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CONTENTS



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# Outbreak of pandemic influenza A (H1N1-2009) in Singapore, May – September 2009

## Introduction

The first influenza pandemic of the 21st century was declared by the World Health Organization (WHO) on 11 June 2009[1]. Prior to that, WHO had progressively elevated its alert phases beginning with a declaration of an outbreak in Mexico and the United States as a public health emergency of international concern on 26 April[2]. WHO then raised its influenza pandemic alert to phase 4 on 27 April[3] and then to phase 5 just two days later on 29 April[4].

## Confirmed cases of influenza A (H1N1-2009) in Singapore from 26 May – 9 July 2009

Epidemiological investigations were carried out on 1,301 laboratory-confirmed cases of H1N1-2009 from 27 May (the first imported case) to 9 July 2009 (when containment measures gave way to mitigation measures). Nearly two-thirds (834 or 64%) were deemed as local transmission while about one-third (467 or 36%) were imported.

### Imported cases

The first imported case of influenza A (H1N1) (also the first known case) was a 22-year old Singaporean female university student. Her infection was detected in Singapore on 26 May 2009 – one month after WHO's announcement of the novel virus outbreak on 24 April 2009. She was in New York from 14 – 24 May and arrived back in Singapore on the morning of 26 May. She had begun to feel unwell on the plane. She consulted a GP upon her return and was sent by a 993 ambulance to Tan Tock Seng Hospital (TTSH) Emergency Department where she was

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subsequently admitted and her diagnosis confirmed as influenza A (H1N1-2009) via polymerase chain reaction (PCR) tests.

Three distinct waves of importation of cases of H1N1-2009 (*Fig. 1*) were noted:

First wave: 27 May - 7 June with cases imported mainly from the United States.

Second wave: 8 - 19 June with cases imported mainly from Australia.

Third wave: 20 June onwards with cases imported mainly from South-East Asia.

The third wave of imported cases coincided with the beginning of local transmission.

Most of the imported cases had travelled to Australia (24%), Philippines (23%), Indonesia (21%), Thailand (10%) and the United States (9%). These five countries accounted for 87% of imported cases reported between 27 May and 9 July 2009. The source of infection of two imported cases was on a cruise ship (*Table 1*).

Not surprisingly, nearly 90% of local cases comprised Singapore residents while foreigners made up over 40% of imported cases. A large proportion of the imported cases were probably tourists, highlighting the vulnerability of Singapore to infectious diseases brought in by travellers (*Table 2*).

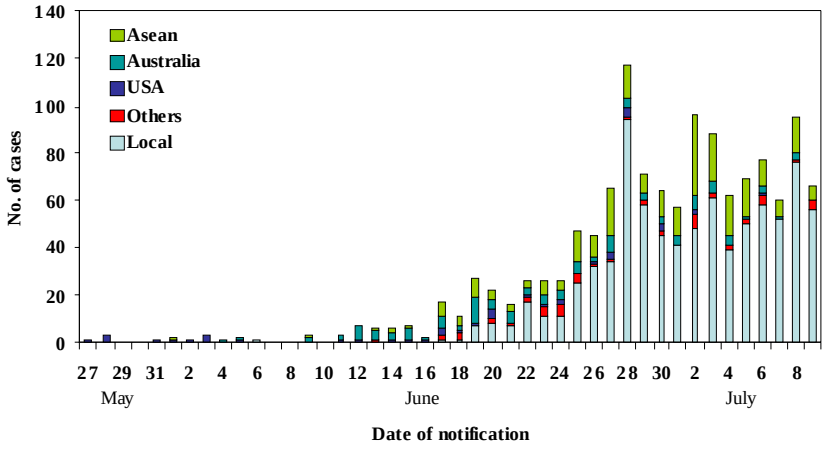
**Table 1**

**Country of origin of imported cases of influenza A (H1N1-2009), May – July 2009**

| Country | Number | % |
|---|---|---|
| Australia | 112 | 24.0 |
| Canada | 2 | 0.4 |
| Chile | 1 | 0.2 |
| China | 2 | 0.4 |
| Cruise | 2 | 0.4 |
| Hong Kong | 16 | 3.4 |
| India | 1 | 0.2 |
| Indonesia | 98 | 21.0 |
| Japan | 4 | 0.9 |
| Korea | 1 | 0.2 |
| Malaysia | 4 | 0.9 |
| New Zealand | 4 | 0.9 |
| Philippines | 106 | 22.7 |
| Thailand | 48 | 10.3 |
| UK | 19 | 4.1 |
| US | 44 | 9.4 |
| Vietnam | 3 | 0.6 |
| Total | 467 | 100.0 |

**Figure 1**

**Imported and local cases of influenza A (H1N1-2009) by date of reporting, 27 May – 9 July 2009**



## Local cases

The first case of H1N1-2009 who was infected locally was reported on 18 June 2009. This case was a 26-year-old Malaysian male who is a permanent resident in Singapore. He departed from Singapore to Kuala Lumpur for work on 14 June. Later that evening, he developed symptoms while he was having dinner at a restaurant. He returned to Singapore from Kuala Lumpur on 17 June. When he arrived at Changi Airport, he immediately approached the airport staff and a 993 ambulance was called to send him to TTSH where he was diagnosed with H1N1-2009.

Of the 834 local cases, 22 were infected from imported cases, 425 were linked to other local clusters and 387 were unlinked. The first local clusters of cases were reported on 20 June. These were clusters arising from a church function held on 13-14 June with total of 10 cases and a night club on 17 June with total of 46 infected. The largest cluster of 116 cases was related to one of the polytechnics. Early clusters were also detected in Singapore Armed Forces (SAF) camps (*Table 3*).

## Age-distribution

Most (84%) of the 1,301 cases were below the age of 30 years. Only 3% were between 50 and 59 years of age and less than 1% were above 60 years of age. A similar age-distribution was seen even when Singapore residents and foreigners were analysed separately (*Table 4*).

**Table 2**

**Nationality of 1,301 imported and local cases of influenza A (H1N1-2009), May – July 2009**

| Nationality | Imported | Local | Total |
|---|---|---|---|
| Singaporeans | 43.5 | 85.1 | 70.2 |
| Singapore permanent residents | 13.1 | 4.2 | 7.4 |
| Foreigners | 43.5 | 10.7 | 22.4 |
| Total | 100.0 | 100.0 | 100.0 |

**Table 3**

**Early local clusters of cases of influenza A (H1N1-2009)**

| Cluster | Cluster size | Earliest date of onset of symptoms |
|---|---|---|
| Polytechnic | 116 | 15 June 2009 |
| SAF Camp 1 | 29 | 15 June 2009 |
| Nightclub (17 June) | 46 | 16 June 2009 |
| Church | 13 | 17 June 2009 |
| SAF Camp 2 | 28 | 18 June 2009 |
| University | 36 | 20 June 2009 |
| SAF Camp 3 | 67 | 24 June 2009 |

Cases in the age group of 10-29 years old made up 80% of local transmission cases compared to 59% among imported cases. The proportion of cases below 10 years of age among imported cases (18%) was more than double that of local transmission cases (8%). This is probably related to parents bringing their young children overseas during the school holidays in June.

### Sex and ethnic group distribution of Singapore residents

Of 1009 cases who were Singapore residents, males were more affected than females (males 60% vs females 40%). Malays and other ethnic groups (besides Chinese, Malay and Indian) were over-represented compared to their distribution in the population (*Table 5*).

