

SPECIAL FEATURE

INDIGENOUS CASES OF CHIKUNGUNYA FEVER IN SINGAPORE, 2013

Introduction

This special feature describes the 2013 experience of indigenous chikungunya virus infection in Singapore.

Epidemiological findings

A total of 1,059 laboratory-confirmed cases of chikungunya fever were reported in 2013, of which 48 were imported cases, involving 19 Singapore residents and 29 foreigners including work permit holders. The remaining 1,011 cases were indigenous cases, bringing the overall incidence rate of chikungunya fever to 18.7 per 100,000 population. 63 (6.2%) of the 1,011 indigenous chikungunya fever cases in 2013 were detected through active case detection carried out as part of epidemiological investigations.

The first indigenous case of chikungunya fever in 2013 was reported on 8 Feb 2013 involving a

41-year-old male Indian national working and living in Kranji who developed onset of symptoms on 4 Feb 2013. This was followed by the notification of the second case of chikungunya fever on 25 Feb 2013, which involved a 41-year-old Indian national crane operator living and working in Sungei Kadut who developed onset of symptoms on 18 Feb 2013. Cases of chikungunya fever were initially confined to the Kranji/Sungei Kadut area, however, on 27 Mar 2013, the first indigenous case of chikungunya fever outside of Kranji/Sungei Kadut was detected in a 37-year-old British housewife residing in Bukit Timah. Indigenous cases of chikungunya fever were subsequently reported in other parts of Singapore such as Jalan Papan, Jalan Lekar, Seletar West Farmway and Tanjong Kling Road, with the highest concentration of cases in Kranji/Sungei Kadut and Bukit Timah.

Figure 1
Time distribution of 1,011 indigenous chikungunya fever cases in Singapore by location, 2013

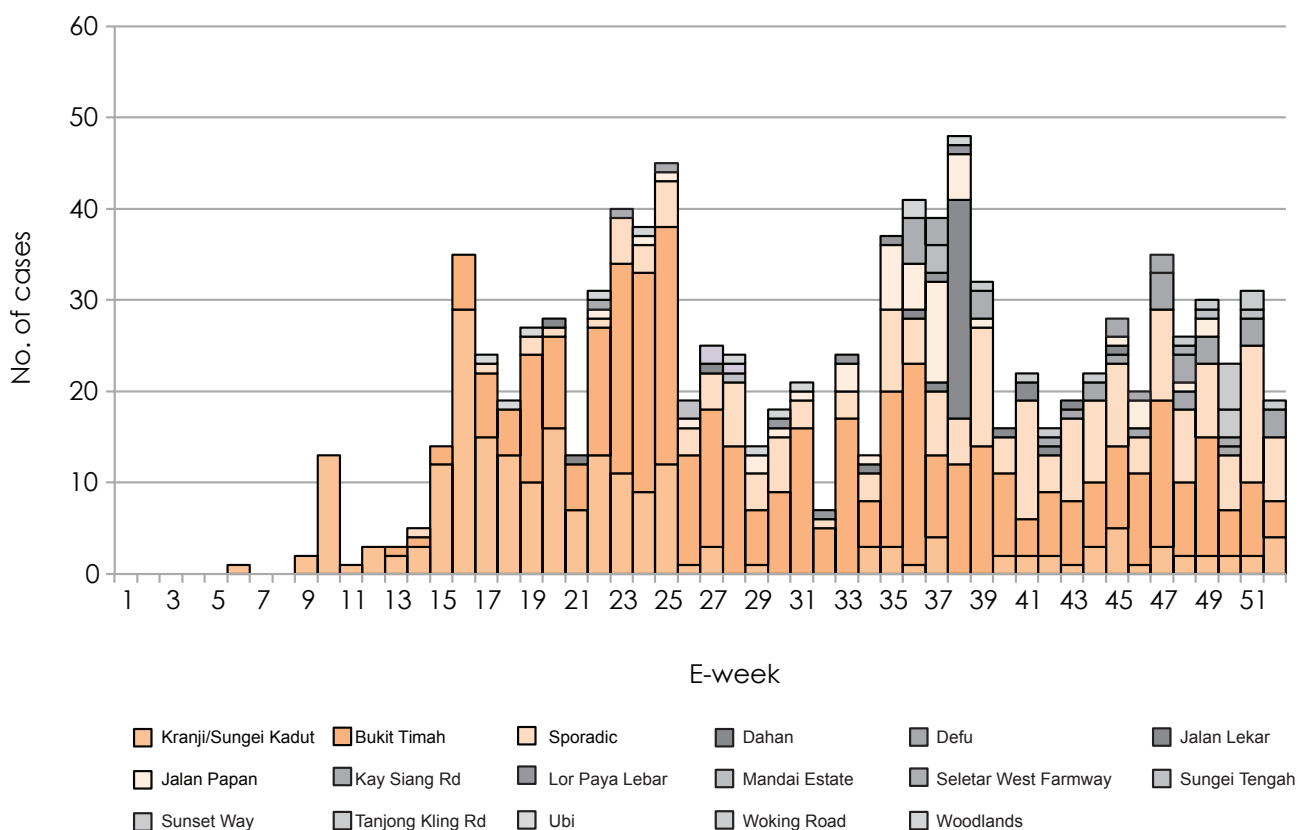


Table 1

Table 1
Distribution of indigenous chikungunya fever cases[^] in Singapore by location, 2013

Area with indigenous cases of chikungunya fever	Number of cases	Percentage of cases
Bukit Timah	418	41.3%
Kranji/Sungei Kadut	219	21.7%
Jalan Papan	48	4.7%
Jalan Lekar	33	3.3%
Defu	21	2.1%
Seletar West Farmway	14	1.4%
Kay Siang Road	11	1.1%
Tanjong Kling Road	10	1.0%
Woodlands	9	0.9%
Sunset Way	7	0.7%
Lor Paya Lebar	6	0.6%
Sungei Tengah	5	0.5%
Dahan Road	3	0.3%
Mandai Estate	3	0.3%
Ubi	3	0.3%
Woking Road	3	0.3%
Sporadic	198	19.6%
Grand Total	1011	100.0%

[^]Cases acquired locally among Singaporeans, permanent and temporary residents.

