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Entomological Investigation and Control of a Chikungunya Cluster in Singapore

Cheong Huat Tan,[1] Pei Sze Jeslyn Wong,[1] Mei Zhi Irene Li,[1] Siok Yin Sharon Tan,[1] Tze Kwang Caleb Lee,[1] Sook Cheng Pang,[1] Sai Gek Lam-Phua,[1] Nasir Maideen,[2] Ah Bah Png,[1] Sin Ying Koou,[1] Deng Lu,[1] and Lee-Ching Ng[1]

Abstract

In August 2008, a team from the National Environmental Agency conducted an entomological investigation of a chikungunya cluster in Singapore, with the primary aim of identifying the vector responsible for the outbreak and to assess the vector control operation. A total of 173 adult mosquitoes were caught using both the sweep-net method and the BG Sentinel Traps in and around the affected workers' quarters. Of these, 120 (69.4%) were *Aedes albopictus* and the rest were *Culex quinquefasciatus*. More than 2700 *Ae. albopictus* larvae were also collected from 33 breeding habitats detected. No *Aedes aegypti* was found. During the preintervention period, 6 (8.4%) out of 71 adult female *Ae. albopictus* were found positive for the chikungunya virus (CHIKV). Vector control measures resulted in a 90% reduction of adult *Ae. albopictus* caught by BG Sentinel Traps. Postintervention surveillance revealed the presence of CHIKV-positive mosquitoes. These findings led to continued intensive vector control operation in the affected area that further reduced vector population and interrupted the transmission of the disease. The E1 gene sequence of the CHIKV was identical to those of CHIKV isolated from human chikungunya cases working in the affected area, and contained the A226V mutation. The incrimination of *Ae. albopictus* as a major vector involved in the transmission of A226V CHIKV had led to the revision of chikungunya control strategy in Singapore. This study suggests the benefit of a vector control program that includes the evaluation of control measures in conjunction to virological surveillance in vector population.

Key Words: *Aedes albopictus*—Chikungunya—Pirimiphos-methyl—Singapore—Vector control.

Introduction

Chikungunya fever is a mosquito-borne disease caused by an *Alphavirus* belonging to the Family Togaviridae. In Africa, though the virus had been transmitted mainly by the sylvatic mosquitoes, *Aedes africanus*, *Aedes furcifer*, *Aedes taylori*, *Aedes leutocephalus*, and *Aedes cordellieri*, involvement of *Aedes aegypti* and *Aedes albopictus* in recent years have been reported (Diallo et al. 1999, Pastorino et al. 2004, Peyrefitte et al. 2007, Sang et al. 2008). In contrast, outbreaks of chikungunya virus (CHIKV) in Asia have mainly been associated with urban *Ae. aegypti* and more recently *Ae. albopictus* (Powers and Logue 2007, Ng et al. 2009b). CHIKV was first isolated in 1953 in the former Tanganyika (present day Tanzania) (Robinson 1955). Between 1960 and 2000, transmissions were evident in numerous countries in Western, Central, and Southern Africa. In the same period, sporadic localized outbreaks have also been reported in South Asia (India, Sri Lanka, and Pakistan) and South East Asia (Malaysia, Philippines, Indonesia, Cambodia, Vietnam, Myanmar, and Thailand) (Halstead 2007, Powers and Logue 2007, Ng et al. 2009a).

Chikungunya had largely been a neglected disease until an unprecedented outbreak occurred in the Indian Ocean Islands in early 2005, when several hundred thousands of cases were reported from Comoros, Reunion, Seychelles, Mauritius, and Mayotte (Chastel 2005, Josseran et al. 2006, Renault et al. 2007, Beesoon et al. 2008, Kariuki Njenga et al. 2008, Sang et al. 2008, Sissoko et al. 2010). Since then, the spread of the virus has caused fresh outbreaks in Asia. India saw an epidemic wave that swept throughout the country, with >1 million cases reported (Yergolkar et al. 2006, Kumar et al. 2008). Chikungunya epidemics were also reported in Sri Lanka, Maldives, Malaysia, and Thailand (Noridah et al. 2007, Sam et al.

[1]Environmental Health Institute, National Environmental Agency, Singapore, Singapore.
[2]South West Regional Office, Environmental Health Department, National Environmental Agency, Singapore, Singapore.