## Indicators of influenza activity

### Acute respiratory infections seen in polyclinics

The number of cases with acute respiratory infection (ARI) seen in polyclinics is monitored on an ongoing basis by the Ministry of Health. Two warning triggers are set based on the mean number of ARI cases seen in the 5 years preceding the current calendar year. These are the "warning level" set at one standard deviation from this mean and the "epidemic level" set at two standard deviations from the mean. The

**Table 4**
**Age distribution (%) of 1,301 local and imported cases of influenza A (H1N1-2009) by residency**

| Age group (years) | Singapore residents | | | Foreigners | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Imported | Local | All | Imported | Local | All | Imported | Local | All |
| 0-9 | 17.8 | 8.2 | 10.7 | 19.2 | 5.6 | 15.1 | 18.4 | 7.9 | 11.7 |
| 10-19 | 31.1 | 33.8 | 33.1 | 31.5 | 39.3 | 33.9 | 31.3 | 34.4 | 33.3 |
| 20-29 | 28.0 | 46.6 | 41.7 | 26.6 | 40.4 | 30.8 | 27.4 | 45.9 | 39.3 |
| 30-39 | 9.1 | 4.4 | 5.6 | 10.3 | 12.4 | 11.0 | 9.6 | 5.3 | 6.8 |
| 40-49 | 10.2 | 3.6 | 5.4 | 6.9 | 2.2 | 5.5 | 8.8 | 3.5 | 5.4 |
| 50-59 | 2.7 | 2.7 | 2.7 | 5.4 | 0.0 | 3.8 | 3.9 | 2.4 | 2.9 |
| 60+ | 1.1 | 0.7 | 0.8 | 0.0 | 0.0 | 0.0 | 0.6 | 0.6 | 0.6 |
| All | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

**Table 5**
**Ethnic distribution of 1009 confirmed cases of influenza A (H1N1-2009) who were Singapore residents, May – July 2009**

| Ethnic group | No. of cases | Distribution among cases (%) | Distribution in the resident population 2009 (%) |
|---|---|---|---|
| Chinese | 598 | 59.3 | 74.2 |
| Malay | 241 | 23.9 | 13.4 |
| Indian | 59 | 5.8 | 9.2 |
| Others | 111 | 11.0 | 3.2 |
| Total | 1009 | 100.0 | 100.0 |

number of ARI cases exceeded the warning level during epidemiological (E)-week 26 (28 June – 4 July 2009), exceeded the epidemic level during E-week 27 (5 - 11 July) and peaked during E-week 30 (26 July – 1 August). It subsequently fell below the epidemic level during E-week 32 (9 – 15 August) and then below the warning level during E-week 34 (23 – 29 August). The number of ARI cases seen in polyclinics remained below the warning level from E-week 34 to E-week 39 (27 September – 3 October) (*Fig. 2*)

## Proportion of cases with influenza-like illness among ARI cases seen in polyclinics

The proportion of cases with influenza-like illness (ILI) among ARI cases seen in polyclinics rose from 14.8% during E-week 28 (12 – 18 July 2009) to a peak of 22.9% during E-week 30 (26 July – 1 August). It then fell below 20% during the following week and then below 10% during E-week 36 (6 – 12 September). The proportion of ILI cases was 5.8% during E-week 39 (27 September – 3 October) (*Fig. 3*)

**Figure 2**
**Weekly polyclinic attendances of acute respiratory infection (ARI), year 2007 to 2009**

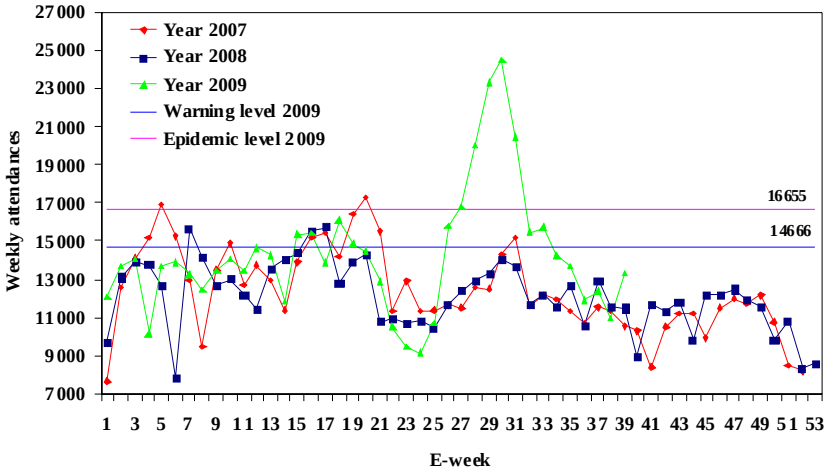
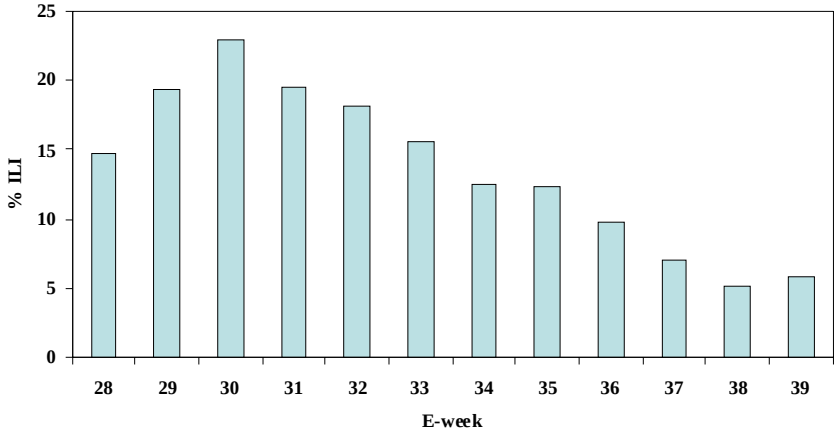



**Figure 3**
**Weekly proportion of influenza-like-illness (ILI) among ARI polyclinic attendances in 2009**



### Prevalence of influenza A (H1N1) among cases with influenza-like illness

Influenza A (H1N1-2009) was first detected among surveillance samples of patients with influenza-like illness (ILI) seen at polyclinics and GP clinics during E-week 24 (14 – 20 June 2009) when it was 1%. It then steadily rose to go beyond 50% in E-week 28 (12 – 18 July) to reach a peak of 65.5% (95% CI: 62.0% -68.8%) in E-week 31 (2 – 8 August). The prevalence of H1N1-2009 among ILI cases seen in polyclinics and GP clinics stayed above 50% for nine consecutive weeks from E-week 28 to E-week 36 (6 – 12 September). The prevalence fell to 18.1% (95% CI: 13.0% - 24.6%) in E-week 39 (27 September – 3 October) (*Fig. 4*).

## Estimated number of cases of H1N1-2009 from June – September 2009

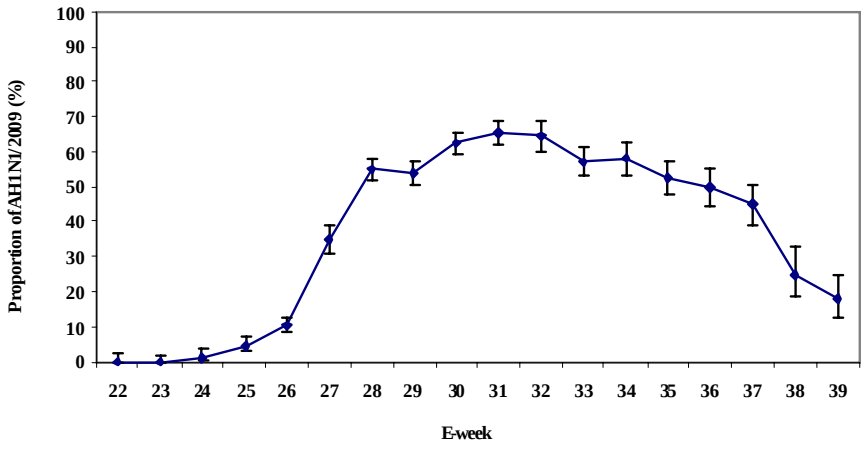
The daily public updates of the number of confirmed cases stopped on 9 July 2009 when containment measures were gradually replaced by mitigation measures. Contact tracing and other containment measures were stopped in recognition of widespread community transmission by then. The cumulative number of cases of H1N1-2009 was then estimated as follows:

(i) The number of acute respiratory infections seen in polyclinics each week were separated into cases with influenza-like illness (ILI) (fever >38 deg C and cough and/or sore throat) and those without.