Of the 1,011 indigenous chikungunya fever cases reported in 2013, 219 (21.7%) were linked through epidemiological investigations to the Kranji/Sungei Kadut area. As part of epidemiological investigations, active case detection was performed and detected 16.4% (36) of the 219 cases linked to the Kranji/Sungei Kadut area. Of the cases detected through active case detection, 11(30.6%) were positive by polymerase chain reaction (PCR) and were viraemic at the point of testing. Majority of the cases in the Kranji/Sungei Kadut area involved males aged between 25 and 34 years of age (41.6%). 85.4% of

cases linked to the Kranji/Sungei Kadut area were foreigners living and/or working in the area.

Cases linked to the Kranji/Sungei Kadut area were reported beginning from E-week 6 and was increasing significantly before peaking in in E-week 16. The number of cases reported then declined sharply for the rest of the year.

Figure 2

Time distribution of 219 indigenous chikungunya fever cases in Kranji/Sungei Kadut, 2013

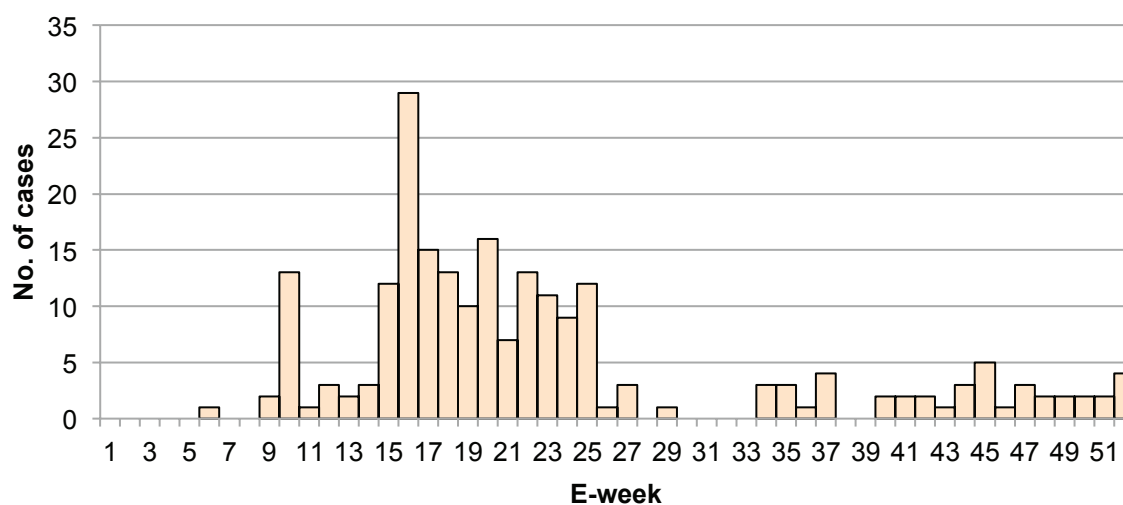


Table 2

Age-gender distribution and age-specific incidence rate of indigenous chikungunya fever cases[^] in Kranji/Sungei Kadut, 2013

Age (Yrs)	Male	Female	Total (%)
0 – 4	0	0	0 (0.0)
5 – 14	0	0	0 (0.0)
15 – 24	21	0	21 (9.6)
25 – 34	91	0	91 (41.6)
35 – 44	2	73	75 (34.2)
45 – 54	19	0	19 (8.7)
55+	13	0	13 (5.9)
Total	146	73	219 (100.0)

[^]Cases acquired locally among Singaporeans, permanent and temporary residents.

Table 3

Ethnic-gender distribution and ethnic-specific incidence rate of indigenous chikungunya fever cases[^] in Kranji/Sungei Kadut, 2013

	Male	Female	Total (%)
Singapore Resident			
Chinese	28	1	29 (13.2)
Malay	1	0	1 (0.5)
Indian	2	0	2 (0.9)
Others	0	0	0 (0.0)
Foreigner	186	1	187 (85.4)
Total	217	2	219 (100.0)

[^]Cases acquired locally among Singaporeans, permanent and temporary residents.

Of the 1,011 indigenous chikungunya fever cases reported in 2013, 418 (41.3%) were linked through epidemiological investigations to the Bukit Timah area, of which 4 (1.0%) were detected through active case detection. None of the cases detected through active case detection were viraemic at the point of testing. Majority of these cases involved were aged between 35 and 34 years of age (41.6%). Residents and foreign domestic helpers constituted 63.2% (264) and 28.2% (118) of the cases reported in Bukit Timah respectively.

Cases linked to Bukit Timah were first reported in E-week 13 and gradually increased. At its peak during E-week 26, 25 cases were reported before the number of cases reported for subsequent weeks was observed to decrease, but was maintained at a higher level than what was seen after the cases linked to Kranji/Sungei Kadut peaked in E-week 16.