2009, Hapuarachchi et al. 2010). In 2007, >200 cases of chikungunya were reported in Italy, indicating that the disease was not restricted to developing tropical countries (Rezza et al. 2007).

The first documented emergence of chikungunya in Singapore in January 2008 was limited to a cluster of 13 reported cases, which was successfully contained by combining aggressive vector control operations with active case detection and isolation of patients (Ministry of Health 2008, Leo et al. 2009, Ng et al. 2009b). The virus belonged to the East, Central, and South African (ECSA) genotype and adult vector surveillance conducted in the cluster area only yielded *Ae. aegypti.* After 3 months of quiescence, local outbreaks of chikungunya resurfaced in May 2008 and the circulating virus was found to be the A226V variant of the ECSA genotype.

In August 2008, a team comprising field and laboratory personnel from the National Environmental Agency conducted an entomological investigation and control of a chikungunya cluster in an industrial area of Singapore. This study suggests the importance of integrating field and laboratory tools in the control, and the value of evaluation of control operations in the management of a chikungunya cluster.

Methods

Study site and chikungunya cases

Twenty-six cases of chikungunya were reportedly linked to a concrete slabs factory with 70 workers and scores of people visiting the factory each day (Fig. 1). The premises of the factory, which included a workers' dormitory, was located in Kranji (1°25′30″N, 103°45′43″E), an industrial estate in the rural part of northwest Singapore. The vegetation (grass, banana, and papaya plants) within the 20 ha premises was sparse and mostly found along the perimeter of the premise.

Mosquito collections

Adult mosquito surveillance. Adult mosquito surveillance was first carried out to determine the vector involved in the chikungunya cluster, and subsequently to evaluate the impact of vector control intervention. Due to the urgency in managing the cluster, the preintervention surveillance was carried out within 16 h (14:00 h of day 0 to 06:00 h of day 1) using two methods—sweep-net method for collecting resting mosquitoes and BG Sentinel mosquito trap (BGS Trap) (Biogents GMbH) with BG-Lure™ for host-seeking mosquitoes.

