Ornithodoros spp. soft ticks was mainly recorded in the western U.S. These findings, together with records of human parasitism by other tick species in the U.S., are briefly discussed with respect to tick-borne disease risk.

Keyword Index: Ticks, human parasitism, United States.

INTRODUCTION

A state-by-state comparison of the various species of ticks recovered from humans in the United States has not previously been undertaken. This information would be useful to public health workers who may need to assess potential disorders caused by attaching ticks, especially those resulting from tick-transmitted pathogens. Many ticks attack humans every year in the United States, often resulting in outbreaks of tick-borne diseases, such as Colorado tick fever, ehrlichiosis, tick-borne typhus (Rocky Mountain spotted fever), Lyme borreliosis, relapsing fever, and babesiosis. These diseases are often debilitating and some can be fatal. Although some information is available on the more common species of ticks which parasitize humans in the U.S., detailed data exist only for a few states. For example, in West Virginia, Hall et al. (1991) reported human parasitism by the carnivore tick, Ixodes cookei Packard, and suggested that it could be a vector of the agent of Lyme borreliosis. Falco and Fish (1988) reported human parasitism by ticks in a Lyme borreliosis-endemic area of New York state, including many records of Ixodes scapularis Say, the blacklegged tick, which is the main vector in that region. In South Carolina, Burgdorfer et al. (1975) documented human parasitism by ticks in relation to Rocky Mountain spotted fever epidemiology and found that the main eastern vector, the American dog tick, Dermacentor variabilis Say, frequently parasitized people. The lone star tick, Amblyomma americanum (L.), the implicated vector of the agent of human monocytic ehrlichiosis, commonly bites humans in South Carolina and Georgia; a recent study showed that 83% of the ticks recovered from humans in these states were A. americanum (Felz et al. 1996). In San Diego County, California, Lang (1999) recovered the following five species of ticks from humans, listed in order of prevalence (highest to lowest): western blacklegged tick, Ixodes pacificus Cooley and Kohls; D. variabilis; Pacific Coast Tick, Dermacentor occidentalis Marx; Dermacentor hunteri Bishop; and A. americanum.
A more widespread survey has been completed on selected U.S. Air Force Bases with respect to ticks parasitizing humans in several different parts of the country (Campbell and Bowles 1994). That four year survey included a broad geographic area and provided some detailed information on tick parasitism of humans in the United States. More than half a century ago, Cooley and Kohls (1945) recorded five Ixodes spp. ticks (I. angustus Neumann, I. cookei, I. pacificus, I. scapularis, and I. uriae White) from humans in the United States. Also, Bishop and Trembley (1945) recorded two species of soft ticks (Argas miniatu Koch and Otobius megnini Dugès) and 14 species of hard ticks (A. americanum, Amblyomma cajennense (F.), Amblyomma maculatum Koch, Dermacentor albipictus Packard, Dermacentor andersoni (Stiles), the Pacific Coast tick, Dermacentor occidentalis Marx, D. variabilis, Haemaphysalis chordelis Packard, I. angustus, I. cookei, Ixodes muris Bishop and Smith, I. pacificus, I. scapularis, and Ixodes scapularis Neuman) from humans in North America. However, records of human parasitism by ticks have not been reported for all 50 states in the country. We have therefore gathered records from 49 of the 50 states for which we could locate records, and established a profile of human parasitism by ticks for these states.

MATERIALS AND METHODS

Data were retrieved from the Smithsonian Institution’s Tick Database that stores information for the more than 122,000 collections accessioned into the U.S. National Tick Collection, curated at Georgia Southern University. These data were accessed from a computer link to the Smithsonian database at Georgia Southern University. Data on ticks recovered from humans were searched for all 50 states in the U.S. Tick species, stages recovered, and the number of collections from humans were collated for each state. All specimens were identified by current or previous curators of the U.S. National Tick Collection.

RESULTS AND DISCUSSION

Collections of ticks from humans were recorded for 49 of the 50 United States; no collections were available for Delaware. TABLES 1-7 list the tick collections from humans that were recorded for these 49 states. States are grouped by region with seven states listed in each Table.

TABLE 1 lists eight species of hard ticks (Ixodidae) recovered from humans in southeastern states. In this region, A. americanum (all stages), accounted for most tick collections, followed by I. scapularis (adults only). Interestingly, larvae of the gopher tortoise tick, Amblyomma tuberculatum Marx, also were relatively common as parasites of humans in this region. Amblyomma triguttatum Koch is an exotic species from Australia and represents a case of tick parasitism in a traveler returning from that continent.

TABLE 2 documents 11 species of hard ticks and one species of soft tick (Argasidae) recovered from humans in northeastern states. The blacklegged tick (all stages) was the most commonly collected tick species in this region followed by I. cookei, A. americanum, and D. variabilis. Some unusual tick collections are apparent for this region. For example, the Cayenne tick, A. cajennense, is native to Central America and southern Texas, Ixodes banksi Bishop typically parasitizes beavers, Ixodes marxi Banks is principally a squirrel tick, I. uriae is a seabird tick, and the spinose ear tick, O. megnini, is native to the southwestern states and Central America.

TABLE 3 lists nine species of hard ticks recovered from humans in seven eastern states. Here, A. americanum (all stages), followed by D. variabilis (adults only) and I. scapularis (adults and nymphs), were the most commonly recorded ticks. Again, some unusual records are apparent; the tropical bont tick, Amblyomma variegatum (F.-), is an African species that was recovered from a returning traveler, I. marxi is mainly a squirrel tick, and Ixodes texanus Banks typically parasitizes carnivores such as raccoons.

TABLE 4 lists eight species of hard ticks and one species of soft tick recovered from humans in seven midwestern states. In this region, D. variabilis (adults only) was the most common tick attacking humans. Unusual records include the Asian Dermacentor auratus Supino, the African Rhipicephalus pulchellus Gerstäcker, both of which were recovered from returning travelers, and the soft tick Ornithodoros kelleyi Cooley and Kohls, which normally feeds on bats.

TABLE 5 lists 14 species of hard ticks and six species of soft ticks recovered from humans in seven southern/southwestern states. Amblyomma americanum (all stages), followed by the brown dog tick, Rhipicephalus sanguineus (Latreille) (adults and nymphs), and D. variabilis (adults only) were the most commonly collected ticks. The remaining tick species recorded include the Rocky Mountain wood tick, D. andersoni, and species that normally parasitize bats (Ornithodoros stagleri Cooley and Kohls), birds (A. miniatu), both bats and birds (Ornithodoros concanensis Cooley and Kohls), or rabbits (Dermacentor parumapertus Neumann, and Otobius lagophilus Cooley and Kohls).
TABLE 6 lists 11 species of hard ticks and two species of soft ticks recorded from humans in five northwestern states, in Alaska, or in Hawaii. By a wide margin, *D. andersoni* (all stages) was the most common tick recovered from humans in the northwestern states, especially in Montana, Washington, and Wyoming. However, *D. variabilis* (adults only) was also collected quite frequently, and the western blacklegged tick, *I. pacificus* (adults only), was recorded from humans in Washington. Unusual records include the southern/southeastern *A. americanum* in Wyoming, *I. uriae* from Alaska, which normally parasitizes seabirds, and *O. concanensis* from South Dakota, which normally parasitizes birds or bats. The only tick recorded from humans in Hawaii was *Ornithodoros capensis* Neumann, a wide-ranging soft tick associated with seabirds.

TABLE 7 lists 13 species of hard ticks and five species of soft ticks recorded from humans in seven western states. *Dermacentor andersoni* (adults and nymphs) dominated these collections, followed by *I. pacificus* (adults and nymphs). Unusual records for this region include *Argas sanchexii* Dugès, a soft tick usually associated with chickens, *A. americanum*, a southern/southeastern species, *D. hunteri* which usually parasitizes bighorn sheep, *D. parumapertus* which is a rabbit tick, and *L. sculptus, Ixodes spinipalpis* Hadwen and Nuttall, *Ornithodoros hermsi* Wheeler, Herms, and Mayer, and *Ornithodoros parkeri* Cooley, all of which are usually recovered from rodents or their burrows.

