Population Dynamics of Blood-Fed Female Mosquitoes and Comparative Efficacy of Resting Boxes in Collecting them from the Northwestern Part of Riverside County, California

Tejbir S Sandhu¹, Gregory W Williams¹, Bryan W Haynes¹, Major S Dhillon¹

¹Northwest Mosquito and Vector Control District, 1966 Compton Ave., Corona, California, USA, ²Department of Zoology, Punjabi University, Patiala, Punjab, India

ABSTRACT

Objectives: Testing of blood-fed mosquitoes plays an integral role in arbovirus surveillance and in understanding its interaction mechanisms between host, vector and reservoir. The present study was undertaken to evaluate the efficacy of two different traps (gravid and resting boxes) for collection of blood-fed mosquitoes in the northwestern part of Riverside County.

Materials and Methods: Three trapping sites were selected in the Northwest Mosquito and Vector Control District of Riverside County, California. At each site resting boxes and gravid traps were set; and mosquitoes were collected on a weekly basis between July-December 2009. Mosquitoes were transported over blue ice, identified up to species level on chill table, and classified as male, female and blood-fed females.

Results: During this study period, 3953 mosquitoes (826 blood-fed females) belonging to three different genera and eight species were collected; resting boxes collecting maximum number (seven) of mosquito species. Overall as well as individually in each trap kind, the most abundant mosquito species collected was Cx. quinquefasciatus. The proportion of blood-fed females of the Culex species collected in resting boxes was 28.8 times more, while of blood-fed females of Cx. quinquefasciatus was 32.2 times more than the proportion collected from gravid traps.

Conclusions: Overall, the proportion of blood-fed female mosquitoes collected for each species trapped was highest in resting boxes. Additionally, resting boxes showed the advantage of extremely low running and maintenance cost; generation of no hazardous waste; quick turnaround time in terms of mosquito collection per man-hour spent; and they were less prone to vandalism or thefts.

Key words: Arbovirus surveillance, Blood-fed mosquito, Blood-meal analysis, Resting box

INTRODUCTION

Testing of blood-fed mosquitoes plays an integral role in the surveillance for arboviruses—to understand the interaction mechanisms between host, vector and reservoir, and to identify and evaluate the role of potential bridge vector species in transmission of pathogens of public health importance. Additionally, blood-fed mosquitoes can give us information regarding the feeding preference, seroconversion status of that host, infectivity level of the reservoir host, etc., which immensely helps researchers in understanding the ecology of arboviruses. Sampling a blood-fed mosquito is equivalent to sampling a host-seeking mosquito as well as a wild bird simultaneously.

For the purpose of arbovirus surveillance this fact is very important.

Different types of traps are available in the market for trapping mosquitoes which can be basically grouped into three main categories. EVS traps collect host-seeking mosquitoes but as they have not yet taken blood-meal the majority of them are sterile/non-infective and non-blood-feds. Gravid traps are attractant towards oviposition females after they have digested the blood-meal and are ready to lay eggs. Resting boxes are attractant to mosquitoes after they have taken blood-meal and should be ideal for collection of blood-fed mosquitoes. Surprisingly, review of the literature revealed that on the US east coast, resting boxes were the least successful for disease surveillance purposes because of their low mosquito trap count.[1] The present study was conducted to evaluate the efficacy of resting boxes in comparison with gravid traps for collecting blood-fed female
mosquitoes of different species in the northwestern part of the Riverside County of California.

MATERIALS AND METHODS

Three trapping sites were selected in the Northwest Mosquito and Vector Control District (NWMVCD) of the Riverside County of California based upon their resemblance to semi-urban and rural topographies [Figure 1]. These sites had a mixed resident population of horses, dogs, birds and humans. One resting box measuring H × D × W: 6' × 6' × 4' [Figure 2] was placed at each selected site, in shade under a tree, with its open end facing south. Resting mosquitoes were aspirated once a week with in-house-developed battery-operated mechanical aspirator [Figure 3]. Gravid traps[2] were set in the afternoons and were collected the next morning. Overnight trappings were done on a weekly basis between July-December 2009. To maintain consistency trap deployment and mosquito collection was done by the same individual each time. Mosquitoes were collected the next morning at the same time during cooler hours (before 9 AM); transported to the NWMVCD laboratory over blue ice; identified up to species level on chill table; and classified as male, female and blood-fed females.[3] Blood-fed mosquitoes were stored individually in cryogenic vials at -80°C. The data was stored in Microsoft Excel spread data sheet and statistically analyzed for percentage proportion of species, sex and blood-fed females of each species collected per trap type.