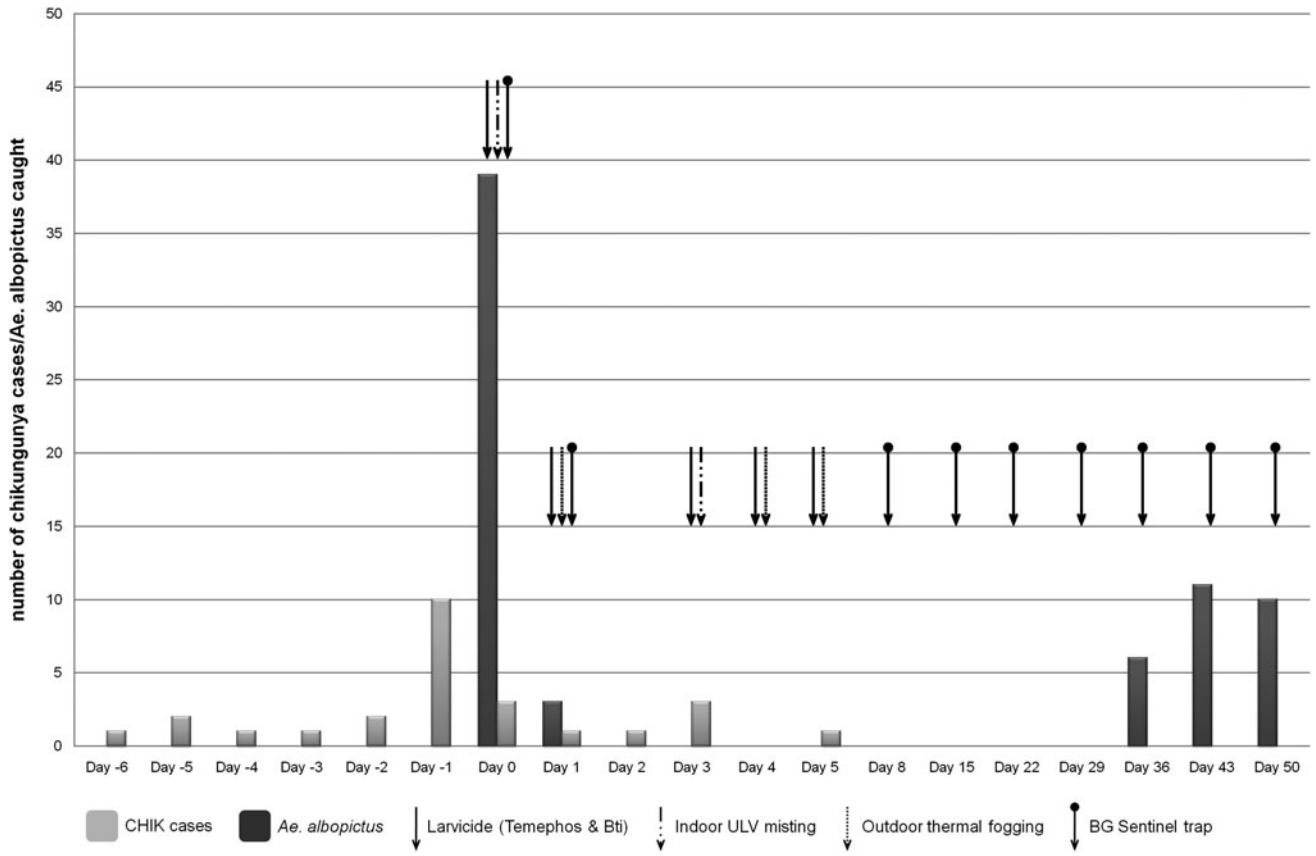

FIG. 1. Number of chikungunya cases, number of *Aedes albopictus* caught with BG Sentinel Traps, and vector control activities at the study site (July–September 2008). In response to a notification of a cluster on day 0, adult mosquito surveillance using sweep net and search and destroy of breeding habitats were concurrently conducted in the afternoon. By 18:00 h of day 0, BG Sentinel Traps were set up and indoor ULV misting was carried out. On day 1, mass outdoor thermal fogging was conducted after removal of the traps. Finding of continued presence of infected mosquitoes in the morning of day 2 triggered more vector control activities on days 3, 4, and 5. Meanwhile, search and destroy of breeding sites continued daily for a month (not shown).

The sweep-net method was performed by 12 officers from 14:00 to 17:00 h (day 0, preintervention), before the start of chemical fogging and misting activities. Nets were moved swiftly over tables, beds, clothing, vegetation, and so on, and were periodically checked for any mosquitoes captured. In areas where the net could not access, the surrounding area was tapped/disturbed to cause resting mosquitoes to escape to the open. Escaping mosquitoes were then caught with the nets. All mosquitoes caught were transferred to screw-cap plastic vials and labeled accordingly. Sweep-net catches were done inside and around the workers' quarters, in an adjacent open shed that houses machineries and in nearby vegetation opposite the workers' quarters. For the collection of host-seeking mosquitoes, four BGS Traps were set outdoor, around the vicinity of the workers' quarters and in the open shed, according to the manufacturer's recommendation, previously described (Maciel-de-Freitas et al. 2006). During the pre-intervention phase (day 0), the four BGS Traps were set at 17:00 h and retrieved at 06:00 h, the following day (day 1). Catch bags were checked for the presence of mosquitoes, and all mosquitoes trapped were transferred by a mechanical aspirating device to screw capped vials. Identification and analysis were performed by the Environmental Health Institute (EHI), a mosquito reference laboratory in Singapore. To determine the effectiveness of the vector control activities, the same number of BGS Traps was placed at the exact location and the area was monitored weekly for 7 weeks (Fig. 1). During this period, the traps were set from 16:00 to 10:00 h the following day.

Adult mosquito processing and identification

Mosquitoes were freeze-killed, at −20°C, sorted according to sex and species, and identified using the taxonomic key of Rueda (2004). All *Aedes* sp. mosquitoes were tested for CHIKV by reverse transcription polymerase chain reaction (RT-PCR) on the day that they were caught. Individual mosquitoes or pools of male mosquitoes were homogenized in 200 μL of MEM with Earle's Salts (PAA Laboratories) supplemented with 1× fungizone. The homogenate was clarified by centrifugation at 14,000 rpm for 10 min, before 140 μL of the supernatant was used for the viral RNA extraction.