(ii) The estimated number of H1N1-2009 cases among cases with ILI seen in the polyclinics each week were calculated by multiplying the number of ILI cases with the prevalence of H1N1-2009 from the biosurveillance survey for the corresponding week.

(iii) Step (ii) was repeated for non-ILI cases of acute respiratory infection taking the prevalence of H1N1 as one-quarter that of ILI cases for the corresponding week. This ratio was determined through a study of cases of acute respiratory infection which did not fulfill the criteria for ILI. The study was done during the week 3-7 August 2009. The study was repeated during the week 5 - 9 October 2009. The ratio of 1:4 among non-ILI and ILI cases was found in both periods.

**Figure 4**
**Prevalence of influenza A (H1N1-2009) among ILI cases by E-week in 2009**



(iv) The number of H1N1 cases seen in GP clinics were then estimated by assuming that 86% of all cases of acute respiratory infection are seen in GP clinics. This ratio comes from the Primary Medical Care Survey 2005[5]. Steps (i) to (iii) are repeated to derive the estimated number of H1N1-2009 cases seen in GP clinics each week.

Using the methodology described above, it is estimated that between E-week 24 (14 – 20 June 2009) and E-week 39 (27 September – 3 October) a total of 270,000 cases of H1N1-2009 were seen in polyclinics and GP clinics throughout Singapore. As the total population of Singapore at the end of June 2009 was reported as 4.99 million, this would give a cumulative clinical attack rate of 5.4% by the beginning of October 2009. If we further assume that only half of clinical cases sought care in polyclinics and GP clinics, the total number of cases would double to 540,000 and the clinical attack rate would also double to nearly 11%.

The peak number of cases occurred during E-week 30 (26 July – 1 August) when an estimated 45,000 cases were seen in polyclinics and GP clinics.

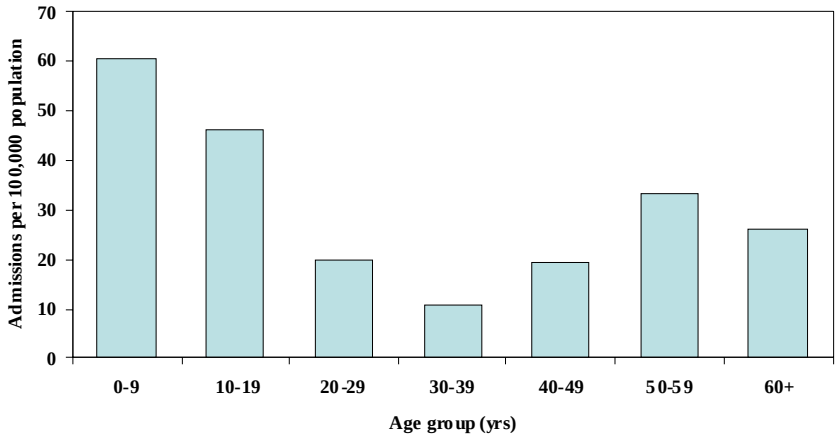
## Hospitalised cases

All hospitals were required from 15 July 2009 to report confirmed H1N1-2009 cases who had been admitted for medical indications. Between 15 July and 28 September 2009, a total of 1,348 confirmed cases, who were admitted into hospitals, were reported. This represents about 0.6% of the estimated number of H1N1-2009 infections seen in polyclinics and GP clinics (N=245,000 between E-week 28 and E-week 38; i.e. between 12 July – 26 September 2009) or a rate of 6 per 1000 cases.

The median age among hospitalised cases was 25 years with a range of 24 days to 95 years. Hospitalisation rates were highest among young children aged below 10 years (60.6 per 100,000 population) and older children aged 10-19 years (46.1 per 100,000 population). In contrast, the hospitalisation rate among the elderly aged 60 and above was 26.1 per 100,000 population (*Fig. 5*).

Of the 1255 hospitalised cases for whom data were available, the commonest co-morbid conditions

**Figure 5**
**Age-specific hospital admission rates of influenza A (H1N1-2009) (n=1348) as of 28 September 2009**



were asthma / chronic obstructive pulmonary disease (COPD) (20%), diabetes (13%) and cardiovascular disease (7%). 42% had no known co-morbid conditions or risk factors (*Table 6*).

The median length of stay in hospital was 3 days. 89% of patients stayed 7 days or less.

## Severe cases

Hospitals were required to report all severe cases (i.e. admitted into intensive care units (ICU) or who died) to the Ministry of Health since the epidemic began locally. The Ministry received reports on 92 severe cases. This represented about 6 - 7% of hospitalised cases (including some cases hospitalised for medical indications before formal reporting began on 15 July) or a rate of about 0.3 per 1000 cases (with N=270,000 estimated clinical cases as the denominator). While the hospitalisation rates were highest among the children, the incidence of severe cases was the highest among adults aged 50-59 (3.4 cases per 100,000 population) and elderly aged 60 and above (3.1 cases per 100,000 population) (*Fig. 6*). About half (54%, n= 50) required mechanical ventilation. The median age among cases admitted into ICU was 44 years with a range of 3 months to 95 years.

Of the 80 severe cases for whom data were available from medical case notes, the commonest co-morbid conditions were cardiovascular disease (24%), asthma / COPD (23%), neuromuscular disorders / epilepsy (19%), metabolic diseases (including diabetes) (18%), and chronic renal disease (14%). 21% had no known co-morbid conditions or risk factors (*Table 7*). One-fifth (16 / 79) of the severe cases were reported to have acute respiratory distress syndrome.

## Deaths

The first death from H1N1 was reported on 18 July 2009. This was a 49-year-old man with multiple

**Table 6**
**Co-morbid conditions or risk factors of 1255 hospitalised cases of influenza A (H1N1-2009), 15 July – 28 September 2009**

| Condition | Number | % |
|---|---|---|
| Asthma / COPD | 255 | 20.3 |
| Diabetes | 159 | 12.7 |
| Cardiovascular diseases | 89 | 7.1 |
| On immunosuppressive therapy | 65 | 5.2 |
| Renal failure | 57 | 4.5 |
| Malignancy | 47 | 3.7 |
| Auto-immune diseases | 42 | 3.3 |
| Pregnancy | 42 | 3.3 |
| Neuromuscular disorder / epilepsy | 37 | 2.9 |
| Obesity | 11 | 0.9 |
| Age 5 years and younger | 163 | 13.0 |
| Age 65 years and older | 95 | 7.6 |
| With one or more of the above | 727 | 57.9 |

