Dengue Fever Vector Composition and Pesticide Residues in Yiwu, Zhejiang Province, China[1]

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Abstract In 2009, an outbreak of dengue fever occurred in Yiwu, the major international market in Zhejiang province, China. A mosquito vector eradication program was undertaken using several insecticides to limit further transmission of the dengue virus. At the conclusion of this effort, the affected area was surveyed for dengue fever vector species, and mosquito-breeding area water samples were collected and analyzed for residual pesticide content. The survey detected the Asian tiger mosquito, *Aedes albopictus* (Skuse), in 19 villages in Yiwu, accounting for 51.7% of the mosquitoes in the 89 samples collected. Other species collected included *Armigeres subalbatus* (Coquillett), *Culex pipiens quinquefasciatus* Say, *Cx. tritaeniorhynchus* Giles, *Cx. mimulus* Edwards, and *Anopheles sinensis* Wiedemann. Analysis of water from 45 mosquito-breeding sites using gas chromatography-mass spectrometry detected a total of 7 pesticides, including the herbicide prometryn, 2 organophosphate insecticides (chlorpyrifos and triazophos), and 3 pyrethroid insecticides (cyhalothrin, cypermethrin, and permethrin). The maximum level of permethrin detected was 323.9 µg/L in breeding waters in which *Ae. albopictus* and *Ar. subalbatus* coexisted.

Key words dengue, mosquito, vectors, pesticide residue, water analysis

Dengue fever is one of the most important and escalating health problems in the world (World Health Organization 2009). Over the past few years, some dengue cases were reported in Zhejiang province, China (e.g., Cixi in 2004) (Yang et al. 2009). In 2009, an outbreak of dengue fever reportedly affected more than 190 individuals in Yiting Town, southwest of Yiwu City, a major international commodities market in China, located in the central area of Zhejiang. The local government allocated ¥70 million Chinese RMB directed to the management of dengue fever mosquito vectors, primarily using insecticides. Approximately 20 pest control operations (PCOs) teamed together to implement the mosquito eradication plan using organophosphates, pyrethroids, and pyrethrins targeted at adult and larval mosquitoes. However, concerns have been voiced about the threat to human and environmental health over the indiscriminant use of such pesticides that may contaminate water, food, plants, and animals (Crinnion 2009, Tulve et al. 2010, van Dyk et al. 2010). Furthermore, the continuous use of mosquito repellents and their subsequent deposition in the

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environment may influence the health of the users, especially children, through skin adsorption, inhalation, or indirect ingestion (Ramesh and Vijayalakshmi 2002). Ostrea et al. (2008) even discovered propoxur in meconium and cord blood and chlorpyrifos in the hair of infants.

This study was undertaken to (1) assess the impact of the intensive eradication program on the occurrence of the mosquito vectors of dengue fever including the Asian tiger mosquito, *Aedes albopictus* (Skuse), and (2) identify and measure pesticide residues in mosquito-breeding waters in the targeted area. Ultimately, these results may assist in modifying application rates, schedules and targeted areas to further minimize risks associated with dengue fever outbreaks and pesticide exposure.

Materials and Methods

Mosquito survey. Mosquito larvae were initially collected from randomly selected breeding sites in Qingsu and Fantianzhu in Zhejiang province. The survey was expanded to 6 adjacent towns and 7 residential districts (29°18′N; 120°04′E) from 2009 - 2010. The location coordinates of each sample site were determined using a hand-held MobileMapper 6.0 GPS (Magellan, The Philippines). Elevation was measured using a 690 Altimeter-Barometer (Ever-Trust, Japan). Larvae and pupae were collected with an 800-mL dipper or pipetted by washing with a 30-mL aspirator in smaller samples of water. The volume of the water sample was at least 50 mL, and any extraneous debris collected in the sample was filtered with a 40-mesh standard copper sieve (0.45 μm, ø = 10 cm). The filtrate was then poured into a 250-mL transparent plastic bottle which was labeled and eventually transported to the laboratory.

Larvae and pupae returned to the laboratory were reared to adults in a BoXun artificial climate incubator SPX-300IC (Sanyo, China) maintained at 25°C ± 1°C, 50 - 70% RH, and a light and dark (L/D) cycle of 12:12 h. At regular intervals, adult mosquitoes were collected using small nylon reticules. Species were determined with the aid of an EZ4D dissecting microscope (Leica, China), and the male–female ratio was determined for each species.