The brief description about the three study sites is as under:

Valley view ave

Located in the city of Norco (Latitude: 33°56′23.7″N, Longitude: 117°32′59.4″W, Altitude: 504 feet, semi-urban type), this site provides indoor and outdoor boarding facility to 55 horses. In addition to horses, there were over 300 chicken roosters (Gallus gallus), 10 goats (Capra hircus), 4 watchdogs (Corgi mixed breed) and 8 human attendants living on the property. Three sides of this ranch have boundaries with neighboring horse-boarding facilities.

Corona ave

Located in the city of Norco (Latitude: 33°54′11.05″N, Longitude: 177°33′12.76″W, Altitude: 533 feet, semi-urban type), this is a horse boarding place with indoor and outdoor riding arenas. Thirty-five horses were living in this place with four dogs (Labrador mix breed) and four humans. The east side of this property has an approach road while the west side of the property has a huge pond filled with waste runway rainwater.

Limonite ave

Located in the city of Mira Loma (Latitude: 33°58′26.28″N, Longitude: 177°29′59.04″W, Altitude: 559 feet, Rural type),
this site has outdoor horse boarding with 40 horses in addition to 5 dogs (3: Pomerians, 2: Labrador mix breed). This site mimics a rural setup with the south end touching the Basin of Santa Ana River and having floodwater wash on the east side.

RESULTS

During this study period (July to December 2009), 3953 mosquitoes (826 blood-fed females) belonging to three different genera and eight species were collected in various traps. Resting boxes (RB) collected maximum variety of different mosquito species (RB: seven, gravid: one, EVS: three species). The most abundant mosquito species collected in each type of trap was *Culex quinquefasciatus* (RB: 70.7% and gravid: 97.4% of their total respective mosquito collection). *Culex quinquefasciatus* is one of the local competent vectors for West Nile virus (WNV) and other arboviruses[4].

During this study period, out of the total 2474 mosquitoes collected from resting boxes, the majority (98.3%) of the females belonged to the *Culex* genus comprising five species (*Cx. quinquefasciatus*, *Cx. tarsalis*, *Cx. stigmatosoma*, *Cx. thriambus*, *Cx. erythrothorax*). A majority (70.7%) of these *Culex* species were females as blood-fed. The proportion of blood-fed females of the *Culex* species collected in resting boxes was 28.8 times more, while of blood-fed females of *Cx. quinquefasciatus* was 32.2 times more than the proportion of *Cx. quinquefasciatus* collected from gravid traps. Although 66.7% of the total *Cx. tarsalis* mosquitoes collected in gravid traps were females, no blood-fed *Cx. tarsalis* female was collected from these traps. Overall, the proportion (63.3%) of the total 1273 female mosquitoes collected of blood-fed female mosquitoes collected was the highest in resting boxes followed by gravid traps with 2.2% of their individual total female count. Species-wise, 63.4% of *Culex*, 50% of *Culiseta* and 61.35% of *Anopheles* females collected in resting boxes were blood-fed while the proportion of blood-fed females of these three genera in gravid traps was very low [Table 1].