CHIKV detection by RT-PCR

The presence of CHIKV in mosquito samples was determined by RT-PCR detection of CHIKV nonstructural protein 1 gene (Ng et al. 2009b). Briefly, CHIKV RNA was extracted from mosquito homogenate by QIAamp viral RNA minikit (Qiagen), and the amplification was performed in a LightCycler 2.0 system by using LightCycler RNA Master SYBR Green Kit I (Roche Diagnostics, GMbH) according to the manufacturer's recommendations.

CHIKV isolation

CHIKV isolation was performed on all samples that were positive by RT-PCR assay. Fifty microliters of each mosquito supernatant was inoculated into a 25-cm^3 flask with 90% confluent monolayer Vero cells. The cells were maintained on M199 medium; supplemented with 2% heat-inactivated fetal bovine serum, 1% HEPES buffer, 1% penicillin, 1% streptomycin, 1% L-glutamine, 1% nonessential amino acids, and 1% sodium pyruvate; and incubated at 37°C in a 5% CO_2 incubator. The supernatant was harvested when 75% of the cells showed cytopathic effects, and stored at −80°C. CHIKV in the cell supernatant was confirmed by the RT-PCR assay mentioned above.

Sequencing of the CHIKV E1 gene

CHIKV E1 cDNA was synthesized directly from the RNA extracted from each or pooled *Ae. albopictus* isolates using the Superscript III First-Strand synthesis system (Invitrogen Corp.) and the PCR products were purified using QIAquick PCR purification kit (Qiagen) following the manufacturer's protocol. Sequencing of CHIKV E1 genes was performed as previously described (Ng et al. 2009b).

Sequence and phylogenetic analyses of the CHIKV E1 gene

Consensus sequences were obtained by assembling a contiguous sequence from raw sequencing data using Seqman software (Lasergene, DNASTAR) and aligned using Clustal W (MegAlign software; Lasergene, DNASTAR). The sequences of CHIKV E1 gene from mosquito samples were compared with those from the local human cases from the premises, and sequences obtained from the GenBank database. The phylogenetic tree was constructed according to the neighbor-joining method (Saito and Nei 1987) using software MEGA version 3. The model used for all analyses was Kimura-2-parameter, including transition and transversion. Sites containing missing data and alignment gaps were removed before the analysis begin. Reliability of the internal nodes of the tree was assessed by the bootstrap method based on 1000 replicates.

Vector control operations

On notification of a cluster of 22 chikungunya cases on 1 August 2010 (day 0), a total of 70 officers from National Environmental Agency were deployed on day 0 (13:00–17:00 h) to conduct search and destroy of mosquito-breeding habitats in the premises of the factory and other surrounding factories. All detected mosquito larvae and/or pupae were collected in screw-cap plastic vials, labeled according to the type of habitat, and sent to EHI for identification. Larviciding using Abate 1% SG (Temephos) was dispensed in all breeding sites found, at 1 g/10 L of water. Bti (Vectobac WG) misting using water-dispersable granules formulation was carried out in areas (500 g/ha) where potential breeding sites can be observed but inaccessible. Overgrown vegetation was also trimmed or removed. On the same day, after the initial adult mosquito surveillance was conducted as described above, indoor ultra low volume (ULV) misting of workers' quarters was carried out using Actellic 50EC (Pirimiphos-methyl) at an application rate of 200 g active ingredient/ha. In addition, extensive outdoor thermal fogging was carried out the next morning at 06:00 h (Fig. 1), with the same insecticide dispensed by 48 portable and one vehicle mounted with thermal fogging machines at an application rate of 100 g a.i./ha. Approximately 180 ha covering the factory premise and eight other industrial premises were targeted.

In response to results from postintervention evaluation, chemical larviciding and adulticiding were repeated on days 3, 4, and 5 (Fig. 1). An average of 24 personnel were also

deployed daily continue the search and destroy operation for a month.

Results

Adult mosquito surveillance

During the study, a total of 173 adult mosquitoes, comprising 120 *Ae. albopictus* and 53 *Culex* sp. (Table 1), were collected around the workers' quarters, adjacent shed, and the nearby vegetation and drains. Before the commencement of vector control activities (day 0), a total of 140 mosquitoes were caught using both the sweep-net method and the BGS Trap. Of these, 64.3% were *Ae. albopictus* and 35.7% were *Culex* sp. (Table 1).