In total, 44 species of ticks were recorded from humans in the United States in this survey (11 species of soft ticks and 33 species of hard ticks, including four exotic species). Therefore, we recorded 40 of the 84 species of U.S. ticks as ectoparasites of humans. We assume that most of these tick specimens had attached to humans (as opposed to crawling on skin or clothing) and, in most cases we could confirm this.

The number of tick species recovered from humans per state ranged from one in each of Hawaii and Illinois, to 10 in California, and 14 in Texas. It should be emphasized that these records reflect only those specimens accessioned into the U.S. National Tick Collection, where the number of ticks recovered from humans varies greatly by state for various reasons. Nevertheless, our data represent the first compilation of human parasitism by ticks across the United States, and are therefore useful for demonstrating trends in tick parasitism and risk of exposure to tick-borne pathogens by region. **TABLE 8** lists the seven most important human-biting tick species in the United States together with their geographical distributions and lists of the pathogens they transmit. In addition to their importance as vectors, attached females of some of these ticks,
<table>
<thead>
<tr>
<th>Species</th>
<th>Connecticut</th>
<th>Maine</th>
<th>Massachusetts</th>
<th>New Hampshire</th>
<th>New York</th>
<th>Rhode Island</th>
<th>Vermont</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amblyomma americanum</em></td>
<td>4 (1M, 3F)</td>
<td>4 (2M, 1F, 1N)</td>
<td>1 (6L)</td>
<td>2 (2N)</td>
<td>1 (1F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Amblyomma cajennense</em></td>
<td>1 (1N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dermacentor albipictus</em></td>
<td>1 (5L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dermacentor variabilis</em></td>
<td></td>
<td>2 (3M, 2F)</td>
<td>3 (7M, 8F)</td>
<td>2 (2F)</td>
<td>1 (1F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ixodes banksi</em></td>
<td></td>
<td></td>
<td></td>
<td>1 (1M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ixodes cookei</em></td>
<td>1 (1N)</td>
<td>4 (1F, 3N)</td>
<td>1 (1N)</td>
<td>5 (1F, 2N, 2L)</td>
<td>3 (3F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ixodes dentatus</em></td>
<td>1 (1N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ixodes marxi</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (1F)</td>
<td></td>
</tr>
<tr>
<td><em>Ixodes muris</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 (1F, 5N)</td>
<td>1 (1N)</td>
</tr>
<tr>
<td><em>Ixodes scapularis</em></td>
<td>3 (1M, 2F)</td>
<td>1 (1F)</td>
<td>9 (30M, 62F, 3N, 6L)</td>
<td>1 (1F)</td>
<td>3 (1M, 3F, 1N, 1L)</td>
<td>1 (1F)</td>
<td></td>
</tr>
<tr>
<td><em>Ixodes uriae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Otodius megnini</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>9 (2M, 5F, 2N)</td>
<td>12 (2M, 3F, 7N, 5L)</td>
<td>11 (33M, 64F, 3N, 6L)</td>
<td>6 (7M, 9F, 1N, 6L)</td>
<td>18 (2M, 7F, 11N, 3L)</td>
<td>3 (2F, 1N)</td>
<td>5 (5F)</td>
</tr>
</tbody>
</table>

M = Male(s); F = Female(s); N = Nymph(s); L = Larva(e)
### TABLE 3. Ticks recovered from humans in the eastern United States.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Collections</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amblyomma americanum</strong></td>
<td>Kentucky: 1 (1M)</td>
</tr>
<tr>
<td></td>
<td>Maryland: 7 (2M, 1F, 2N, 6L)</td>
</tr>
<tr>
<td></td>
<td>New Jersey: 3 (4M, 3N)</td>
</tr>
<tr>
<td></td>
<td>Ohio: 1 (1F)</td>
</tr>
<tr>
<td></td>
<td>Pennsylvania: 12 (1M, 2F, 8N, 25L)</td>
</tr>
<tr>
<td></td>
<td>Virginia: 1 (1M)</td>
</tr>
<tr>
<td></td>
<td>West Virginia: 1 (1N)</td>
</tr>
<tr>
<td><strong>Amblyomma variegatum</strong></td>
<td>1 (1N)</td>
</tr>
<tr>
<td><strong>Dermacentor variabilis</strong></td>
<td>Kentucky: 1 (1M)</td>
</tr>
<tr>
<td></td>
<td>New Jersey: 6 (8M, 3F)</td>
</tr>
<tr>
<td></td>
<td>Ohio: 1 (1F)</td>
</tr>
<tr>
<td></td>
<td>Pennsylvania: 1 (1M, 1F)</td>
</tr>
<tr>
<td></td>
<td>Virginia: 7 (1M, 6F)</td>
</tr>
<tr>
<td></td>
<td>West Virginia: 1 (1M)</td>
</tr>
<tr>
<td><strong>Ixodes cookei</strong></td>
<td>1 (1L)</td>
</tr>
<tr>
<td><strong>Ixodes dentatus</strong></td>
<td>2 (2N)</td>
</tr>
<tr>
<td><strong>Ixodes marxi</strong></td>
<td>1 (1F)</td>
</tr>
<tr>
<td><strong>Ixodes muris</strong></td>
<td>1 (1F)</td>
</tr>
<tr>
<td><strong>Ixodes scapularis</strong></td>
<td>Kentucky: 13 (5M, 7F, 1N)</td>
</tr>
<tr>
<td></td>
<td>New Jersey: 2 (2F)</td>
</tr>
<tr>
<td></td>
<td>Ohio: 1 (1F)</td>
</tr>
<tr>
<td><strong>Ixodes texanus</strong></td>
<td>1 (1F)</td>
</tr>
<tr>
<td></td>
<td>West Virginia: 1 (2L)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2 (2M)</td>
</tr>
<tr>
<td></td>
<td>26 (15M, 11F, 3N, 6L)</td>
</tr>
<tr>
<td></td>
<td>6 (4F, 3F, 3N)</td>
</tr>
<tr>
<td></td>
<td>5 (4F, 1N)</td>
</tr>
<tr>
<td></td>
<td>4 (1M, 2F, 1N, 1L)</td>
</tr>
<tr>
<td></td>
<td>19 (2M, 8F, 8N, 25L)</td>
</tr>
<tr>
<td></td>
<td>4 (1M, 2L)</td>
</tr>
</tbody>
</table>

M = Male(s); F = Female(s); N = Nymph(s); L = Larva(e).
TABLE 4. Ticks recovered from humans in the midwestern United States.

<table>
<thead>
<tr>
<th>Species</th>
<th>Illinois</th>
<th>Indiana</th>
<th>Iowa</th>
<th>Michigan</th>
<th>Minnesota</th>
<th>Missouri</th>
<th>Wisconsin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amblyomma americanum</em></td>
<td>2 (2F)</td>
<td></td>
<td></td>
<td>3 (4N)</td>
<td>1 (1N)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dermacentor albipictus</em></td>
<td>1 (1M)</td>
<td></td>
<td></td>
<td>1 (1N)</td>
<td>11 (20M, 40F)</td>
<td>1 (1N)</td>
<td></td>
</tr>
<tr>
<td><em>Dermacentor variabilis</em></td>
<td>12 (18M, 18F)</td>
<td>1 (1F)</td>
<td>1 (1F)</td>
<td>18 (47M, 7F)</td>
<td>3 (1M, 3F)</td>
<td>1 (1F)</td>
<td>1 (1F)</td>
</tr>
<tr>
<td><em>Ixodes dentatus</em></td>
<td>1 (1N)</td>
<td></td>
<td></td>
<td>1 (1N)</td>
<td>1 (1F)</td>
<td>1 (1F)</td>
<td></td>
</tr>
<tr>
<td><em>Ixodes scapularis</em></td>
<td>2 (2F)</td>
<td></td>
<td></td>
<td>1 (1N)</td>
<td>1 (1F)</td>
<td>1 (1F)</td>
<td></td>
</tr>
<tr>
<td><em>Ornithodoros kelleyi</em></td>
<td>1 (1N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rhipicephalus pulchellus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rhipicephalus sanguineus</em></td>
<td>1 (5M, 1F)</td>
<td></td>
<td></td>
<td>1 (1F)</td>
<td>1 (1F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>12 (18M, 18F)</td>
<td>3 (3F)</td>
<td>3 (5M, 2F, 1N)</td>
<td>25 (48M, 10F, 3N)</td>
<td>4 (2M, 3F)</td>
<td>4 (1F, 4N)</td>
<td>13 (20M, 41F, 1N)</td>
</tr>
</tbody>
</table>

M = Male(s); F = Female(s); N = Nymph(s); L = Larva(e).
### TABLE 5. Ticks recovered from humans in the southern United States.