Pesticide residue analysis. Pesticide residues in the mosquito-breeding waters were identified and measured using a gas chromatography-mass spectrometry (GCMS-QP2010 Plus; Shimadzu, Japan) operated in electron ionization mode. Gas chromatography-mass spectrometry has been widely used in pesticide monitoring in complex environmental samples because of its high sensitivity and specificity (Konda and Pásztor 2001), Barr and Needham 2002, Gonçalves and Alpendurada 2004, Amoo et al. 2008, Yoshida 2009, Feo et al. 2010). Basically, a 1.0-μL aliquot of the water sample was injected in splitless mode at 230°C. The separation was performed on a chromatographic column (30 m × 0.25 mm i.d. × 0.25 μm df, DB-5ms, J&W). The GC oven temperature was set to 70°C for 2 min, which was increased to 280°C at a rate of 10°C/min for 15 min. Helium (99.999%) was used as the carrier gas in the collision cell; the column sampling inlet pressure was 60 psi in constant voltage mode. The GC/MS ion source was set to 200°C, the transmission-line temperature to 270°C, and the electron energy to 70 eV. The range of mass from 60 - 500 amu was fully scanned, and the solvent delay time was held for 3.5 min. The quadrupole mass spectrometer was auto-tuned and calibrated using perflurotributylamine before the start of each sample sequence. Data acquisition used multiple reaction monitoring according to standard reference (Trenholm et al. 2008, Yoshida 2009, Rashid et al. 2010). More than 100 pesticides, including organophosphates, organochlorines, pyrethroids, pyrethrins, carbamates, and herbicides, based on agrochemicals information used in the local market, were mixed and applied as the standard tracer liquid during the residue analysis of mosquito-breeding water.

Results

Mosquito survey. *Aedes albopictus*, *Armigeres subalbatus* (Coquillett), *Culex pipiens quinquefasciatus* Say, *Cx. tritaeniorhynchus* Giles, *Cx. mimulus* Edwards, *Cx. fuscanus* Wiedemann, and *Anopheles sinensis* Wiedemann were identified from the 89 samples collected throughout the region (Fig. 1). *Aedes albopictus* was the most abundant species and most widely distributed of these species with specimens recovered in Chi'an, Dachen, Futang, Shangxi, Suxi, and Yiting towns, as well as in Beiyuan, Choujiang, Houzhai, and Jiangdong residential districts (Fig. 1). It accounted for 51.7% of the total mosquitoes collected in the 89 samples. Of those mosquitoes reared to adulthood, females outnumbered males 2.2 - 1.

Culex tritaeniorhynchus ranked second in terms of abundance and was recovered from Fantianzhu, Jiting, Laishan, Lizhao, Maohou, Qingsu, and Xiazhai districts (Fig. 1). Only 28 individuals emerged as adults with a 13:1 female-to-male ratio. *Culex pipiens quinquefasciatus* was recovered from Hesilu, Jiting, and Xi'an villages. Fewer females than males were observed. *Culex mimulus* and *Cx. fuscanus* were limited to Lizhao and Hesilu, with *Cx. mimulus* more abundant than the latter. Very few *Ar. subalbatus*

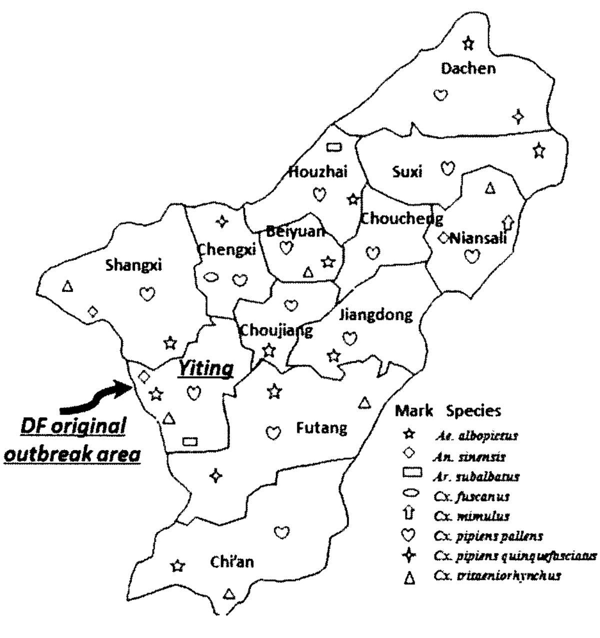

Fig. 1. Primary distribution area of mosquito species in the Yiwu region (the source of *Cx. pipiens pallens* were collected by light traps).

were found in Fantianzhu and Siqian villages, similar to the *An. sinensis* collected from Fantianzhu, Lizhao, and Xiazhai (Fig. 1).