Figure 3
Time distribution of 418 indigenous chikungunya fever cases in Bukit Timah, 2013

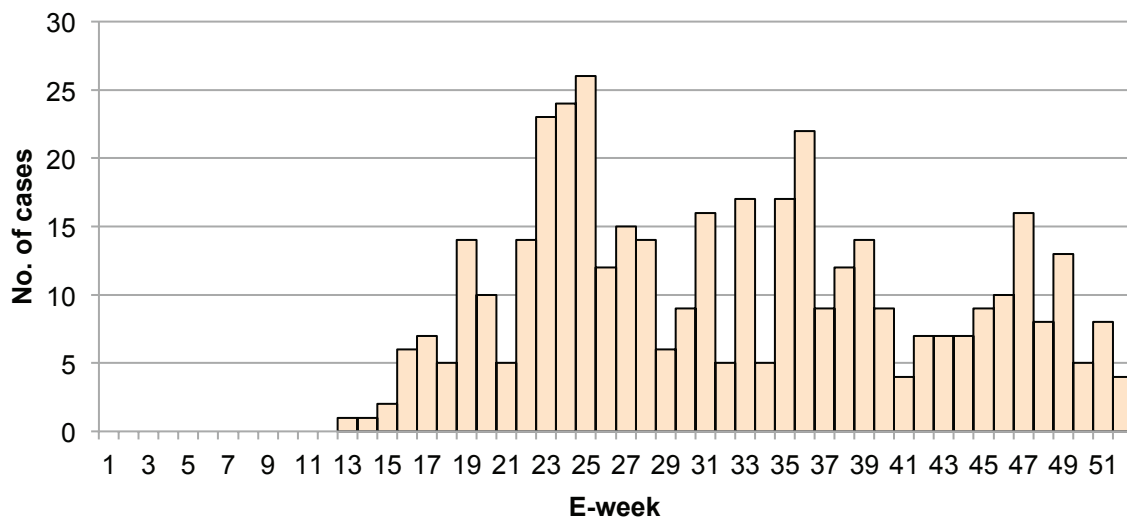


Table 4
Age-gender distribution and age-specific incidence rate of indigenous chikungunya fever cases[^] in Bukit Timah, 2013

Age (Yrs)	Male	Female	Total (%)
0 – 4	2	1	3 (0.7)
5 – 14	15	6	21 (5.0)
15 – 24	16	9	25 (6.0)
25 – 34	31	50	81 (19.4)
35 – 44	36	98	134 (32.0)
45 – 54	22	36	58 (13.9)
55+	47	49	96 (23.0)
Total	169	249	418 (100.0)

[^]Cases acquired locally among Singaporeans, permanent and temporary residents.

Table 5
Ethnic-gender distribution and ethnic-specific incidence rate of indigenous chikungunya fever cases[^] in Bukit Timah, 2013

	Male	Female	Total (%)
Singapore Resident			
Chinese	63	67	130 (31.1)
Malay	7	0	7 (1.7)
Indian	3	2	5 (1.2)
Others	12	11	23 (5.5)
Foreigner	84	169	253 (60.5)
Total	169	249	418 (100.0)

[^]Cases acquired locally among Singaporeans, permanent and temporary residents.

Although most cases were found to be associated with clusters of indigenous chikungunya fever cases, 19.6% of the indigenous cases reported in 2013 were sporadic and could not be linked to any of the other known clusters. The demographics of sporadic cases was also observed to be more diverse than those seen in Kranji/Sungei Kadut and Bukit Timah, and involved more Singapore residents (49.0% compared to 14.6% and 39.5% in Kranji/Sungei Kadut and Bukit Timah respectively).

Sporadic cases were first reported in E-week 14 but was noted to pick up towards E-week 23, gradually increasing and peaking in E-week 51. Despite extensive investigations to elucidate possible links to other known clusters of cases, cases were reported having no recent movement history to other clusters.

Figure 4
Time distribution of 198 sporadic indigenous chikungunya fever cases, 2013

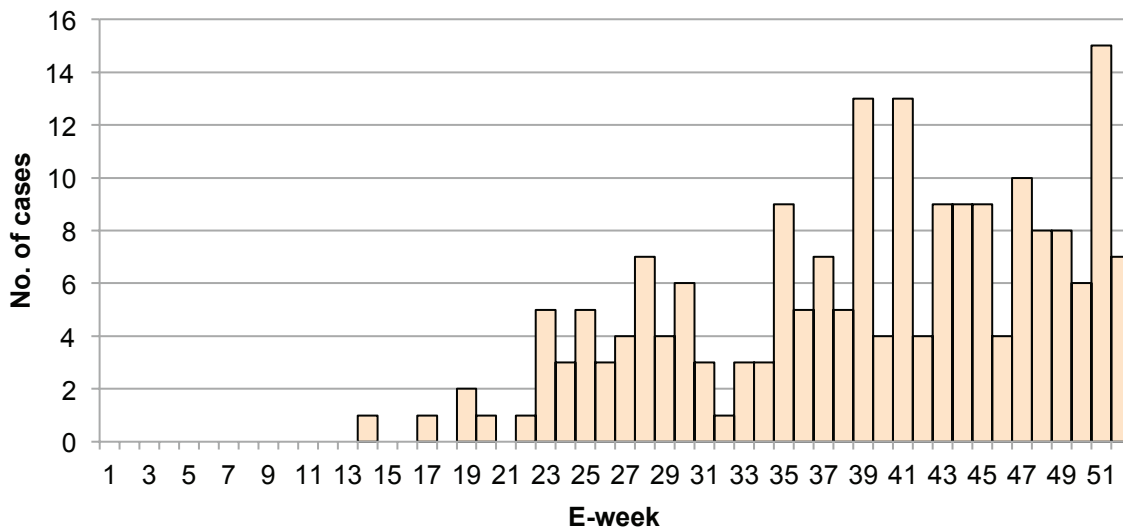


Table 6
Age-gender distribution and age-specific incidence rate of sporadic indigenous chikungunya fever cases[^], 2013

Age (Yrs)	Male	Female	Total (%)
0 – 4	0	1	1 (0.5)
5 – 14	3	2	5 (2.5)
15 – 24	11	3	14 (7.1)
25 – 34	42	12	54 (27.3)
35 – 44	33	9	42 (21.2)
45 – 54	27	12	39 (19.7)
55+	28	15	43 (21.7)
Total	144	54	198 (100.0)

[^]Cases acquired locally among Singaporeans, permanent and temporary residents.