Out of the 90 *Ae. albopictus* caught before intervention, 51 were caught using the sweep-net method and the rest ($n = 39$) were trapped using BGS trap (Table 1 and Fig. 1). Among those caught with sweep net methods, 35 (68.6%) were female *Ae. albopictus*; of these, 21 (60%) were caught outdoor (Table 1). Ten (62.5%) of the male *Ae. albopictus* caught using the sweep-net methods were from outdoor locations. Among the 39 caught with BGS trap, 36 (92.3%) were females. Majority ($n = 26$) of the females were caught near the vicinity of the workers' living quarters, whereas the rest was caught in the open shed. No *Ae. aegypti* was caught throughout the study.

CHIKV detection by RT-PCR and virus isolation

All female *Ae. albopictus* were individually tested for the presence of CHIKV using the real-time RT-PCR assay. For male *Ae. albopictus*, nine were individually assayed, whereas the rest were pooled with two or four mosquitoes per pool. None of the male *Ae. albopictus* was found to be positive for the virus. During the initial preintervention surveillance, six (9.1%) female *Ae. albopictus* were found positive with the CHIKV (Table 1). Three of the positive *Ae. albopictus* were analyzed using whole mosquitoes; we thus could not confirm whether these mosquitoes would have disseminated CHIKV in their body (Table 2). However, the other three positive mosquitoes were found to have disseminated viral infection, as shown by the presence of CHIKV RNA in the head and thorax region (Table 2). Four of the six CHIKV-positive mosquitoes were caught outdoor, whereas two were caught in the living quarters of the workers, including a case's quarter.

Among the CHIKV-disseminated mosquitoes, the viral load was found to be in the range of 50 pfu to 5×10^4 pfu per mosquitoes, determined by PCR using an external standard curve generated by plotting 10-fold serially diluted virus from a concentration of 10^8 pfu/mL. CHIKV from all RT-PCR-positive *Ae. albopictus* were successfully isolated. Together with the larvae surveillance (described below), the results from the adult surveillance strongly suggest that *Ae. albopictus* was the vector responsible for the CHIKV transmission.

Sequence and phylogenetic analyses of the CHIKV E1 *gene*

Sequencing of the *E1* gene was performed on all seven RT-PCR-positive mosquitoes. Phylogenetic inferences (Fig. 2) showed that all CHIKV isolated from *Ae. albopictus* clustered with those sequences obtained from human cases from the most recent outbreak (Ng et al. 2009b), including those isolated from human cases working in the concrete slabs-manufacturing factory. They belonged to the ECSA genotype, and had a valine at residue 226 in the E1 gene.

Search and destroy operations cum larvae surveillance

During the first 4 days of operation (days 0–4, excluding day 2), a total of about 2275 *Ae. albopictus* larvae was collected from 34 breeding habitats detected in the factory premise. The most common breeding habitats were discarded receptacles, canvas/plastic sheets, and domestic containers. A summary of the *Ae. albopictus* larval habitats and average number of

Table 1. The Species, Number of Mosquitoes Collected by Sweep-Net Method and BG Sentinel Mosquito Trap, and the Number of *Aedes albopictus* Found Positive for the Chikungunya Virus

Week of collection	*Method of collection*	*Mosquitoes collected*				*Number of CHIKV-positive* Ae. albopictus
		Aedes albopictus				
		Female (%)	*Males (%)*	*Total*	*Culex* sp.	
Day 0[a]	Sweep-net	35 (68.6)[b]	16 (31.4)[c]	51	30	5
	BGS Trap	36 (92.3)	3 (7.7)	39	20	1
Day 1[d]	BGS Trap	3 (100)	0	3	3	1
Day 8	BGS Trap	0	0	0	0	0
Day 15	BGS Trap	0	0	0	0	0
Day 22	BGS Trap	0	0	0	0	0
Day 29	BGS Trap	0	0	0	0	0
Day 36	BGS Trap	5 (83.3)	1 (16.7)	6	0	0
Day 43	BGS Trap	8 (72.7)	3 (27.3)	11	0	0
Day 50	BGS Trap	10 (100)	0	10	0	0
Total		97	23	120	53	7

[a]Preintervention.
[b]Fourteen (40%) and 21 (60%) were caught indoor and outdoor, respectively.
[c]Four (25%) and 12 (75%) were caught indoor and outdoor, respectively.
[d]Postintervention (initial thermal fogging activity).
BGS, BG Sentinel mosquito Trap; CHIKV, chikungunya virus.