<table>
<thead>
<tr>
<th></th>
<th>Number of Collections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arizona</td>
</tr>
<tr>
<td><em>Amblyomma americanum</em></td>
<td>10 (3M, 2F, 5N, 2L)</td>
</tr>
<tr>
<td><em>Amblyomma cajennense</em></td>
<td></td>
</tr>
<tr>
<td><em>Amblyomma imitator</em></td>
<td></td>
</tr>
<tr>
<td><em>Amblyomma inornatum</em></td>
<td></td>
</tr>
<tr>
<td><em>Amblyomma maculatum</em></td>
<td>1 (1M)</td>
</tr>
<tr>
<td><em>Argas miniatus</em></td>
<td></td>
</tr>
<tr>
<td><em>Boophilus annulatus</em></td>
<td></td>
</tr>
<tr>
<td><em>Dermacentor albipictus</em></td>
<td>2 (2M)</td>
</tr>
<tr>
<td><em>Dermacentor andersoni</em></td>
<td>1 (1F)</td>
</tr>
<tr>
<td><em>Dermacentor parumapertus</em></td>
<td>1 (1N)</td>
</tr>
<tr>
<td><em>Dermacentor variabilis</em></td>
<td>1 (1F)</td>
</tr>
<tr>
<td><em>Ixodes cookei</em></td>
<td></td>
</tr>
<tr>
<td><em>Ixodes kingi</em></td>
<td></td>
</tr>
<tr>
<td><em>Ixodes scapularis</em></td>
<td>4 (2M, 3F)</td>
</tr>
<tr>
<td><em>Ornithodoros concanensis</em></td>
<td>1 (1M)</td>
</tr>
<tr>
<td><em>Ornithodoros stageri</em></td>
<td></td>
</tr>
<tr>
<td><em>Ornithodoros turicata</em></td>
<td></td>
</tr>
<tr>
<td><em>Otodius lagophillus</em></td>
<td></td>
</tr>
<tr>
<td><em>Otodius megnini</em></td>
<td>2 (1F, 1N)</td>
</tr>
<tr>
<td><em>Rhipicephalus sanguineus</em></td>
<td>2 (2F, 1N)</td>
</tr>
<tr>
<td>Total</td>
<td>9 (2M, 5F, 3N)</td>
</tr>
</tbody>
</table>

*M = Male(s); F = Female(s); N = Nymph(s); L = Larva(e).*
TABLE 6. Ticks recovered from humans in the northwestern United States, Alaska, and Hawaii.

<table>
<thead>
<tr>
<th>Number of Collections</th>
<th>Alaska</th>
<th>Hawaii</th>
<th>Montana</th>
<th>North Dakota</th>
<th>South Dakota</th>
<th>Washington</th>
<th>Wyoming</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amblyomma americanum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (1F)</td>
<td></td>
</tr>
<tr>
<td><em>Dermacentor albipictus</em></td>
<td>1 (2M, 8F)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dermacentor andersoni</em></td>
<td>121 (124M, 195F, 6N, 3L)</td>
<td>2 (2F)</td>
<td>7 (3M, 3F, 1N)</td>
<td>56 (53M, 70F)</td>
<td>23 (44M, 11F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dermacentor parumapertus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (1F)</td>
<td></td>
</tr>
<tr>
<td><em>Dermacentor variabilis</em></td>
<td>1 (1M)</td>
<td>3 (1M, 3F)</td>
<td>2 (1M, 2F)</td>
<td>3 (1M, 4F)</td>
<td>20 (12M, 18F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ixodes angustus</em></td>
<td>1 (1F, 1N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15 (15F)</td>
<td></td>
</tr>
<tr>
<td><em>Ixodes pacificus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26 (11M, 37F)</td>
<td></td>
</tr>
<tr>
<td><em>Ixodes scapularis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (1N)</td>
<td></td>
</tr>
<tr>
<td><em>Ixodes spinipalpis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 (10F, 4N)</td>
<td></td>
</tr>
<tr>
<td><em>Ixodes uriae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (1F)</td>
<td></td>
</tr>
<tr>
<td><em>Ixodes woodi</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (1N)</td>
<td></td>
</tr>
<tr>
<td><em>Ornithodoros capensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (3N)</td>
<td></td>
</tr>
<tr>
<td><em>Ornithodoros concanensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (1M)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3 (1M, 2F, 1N)</td>
<td>1 (3N)</td>
<td>126 (127M, 206F, 7N, 3L)</td>
<td>5 (1M, 4F, 1N)</td>
<td>11 (5M, 7F, 1N)</td>
<td>122 (76M, 151F, 4N)</td>
<td>25 (44M, 12F, 1N)</td>
</tr>
</tbody>
</table>

*M* = Male(s); *F* = Female(s); *N* = Nymph(s); *L* = Larva(e).
<table>
<thead>
<tr>
<th>Tick Species</th>
<th>Number of Collections</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amblyomma americanum</strong></td>
<td>2 (2M)</td>
</tr>
<tr>
<td><strong>Amblyomma cajennense</strong></td>
<td>1 (1N)</td>
</tr>
<tr>
<td><strong>Argas sancti</strong></td>
<td>10 (3M, 10F)</td>
</tr>
<tr>
<td><strong>Dermacentor albipictus</strong></td>
<td>25 (12M, 34F)</td>
</tr>
<tr>
<td><strong>Dermacentor andersoni</strong></td>
<td>40 (55M, 85F)</td>
</tr>
<tr>
<td><strong>Dermacentor hunteri</strong></td>
<td>3 (3M, 3F)</td>
</tr>
<tr>
<td><strong>Dermacentor parumapertus</strong></td>
<td>64 (61M, 85F, 1N)</td>
</tr>
<tr>
<td><strong>Dermacentor occidentalis</strong></td>
<td>11 (34M, 2F)</td>
</tr>
<tr>
<td><strong>Dermacentor variabilis</strong></td>
<td>31 (37M, 44F)</td>
</tr>
<tr>
<td><strong>Ixodes kingi</strong></td>
<td>2 (1M, 1F)</td>
</tr>
<tr>
<td><strong>Ixodes pacificus</strong></td>
<td>33 (36M, 61F)</td>
</tr>
<tr>
<td><strong>Ixodes sculptus</strong></td>
<td>7 (5M, 3F)</td>
</tr>
<tr>
<td><strong>Ixodes spinipalpis</strong></td>
<td>4 (1M, 4F)</td>
</tr>
<tr>
<td><strong>Ornithodoros coriaceus</strong></td>
<td>4 (1M, 1F)</td>
</tr>
<tr>
<td><strong>Ornithodoros hermsi</strong></td>
<td>2 (1M, 1F)</td>
</tr>
<tr>
<td><strong>Ornithodoros parkeri</strong></td>
<td>17 (15M, 21F, 1N)</td>
</tr>
<tr>
<td><strong>Otobius megnini</strong></td>
<td>25 (5M, 28F)</td>
</tr>
<tr>
<td><strong>Rhipicephalus sanguineus</strong></td>
<td>4 (4F)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>74 (59M, 108F, 8N)</td>
</tr>
</tbody>
</table>