Note: one patient may be listed more than once in the above categories

**Figure 6**
**Age-specific incidence of severe cases of influenza A (H1N1-2009) (n=92) as of 28 September 2009**

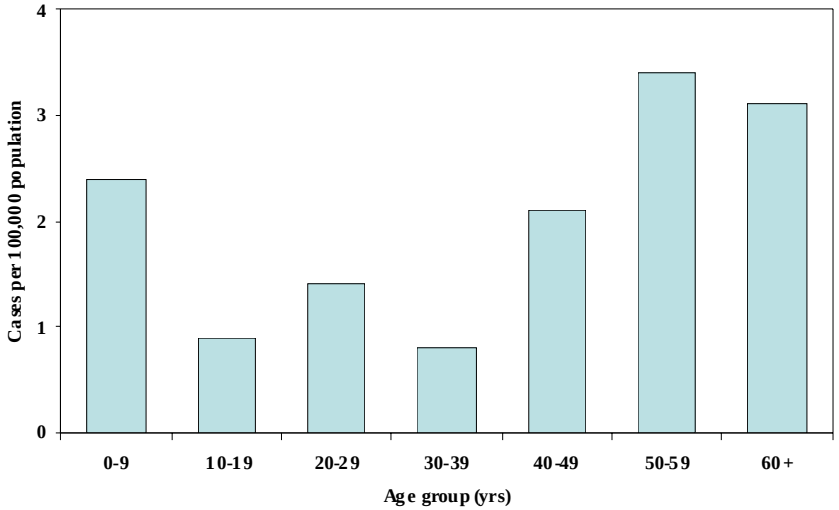



**Table 7**

**Co-morbid conditions or risk factors of 80 severe cases, including 17 deaths, of influenza A (H1N1-2009)**

| | Severe cases (n=80) | | Deaths (n=17) | |
|---|---|---|---|---|
| Condition | No. | % | No. | % |
| Cardiovascular diseases | 19 | 23.8 | 4 | 23.5 |
| Asthma / COPD | 18 | 22.5 | 3 | 17.6 |
| Neuromuscular disorder / epilepsy | 15 | 18.8 | 3 | 17.6 |
| Metabolic diseases | 14 | 17.5 | 3 | 17.6 |
| Renal diseases | 11 | 13.8 | 0 | 0 |
| On immunosuppressive therapy | 8 | 10.0 | 2 | 11.8 |
| Obesity | 7 | 8.8 | 2 | 11.8 |
| Malignancy | 3 | 3.8 | 2 | 11.8 |
| Auto-immune diseases | 3 | 3.8 | 0 | 0 |
| Pregnancy | 2 | 2.5 | 0 | 0 |
| Age 5 years and less | 5 | 6.3 | 0 | 0 |
| Age 65 years and above | 12 | 15.0 | 5 | 29.4 |
| With one or more of the above | 63 | 78.8 | 14 | 82.4 |

Note: one patient may be listed more than once in the above categories

co-morbidities (diabetes, hypertension, and high cholesterol). He was admitted to Changi General Hospital (CGH) on 16 July after 4 days of flu-like symptoms. He died of acute myocardial infarction, contributed by severe pneumonia with underlying influenza A (H1N1-2009) infection.

A total of 18 H1N1-related deaths were reported up to 28 September 2009. This represents slightly more than 1% of hospitalized cases and a case fatality rate of 6.7 per 100,000 cases (using N= 270,000 estimated clinical cases as the denominator). The median age among H1N1-related deaths was 50 years with a range of 7 years to 95 years. The commonest co-morbid conditions among the first 17 fatal cases were cardiovascular disease (24%), asthma / COPD (18%), neuromuscular disorders / epilepsy (18%), and metabolic diseases (including diabetes) (18%). 18% had no known co-morbid conditions or risk factors (*Table 7*).

*(Reported by Cutter J, Ang L W, Lai F, Subramony H, Ma S & James L, Communicable Diseases Division, Ministry of Health)*

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4 *World Health Organization. Statement by WHO Director-General Dr Margaret Chan 29 April 2009 . Available at http://www.who.int/mediacentre/news/statements/2009/h1n1_20090429/en/index.html (accessed 15 October 2009).*

5 *Ministry of Health 2006. Primary Medical Care Survey, Singapore, 2005.*


# An outbreak of *Vibrio parahaemolyticus* traced to consumption of Indian 'rojak'

## The outbreak

On 3 April 2009, the Ministry of Health (MOH) was notified by the Emergency Department of a local hospital of some 20 patients with food poisoning. Notably, all of them presented with severe gastroenteritis symptoms after consuming Indian 'rojak' bought from a market at Geylang Serai.

Investigations were conducted immediately by MOH at the hospital. The source of the Indian 'rojak' was identified to be from a stall located at Eunos Road. Officers from MOH and the National Environment Agency (NEA) subsequently went down to the stall to carry out a joint inspection. The stall had been closed that morning after the operator separately learnt about the food poisoning incident from some of his affected

customers. NEA subsequently instructed the stall operator that the stall stayed closed until further notice.

Members of the public were alerted to the outbreak and updated by daily press releases. They were advised to discard any unconsumed food items purchased from the implicated stall. They were also advised to seek immediate medical attention if they had consumed food from the stall and subsequently developed symptoms of food poisoning.

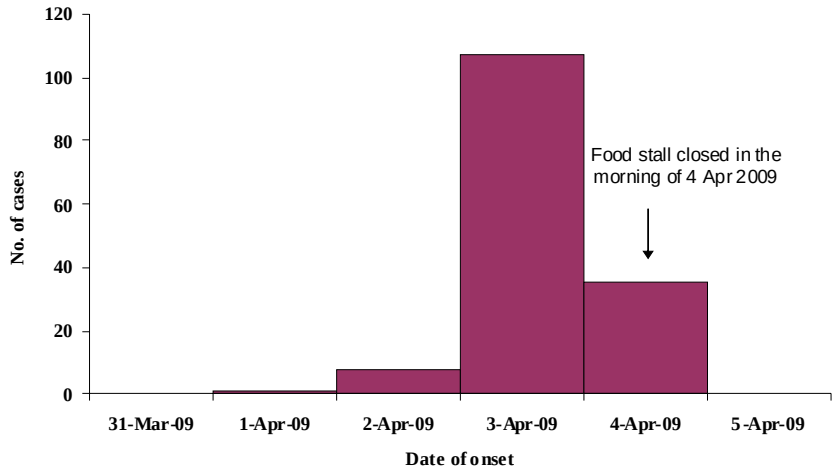
## Clinical and epidemiological findings

There were a total of 154 cases notified to MOH. The presenting symptoms reported by the cases, in order of decreasing frequency, were: watery diarrhoea (99%), abdominal cramps (88%), vomiting (87%), fever (55%), chills/rigors (29%), and bloody stools (11%). 48 cases were hospitalized, while the rest received outpatient treatment at various emergency departments and clinics. Two of the hospitalized cases died.

Members of the Malay-Muslim community who frequently patronized the Geylang Serai area constituted the high-risk population in this outbreak. Cases comprised 149 (96.8%) Malays, 3 (1.9%) Chinese, and 2 (1.3%) Indians. Their ages ranged from 2 to 95 years. There were 97 females and 57 males, giving a female to male ratio of 1.7: 1.

All 154 cases gave a positive food history associated with the stall and developed onset of illness between 1 and 4 April 2009. The median incubation period based on the interval between time of consumption and onset of illness was established to be 6 hours, while the mean incubation period was 7.2 hours (range 1-24 hours). The shape of the epidemic curve was consistent with a common source outbreak. Transmission ceased following closure of the stall on 4 April 2009 (*Fig. 7*).