Table 2. The Crossing Point Value: Methods, Place, and Timing of Collection of Chikungunya Virus-Infected *Aedes albopictus* and Parts of the Mosquitoes Used in the Detection and Isolation of Chikungunya Virus

Mosquito	*CP[a] value*	*Method of collection*	*Place caught*	*Pre-/postintervention*	*Parts of mosquitoes used in the detection of CHIKV*
M-Kr15	19	Sweep-net	Outdoor, nearby vegetation	Preintervention	Whole mosquitoes
M-Kr2	21	Sweep-net	Outdoor, living quarters	Preintervention	Whole mosquitoes
M-Kr42	25	Sweep-net	Outdoor, nearby vegetation	Preintervention	Whole mosquitoes
M-Kr58	30.78	Sweep-net	Outdoor, living quarters	Preintervention	Head/thorax only
M-Kr71	21.01	Sweep-net	Indoor, living quarters	Preintervention	Head/thorax only
M-Kr79	19.55	BGS Trap	Indoor, living quarters	Preintervention	Head/thorax only
M-Kr84	30.5	BGS Trap	Outdoor, living quarters	Postintervention	Head/thorax only

[a]Crossing point (CP) (equivalent to Ct value) derived from the Roche Lightcycler. CP represents the cycle number that generates a threshold amount of product, and correlates inversely to the amount of virus in the sample.

immature per habitat found in the study site is listed in Table 3. No *Ae. aegypti* was found.

Assessment of vector control operations

To assess the impact of the vector control measures on *Ae. albopictus* population, the same number of BGS traps were set up at the same locations. Postintervention surveillance using the BG Trap (days 1–2) showed a reduction of 92.3% in the number of female *Ae. albopictus* caught (Table 1 and Fig. 1), demonstrating a significant reduction in the *Ae. albopictus* population. However, RT-PCR revealed that one of the three *Ae. albopictus* caught was infected and had disseminated CHIKV (Tables 2 and 3). Despite a reduction in mosquito population, there was still evidence of infected mosquitoes. The findings led to a follow-up indoor ULV misting and outdoor thermal fogging on days 3, 4, and 5 to further reduce the risk of transmission. Subsequently, adult mosquito