M = Male(s); F = Female(s); N = Nymph(s); L = Larval(e).
especially *D. andersoni* and *D. variabilis*, can cause tick paralysis. As expected, the vectors for Rocky Mountain spotted fever, *D. andersoni* and *D. variabilis*, commonly parasitize humans in the western and eastern United States, respectively. *Amblyomma americanum*, the suspected vector of the agent of human monocytic ehrlichiosis and Lyme-like disease (thought to be caused by *Borrelia lonestari* Barbour, Maupin, Teltow, Carter and Piesman), parasitizes humans in the South and along the east coast. High risk areas for babesiosis, Lyme disease, and human granulocytic ehrlichiosis are prominent in the Midwest and eastern U.S. because the principal vector, *I. scapularis*, bites humans in those areas. However, *I. scapularis* is also commonly recorded as an ectoparasite of humans in the southern United States, where these tick-borne diseases are less frequently reported, suggesting that intensified surveillance in the South might reveal more human cases. In the Pacific states, another vector of the Lyme disease spirochete, *I. pacificus*, is a widespread ectoparasite of humans. Other high-risk areas for tick-borne pathogens include the Rocky Mountain region for Colorado tick fever virus transmitted mainly by *D. andersoni*, much of the western U.S. for relapsing fever spirochetes transmitted by *Ornithodoros* spp. soft ticks, and parts of the Northeast and Midwest for Powassan encephalitis virus transmitted mainly by *I. cookei*. We recorded all of these ticks as ectoparasites of humans in these respective regions.

Our data on ticks recovered from humans compare favorably with most other surveys of a similar nature pertaining to various parts of the U.S. For example, in their analysis of ticks recovered from humans in Georgia and South Carolina, Felz et al. (1996) found that *A. americanum* (83% of 913 ticks) accounted for most tick bites in these states, with *D. variabilis* (11.4%), *I. scapularis* (3.9%), the Gulf Coast tick *A. maculatum* (1.0%), and *R. sanguineus* (0.7%) being recovered much less frequently. Severe human infestation by *A. americanum*, especially by larvae, has also been reported by Jones (1981) in North Carolina, Duckworth et al. (1985) in Virginia, Goddard and McHugh (1990) in Arkansas, and Campbell and Bowles (1994) in several southern states. In eastern North America, *D. variabilis* has been reported as a common ectoparasite of humans by Burgdorfer et al. (1975) in South Carolina, Scholt (1977) in Ontario, Falco and Fish (1988) in New York, Slaff and Newton (1993) in North Carolina, and Campbell and Bowles (1994) for several U.S. states. Although our data show that *I. scapularis* clearly attacks humans in the northeastern, midwestern, and southern states, previously published records of human ectoparasitism by this medically important tick are mainly from New York (Falco and Fish 1988), Rhode Island (Yeh et al.
1995). Georgia and South Carolina (Felz et al. 1996), and various Atlantic states from South Carolina north to New York (Campbell and Bowles 1994). *Ixodes cookei*, a vector of Powassan virus, has previously been reported as a parasite of humans in Ontario (Scholten 1977, Wyoming (Hall et al. 1991), New York and New Jersey (Campbell and Bowles 1994), and North Carolina (Harrison et al. 1997). In San Diego County, California, Lang (1999) recorded similar data to those reported here for California (TABLE 7). He found adults of *I. pacificus* to be the most prevalent (48%) tick attacking humans followed by *D. variabilis* (33%) and *D. occidentalis* (19%), whereas, werecorded *D. occidentalis*, *I. pacificus*, and then *D. andersoni* to be the most frequent ticks recovered from humans (TABLE 7).

In their survey of human parasitism by ticks at U.S. Air Force Bases in the contiguous United States, Campbell and Bowles (1994) also highlighted *D. variabilis* in west coast states (California, Oregon, and Washington), *D. andersoni* in the West and Rocky Mountain states (California, Washington, Idaho, Wyoming, and Utah), and *I. pacificus* in California. Further, *R. sanguineus* has been reported as a relatively infrequent but widespread parasite of humans in several states (Burgdorfer et al. 1975, Goddard 1989, Carpenter et al. 1990, Campbell and Bowles 1994).

Because soft ticks typically feed rapidly, there are relatively few reports of human parasitism by these ticks (Bishop and Trembley 1945). Instead, parasitism is usually inferred after cases of relapsing fever, for which the causative agents must have been transmitted by soft ticks, are diagnosed. An exception to the rare findings of soft ticks parasitizing humans involves the spinose ear tick, *O. megnini*, in which the immature stages may remain lodged in the ear for several days (Jensen 1982, Eads and Campos 1984, Harrison et al. 1997).

Our data extend the records of Bishop and Trembley (1945) who provided separate distribution maps and records of tick species recovered from various hosts, including humans, in North America. In agreement with our findings, they reported *A. americanum* (159 collections: 314 males, 328 females, 319 nymphs, 145 larvae), *D. andersoni* (270 collections: 513 males, 614 females, 2 nymphs), and *D. variabilis* (113 collections: 48 males, 70 females) to be common ticks on humans in their respective ranges. They also found *D. occidentalis* (44 collections: 59 males, 81 females), *I. pacificus* (22 collections: 14 males, 3 females) and *I. scapularis* (21 collections: 19 males, 29 females, 4 nymphs) to be relatively common as parasites of humans within their ranges.

Some other hard ticks that have previously been reported as occasional ectoparasites of humans in North America are the rabbit ticks *Haemaphysalis leporispalustris* (Packard), and *Ixodes dentatus* Marx (Sollers 1955, Harrison et al. 1997), the avian tick *H. chordeiles* (Bishop and Trembley 1945), and *I. angustus*, *Ixodes muris* Bishop and Smith, *Ixodes woodi* Bishop, and *I. scapularis*, all four of which usually parasitize small mammals (Bishop and Trembley 1945, Scholten 1977, Robbins and Keirans 1992). *Ixodes dentatus*, *I. muris*, and *I. scapularis* were also recorded as occasional parasites of humans in this survey.

The four species of exotic ticks that we recorded from humans are of interest because they were attached to travelers who had returned from overseas. One of these tick species, *A. variegatum*, which was collected in Pennsylvania (TABLE 3) is a vector of *Cowdria ruminantium* (Cowdry), the rickettsial agent of heartwater fever of African ungulates. This economically important livestock disease is presently absent from the United States, but accidental introductions of *A. variegatum* into North America could jeopardize this status. Other exotic tick species have been recovered previously from humans and imported animals in North America (e.g., Beckland 1968; Scholten 1976, 1977; Mertins and Schlater 1991).

Our data clearly demonstrate that, although less than 10 species of ticks commonly attack humans in the United States, at least 40 native tick species will bite humans. Many of these tick species are known or suspected vectors of pathogens. Further, exotic tick species are occasionally brought into the United States as ectoparasites attached to travelers, and pose a potential threat for introducing exotic pathogens of humans or animals.

Acknowledgments

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Musca domestica as a Mechanical Carrier of Bacteria in Chiang Mai, North Thailand

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ABSTRACT: The common house fly, Musca domestica L., was assessed for its potential as a mechanical carrier of bacteria in urban areas of Chiang Mai province, north Thailand. Sixty-one specimens (61.0%) were found to carry bacteria, with each harboring 1-5 bacteria. No significant difference between the sex of flies for carrying bacteria was found. A total of 21 bacteria was isolated, of which the most common was coagulase negative staphylococci (n = 38), followed by Viridans streptococci (n = 9). The highest bacterial load for all bacteria isolated was 10^4-10^6 (67%), followed by >10^4 (26%) colonies per fly. It is recommended that fly control management measures, including sanitation improvement, should be implemented.

Keyword Index: House fly, vector, bacteria, Thailand.