**Figure 7**
**Time distribution of 151* food poisoning cases associated with consumption of Indian 'rojak' from the implicated stall**




* 3 cases could not recall their exact dates of onset

Two fatalities occurred in the outbreak. The first was a 57-year-old female who was admitted for gastroenteritis symptoms on 4 April 2009, and died on 6 April 2009. The second was a 59-year-old female with comorbidities who was admitted on 4 April 2009, and died on 8 April 2009. Both died from septicemia and multi-organ failure, and infectious enterititis.

## Food preparation

Routinely, the preparation of Indian 'rojak' would begin daily at 0400 hours when two of the food handlers would open the stall and go to the wet market to purchase raw ingredients. The 'rojak' gravy was made from boiled sweet potatoes, chilli powder, tamarind, sugar, salt, pepper, sesame seeds, peanuts, artificial colouring and water. Other food items would be prepared at 0530 hours after the 'rojak' gravy was prepared. Fried food items consisted of prawn fritters, soya bean curd (*tempeh*), eggs, fish balls, fish fillet, fish cakes, beef lung, spleen and liver. Boiled items included potatoes, fish balls, fish cakes and cuttle fish. These food items were displayed within the food showcase and sales commenced at 0700 hours. Upon receiving orders, the food items, chilli, cucumber and onion were cut and placed on the plate for serving with a bowl of 'rojak' gravy. The stall was usually closed by 1900 hours.

Owing to booming business, there was no reheating of food items prior to serving. Some lapses in hygiene were detected at the stall: cockroach infestation and no refrigerator for the storage of perishable food items. There were also inconsistencies in the statements provided by the operator and the food handlers. In the initial interview, a food handler admitted to reusing a portion of the gravy for sale the following day but this statement was denied in subsequent interviews. In addition, the food handlers of the implicated 'rojak' stall had used the refrigerator of the neighboring stall to store seafood items, and to wash and store their equipment there. It was later discovered that the licensee of the neighbouring stall had sublet his premises to the licensee of the 'rojak' stall.

Although prompt investigation was carried out, no food remnants were available for sampling as the food handlers had discarded all the food items and cleaned up the premises as soon as complaints from customers were received.

## Microbiological findings

Stool samples were obtained from 27 cases. Of the samples, *V. parahaemolyticus* was isolated from 13 of the cases, including the first fatal case. All the 5 food handlers from the 'rojak' stall as well as the foodhandler of the adjacent stall were tested negative for *V. parahaemolyticus*. There was an incidental finding of rotavirus in one of the food handlers. Two spouses of the food handlers were found to be positive for *V. parahaemolyticus*. However, they had denied any involvement in the preparation or consumption of 'rojak' sold at the stall.

All *V. parahaemolyticus* isolates (from the 13 cases and the two spouses of the foodhandlers) were of the O4:K55 serotype. Temporal and geographical isolates were also included in the study as negative controls. These were found to be of a different serotype, suggesting that the common serotype among the cases was not a coincidental finding. Toxin gene analysis also revealed that all the isolates from the cases and spouses were positive for thermostable direct hemolysin (TDH) and negative for TDH-related hemolysin (TRH).

No *V. parahaemolyticus* was detected in two food samples and four environmental swabs subsequently taken from the 'rojak' stall. However, high faecal coliform count was found in a sample of raw vegetable and *Staphylococcus aureus* and faecal coliform bacteria from the chopping board. *Salmonella* bacteria were also detected in environmental swabs taken from the refrigerator tray. Additionally environmental swabs of the refrigerator door, a tray used to contain fried food items and a knife from the adjacent stall, all revealed high total plate counts.

## Comments

The clinical and epidemiological findings of this common source outbreak are suggestive of *V. parahaemolyticus* food poisoning. The aetiology was confirmed when the causative bacteria were detected in the stools of hospitalized cases.

*V. parahaemolyticus* is a naturally occurring inhabitant of the marine environment[1] and is a common cause of bacterial food poisoning[2]. It causes watery diarrhoea and abdominal cramps in nearly all cases, usually with nausea, vomiting, fever and headache. Typically, it is a disease with a mean incubation period of 15 hours (range from 4 to 96 hours) and of moderate severity which lasts from 1-7 days. Systemic infection and death rarely occur [1, 3]. The severity of illness and short incubation period of some of the cases in this outbreak suggest ingestion of high doses of *V. parahaemolyticus* in the implicated food.

Results from serotyping and toxin gene analysis are consistent with a common source outbreak. TDH is a putative virulence factor and has been associated with the infection being lethal, hemolytic, cytotoxic, cardiotoxic, and enterotoxic [5-7].

Our investigations pointed strongly to the likelihood of cross-contamination between ready-to-serve food items and raw seafood ingredients during preparation and storage. This is evident from the widespread contamination of food poisoning bacteria in food and environmental samples. It also indicates lapses in food and personal hygiene practices. Storage of contaminated food at ambient temperatures further contributed to the rapid multiplication of *V. parahaemolyticus* to reach high infectious doses.

A similar outbreak of 34 cases of *V. parahaemolyticus* food poisoning was reported in 1983. It was also traced to the consumption of Indian rojak from a market stall, coincidentally also at Geylang Serai[8]. In that outbreak, seven cases were hospitalized and 27 cases sought outpatient treatment. All of them recovered. The incubation period ranged from 5.5 -18.5 hours. Investigations showed that raw and cooked foods were haphazardly stored in an overcrowded refrigerator maintained at 10 degrees Celsius. Abundant drippings from raw cuttlefish were observed to contaminate cooked gravy stored in uncovered containers on the lower shelves. Swabs taken from the drippings and contaminated gravy tested positive for *Vibrio parahaemolyticus*. The bacteria were also isolated from one of the cases hospitalized.

The two deaths associated with this outbreak are reminiscent of the fatalities due to *V. parahaemolyticus* reported in the 1950 outbreak in Osaka, where autopsies revealed extensive damage to the stomach, other components of the gastrointestinal tract, and other internal organs. That outbreak was associated with the consumption of shirasu, small semi-dried fish [5, 9].

To prevent a recurrence of the current outbreak, food handlers should be constantly reminded to observe good food and personal hygiene practices at all times. The public should also take note of the hygiene and general cleanliness of the food premises when eating out.

*(Reported by Suhana S, Tang ZC, Mak TM, Foong BH, Ooi PL and James L, Communicable Diseases Division, Ministry of Health)*

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# HIV-positive cases detected during medical care versus voluntary HIV screening in Singapore – how are they different?

The notification rate of human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) in Singapore has been on the rise from 3.9 per million population in 1987 to 71.9 per million population in 2003 and further increased to 117.8 per million population in 2007. Men constituted about 90% of all HIV-infected cases. Since 1985, the majority of cases had been infected through sexual con-

tact (heterosexual 68%; homosexual 19%; bisexual 7%). More than 70% of the cases were detected during medical care, while voluntary HIV screening constituted about 10%, contact tracing 7% and the others were mainly from HIV testing in military settings, prisons and drug rehabilitation centres (*Fig.* 8).

A retrospective study was conducted to examine the differences in epidemiological profile of HIV-positive cases detected during medical care and those detected as a result of voluntary HIV screening. The findings would help in the formulation of targeted and focused public health initiatives to educate and increase awareness of the disease and to encourage HIV screening.