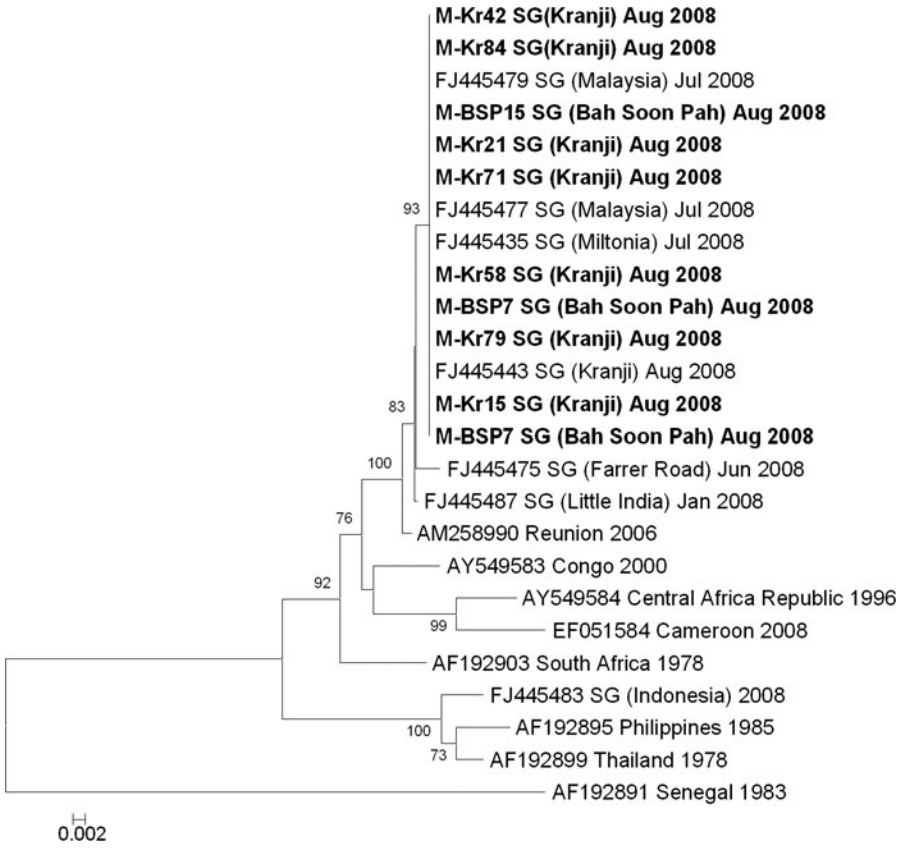

FIG. 2. Phylogenetic tree based on the E1 envelope glycoprotein genes of chikungunya produced by the neighbor-joining method. Sequences highlighted in bold are obtained from *Ae. albopictus* and those underlined are from human cases detected in Singapore. Whenever available, all sequences are labeled with GenBank accession numbers and country of origin and isolated by year/month. In addition, Singapore isolates are labeled with the reported area or country (imported human cases). Figures on the branches are bootstrap percentages based on 1000 replicates and only those above 70% are shown. SG, Singapore.

Table 3. Summary of *Aedes albopictus* Larval Habitats and Average Number of Immatures per Habitat Detected in the Premises of the Concrete Slab Manufacturing Factory During the 3 Days of Search and Destroy Operation

Larval habitat	*Number of habitats found with* Ae. albopictus *larvae*	*Average number of immatures per habitat*
Discarded receptacles	8	43
Canvas/plastic sheets	6	70
Domestic containers	8	93
"U"-channel metal racks	3	83
Discarded boots	2	30
Tires	2	35
Machinery parts	1	30
Concrete slab	1	50
Safety barrier	1	200
Ground puddle	1	50
Coconut husk	1	50

surveillance using the BGS Trap did not yield any adult mosquitoes for 4 weeks (Table 2 and Fig. 1). However, on week 5 (day 36), six adult *Ae. albopictus* were caught and the number of *Ae. albopictus* caught increased in the following weeks (Table 2). The adult mosquito surveillance was discontinued after week 7 (day 50). All *Ae. albopictus* collected from day 36 onward were found to be negative for the CHIKV. In addition, no further cases were notified after day 5 (Fig. 1).

Discussion

The interruption of transmission at the study site showed that the comprehensive combination vector control strategies were effective in reducing the risk of chikungunya. Chemical adulticiding was to effect an immediate sharp reduction of *Ae. albopictus* population; of particular importance was to remove infective vectors. Thorough source reduction and larviciding were to minimize emergence of new mosquitoes that could cause a next wave of transmission through acquiring the virus from viraemic patients in the area. Clearing of unkempt vegetations and general housekeeping aimed to minimize further breeding of vectors. A similar integrated approach has previously been reported to be successful for malaria control in forested areas in Singapore (Lee et al. 2010). Although it was not feasible to quantify the contribution of each component to the reduction in *Aedes* population, it is unlikely that the weekly use of four BGS traps had any trap-out effect. The total number of 70 *Ae. albopictus* adults caught by the traps is small compared to the >2200 larvae found and destroyed (Table 3). It is also supported by other studies on *Ae. albopictus* in Singapore (C.H. Tan, unpublished data) and a previous report on *Ae. aegypti* (Williams et al. 2007), where the population of the *Aedes* mosquitoes were not reduced by weekly and long-term use of the trap.

Although five more cases were detected postintervention from day 2 to 5 (Fig. 1), it is unlikely that the cases were infected postintervention. By day 2, they were likely already in the incubation period, which usually lasts 3–7 days. No further cases were reported from the study site.

Entomological investigation conducted at the concrete slab factory yielded only *Ae. albopictus* and *Culex* sp. mosquitoes. No *Ae. aegypti* larvae or adults were found. The finding of *Ae. albopictus* females disseminated with CHIKV that had the *E1* gene sequence identical to those isolated from the patients from the study area confirmed the vector role of *Ae. albopictus*. It led to the revision of chikungunya control strategy in Singapore (Ng et al. 2009b). As *Ae. albopictus* are generally exophagic and exophilic mosquitoes in contrast to *Ae. aegypti*, we included measures such as outdoor thermal fogging and expansion of source reduction effort to include *Ae. albopictus* habitats, in controlling this and subsequent clusters.