INTRODUCTION

The common house fly, Musca domestica L., is an insect of considerable medical importance worldwide. In addition to causing myiasis in humans and animals (Zumpt 1965), it has also been reported to be a mechanical carrier and reservoir of various pathogenic organisms, including bacteria, viruses, protozoan cysts, and helminth eggs, which cause disease in humans (Greenberg 1973, Dipelo 1977, Bidawid et al. 1978, Echeverria et al. 1983, Adeyemi and Dipelo 1984, Umeche and Mandah 1989, Monzon et al. 1991, Levine and Levine 1991, Fotedar et al. 1992, Tan et al. 1997, Iwasa et al. 1999). Among these organisms, pathogenic bacteria seem to be extremely prevalent, and humans appear to be easily exposed to these organisms via their association with flies.

The common house fly has been reported to be the most abundant of adult flies collected in Thailand, especially in urban areas (Sucharit et al. 1976, Tumrasvin et al. 1978, Sucharit and Tumrasvin 1981). However, no information regarding the role of the house fly as a mechanical carrier of bacteria in such areas is available. This study was, therefore, undertaken as a pilot investigation in the urban areas of Chiang Mai province, the biggest city of north Thailand and one of the country’s prime tourist attractions.

MATERIALS AND METHODS

Four fresh-food markets in the urban area of Chiang Mai province (north latitude 17-21 and east longitude 98-99) were selected as the collection sites where numerous adult house flies occur. One hundred adult house flies were collected with insect sweep nets from these markets from April-May 1999. Fifty males were collected in the first batch then another 50 females in the second batch. All fly specimens captured in the field were separated individually into 20 ml sterile glass vials (2.5-cm diameter, 6-cm height) that were immediately covered with sterile caps. The vials of flies were transported within one-half hour to the laboratory of the Department of Parasitology, Faculty of Medicine, Chiang Mai University. All flies were killed by placing the vials in a freezer set at -20°C for 30 min. and then identified under a stereomicroscope while still within the vials. The keys of Greenberg (1971) and Tumrasvin and Shimonaga (1978) were used to identify them as M. domestica.

After identification, 1 ml of sterile physiological
A saline solution was added to each vial and then the vial was shaken vigorously for 1 min. with the fly remaining in the vial. The solutions were then immediately examined for the presence of bacteria at the Microbiology Section, Central Laboratory, Faculty of Medicine, Chiang Mai University. The saline wash solution in each vial was inoculated in the following plates: a phenyl ethyl alcohol agar plate (for the isolation of Staphylococcus spp. and Enterococcus spp.); a MacConkey plate (for the isolation of gram negative bacilli, Shigella spp. and Salmonella spp.); Salmonella and Shigella (SS) agar plates and Selenite - F broth (for the isolation of Salmonella spp. and Shigella spp.); a thiosulfate citrate bile salt agar plate and alkaline peptone water (for the isolation of Vibrio spp.). The inoculated plates were subsequently incubated for 24-48 hr. at 37°C in a normal atmosphere. Resulting isolates were characterized morphologically and further identifications were carried out following the methods of Koneman et al. (1992).

A Chi-square test was employed to determine if the number of positive male and female flies carrying bacteria differed significantly. A $P$ value of $<0.05$ was deemed significant. Analysis was carried out using the SPSS program version 7.5.1 for Windows.

**RESULTS AND DISCUSSION**

Sixty-one (61.0%) out of the 100 adult house flies collected in the markets were bacterial carriers based on bacteriological isolation results (TABLE 1). Positive male (58.0%; 29/50) and female (64.0%; 32/50) flies were not significantly different as to the number found carrying bacteria ($\chi^2 = 0.168; \text{df} = 1; \ P = 0.682$). As shown in TABLE 1, each positive fly harbored from 1-5 bacterial species. A total of 21 bacterial species was isolated (TABLE 2), the most common being coagulase negative staphylococci ($n = 38$), followed by Viridans streptococci ($n = 9$).

Results of the quantitative examination of bacteria isolated from house flies are shown in TABLE 3. The most common bacterial load was $10^5-10^4$ (67%; 73/109) colonies per fly followed by $>10^4$ (26%; 28/109) and $10^3-10^1$ (7%; 8/109) colonies per fly, respectively.

Besides those causing diarrhea, many bacteria isolated from this study have been previously reported to be the causative agents of several diseases reported elsewhere; e.g., endophthalmitis caused by Enterobacter cloacae (Okrhavi et al. 1998), pneumonia and extensive hemorrhagic necrotizing consolidation of the lung caused by Klebsiella pneumoniae (Jawetz et al. 1980), eczema gangrenosum-like eruption and opportunistic pathogenic infection caused by Morganella morganii (Del Pozo et al. 1998, Gebhart-Mueller 1998), etc. In this regard, the house fly can carry bacterial species causing such diseases. Thus, house fly management is required, especially in areas where sanitation is improperly managed and when house flies are abundant. To be totally effective, fly control strategies must be performed together with sanitation improvement.

**TABLE 1.** Bacterial carrying rates for house flies (M. domestica) collected from an urban area of Chiang Mai province, north Thailand.

<table>
<thead>
<tr>
<th>No. of Bacterial Species Isolated From Each Fly</th>
<th>No. of Flies Examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Total positive flies$^a$</td>
<td>29</td>
</tr>
<tr>
<td>Total negative flies</td>
<td>21</td>
</tr>
<tr>
<td>Total flies examined</td>
<td>50</td>
</tr>
</tbody>
</table>

$^a$No significant difference was found between the numbers of positive male and female flies ($\chi^2 = 0.168; \text{df} = 1; \ P = 0.682$).
### TABLE 2. Bacteria isolated from house flies (*M. domestica*) collected from an urban area of Chiang Mai province, north Thailand.

<table>
<thead>
<tr>
<th>Bacteria Isolated</th>
<th>Bacterial Species Isolated No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>38</td>
<td>(35)</td>
</tr>
<tr>
<td><em>Viridans</em> streptococci</td>
<td>9</td>
<td>(8)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>8</td>
<td>(7)</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>7</td>
<td>(6)</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>7</td>
<td>(6)</td>
</tr>
<tr>
<td>Streptococcus group D non-enterococci</td>
<td>6</td>
<td>(6)</td>
</tr>
<tr>
<td>Non-fermentative gram negative bacilli</td>
<td>4</td>
<td>(4)</td>
</tr>
<tr>
<td><em>Providencia stuartii</em></td>
<td>4</td>
<td>(4)</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp.</td>
<td>4</td>
<td>(4)</td>
</tr>
<tr>
<td><em>Enterobacter agglomerans</em></td>
<td>4</td>
<td>(4)</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>2</td>
<td>(2)</td>
</tr>
<tr>
<td><em>Providencia alcalifaciens</em></td>
<td>2</td>
<td>(2)</td>
</tr>
<tr>
<td><em>Morganella morganii</em></td>
<td>2</td>
<td>(2)</td>
</tr>
<tr>
<td><em>Micrococcus</em> spp.</td>
<td>2</td>
<td>(2)</td>
</tr>
<tr>
<td>Mixed gram negative bacilli</td>
<td>2</td>
<td>(2)</td>
</tr>
<tr>
<td><em>Citrobacter amalonaticus</em></td>
<td>2</td>
<td>(2)</td>
</tr>
<tr>
<td><em>Bacillus</em> spp.</td>
<td>2</td>
<td>(2)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>1</td>
<td>(1)</td>
</tr>
<tr>
<td><em>Providencia rettgeri</em></td>
<td>1</td>
<td>(1)</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp.</td>
<td>1</td>
<td>(1)</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>1</td>
<td>(1)</td>
</tr>
</tbody>
</table>

Total Bacteria Isolated From 61 Positive Flies: 109 (100)

### TABLE 3. Bacterial load for all species of bacteria isolated from house flies (*M. domestica*) collected from an urban area of Chiang Mai province, north Thailand.