Table 7
Ethnic-gender distribution and ethnic-specific incidence rate of sporadic indigenous chikungunya fever cases[^], 2013

	Male	Female	Total (%)
Singapore Resident			
Chinese	57	22	79 (39.9)
Malay	6	1	7 (3.5)
Indian	2	1	3 (1.5)
Others	3	5	8 (4.1)
Foreigner	76	25	101 (51.0)
Total	144	54	198 (100.0)

[^]Cases acquired locally among Singaporeans, permanent and temporary residents.

Virological and entomological findings

The genetic analysis of envelope 1 (E1) gene of Chikungunya virus (CHIKV) included virus strains obtained from 487 indigenous and eight imported cases. In addition, three virus strains isolated from adult *Aedes albopictus* mosquitoes in two clusters during the early phase of the outbreak were also included in the analysis. Mosquito-derived E1 gene sequences were identical to those of human viruses from the two clusters, indicating the role of *Ae. albopictus* in virus transmission. The same finding confirmed establishment of indigenous transmission of outbreak strains. Furthermore, adult mosquito surveillance in clusters captured only *Ae. albopictus* further strengthening its role in CHIKV transmission during the outbreak.

Phylogenetic analysis revealed that all locally-transmitted CHIKV strains clustered into a monophyletic group within the East, Central and South African (ECSA) genotype. They shared an Indian sub-continent ancestry.

Imported virus strains in 2013 belonged to the ECSA and Asian genotypes. Both groups of viruses did not possess E1-A226V substitution. Imported ECSA strains shared an Indian sub-continent ancestry,

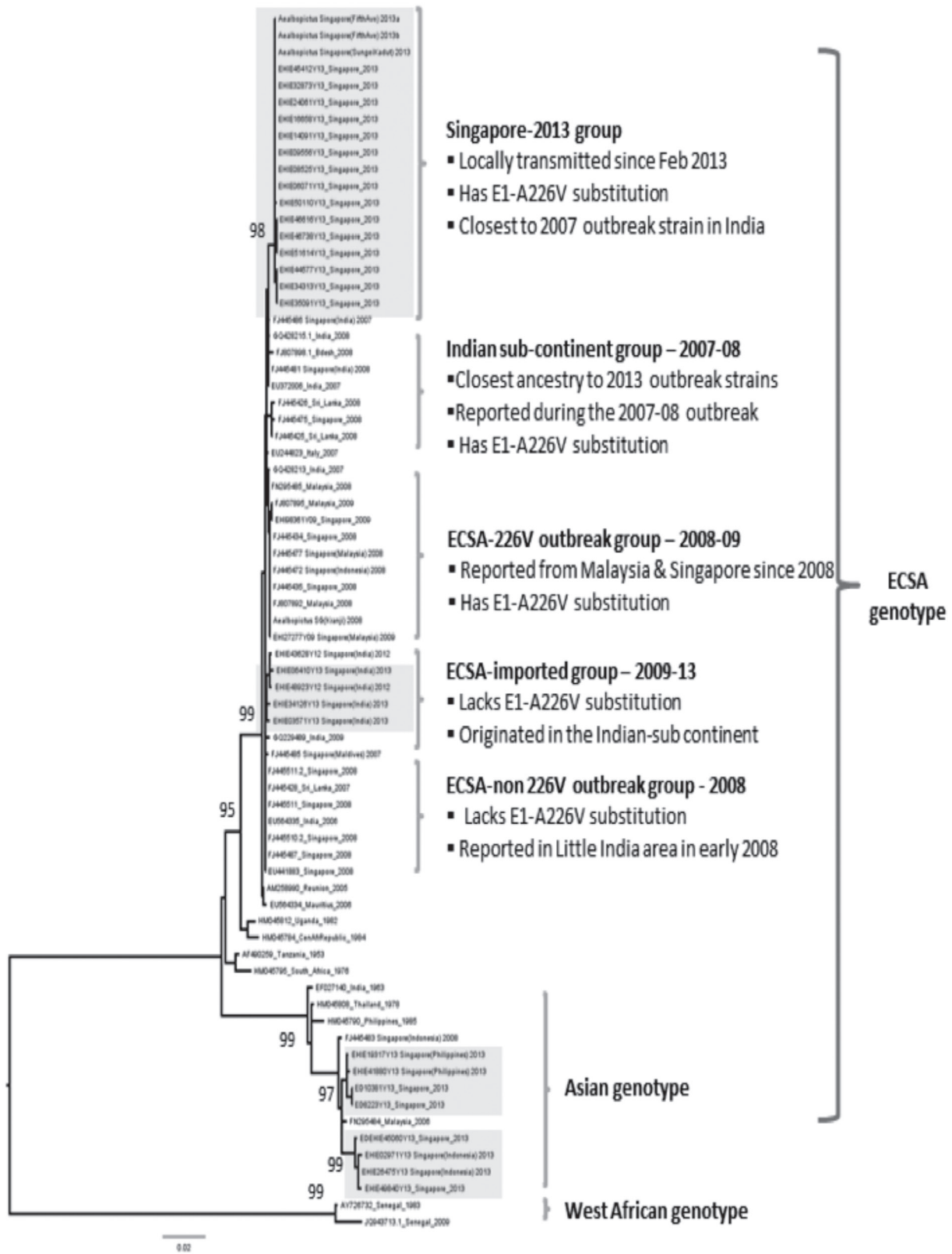
but clustered separately from outbreak strains and were repeatedly detected as sporadic cases during 2009-2013 periods. On the other hand, Asian genotype strains were imported from Philippines and Indonesia. As shown in Figure 5, high bootstrap

support indicated that virus strains from those two countries were genetically distinct. Those that belonged to Philippines lineage established local transmission for a brief period, causing a cluster of six cases in Lorong Paya Lebar in October 2013.