## Materials and methods

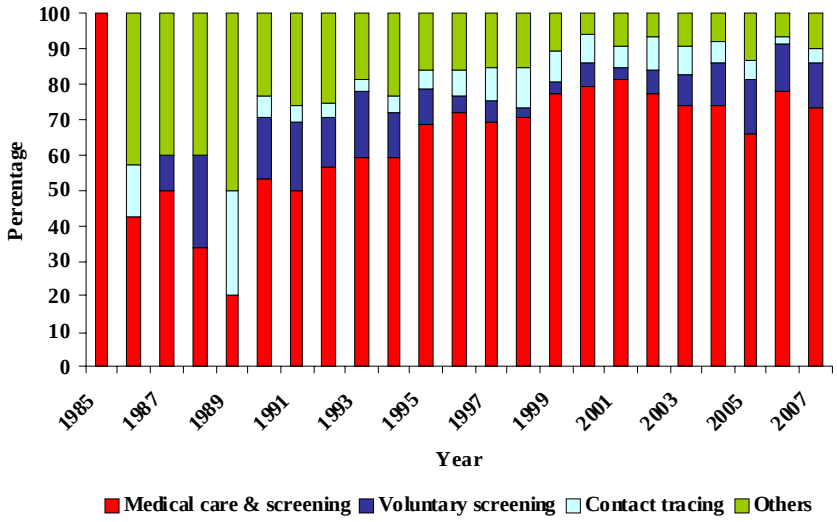
The data for the retrospective study was obtained from the national HIV registry. We included 2484 male cases infected via the sexual route and diagnosed from 1985 to 2007.

Associations between HIV-positive male cases detected during medical care versus those diagnosed as a result of voluntary testing and various epidemiological characteristics were examined using Chi-square test. We used the Kaplan-Meier method to describe the survival pattern of patients in these two groups. Multivariate logistic regression was used to determine independent epidemiological risk factors for diagnosis of HIV infection via medical care or voluntary screening, using backward stepwise selection based on maximum partial likelihood estimates. In the final model, all remaining predictors were significant at $p < 0.05$.

### Results

The cumulative proportion of HIV-infected male cases detected during medical care surviving till the 8th year since diagnosis was 51.8%, compared to 69.1% among HIV-infected male cases diagnosed as a result of voluntary screening. Bivariate analyses showed that cases detected during medical care were

**Figure 8**
**Distribution (%) of HIV-infected cases by mode of detection, 1985-2007**



more likely to be 30 years or older at diagnosis, of Chinese ethnic group, single, belonged to lower social-economic status (based on occupation), infected via heterosexual mode of transmission, had escorts and sex workers as sexual partners, and were diagnosed at late-stage HIV infection ($p$ <0.005) (*Table 8*). On the other hand, cases diagnosed as a result of voluntary HIV screening were more likely to enjoy higher social economic status, infected via homosexual/bisexual route, had regular and casual sexual partners only, and were not diagnosed at late-stage HIV infection.

Independent factors significantly associated with HIV detection through medical care as opposed to voluntary screening were age of diagnosis at 30 years and older, blue-collar workers or unemployed, infection via heterosexual transmission, sexual partners

**Table 8**

**Epidemiological characteristics (%) of HIV-infected male cases**

| Characteristics | Medical care (n=2174) | Voluntary screening (n=310) | $p$-value |
|---|---|---|---|
| **Age at diagnosis** | | | <0.0005 |
| Less than 30 years | 11.2 | 29.0 | |
| 30 years or older | 88.8 | 71.0 | |
| **Ethnic group** | | | 0.005 |
| Chinese | 88.8 | 84.2 | |
| Malay | 7.4 | 7.7 | |
| Indian | 2.8 | 6.5 | |
| Others | 1.0 | 1.6 | |
| **Marital status** | | | <0.0005 |
| Single | 56.7 | 74.8 | |
| Married | 29.7 | 17.4 | |
| Divorced/separated | 10.8 | 6.8 | |
| Widowed | 2.8 | 1.0 | |
| **Occupation** | | | <0.0005 |
| Professional / executive | 14.5 | 27.7 | |
| Administrative / service-oriented | 29.7 | 41.0 | |
| Blue-collar worker | 32.8 | 14.5 | |
| Unemployed | 16.8 | 8.1 | |
| Others | 6.2 | 8.7 | |
| **Mode of transmission** | | | <0.0005 |
| Homosexual/bisexual | 24.6 | 60.3 | |
| Heterosexual | 75.4 | 39.7 | |
| **Type of sexual partners** | | | <0.0005 |
| Regular only | 5.1 | 8.1 | |
| Regular and casual only | 20.2 | 51.9 | |
| Escorts and sex workers | 74.7 | 40.0 | |
| **Stage of HIV infection at diagnosis*** | | | <0.0005 |
| Without late-stage | 34.1 | 78.7 | |
| Late-stage | 65.9 | 21.3 | |

* Late-stage HIV infection was defined as having a CD4 cell count of less than 200 per $mm^3$ or developing AIDS-defining opportunistic infections at first diagnosis or within one year after HIV diagnosis when the cases were diagnosed.

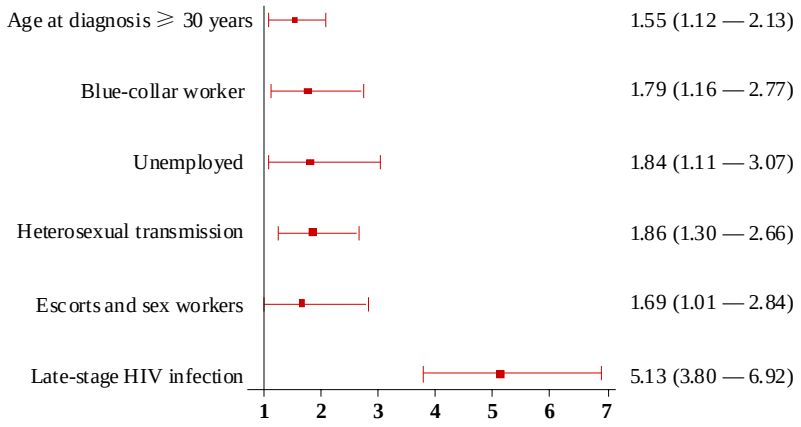
involving escorts and sex workers, and diagnosis at late-stage of the disease (*Fig.* 9).

## Comments

In September 2006, the US Centers for Disease Control and Prevention (CDC) issued its "Revised recommendations for HIV testing of adults, adolescents, and pregnant women in health-care settings", in which it recommended that screening for HIV infection should be performed routinely for all patients aged 13-64 years, and that health-care providers should initiate screening unless prevalence of undiagnosed HIV infection in their patients has been documented to be <0.1%.[1] To determine the HIV seroprevalence among hospital patient population, the Ministry of Health (MOH) undertook an unlinked HIV surveillance study of more than 3000 anonymous blood samples collected in hospitals from February to March 2007. It was found that 0.28% of the patients who were not previously known to be HIV-positive were actually infected.[2] In view of the findings from this study and that more than half of the HIV-positive cases had late diagnosis in Singapore, Changi General Hospital started to offer HIV screening for adult inpatients on a voluntary opt-out basis since 17 December 2007, followed by other acute public sector hospitals by the end of 2008. The objective of encouraging more HIV testing is to increase the proportion of individuals who are aware of their HIV status and hence reduce the number of HIV-infected patients who present with late-stage HIV infection.