The viruses isolated in this cluster were of the A226V variant of the ECSA genotype. Globally, the A266V mutation has been acquired by CHIKV in at least three independent events (de Lamballerie et al. 2008, Hapuarachchi et al. 2010), in Reunion, India, Cameroon, and possibly Sri Lanka. It has been shown that the single A226V mutation in the CHIKV has increased the susceptibility of *Ae. albopictus* to the CHIKV. This effective partnership between *Ae. albopictus* and the A226V mutant CHIKV conferred a higher vector competence to *Ae. albopictus* when compared to *Ae. aegypti* (Tsetsarkin et al. 2007, Vazeille et al. 2007). Studies have also shown the short extrinsic incubation period of the mutant CHIKV, which can be detected virus in the saliva of *Ae. albopictus* and *Ae. aegypti* 2 days after oral infection (Dubrulle et al. 2009). EHI has also observed that 33.3% of *Ae. aegypti* and 60% of *Ae. albopictus* had disseminated A226V CHIKV in the salivary glands on day 3 postinfectious blood meal (C.H. Tan, unpublished data). Together, these suggested a high epidemic potential of the *Ae. albopictus*–CHIKV–A226V partnership.

The high epidemic potential of CHIKV in *Ae. albopictus* was also reflected in the high infection rate (9.5%) of the female *Ae. albopictus* caught in this study. In another cluster (Bah Soon Pah area), 13% of female *Ae. albopictus* caught were found to have disseminated infection (C.H. Tan, unpublished data).

Like *Ae. aegypti*, *Ae. albopictus* is highly anthropophagic mosquitoes. *Ae. albopictus* has been shown to prefer human as their source of blood meal (Ponlawat and Harrington 2005). The relatively high proportion of *Ae. albopictus* caught in and around living quarters in our study further demonstrated such feeding behavior. Their multiple feeds within a single gonotrophic cycle (Ponlawat and Harrington 2005) coupled with a short extrinsic incubation period for CHIKV makes it a highly efficient vector to transmit the virus. In the Reunion island, 35% of 770,000 residents were infected within 15 months. Similarly, in India, Mayotte, Lamu Island, and Comoros, attack rate ranges from 37.5% to 75% (Ng et al. 2009a).

In Singapore, large clusters of chikungunya attributable to the A226V strain were seen in less urbanized areas of Singapore where *Ae. albopictus* was the predominant species (Ng et al. 2009b). Although sporadic cases of chikungunya fever caused by the mutant strain have also been reported in urban parts of Singapore where *Ae. aegypti* is the predominant mosquitoes, local transmission in these areas was limited. This could be due to *Ae. aegypti* being a less competent vector and/or the aggressive dengue control program, which mainly targets the urban and suburban parts of Singapore where dengue fever is transmitted by *Ae. aegypti*.

The success in interrupting transmission in Kranji demonstrated the benefit of a program that includes good coordination between field and laboratory personnel. The data gathered using field and laboratory tools had assisted in situation assessment on the ground, and in operational decision-making in controlling the spread of chikungunya in

Singapore. First, through the field and laboratory investigation, it became clear that we had to target *Ae. albopictus* that were resting both indoors and outdoors. Second, the post-intervention evaluation that found infected mosquitoes revealed the need for more intervention, without which, we would probably have seen more cases due to the continued presence of infected mosquitoes in the area.

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Authors' Contributions

C.H.T. led the field investigation and the molecular aspect of the laboratory investigation, and prepared the article. P.S.W. and M.Z.I.L. participated in the field and laboratory investigations. S.C.P. and S.G.L.P. were involved in the field investigation and mosquito identification. S.Y.S.T., T.K.C.L., A.B.P., S.Y.K., and D.L. participated in the field investigation. NM coordinated the vector control operations. L.C.N. conceptualized and oversaw the study, and participated in the field investigation and the writing of article.

Disclosure Statement

No competing financial interests exists.

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Address correspondence to:
Lee-Ching Ng
Environmental Health Institute
National Environmental Agency
Singapore 138667
Singapore

E-mail: ng_lee_ching@nea.gov.sg