Table 8
Summary of entomological surveillance findings in 2013

Year	Location	Type	Adult mosquito collection		CHIKV infected
			<i>Aedes aegypti</i>	<i>Aedes albopictus</i>	
2013	Sungei Kadut	Rural/sub-urban	0	6	1
2013	Sixth Avenue	Sub-urban	0	32	0
2013	Fifth Avenue	Sub-urban	0	82	2

Figure 5
Phylogenetic analysis of CHIKV envelope 1 (E1) gene sequences.



The maximum likelihood tree was constructed using MEGA 6.06 software [86] based on the general time reversible (GTR) model with gamma distribution and invariant sites. The robustness of the original tree was tested with 1000 bootstrap replications. Sequences

of outbreak strain in 2013 are shaded. Figures on branches are bootstrap support values and only those on major nodes are shown. All GenBank-derived sequences are named with accession number, country of origin and year of isolation.

Discussion

Chikungunya fever is a new emerging disease to Singapore, with the first outbreak of chikungunya fever in Singapore having occurred in 2008, where there were two episodes of indigenous transmission. During the 2008 outbreak, there was an initial wave of cases that occurred in Little India (Jan – Feb 08) while the second episode was initiated by a wave of imported cases from Malaysia.

In 2013, cases were first detected in the semi-rural, industrial area of Kranji/Sungei Kadut and were largely confined to foreigners living and working there. It was hypothesized that the movement of human traffic in and out of the Kranji/Sungei Kadut area eventually resulted in the introduction of cases in the Bukit Timah area almost 2 months later. This hypothesis was further strengthened through virological findings where analysis of the E1 genetic sequences obtained from CHIKV virus strains from cases in both Kranji/Sungei Kadut and Bukit Timah were found to be closely related. This eventually resulted in the introduction of cases in other parts of Singapore, resulting in smaller clusters of cases as well as the reporting of sporadic cases of chikungunya fever which could not be linked to clusters despite extensive investigations. All samples from indigenous cases of chikungunya fever in 2013 were of the ECSA genotype and were closely related to the 2007 outbreak strain in India. Evidence of local transmission was further observed through the capture of mosquitoes carrying the CHIKV virus,

which were found to be identical to what was isolated from human cases.

Despite belonging to the same genotype as virus strains involved in the first chikungunya outbreak in Singapore in 2008, indigenous strains in 2013 possessed a signature of two synonymous substitutions (C639T + C816A) in E1 gene, making them a genetically distinct group. These observations, together with the long-term absence of CHIKV transmission in the country at an outbreak scale, supported a viral introduction event prior to the establishment of indigenous transmission during the CHIKV outbreak in 2013. Outbreak strains possessed E1-A226V substitution, an adaptive substitution to *Ae. albopictus*, further supporting the potential role of *Ae. albopictus* in transmitting chikungunya fever in 2013.

The 2013 experience of indigenous cases of chikungunya fever demonstrates Singapore's vulnerability towards the importation of infections such as chikungunya fever due to our open borders as well as the presence of the vectors required for transmission. Close cooperation between the relevant authorities as well as the community is required for prompt identification of cases and clusters, leading to the implementation of measures such as employing effective vector control measures to stop the chain of transmission.

OUTBREAK OF GASTROENTERITIS CAUSED BY *SALMONELLA* ENTERITIDIS ASSOCIATED WITH THE CONSUMPTION OF FOOD FROM A RESTAURANT IN SINGAPORE

Introduction

Salmonellosis is one of the commonest food-borne infections worldwide¹. An estimated 1.4 million cases of *Salmonella* infections are reported in the US yearly², while 1374 cases of salmonellosis were reported in Singapore in 2011³.

Salmonella Enteritidis is one of the most common serotypes worldwide, particularly in developed countries^{4,5}. In Asia it has also emerged as the most common serotype in Japan, the Republic of Korea, Thailand¹ and Singapore³.

Two incidents of gastroenteritis linked to consumption of food in a restaurant were notified to the Ministry of Health (MOH) on 12 May 2013. We report the findings of our epidemiological, microbiological and environmental investigations, and highlight the importance of molecular typing in establishing the source of infection.

Notification

The first incident involved 2 adults who developed diarrhoea and fever after their meal at the restaurant on 10 May 2013 at 1800 hrs. The second incident involved 4 adults who developed diarrhoea, fever and nausea after consuming food at the same restaurant on 10 May 2013 at 2030hrs.

Methods

A case was defined as a previously well individual who developed watery diarrhoea (two or more episodes in 24 hours) with/without fever, after consuming food from the restaurant from 10-13 May 2013. All the cases were interviewed and relevant clinical and epidemiological data such as age, sex, ethnicity, clinical symptoms, date of onset of symptoms, food items eaten and medical treatment sought were obtained.

A site visit was immediately made to the implicated restaurant to identify the source of infection and mode of transmission. The food preparation process was also reviewed with the management. Stool from the cases as well as food and environmental

samples were taken for microbiological analysis (*Campylobacter*, *Salmonella*, *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli*, *rotavirus* and *norovirus*). All implicated food handlers were referred to the Communicable Disease Centre and screened for enteropathogens.