<table>
<thead>
<tr>
<th>Bacteria Colony Counts Per Fly</th>
<th>Bacteria Isolated No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^2$-$10^3$</td>
<td>8</td>
<td>(7)</td>
</tr>
<tr>
<td>$10^3$-$10^4$</td>
<td>73</td>
<td>(67)</td>
</tr>
<tr>
<td>$&gt;10^4$</td>
<td>28</td>
<td>(26)</td>
</tr>
</tbody>
</table>

Total No. Bacteria Isolated From 61 Positive Flies: 109 (100)
Acknowledgments

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Autogeny in Culex pipiens quinquefasciatus Say

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Received 29 November 1999; Accepted 23 February 2000

ABSTRACT: Autogeny was observed in a laboratory colony of Culex pipiens quinquefasciatus Say originating from Hyderabad, India. In a strain selected for autogeny for 15 generations, all females, both mated and unmated, were able to mature eggs although the unmated females were unable to oviposit them. Ingesting glucose had little effect on the expression of autogeny and on the number of eggs in rafts in the laboratory experiments. Most autogenous females can ingest blood during first gonotrophic cycle, if the host is available. The blood feeding that occurred during the first cycle to initiate the second cycle was not confirmed, however, we cannot exclude this possibility.

Keyword Index: Mosquito, Culex pipiens quinquefasciatus, autogeny, gonotrophic cycle.

INTRODUCTION

Mosquitoes in the Culex pipiens complex have a cosmopolitan distribution and are among the most variable in terms of the number of synonyms (Knight and Stone 1977, Vinogradova 1997). The most often mentioned forms, Culex pipiens Linnaeus, 1758 (s. str.), Cx. p. quinquefasciatus Say, 1823 and Cx. p. molestus Forskal, 1775 differ from each other primarily in their physiology, behavior, and distribution.

The ability of a hematophagous species to lay its initial (rarely also the second) egg clutches on a blood-free diet was first mentioned by Theobald (1901), and Roubaud (1929) suggested the term autogeny for this phenomenon. In Culex pipiens complex this phenomenon is usually linked only with the form molestus. However, three cases of autogeny have been reported in Cx. p. quinquefasciatus: one in India, where the species was not conclusively identified, one in California, where populations are intermediate between pipiens (probably form molestus) and quinquefasciatus, and one from Tanzania (Eastern Africa) (Bushrod 1978). This last report suggests that "...the autogeny in this subspecies was caused by a long rainy season and none of the F₁ females proved to be autogenous."

In 1996 we obtained a population of Cx. p. quinquefasciatus originating from Hyderabad, India. Autogenous egg rafts were observed during several generations in the insectary at the Institute of Parasitology, České Budejovice. As C. p. quinquefasciatus is considered the major vector of bancroftian filariasis in Asia, the existence of autogeny is an important phenomenon which can influence the epidemiological significance of the species.

MATERIAL AND METHODS

The Cx. p. quinquefasciatus Say, Hyderabad strain was obtained from the Department of Applied Genetics, Bangalore University, India, and was reared in České Budejovice separately from the other strains of the same species. The adults were kept in Plexiglas boxes (Olejnícek, 1993). The preimaginal stages were kept in covered dishes to prevent possible contamination by other strains of the complex. Larvae were fed lyophilized yeast Pangamin® ad libitum, similar to what had been used in the Bangalore University. Adults were offered anesthetized laboratory mice for blood-feeding twice a week. The stock colony and the experimental mosquitoes were held under 12L:12D photoperiod at 25°C and 80% RH. Sugar water (10% glucose) was available to adults at all times. In addition to the stock colony (designated as CqH) a new colony was established. The first autogenous egg rafts were used for initiation of a new, separate strain (designated as CqH-A) and the adults were maintained without them feeding
on blood for 15 generations. Larvae of both colonies were reared at a density of about 100 larvae/1 dm$^2$ of water surface, with a 2 cm depth. These standard conditions were important to eliminate possible differences in the accumulation of reserves (Timmermann and Briegel, 1993).

The pupae were removed from the stock colony and placed in the rearing box in a polystyrene dish (the cases with random sex ratio) or they were placed individually into test tubes capped with cotton plugs (the cases with sex ratio 2M:1F or 30M:20F) so that experiments could be initiated with virgin specimens of about the same age.

One group of adults was kept without access to blood, while the second group was allowed to feed on mice daily. In this case (2M:1F which were kept separately in smaller boxes) the mice were available until the first egg raft appeared or until the females were found to feed on blood.

The ability of unfertilized females to produce eggs autogenously was tested on four groups of 40 virgin females of both CqH and CqH-A strains (without males). The virgin females were kept in the boxes and the observation was discontinued when the last female died or after 10-15 days, when the females were dissected and their ovaries examined.

**RESULTS**

The numbers of females that oviposited autogenously and the numbers of eggs in the egg rafts are shown in **TABLE 1**. Although all experimental groups were reared under the same conditions (temperature, relative humidity, light, food availability, larval density), the number of females that laid autogenously as well as the numbers of eggs, varied considerably (**TABLES 1 and 2**).

**TABLE 1.** Expression of autogeny in *CqH* and selected (CqH-A) strains. M:F - number of specimens (males:females) in particular samples.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sample No.</th>
<th>Males:Females</th>
<th>No. of autogenous egg rafts</th>
<th>% autogyogeny</th>
<th>Eggs / autogenous female (Mean ± SE)</th>
<th>Sugar available Y/N</th>
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<tbody>
<tr>
<td>CqH</td>
<td>1</td>
<td>157M:46F</td>
<td>14</td>
<td>30.4</td>
<td>37 ± 19</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>107M:159F</td>
<td>23</td>
<td>14.5</td>
<td>38 ± 17</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>169M:66F</td>
<td>10</td>
<td>15.2</td>
<td>42 ± 14</td>
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<tr>
<td></td>
<td>4</td>
<td>123M:87F</td>
<td>6</td>
<td>6.9</td>
<td>41 ± 14</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>125M:159F</td>
<td>24</td>
<td>15.1</td>
<td>43 ± 18</td>
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<td></td>
<td>6</td>
<td>72M:50F</td>
<td>6</td>
<td>12</td>
<td>43 ± 18</td>
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<tr>
<td></td>
<td>7</td>
<td>82M:107F</td>
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<td>17.8</td>
<td>40 ± 19</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
<td>835M:674F</td>
<td>102</td>
<td>15.1</td>
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<tr>
<td>CqH</td>
<td>8</td>
<td>88M:130F</td>
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<td>8.5</td>
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<td>Y</td>
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<tr>
<td></td>
<td>9</td>
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<td>25.8</td>
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<tr>
<td></td>
<td>10</td>
<td>99M:55F</td>
<td>13</td>
<td>23.6</td>
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<tr>
<td></td>
<td>11</td>
<td>139M:52F</td>
<td>11</td>
<td>21</td>
<td>37 ± 21</td>
<td>Y</td>
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<tr>
<td></td>
<td>12</td>
<td>10M:10F</td>
<td>0</td>
<td>0</td>
<td>0 ± 0</td>
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<td>13</td>
<td>30M:20F</td>
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<td>14</td>
<td>10 x 2M:1F</td>
<td>4</td>
<td>40</td>
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<tr>
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<td>Total</td>
<td>433M:343F</td>
<td>68</td>
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<tr>
<td>CqH-A</td>
<td>15</td>
<td>30M:20F</td>
<td>6</td>
<td>30</td>
<td>45 ± 16</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>16 x 2M:1F</td>
<td>6</td>
<td>37.5</td>
<td>42 ± 13</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>10 x 2M:1F</td>
<td>7</td>
<td>70</td>
<td>31 ± 10</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>66M:46F</td>
<td>19</td>
<td>41.3</td>
<td>39 ± 14</td>
<td>Y</td>
</tr>
</tbody>
</table>
Expression of Autogeny in the Stock Population

TABLE 1 shows the expression of autogeny in 14 samples taken from the stock colony. The percent of females able to lay an autogenous egg raft varied from 0 to 60 and the number of eggs in a raft varied from 11 to 76. The females laid between the 5th and 10th days regardless of whether glucose solution was available in the rearing box. The numbers of eggs in the rafts were almost the same for both groups (sugar and no sugar available), however, the percentage of autogeny was somewhat higher in the group provided with glucose.