Late-stage HIV infection in Singapore was mainly detected through diagnostic testing as a result of illnesses, similar to findings from other studies[3,4]. Regular HIV testing can help limit the spread of infection. Only 13% of the newly diagnosed cases were detected through voluntary testing in 2007, unchanged from 2006. Lack of anonymity in HIV testing is often cited as a reason for poor take-up rate of

**Figure 9**
**Adjusted odds ratios (95% CI) for HIV diagnosis during medical care versus voluntary screening***




* The referent category is the first row of the respective characteristic in Table 8.
CI: Confidence interval.

voluntary screening. To encourage more to come forward for voluntary screening, since August 2007, MOH has allowed medical clinics to offer HIV testing using oral-fluid or blood-based rapid HIV test kits, which can produce results in approximately 20 minutes.[5] The number of anonymous HIV test sites in Singapore has also increased from three to seven, with effect from 1 November 2008.[6]

This study examined the profile of HIV-infected cases diagnosed during medical care and voluntary screening. Educational campaigns concerning voluntary HIV screening could be targeted at specific groups of individuals at risk, so that those at higher risk of transmission could be detected early rather than during medical care at late-stage HIV infection.[7] Late-stage diagnosis arises mainly from HIV-related stigma and discrimination, which are the greatest barrier that discourages individuals from HIV testing and seeking treatment.[8] The Health Promotion Board (HPB) offers a range of HIV prevention and education programmes and has intensified its efforts. The findings from this study serve as important references for planning of targeted public health initiatives to facilitate early detection and treatment of HIV infection, so as to significantly delay the onset of AIDS and achieve better overall survival for people living with HIV/AIDS in Singapore.

*(Reported by Ang LW, Tey SH, James L, Communicable Diseases Division, Ministry of Health)*

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# Epidemiological surveillance of chikungunya in Singapore

## Introduction

Chikungunya is a mosquito-borne infectious disease caused by the chikungunya virus (CHIKV), an *Alphavirus* of *Togaviridae* family. Classically, there are 3 genetic lineages of CHIKV; West African, Asian and East, Central and South African (ECSA). The Asian lineage circulated in Asia until it was replaced by a newer strain of the ECSA type, which is believed to have emerged in Kenya in 2004. CHIKV causes a non-fatal, self-limiting disease characterized by abrupt onset of high fever, severe arthralgia, or arthritis, often associated with skin rash.

CHIKV was first isolated during an outbreak in Tanzania in 1952-53. The first CHIKV isolation in Asia was in Thailand in 1958. A 2002/2003 serosurvey on 531 healthy young adults in Singapore showed a low prevalence (0.3%) of chikungunya antibodies. Despite several, large-scale epidemics in the past, chikungunya remained largely neglected till its reemergence in the Indian Ocean Islands in early 2005. Since then, CHIKV has caused outbreaks in India, Sri Lanka, Singapore, Malaysia and Italy, focusing global attention to this newly emerging disease.

Unlike in Africa, where the virus is maintained in a sylvatic cycle, chikungunya in Asia has been an urban disease, typically found in dengue-endemic areas and transmitted largely by *Aedes aegypti* mosquitoes. However, the predominant *Aedes* sp. in locations such as Reunion Islands, where chikungunya emerged in 2005, was *Ae. albopictus*. The spread of chikungunya into rural areas during the later stages of outbreaks in India further confirmed the potential of *Ae. albopictus* mosquitoes in transmitting CHIKV. These changes were concurrent with the emergence of a strain having an alanine to valine substitution at codon 226 (A226V) of the envelope 1 (*E1)* gene. This mutation is known to increase the transmissibility of the virus by *Ae. albopictus* mosquitoes.

### Case surveillance

Singapore initiated a chikungunya surveillance system in late 2006. The medical community was apprised by the Ministry of Health (MOH) to look out for chikungunya cases among febrile patients with symptoms and signs (e.g., arthralgia, rash) suggestive of chikungunya. At the Environmental Health Institute (EHI), an active laboratory-based surveillance was set up among a network of general practitioners. Diagnosis of chikungunya was confirmed by detection of CHIKV RNA by a real-time reverse transcription-PCR (RT-PCR) assay. Confirmed cases were categorized as imported or local based on detailed travel history. Sequencing of CHIKV *E1* gene and phylogenetic analyses were also conducted.

### Entomological surveillance

Seven local transmission clusters representing major local outbreaks were selected for entomological investigation: Little India, Queen Street, Teachers' Estate, Kranji, Sungei Kadut, Mandai Estate and Bah Soon Pah Road. The georeferenced *Aedes* larvae collection data from the chikungunya clusters were

extracted from the Geographic Information System (ArcGIS) database of the National Environmental Agency, Singapore. The database was assembled using vector surveillance data obtained daily through area-wide inspection for mosquito breeding. The intensity of the presence of each species of *Aedes* larvae in individual cluster areas was expressed as the ratio between the numbers of *Ae. aegypti* and *Ae. albopictus* larvae collected. The calculation was based on the number of larvae collected 3 months before and after the first case of each cluster, either within 200-m radius of the case (in single case episodes) or within the boundary of cluster areas (widespread clusters).

Adult mosquitoes were also collected in each cluster area by using the sweep-net method, the Biogents (BG) Sentinel Traps (4-15 traps) or both. The adult collections were conducted in and around the location/s with the highest case intensity within 1-week of the outbreak reporting. The adult survey was carried out once in all areas, except for Kranji Way, where it was carried out twice with a gap of 1 week between each collection. Adult *Aedes* mosquitoes were crushed individually and RT-PCR was performed as for serum samples to detect CHIKV. The isolated viruses were sequenced and analyzed.

## Results

### Epidemiological findings

Of 1,375 serum samples tested at the EHI from December 2006 to December 2007, 10 were positive for CHIKV. All of them were imported infections from India, Maldives, Sri Lanka and Indonesia.

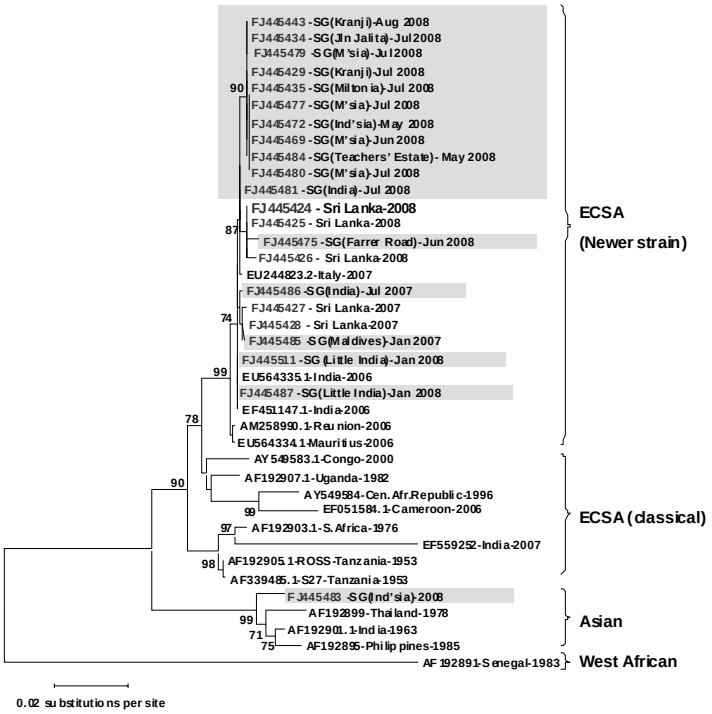
In 2008, more than 7,000 samples from general practitioners, hospitals and active case detection by MOH were tested by the end of September. The first locally acquired chikungunya case was detected in the Little India area in January 2008 by a general practitioner involved in the chikungunya surveillance network. A total of 13 locally acquired chikungunya cases were confirmed by PCR before the outbreak was brought under control in February 2008.