Genotyping of *Salmonella* cultured from stool samples (determined by multiple-locus variable number of tandem repeat analysis, MLVA), was performed by the National Public Health Laboratory (NPHL). Seven variable-number tandem repeats (VNTR) loci selected for MLVA were amplified in a single multiplex PCR⁶. The PCR products obtained were then directly analysed using the QIAxcel High Resolution Kit, in combination with QIAxcel instruments.

Results

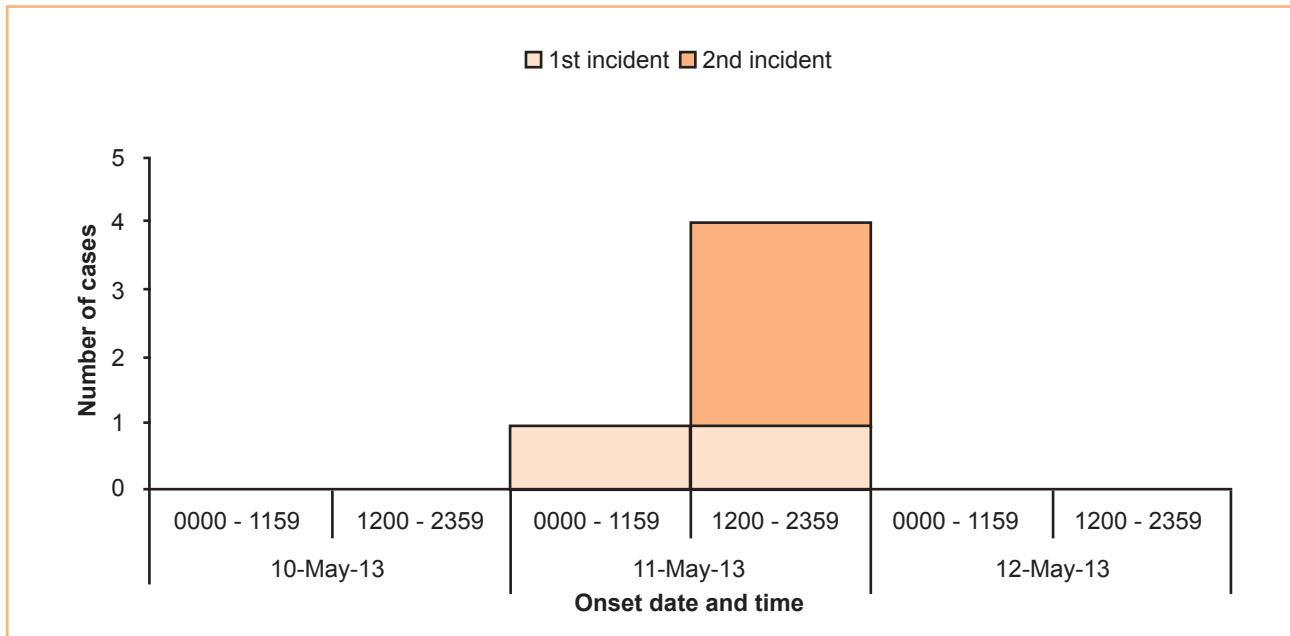
A total of five cases that met the case definition were identified. Two of these cases were from the first incident and the remaining cases were from the second incident. All of them had consumed tiramisu and roast chicken for dinner on 10 May 2013 prior to their onset of symptoms.

All the cases were Singaporean Chinese and 60% were males. The presenting symptoms were watery diarrhoea (100%), fever (100%) and nausea (20%). Of the five cases, one was hospitalised (20%) while the rest sought outpatient treatment (80%). The hospitalised case was admitted on 11 May 2013 and discharged on 14 May 2013.

The onset of illness was from 0200 to 2100 hrs on 11 May 2013 (Figure 1). The mean and median

incubation periods were 16.2 hours and 16 hours respectively, with a range of 8-23 hours.

Figure 1
Onset of illness of 5 reported food poisoning cases who consumed food at a restaurant on 10 May 2013