Expression of Autogeny in the CqH-A Strain

Although the colony was selected for autogeny for 15 generations, no more than 70% of females of the CqH-A strain laid their first egg raft without blood-feeding (TABLE 1, samples 15-17). However, at least 50% of the females of the group that had mice available (TABLE 2, sample 5) ingested blood (for the first gonotrophic cycle) and laid eggs. The relatively small number of females ovipositing autogenously in the strain kept for several generations without a blood meal (TABLE 1, samples 15-17) was somewhat surprising. Because of this, the females that did not oviposit after 15 days were dissected and the ovaries inspected. All of them contained fully developed eggs (Vth stage of Christophers) in the average numbers. This shows that while probably all the females of the autogenous strain are able to develop mature eggs without blood, not all are able to lay egg rafts.

Influence of Mating on Autogeny

Females of both colonies used in the experiment (CqH and CqH-A) were tested for the dependency of autogenous laying on mating. In both colonies, (40 virgin females of the CqH and two times 40 virgin females of CqH-A) were placed into boxes where water for oviposition and glucose solution was available. These replications were finished when the last female died. Only one autogenous egg raft (52 eggs) was recorded in one of the boxes containing the CqH-A females. As a control for the determination of ovarian growth, a further group was initiated; 34 females of CqH-A strain that survived the 12th day of the experiment were dissected. All but two females that were dissected had ovaries with mature eggs (Christophers' stage V). The numbers of mature eggs varied similarly as did the eggs deposited by fertilized females. One female developed only a single ovary, and the second ovary was of 1b stage. One female had undeveloped ovaries (1b of Christophers).
Bloodsucking During the First Gonotrophic Cycle

Newly emerged females were individually placed in two smaller boxes with two newly emerged males and the experiments were replicated five times with various numbers of mosquitoes (73 separately kept CqH strain and 20 CqH-A strain females). The number of females that engorged before the first egg-laying as well as the number of eggs was counted (TABLE 2). Only 15.1% of females of the CqH and 50% of CqH-A strains, respectively, laid autogenously (before the blood feeding) compared with 19.8% and 41.3%, respectively, when females did not have access to blood (TABLE 1). In both cases the percentage of females that laid autogenously was higher in CqH-A strain. The females that blood-fed before their first egg raft did so between the 2nd to 8th days and they laid on the 3rd to 8th day after blood feeding. The egg rafts of these females were considerably larger (TABLE 2). One female in sample 3 (TABLE 2) blood-fed on the third day and laid two batches – the first (38 eggs) on the 6th day and the second (142 eggs) on the 9th day. Because this set of observations finished always when the particular female died, it is obvious that none except for the one female mentioned laid more than one raft. However, the one case with two egg rafts encouraged us to establish an additional test: A mouse was offered daily to a group of freshly emerged adults of the CqH-A strain and 100 of the freshly engorged females were separated into smaller boxes, one female per box. Most of these females (82%) laid eggs. All females that survived to the 8th day after oviposition as well as all the females which did not lay and survived to the 10th day after bloodsucking were dissected. The females that laid eggs never initiated the second gonotrophic cycle, but all the females that did not lay eggs had developed mature ones.

**DISCUSSION**

The expression of autogeny in mosquitoes is controlled genetically (Clements 1992, Spielman 1957), however, this phenotype can be suppressed by various factors (Nasci and Miller 1996). Two types of autogeny are usually mentioned in papers dealing with this phenomenon: obligatory autogeny, which is typical for northern populations of *Wyeomyia smithii*, and so-called facultative autogeny, in which the genetically autogenous female is able, but is not required to ingest blood during the first gonotrophic cycle (O’Meara 1985a, Clements 1992). In the Culex pipiens complex, the form *molestus* is usually noted as an example of obligatory autogeny, but also in this form the obligatory autogeny is dependent on mating with a male of the same subspecific taxon (Olejněček, 1995). Autogeny in *C. p. quinquefasciatus* is very rare and it is not known if it is suppressed by natural climatic conditions or if the autogeny in this subspecies was only overlooked.

Autogeny is influenced by male accessory gland substances (O’Meara and Evans 1977, O’Meara and Petersen 1985). Male accessory gland substances activate egg development also in anautogenous mosquitoes (Klowden and Chambers 1991). In our experimental groups a great part of the CqH and probably all of the CqH-A females were able to mature eggs, while unmated females seldom were able to lay them.

Autogeny in facultatively autogenous females often is influenced by ingestion of carbohydrate (Klowden 1993, Su and Mulla 1997b) The influence of sugar feeding on autogeny varies from negligible to paramount; more often this factor is important when autogeny is facultative (O’Meara 1985b). In our experimental groups glucose ingestion had little effect on autogeny in the facultatively autogenous CqH strain (TABLE 1, samples 1-7 and 8-14).

McGinnis and Brust (1985) mentioned that autogenous females of *Aedes togoi* can feed on blood during the first, autogenous cycle for the second gonotrophic cycle. The case of one female of CqH-a strain that laid two egg rafts after blood-feeding, suggests that something similar can also occur in our population of *C. quinquefasciatus*, but it appears to be very rare.

The question arises why the autogeny was not observed in India, where the CqH strain has been kept in laboratory for a relatively long time. One explanation may be that the change of some life conditions unblocked something that suppressed the display of this phenotype in the country of the origin of the strain. The quality of food can also be of importance, especially because in *Culex* mosquitoes autogeny can be suppressed by food deprivation in the larval stage (Fannig et al. 1992, Su and Mulla 1997a, Kay et al 1986, Tveten and Meola 1988). Autogeny is an important phenomenon which can be exploited in the mosquito control programs (Olejněček and Benedík 1988) and thus it would merit further investigations.

**Acknowledgments**

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Bushrod, F. M. 1978. Autogeny in East African *Culex*
Infection and Seroconversion of Cats Exposed to Cat Fleas 
(Ctenocephalides felis Bouché) Infected with Rickettsia felis

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ABSTRACT: Cats with no prior exposure to cat fleas were exposed to fleas infected with Rickettsia felis and monitored monthly for seroconversion via an indirect florescent assay (IFA). Each of 12 cats continually infested with fleas, seroconverted by four months post-exposure. Three of six cats fed on by 50 fleas for 15 minutes once per week also seroconverted by month four. Rickettsia felis DNA was detected by PCR in the blood of five of the 16 cats.

Keyword Index: Ctenocephalides felis, Rickettsia felis, cats, infection.

INTRODUCTION

Rickettsia felis was discovered in 1990 during studies on potential vectors of Ehrlichia risticii, the causative agent of Potomac horse fever. The bacterium was found in many tissues (gonadal, muscle, and midgut lining) of the cat flea (Ctenocephalides felis Bouché). Until Higgins et al. (1996b) described the organism, R. felis was referred to as the ELB (El Soquel Labs) agent in reference to the colony of cat fleas from which the new rickettsia was first detected (Adams et al. 1990).

Rickettsia felis and Rickettsia typhi both are found in cat fleas in nature, and Adams et al. (1990) indicated that R. felis shares substantial antigenic composition with R. typhi. Higgins et al. (1996b) sequenced and compared PCR products of R. typhi and R. felis and found 32 nucleotide differences between them. Therefore, when differentiation between R. typhi and R. felis is attempted, PCR based analysis is possible (Higgins et al. 1996b). The immunological response of cats exposed to fleas infected with R. felis has not been studied. We report here results of experiments to determine whether cats fed upon by cat fleas infected with R. felis produce antibodies in response to antigens of R. felis.