| Table 1: Proportion of blood-fed female mosquitoes collected in different traps from different study sites |
|---|---|---|---|---|---|---|---|---|
| Site name | Valley view | | | | | | | |
| Trap type | Box | Gravid | Box | Gravid | Box | Gravid | Box | Gravid |
| Species | % of Bf out of total females of this species | % of Bf out of total females of this species | % of Bf out of total females of this species | % of Bf out of total females of this species | % of Bf out of total females of this species | % of Bf out of total females of this species | % of Bf out of total females of this species | % of Bf out of total females of this species |
| Cx. tarsalis | 50 | 0 | 30.15 | 3.08 | 38.03 | 1.92 | 63.4 | 2.2 |
| Cx. quinquefasciatus | 77 | 2.02 | 34 | 3.12 | 0 | 2.08 | 71.3 | 2.21 |
| Cx. stigmatosoma | 71 | 0 | 40.4 | 0 | 16.6 | 0 | 54.1 | 0 |
| Cx. thriambus | 100 | 0 | 33.3 | 0 | 60 | 0 | 63.6 | 0 |
| Total | 75 | 1.99 | 30.15 | 3.08 | 38.03 | 1.92 | 63.4 | 2.2 |
| Cs. particep | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cs. inorata | 0 | 0 | 50 | 0 | 0 | 0 | 33.3 | 0 |
| Total | 50 | 0 | 50 | 0 | 0 | 0 | 50 | 0 |
| An. hermsi | 0 | 0 | 0 | 0 | 61.3 | 0 | 61.3 | 0 |
| Total | 0 | 0 | 0 | 0 | 61.3 | 0 | 61.3 | 0 |
| Grand total | 75 | 1.99 | 30.15 | 3.08 | 38.03 | 1.92 | 63.4 | 2.2 |

DISCUSSION

Historically speaking, since the invasion of Southern California by WNV in 2003,[5] it has been detected in pools of *Cx. quinquefasciatus*, *Cx. tarsalis*, *Cx. stigmatosoma* and *Cx. erythrothorax* in the northwestern part of Riverside County. In the present study, resting boxes collected blood-fed female mosquitoes of all the above species except *Cx. erythrothorax* which are commonly found in the wetland area containing cattails (*Typha spp.*) and bullrush (*Scirpus californicus*). Although in the present study area all the three collection sites were lacking such ecotone, some host-seeking but non-blood-fed *Cx. erythrothorax* females were collected from our traps. The success of resting boxes in collecting such a huge proportion of blood-fed females was due to their strategic locations i.e. they were strategically placed close to a potential source of blood-meals. After taking blood-meal, it is insect physiology that female mosquitoes try to rest at the next available cool dark place for its digestion and production of eggs. A resting box nearby offered such cool dark shade and care was taken to avoid exposure of resting mosquitoes to sunlight from the setting or rising sun by facing the open face of the box towards the south. Another important aspect for a higher proportion of blood-fed mosquitoes from resting boxes was the aspiration time. Resting boxes were aspirated during early morning cooler hours (not later than 9 AM in the present study area) as with the rising
sun, the temperature also rises, resulting in flying away of blood-fed females. During this study, whenever we were in the neighborhood of resting boxes, they were checked for the presence of mosquitoes during mid-day or afternoon hours. Since resting boxes do not have any trap-door or fan to hold back the mosquitoes, they were either found empty or having very few mosquitoes. So if a researcher did not come to collect the mosquitoes from resting boxes during morning cooler hours, he/she would be disappointed with their collection ability. This factor was very crucial for mosquito collection from these boxes. Thus, resting boxes proved to be a huge success in collection of blood-fed mosquitoes in the northwestern part of Riverside County.

CONCLUSIONS

During this study period mosquitoes belonging to three different genera and eight species were collected in various traps. Resting boxes collected the maximum (seven) variety of different mosquito species. The most abundant mosquito species collected in each type of trap was Cx. quinquefasciatus, which is one of the local competent vectors for WNV and other arboviruses. The proportion of blood-fed females of the Culex species collected in resting boxes was 28.8 times more, while of blood-fed females of Cx. quinquefasciatus was 32.2 times more than the proportion of Cx. quinquefasciatus collected from gravid traps.

Additionally, in comparison to gravid traps, resting boxes require no batteries or dry ice to operate, generate no hazardous waste, require less man-hours per mosquito collection, have extremely low running cost and are not prone to thefts or vandalisms. These advantages make the choice of resting boxes extremely beneficial to researchers and public health agencies who have limited resources, especially in developing/underdeveloped countries where the epidemics of arboviruses, e.g. Dengue, Chickungunya, etc. occur frequently.

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