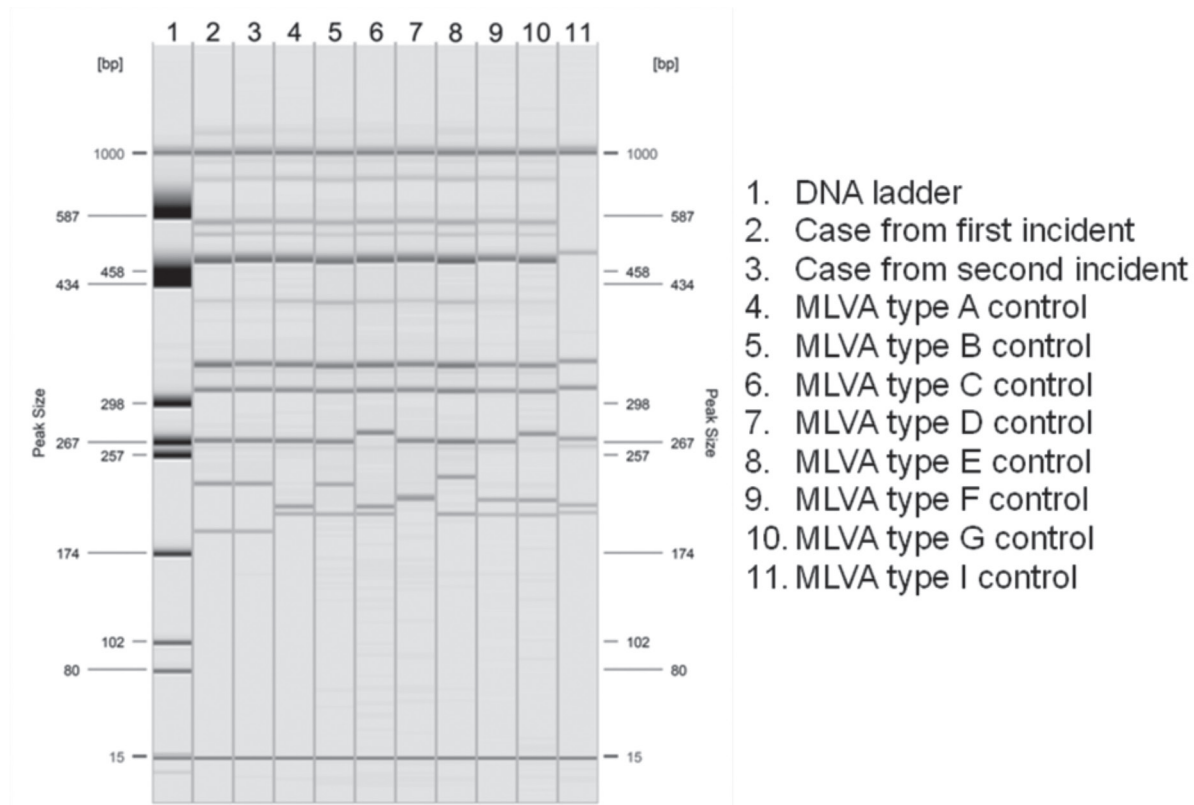


Two of four stool samples obtained from the cases were positive for *Salmonella* Enteritidis, MLVA type J (Figure 2). All the four food handlers from the implicated restaurant tested negative for food-borne pathogens, including norovirus and rotavirus.

found to have high total plate count (2,900,000 CFU/g; limit <100,000 CFU/g) and high total coliform count (1100 MPN/g; limit <50 MPN/g). An environmental swab of the chopping board for roasted chickens tested negative for bacterial food poisoning pathogens.

Of the two food samples collected (tiramisu and poached eggs) for microbial analysis, tiramisu was

Figure 2
MLVA typing of two *Salmonella*-positive cases



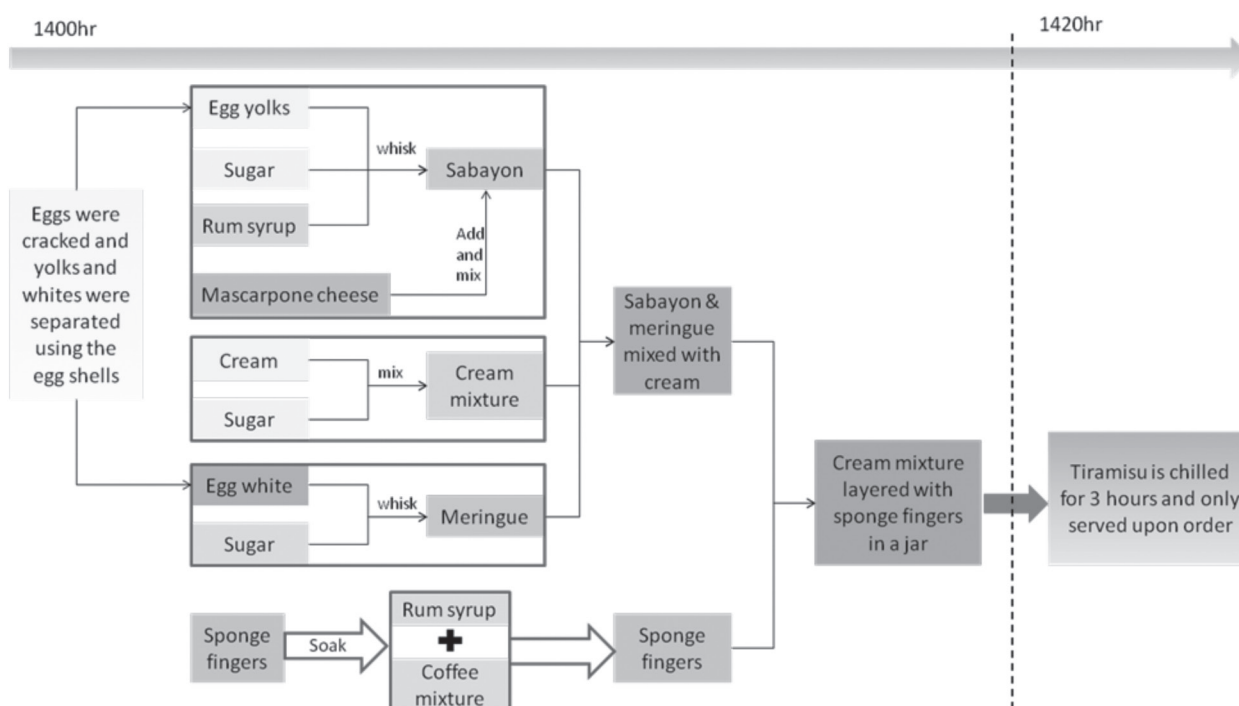
Food preparation process

Two batches of chickens were cooked each day at 0900hrs and 1500hrs. Preparations for roast chickens began at 0900hrs when fresh chickens were delivered to the restaurant. The chickens were marinated in brine with salt and sugar. The first batch of chickens was seared before cooking in the combination oven. The remaining chickens were kept in the refrigerator to be cooked at 1500hrs. After cooking in the combination oven, the chickens were then transferred to the rolling oven for further roasting. The roast chickens were then transferred to the warmer before serving. Sauces were added to the chickens just before serving to the customers. Precautionary measures were taken throughout the food preparation process to prevent cross-contamination between raw and cooked chickens

