Original Article

Biting Density and Distribution of *Aedes albopictus* during the September 2014 Outbreak of Dengue Fever in Yoyogi Park and the Vicinity of Tokyo Metropolis, Japan

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SUMMARY: A total of 160 autochthonous dengue cases transmitted by *Aedes albopictus* were reported between August and October of 2014 in Tokyo Metropolis, Japan. *Ae. albopictus* is a medically important vector of dengue virus, which has expanded its geographic distribution in temperate regions. Understanding the distribution and biting density of *Ae. albopictus* during the 2014 dengue outbreak in Tokyo is important to evaluate the epidemic risks of dengue fever in other highly populated cities in Europe and Asia. Of the 160 patients, 134 visited the same park (Yoyogi park) located in central Tokyo. Mosquitoes infected with dengue virus were collected from this park, suggesting that it was the exclusive location for the transmission of dengue. This study aimed to collect referential data to estimate the transmission threshold of dengue virus in terms of biting density of *Ae. albopictus* and determined high transmission risk areas of dengue virus in Yoyogi Park and its vicinity. The overall mean density of biting *Ae. albopictus* (7.13/man/8 min) was sufficiently high for successful transmission of dengue virus, and areas with biting densities higher than the overall mean density were classified as high risk areas for the transmission of dengue virus in Yoyogi Park.

INTRODUCTION

A total of 160 autochthonous dengue cases were reported between August and October of 2014 in Tokyo Metropolis, Japan (1). Local transmission of dengue had been reported during the 1940s in Japan (2), but no autochthonous dengue cases were reported for the next 70 years. However, annual numbers of imported dengue cases have reached 200 in the recent years (3).

The primary vector mosquito of dengue in Japan is *Aedes albopictus* (Skuse), that is distributed widely across the country (4,5). Geographic distribution of *Ae. albopictus* has been expanding in temperate regions since the 1980s (6) and its medical importance as a vector of chikungunya and dengue viruses has subsequently increased in newly established areas (7). In Tokyo, the *Ae. albopictus* population typically shows an annual geometric growth from May to July, peaking in August, and then decreases rapidly from September to November (8). Parks in urban Tokyo provide ideal habitats for *Ae. albopictus* and the average biting density was reported to exceed 10 females/man/5 min during midsummer in a particular park (9,10). Mark-release-recapture studies on dispersal of *Ae. albopictus* indicated the movement of the mosquito depends on vegetation distribution within a forest or residential area where patchy vegetation distribute in open spaces (11,12).

A proper understanding of the distribution and density of biting *Ae. albopictus* in the area where the dengue outbreak case took place in Tokyo in 2014 could be important in evaluating the epidemic risk of the dengue virus in other highly populated cities. Majority of dengue cases confirmed in this outbreak were linked to Yoyogi Park and its vicinity, which is located in a major commercial and business area in Tokyo. Out of the 160 dengue cases reported, 134 individuals visited Yoyogi Park and were bitten by mosquitoes inside the park. The Bureau of Social Welfare and Public Health (BSWPH) of the Tokyo Metropolitan Government collected mosquitoes in Yoyogi Park in September 2014 and dengue virus was detected by PCR assay (13) from some of the female mosquitoes (14). This fact clearly indicates that Yoyogi Park was the central location of dengue transmission in the outbreak in 2014. In vector control of mosquito-borne diseases, the concept of “transmission threshold” (15) or “disease risk threshold” (16), which means a threshold beyond which a vector-borne disease epidemic can arise, is important to evaluate the epidemic risk in a particular area. We studied the distribution and density of biting *Ae. albopictus* in Yoyogi Park and vicinity during the dengue outbreak in 2014 to identify areas with a high risk of mosquito bites. The association between the biting density of *Ae. albopictus* and transmission risk of dengue virus was also investigated by statistically analyzing of the results of dengue virus detection in mosquitoes collected by BSWPH. The transmission threshold of dengue virus in terms of biting density in Yoyogi Park is discussed.
METHODS