METHODS AND MATERIALS

Adult fleas were obtained from a colony (Henderson and Foil 1993) at Louisiana State Agricultural Center St. Gabriel Research Station (LSUSG). Fleas were periodically assayed via PCR (Azad et al. 1992) for the presence of R. felis DNA using the 17 kDa primer. Approximately 65% of the fleas were found to be positive for R. felis (Wedincamp 1997). The 17 kDa primers are specific only to the generic level, thus, confirmation that products were of R. felis origin was made by restriction fragment length polymorphism (Azad et al. 1992) and DNA sequencing. The 17 kDa PCR product amplified from the fleas was cloned and sequenced using the Invitrogen T/A cloning kit (San Diego, CA). We followed procedures for this assay described by Higgins et al. (1996a).

Three control cats were maintained in a flea-free environment at the LSU School of Veterinary Medicine. Twelve specific pathogen free [(SPF)(flea naive)] cats were fed upon by cat fleas from the R. felis infected LSUSG colony from August 1996 until January 1997. Six cats, group 1 (A-F), were maintained at the LSUSG. This group was infested with approximately 75 fleas weekly to ensure continuous exposure to flea feeding. The other six cats, group 2 (G-L), were housed at the LSU School of Veterinary Medicine in a flea-free environment. These cats were exposed to 50 fleas from the infected colony once per week for 15 minutes using the chambered flea technique (Thomas et al. 1996). As a qualitative test to insure that fleas were feeding, ten fleas from each group of 50 allowed to feed intermittently on each cat in group 2 were tested for the presence of blood using the Hemoccult® screening test (SmithKline...
Diagnostics, San Jose, CA). Fleas that had not fed on cats were also tested. Four SPF cats, group 3, were maintained at LSUSG and infested with approximately 75 fleas from the infected colony weekly from April 1997 until July 1997.

Blood samples from all cats were taken monthly, and serum was stored at -20°C. An indirect florescent assay was used to detect antibody *R. felis* (Philips et al. 1976). IFA microscope slides with rickettsial (*R. felis* and *R. typhi*) antigens were provided by Suzana Radulovic (University of Maryland, Baltimore, MD). Serial dilutions of serum between at 1:64 and 1:2048 were prepared in phosphate buffered saline (PBS). Serially diluted serum was applied to antigen slide wells and allowed to incubate for one hour at room temperature. The slide was rinsed and air dried. Then a secondary antibody (goat anti-cat) labeled with fluorescein isothiocyanate (FITC) was applied to each well and incubated for one hour. The slides were then viewed at 400 X using a fluorescent microscope and titers of 31:64 were considered positive. Instagene® matrix (Bio-Rad Laboratories, Hercules, CA) was used according to manufacturer’s specifications for preparation of *R. felis* DNA from fresh cat blood samples. Controls of serum from cats fed on by uninfected fleas were also screened.

**RESULTS**

All cats in group 1 tested seropositive for *R. felis* antibody by the fourth month (TABLE 1). Three of the cats in group 2 seroconverted by month four, one of which seroconverted in month two. Three of four cats in group 3 seroconverted by the second month, and the fourth cat seroconverted in the third month (TABLE 2). Control cats tested seronegative throughout the study period.

Restriction fragment length polymorphism (RFLP) analysis of amplified PCR products indicated that they were of *R. felis* origin. DNA sequence data comparisons indicated that the PCR products that were amplified had a 99% homology to *R. felis*.

The blood of three of six cats in group 1 and of two of six cats in group 2 were found to contain *R. felis* DNA by PCR and RFLP after three months of exposure. None of the cats in group 3 were found to be positive via PCR and RFLP during the second study.

Fleas that fed on cats intermittently were screened using the Hemoccult® blood screening test (SmithKline Diagnostics, San Jose, CA) and blood was detected in fleas from all dates except for those which fed on one cat on three dates. Fleas that were not fed on cats were also tested, and there were no false positives.

---

**TABLE 1.** PCR detection of *R. felis* DNA and antibody detection in blood samples obtained from cats exposed to the continuous or intermittent feeding of fleas infected with *R. felis*.

<table>
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<tr>
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<th>2</th>
<th>3</th>
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* indicates PCR positive
+ indicates antibody positive
DISCUSSION

This is the first study to demonstrate that cats exposed to cat fleas infected with *R. felis* develop antibodies against rickettsial antigens. Azad and Traub (1985) demonstrated that rats inoculated with the feces of the rat flea, *Xenopsylla cheopis*, infected with *R. typhi* seroconverted within one month. Seven of the 16 cats developed antibodies to *R. felis* by two months.

All cats continually exposed to the feeding of *R. felis*-infected fleas became seropositive while only three of six cats in the intermittently exposed group seroconverted. Similarly, Azad and Traub (1989) reported that increases in the feeding period of *Xenopsylla cheopis* fleas infected with *R. typhi* corresponded with increases in transmission rates to rats.

Price (1954) demonstrated that physiological changes must take place in *D. andersoni* in order for the rickettsiae to become virulent to guinea pigs. These changes might have been induced by molting hormones, a component of the blood meal, and/or increases in temperature. In our study, allowing groups of newly emerged infected fleas to feed on cats weekly for only 15 min. resulted in three of the six cats developing antibodies to *R. felis*. *Rickettsia felis* DNA also was detected in blood samples from two of these seropositive cats. Since flea feces were not visually detected after the fleas were allowed to feed on the cats, our data may indicate that fleas infected with *R. felis* are capable of orally transmitting *R. felis* to cats. Azad and Traub (1989) reported similar results with the transmission of *R. typhi*. Fleas were capable of transmitting the infection to rats by feeding for 45 min. through bolting cloth. Thus, very few *R. felis*-infected fleas present in an area could have a dramatic effect on the seropositivity of cats in that area.

Although we did not examine salivary glands for the presence of *R. felis*, there are two likely methods for *R. felis* transmission by flea bite to cats. The rickettsiae may have been inoculated into the cats along with salivary secretions or regurgitated from the foregut lumen into the bite wound. Adams et al. (1990) reported *R. felis* in midgut, muscle, ovaries, tracheal matrix, hypodermis, and in the epithelial sheath of the testes, but not in the salivary glands of the fleas.

The PCR analysis data of the cat blood samples did not correlate with the presence of *R. felis* antibodies in serum samples, especially in trial 2 where we detected no *R. felis* DNA in any of the cat blood samples but found all cats to be seropositive for *R. felis* by three months post-exposure. However, correlations were perfect for cat D that had detectable *R. felis* in it’s blood in month 2, after which the rickettsiae were cleared and antibodies were continually present. Three other cats (G, K, and L) also provide strong support for the specificity of the PCR assay as all samples were negative for both *R. felis* DNA and antibodies. Two other cats (A and I) provided a good correlation of PCR and serologic data with *R. felis* DNA present only in month 2 and detectable antibodies in months 4 and 5. The apparent insensitivity of the IFA serologic test in this study would be understandable if the antigens were not specific for *R. felis*. Recognition of the antigens shared by *R. felis* and *R. typhi* would vary among individual cats, and it is very conceivable that the quantity of antibodies produced could remain at the level of the cutoff titer. Under these circumstances, antibodies might seem to disappear (as in cats B and E) or disappear and subsequently reappear (as in cats F and J). These data suggest that cats susceptible to *R. felis* mount an immune response that clears the bloodstream of the infection. Because blood samples were taken monthly, there is the possibility that we failed to sample the period of rickettsiemia in some cats.

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<th>Cat</th>
<th>Months Post Exposure</th>
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*test animal was removed from study due to illness.
In this study, we demonstrated that cats seroconvert when exposed to cat fleas infected with *R. felis*. *Rickettsia felis*-infected fleas are found in nature (Sorvillo et al. 1993). Serum surveys on cats reporting rates of *R. typhi* serosensitivity and conclusions regarding the possible role of cats as emerging reservoirs for *R. typhi* should be closely examined (Sorvillo et al. 1993). Previously, a potential connection of *R. felis* to human disease was reported when an individual was found to be PCR positive for *R. felis* DNA (Schriefer et al. 1994).

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