The tiramisu was prepared by the food handlers daily at 1400hrs and it took 20 minutes to complete. There were no specific personnel-in-charge of making

the tiramisu. Eggs were a key ingredient used to make the tiramisu. These were supplied daily to the restaurant at 0900hrs. The egg whites and eggs yolks were manually separated from whole eggs into 2 bowls. Sugar was added to 18 of these pooled egg yolks to be whisked together with rum syrup to make a mousse (sabayon). Mascarpone cheese was then added to the sabayon and this was subsequently chilled in the refrigerator. Similarly, sugar was added to the pooled egg whites and whisked to become a meringue. Fresh cream was mixed with sugar to make a cream mixture. Sponge fingers were separately soaked in rum syrup and coffee mixture. Finally, the meringue, sabayon and cream were mixed together and then layered onto the sponge fingers to make 60 jars of tiramisu. The tiramisu was then chilled for 3 hours before serving. This batch of tiramisu would be served on the same day as well as for lunch on the following day. The preparation process of the tiramisu is depicted in Figure 3.

Figure 3
Preparation process of tiramisu



The restaurant was found to be satisfactorily maintained. However, two hygiene lapses were observed. These included uncovered food items in the refrigerator and a refuse bin without a functional pedal. None of the staff reported being unwell one week prior to the two incidents and the establishment had not received any other complaints of food-borne illness.

Discussion

The epidemiological and clinical findings suggest that this is an outbreak of salmonellosis. The reported symptoms (fever and diarrhoea) with a mean incubation period of 16.2 hours (range 8 to 23 hours) are compatible with the symptoms and known incubation period for *Salmonella* infection. This is further supported by the isolation of *Salmonella* Enteritidis from the stool samples of two cases, one from the first incident and the other from the second incident. The common source of infection from the implicated restaurant was confirmed by the same genetic sequence (MLVA type J).

There were no common meals among the cases other than the dinner at the restaurant on 10 May 2013. Tiramisu and roast chicken were the two common food items consumed by the cases in the 2 incidents.

Salmonellosis is a bacterial disease characterized by acute enterocolitis, with sudden onset of abdominal pain, diarrhoea, nausea and vomiting. The incubation period is usually between 12 and 36 hours but it can range from 6 to 72 hours. Infection can arise from ingestion of the salmonella bacteria in food derived from infected animals or food that is contaminated by faeces of infected animals or humans⁷.

Common implicated food items include raw or inadequately cooked poultry and eggs and dairy products, as well as processed meat products^{7,8}. Its increasing incidence in the United Kingdom and the United States of America in the 1980s was mainly attributed to consumption of raw or undercooked contaminated poultry, hen eggs and egg-containing products^{5,9}. In Singapore, food-borne outbreaks of *Salmonella* Enteritidis have been reported in cream cakes¹⁰, bread¹¹, and an egg-based pancake¹².

Outbreaks of *Salmonella* Enteritidis associated with eggs and desserts are not uncommon. In 2010, Wright Country Egg of Galt, Iowa, conducted a nationwide voluntary recall of shell eggs due to *Salmonella* Enteritidis contaminated egg shells. 1,939 reported cases of salmonellosis were associated with this outbreak⁸. In the United States, eggs or egg-containing foods were implicated in

77% of the outbreaks of *Salmonella* Enteritidis infection in which a food vehicle was identified⁵. In the United Kingdom, desserts were implicated in 19% of outbreaks and eggs were used as an ingredient in 70% of these desserts⁹. The high number of eggs-associated outbreaks may be due to the fact that eggs are often eaten raw or undercooked. Foods that contain eggs, such as tiramisu or ice-creams, are often lightly cooked or uncooked. The practice of pooling a large number of eggs for use in commercial settings may also increase the risk of salmonella-associated outbreaks as one or a few contaminated eggs can accelerate and increase the exposure of consumers to *Salmonella* Enteritidis contamination.

In this outbreak, tiramisu was prepared from unpasteurised raw eggs without further heating or reheating. Any contamination in a single batch of eggs could potentially present a risk to consumers. It is likely that the tiramisu served during the dinner on 10 May 2013 could have been contaminated during its preparation. We noted that the egg shell had been used to manually separate the egg yolk and egg whites during the preparation process. This could have resulted in contamination of the tiramisu if the surface of the egg shells was contaminated with the faeces of an infected animal or after oviposition. Eggs can also be contaminated by direct penetration through the eggshell from the colonised gut of an infected poultry or by direct contamination of the internal contents of the eggs by infected ovaries and oviducts¹³.

The other food item that was common in this outbreak was roasted chicken. However, we believe that it is an unlikely vehicle of transmission as it was cooked at temperatures above 100°C (the temperature settings were checked during the inspection). In addition, as roast chickens accounted for half of the sales, we would have expected more cases linked to this premises.

Other possible sources of contamination included

the cross-contamination of utensils, equipments and work surfaces. Our investigations uncovered hygiene lapses such as ready-to-serve food items that were not properly stored and covered rubbish bin with faulty pedal. *Salmonella* can survive in the environment for several days¹⁴. Therefore, when personal and food hygiene practices are insufficiently observed, there is a possibility that *Salmonella* Enteritidis can be transferred from the contaminated food to other ready-to-serve food through unwashed hands or food preparation surfaces.

Currently, there is no regulation on using pasteurised eggs in the preparation of food in Singapore. Nevertheless, we have advised the management of the implicated restaurant to use pasteurised eggs and to use an egg strainer to separate the yolks from the whites to minimize the risk of contamination. The restaurant was also reminded to ensure that proper personal and food hygiene practices are observed at all times.

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