Study area: Yoyogi Park (YP: 35°40'19.11"N, 139°41'51.67"E) constitutes a large forest of approximately 130 ha (Fig. 1a) and borders the Meiji-Jingu shrine (MJ: 35°40'34"N, 139°41'57"E) and the National Institution for Youth Education (NYC: 35°40'28.7"N, 139°41'36.4"E). For the present study, the entire area was examined. A large part of Yoyogi Park is covered with deciduous trees, evergreen trees, and shrubs, which provide ideal shaded resting sites for *Ae. albopictus*. People visit Yoyogi Park year-round for sightseeing, jogging, bird watching, and exercise. The MJ shrine is surrounded by a dense forest composed of evergreen broad-leaved trees and it provides a good habitat for a variety of plants and animals (17). The western side of MJ bordering on YP and NYC is covered densely with large trees and bushes, and public entrance is not permitted. The NYC is a compound of educational facilities including conference rooms, meeting rooms, and accommodations, and students staying at NYC commonly visit Yoyogi Park for exercise, training, and recreation.

Another park, Shinjuku Central Park (SCP: 35°41'23.3"N, 139°41'21.8"E), is located about 1 km north of YP and was also examined in the present study due to the report of 2 dengue patients who had visited this park and were bitten by mosquitoes at the end of August 2014. The 8.8 ha SCP is situated in a busy business and administrative area with deciduous trees and shrubs growing around the periphery. Many people pass through this park daily for commuting or leisure.

Mosquito collection: Human bait-sweep net collection of biting mosquitoes was carried out at YP and NYC on September 4, 2014 and at MJ and SCP on September 5, 2014. YP and SCP were divided into 6 blocks and 4 or 5 sites were selected for mosquito collection in each block. MJ and NYC were divided into 7 blocks each. Biting mosquitoes were collected in 6 or 7 sites per block in MJ and 2 sites per block in NYC. The total numbers of mosquito samples were 30, 27, 43, and 14 in YP, SCP, MJ, and NYC, respectively. The location of each collection site is depicted for YP, MJ, and NYC in Fig. 1b. One collector was responsible for mosquito collection in a particular block and the location of the collection sites within the block were decided uniformly. All mosquitoes attracted to the collector were captured by a sweep net (36 cm in diameter) for 8 min. A reliable and faster procedure of mosquito sampling was required in this study to provide information of spatial distribution of biting mosquitoes and to carry out the effective control of dengue transmission as soon as possible. In a previous study, it was found that the arrival patterns of *Ae. albopictus* on a person during a 30 min collection were 56% and 68% of the total females collected during the first 5 and 10 min of the mosquito collection, respectively (18). Thus, a 5–10 min time period for mosquito collection is sufficient to obtain a reliable estimate of biting density of *Ae. albopictus* rather than a 30 min collection. In each collection site, it takes about 5 to 7 min to transfer the collected mosquitoes to
a small carrying cage by a vacuum tube with a label distinguishing the collection site and move to the next collection site. Therefore, we fixed the time period of mosquito collection to 8 min in this study and finished the mosquito treatment and movement to the next site within 7 min. In this way, 4 collection sites were examined within 1 h by 1 person. The collected mosquitoes were brought to the National Institute of Infectious Diseases, where they were counted by species and sex. A mosquito repellent (DEET 12%) was used to protect the collectors from mosquito bites.

Data analysis: The mean numbers of *Ae. albopictus* biting per collection site were calculated and compared between YP, NYC, MJ, and SCP using ANOVA. To show places with a high risk of mosquito bites, the mean number of biting *Ae. albopictus* was calculated for the entire Yoyogi area. Each collection site was classified into the following 5 classes based on the overall mean density and biting density of the site, *N*, and is represented by different colors in Fig. 1b: *N* = 0 (white), 0 < *N* ≤ mean (light blue), mean < *N* ≤ 2·mean (blue), 2·mean < *N* ≤ 3·mean (yellow), and 3·mean < *N* (red).

The results of mosquito collection and dengue virus detection conducted in YP by BSWPH on September 4, 11, and 18, 2014 were analyzed to examine the relationship between mosquito density and proportion of infected mosquitoes. The mosquito collection was carried out using CDC traps enhanced with 1 kg of dry ice with 10, 20, or 20 traps distributed in YP on September 4, 11, or 18, respectively. Since mosquito density varied between dates, mean mosquito density was calculated for each collection day, and mosquito samples were classified into 2 density classes: lower or higher than the mean density of the collection day. The data collected from the 3 collection days were combined and the total number of mosquito samples with or without dengue virus was counted for the 2 density classes. The proportion of infected mosquito samples was calculated for each density class and the significance of the difference was tested by Fisher’s exact test.