Pesticide residues. Seven pesticides were detected among the 45 breeding-site water samples collected in 2010. These included the herbicide prometryn, 2 organophosphate insecticides (chlorpyrifos and triazophos), and 3 pyrethroid insecticides (cyhalothrin, cypermethrin, and permethrin) (Table 1). The herbicide was the most prevalent pesticide identified in the samples, being recovered from 4 of the 45 sites. Amounts ranged from 0.035 - 6.300 μg/L in these samples with the higher level detected in the Hangci river in Qingsu. Water from a sugarcane field in Fantianzhu yielded levels of prometryn (0.423 μ/L), chlorpyrifos (2.070 μg/L), and triazophos (0.032 μg/L). Jiting pond in Futang yielded 18.50 μg/L of cyhalothrin and 28.20 μg/L of cypermethrin. Triazophos (7.00 μg/L) also was found in a sample from Tongchun, Suxi, whereas permethrin (323.9 μg/L) was detected in water from a paint kettle in Beiyuan.

Discussion

A primary vector of dengue fever, *Ae. albopictus*, remains prevalent in the area. It was the most frequently encountered mosquito species, being recovered from 19 villages and residential districts. Females of this species readily transmit the dengue fever virus whereas feeding on blood meals (Reiter 2007). Furthermore, we observed that the females outnumbered the males in the Yiwu region. To some extent, the reproductive space and, thus, probability of survival of *Ae. albopictus* could have been enhanced by the practice of applying dry lime on the ground and to pond surfaces to slow the transmission of dengue fever (Gibbons and Vaughn 2002, Benedict et al. 2007).

Seven pesticides were simultaneously detected in nonpotable water samples obtained from drainage areas, ditches, rivers, etc. However, these same pesticides were not found in wine jars, drinking water vats, and potable water sources. High levels were detected in smaller water containers that were likely targeted by pest control operation staff and villagers; some could have been treated on multiple occasions. This unavoidable mistake could have adverse effects on human health (Beard 2006, Crinnion 2009, van Dyk et al. 2010).

Agrochemicals are being used in increasing amounts for agricultural production and for public health and are, therefore, potential environmental contaminants in China (Wang et al. 2007, Liu et al. 2009). Mosquito adulticides and larvicides should be applied as a last resort and should be coupled with other management tactics including environmental management. This might achieve a favorable balance of risk versus benefit in the midst of mosquito-borne disease control (Kolaczinski and Curtis 2004, Rogan and Chen 2005). The monitoring of biological systems for exposure to pesticides (Barr and Needham 2002, Kolaczinski and Curtis 2004, Akogbéto et al. 2006, Arora et al. 2008, Pinheiro and de Andrade 2009) and performing ecological risk assessments for insecticides used in integrated mosquito management is necessary (Davis et al. 2007, Green et al. 2009).

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Table 1. Pesticide residue detection results in water-storage containers of mosquito by GC/MS (μg/L).

No.	Location	Mosquito species	Habit	Cyhalothrin	Cypermethrin	Permethrin	Chlorpyrifos	Triazophos	Prometryn
5	Xiazhai Health Center, Shangxi	*Anopheles* spp.+ *Culex* spp.	drainage ditch						0.035
6	Daxi River of Xiazhai, Shangxi	*An. sinensis*	collection sump						0.038
8	Hangci River, Qingsu	*Cx. tritaeniorhynchus*	river						6.300
9	Sugarcane field, Fantianzhu	*An. sinensis* + *Cx. tritaeniorhynchus*	feed ditch				2.070	0.032	0.423
16	Jingju Temple, Beiyuan	*Ae. albopictus* + *Ar. subalbatus*	paint kettle			323.9			
20	Pond of Jiting, Futang	*Cx. pipiens quinquefasciatus*	accumulator box	18.50	28.20				
31	177# Tongchun, Suxi	*Culex* spp.	urine cannikin					7.000	

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