### RESULTS AND DISCUSSION

Frequency distribution, mean, and standard deviation (SD) of biting densities of *Ae. albopictus* observed in the 4 study sites are summarized in Table 1. The mean biting density in MJ, NYC, YP, and SCP was 5.51, 5.50, 10.20, and 11.43/\text{man/8 min}, respectively, and the differences were not statistically significant (*P* = 0.092). Overall mean and SD of biting density for the entire Yoyogi area was 7.13 and 11.80, respectively. Each collection site in the Yoyogi area was grouped into 5 classes based on the overall biting density mean and the results are represented by different colors in Fig. 1b. The sites with no biting mosquitoes (white color) and those with lower biting density than the overall mean density (light blue color) are distributed fairly uniformly throughout the entire study area. On the other hand, sites with a higher biting density than the overall mean density (blue, yellow, and red colors) are distributed along the borders between YP, NYC, and MJ. The clustered distribution of biting *Ae. albopictus* may be explained by the vegetation-dependent movement of the females. The association between vegetation and the distribution of *Ae. albopictus* has been reported in many studies (19–25). However, most of the previous studies examined the spatial distribution of *Ae. albopictus* eggs using ovitraps, and studies focusing on the spatial distribution of *Ae. albopictus* biting are limited. Experimental studies conducted in Japan on the vegetation-dependent movement of individually marked *Ae. albopictus* demonstrated that resting/searching *Ae. albopictus* females tend to stay and accumulate in particular sites during directional movement through the surrounding habitat (11,12). The size of the forest in the entire Yoyogi area is large (the distance from the fringe to the center of the forest is approximately 500 m to 700 m) while the dispersal ability of biting *Ae. albopictus* was observed to be 67 m to 104 m per day (20,25). Thus, females may be able to reach the center of the forest within 5 to 10 days with the chance to encounter suitable resting/searching sites during their movements.

The relationship between mosquito density and proportion of mosquito samples with infected mosquitoes is shown in Table 2. Infection rates of the mosquito samples were 12.5% and 38.9% for the lower and higher than mean density classes, respectively, and the difference was statistically significant (Fisher’s exact test, *P* = 0.041). Therefore, it is suggested that the probability of receiving an infectious bite was 3.11 (0.389/0.125) times higher in places with higher than mean mosquito density in YP. Theoretically there is a high probability of detecting an infected mosquito in a high density area (26), and a similar correlation between density and infection rate of mosquitoes collected by the
human bait-sweep net method is also expected. Since 3 areas, A, B, and C (Fig. 1b), where biting densities were higher than the overall mean, were considered as high risk areas for the transmission of dengue virus, we applied adulticide in these areas 1 day after our vector survey. We also recommended an extensive adulticide application, which covers a wider area than the high transmission risk areas, but the actual coverage of adulticide application was decided on by the responsible office while taking into account the adverse effects of adulticide on the environment.

A transmission threshold, beyond which a vector-borne disease epidemic may take place, is an important parameter to understand the prevalence of vector-borne diseases and evaluate the epidemic risk of a disease in a particular area. The transmission threshold of dengue virus has been estimated for *Ae. aegypti* by using a simulation model (15); however, no similar attempts have been made for *Ae. albopictus*. The present study aimed to collect referential data to estimate the transmission threshold of dengue virus in terms of biting density of *Ae. albopictus*. The epidemic curve of the dengue outbreak in YP shows that new human infections were continuing to occur while our mosquito survey was being carried out (27). The epidemiological situation in SCP was nearly identical to that in YP, 2 and 9 dengue cases were reported before and after the mosquito collection of this study, respectively. Thus, the observed density of *Ae. albopictus* was sufficiently high to circulate the dengue virus successfully. In these cases, the observed value of the mean biting densities was 7.13/man/8 min in YP and 11.43/man/8 min in SCP. However, the actual transmission thresholds of dengue virus in the 2 parks should be lower than those values. The ordinary vector control that keeps a lower biting density of *Ae. albopictus* than the observed mean biting density will be required to reduce the risk of an dengue fever outbreak in Japan.

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**Conflict of interest** None to declare.

**REFERENCES**