After the first episode of transmission, the local scenario was quiet until May 2008, except for 6 cases imported from Sri Lanka (n=2), Indonesia (n=3) and Malaysia (n=1). During May-June period, 2 brief episodes of local transmission occurred in suburban residential areas; Teachers' Estate area in late May (2 cases) and Farrer Road area in early June (1 case). There were no additional, locally acquired cases associated with those 2 episodes. The number of indigenous cases escalated in July 2008 coinciding with a rise in imported cases from Malaysia. In overall, 231 cases comprising of 108 imported and 123 indigenous infections were reported by the end of September 2008. Of the imported cases, 92% (n = 99) had travel history to Malaysia, largely to the state of Johor, whereas the 123 local cases were distributed across 25 different locations, mainly in rural industrial and farming areas of Singapore such as Kranji, Sungei Kadut and Bah Soon Pah Road.

### Virological findings

Phylogenetic analysis of *E1* gene of CHIKV from 85 imported and locally acquired infections showed that 84 infections were due to viruses of the newer strain of ECSA genotype (*Fig. 10*). CHIKV isolated from the remaining infection was of Asian lineage and was imported from Indonesia (*Fig. 10*). All ECSA-type viruses formed a monophyletic clade together with isolates from India, Sri Lanka, Italy and the Indian Ocean Islands (*Fig. 10*). The viruses iso-

**Figure 10**
**Phylogenetic analysis of the CHIKV envelope 1 (*E1*) gene.**




*The maximum likelihood method was used to construct the phylogenetic tree using 1002 nucleotides of the sequence of* E1 *gene, spanning from codons 91 to 424. The tree included 17 isolates detected in Singapore (shaded), 5 Sri Lankan isolates sequenced at the EHI, and 17 sequences retrieved from the GenBank database to represent all known phylogenetic lineages. All isolates emerged in Indian Ocean Islands and Asia after 2005 are shown under ECSA (Newer strain). In the tree, all sequences are labelled with GenBank accession numbers, country of origin, and are isolated by year/month. In addition, all locally acquired and imported Singapore isolates are labelled with the reported area and country of origin, respectively within parentheses. Only the bootstrap values >70 are shown on branches. ECSA = East, Central and South African genotype, Ind'sia = Indonesia, M'sia = Malaysia, SG = Singapore.*

lated during the first outbreak in the Little India area clustered closely with Indian and Maldivian isolates reported in 2006. These viruses were wild-type (alanine) at amino acid residue 226 (A226) of the *E1* gene. In contrast, viruses isolated from all areas after May 2008, except for the isolate from the third episode in Farrer Road area, clustered with those imported from Malaysia. The third local episode was due to a CHIKV isolate closely related to Sri Lankan strains (*Fig. 10*). All viruses reported after May 2008 possessed the valine substitution (A226V) at amino acid residue 226 of the *E1* gene.

Besides A226V, viruses of the second local outbreak showed a combination of two synonymous mutations uniquely seen in CHIKV of Malaysian origin; C300T and A363G of the *E1* gene. On the other hand, CHIKV isolated during the third local episode showed a unique combination of mutations typically seen in Sri Lankan isolates; 2 synonymous (A105G and C1308T) and a non-synonymous mutation [A633C (K211N)] of the *E1* gene. These observations demonstrated that the first, second and third indigenous episodes of chikungunya transmission in Singapore were most likely due to independent importations of genetically and geographically distinct CHIKV from the Asian region.

### Entomological findings

*Aedes* larval collection data showed that *Ae. albopictus* was the predominant species in all cluster areas, except Little India and Clive Street areas, where the first outbreak occurred (*Table 9*). To strengthen this observation, the adult mosquito surveillance also yielded *Ae. aegypti* only in the Little India cluster. Adult *Ae. albopictus* mosquitoes from the Kranji Way (9.1%, 7/77) and Bah Soon Pah Road (15.8%, 6/38) areas were positive for CHIKV by RT-PCR. The *E1* gene sequences of those 13 *Ae. albopitcus*-borne CHIKV were identical to sequences of strains imported from Malaysia. All mosquito-borne viruses possessed the A226V substitution.

## Discussion

Chikungunya is an emerging infectious disease of public health importance in Singapore. Owing to intensive human trafficking, population density and vector availability in Singapore, timely and effective disease control is required to minimize the risk of chikungunya outbreaks. Since its emergence in the local scene in January 2008, entomological and virological investigations have assisted in the epidemiological assessment of CHIKV spread in Singapore.

Phylogenetic data indicated that the first, second and third episodes of local transmission from January to June 2008 were due to 3 genetically distinct viruses largely circulated in India, Malaysia and Sri Lanka respectively. The lack of local CHIKV transmission between the first three indigenous outbreaks and unique genetic characteristics of viruses involved in each episode strongly indicated the possibility of independent importations of CHIKV. This finding was further supported by the fact that 6 imported cases reported during the first and second episodes included cases imported from Malaysia and Sri Lanka. However, all cases reported after July 2008 were due to a single strain of Malaysian origin, which was highly similar to CHIKV strain of the second outbreak. It is now known that 2006-2008 chikungunya outbreaks in Malaysia were due to a virus of the ECSA lineage. This evidence points to the interconnectedness of the simultaneous chikungunya

outbreaks in Singapore and Malaysia, which is not unexpected given the close proximity and porous borders between these 2 countries.

Entomological surveillance revealed a difference in the vector species involved in the first and subsequent outbreaks in Singapore. Adult mosquito and larval surveillance in the vicinity of the first outbreak area (Little India and Clive Street) predominantly yielded *Ae. aegypti* (*Table 9*). Both Little India and Clive Street areas are generally highly urbanized areas with sparse vegetation, which could explain the abundance of *Ae. aegypti*. However, large clusters of chikungunya were generally seen in less urbanized areas (*Table 9*) where *Ae. albopictus* was typically the predominant vector species. Detection of CHIKV in *Ae. albopictus* mosquitoes further confirmed its role in CHIKV transmission in less urbanized areas of Singapore.

Of note, CHIKV strains isolated from *Ae. aegypti* abundant, urban areas showed alanine at codon 226 (A226) of the *E1* gene. In contrast, all CHIKV strains isolated from semi-urban and rural areas, from where *Ae. albopictus* was largely found, showed A226V substitution. Recently, It was shown that CHIKV strains with A226V substitution replicate better than the wild-type virus in *Ae. albopictus*. The current evidence further indicate that though the transmission potential of the wild-type virus is optimum for *Ae. aegypt* mosquitoes, A226V substitution confers greater vector competence in *Ae. albopictus* mosquitoes, making the latter a better vector of the mutated strain than *Ae. aegypti*. This finding may result in selection for the mutated strain in areas where *Ae. albopictus* mosquitoes are abundant. Although the competence of *Ae aegypti* mosquitoes in transmitting the virus with A226V in Singapore remains uncertain,

**Table 9**

**Summary of the entomological surveillance findings**

| Location | Type | No. of cases[a] | Adult mosquito collection[b] | | *Aedes* larval ratio[c] | |
|---|---|---|---|---|---|---|
| | | | ***Ae. aegypti*** | ***Ae. albopictus*** | | |
| Little India | Urban | 13 | 10 | 0 | 1.77:1 | (826:466) |
| Queen Street | Urban | 1 | 0 | 2 | 0:1 | (0:127) |
| Teacher's Estate | Suburban | 1 | 0 | 10 | 0.03:1 | (40:1261) |
| Kranji Way | Rural | 41 | 0 | 77 | 0.04:1 | (1129:26546) |
| Sungei Kadut | Rural | 33 | 0 | 7 | 0.001:1 | (70:77086) |
| Mandai Estate | Rural | 11 | 0 | 23 | 0.02:1 | (30:1260) |
| Bah Soon Pah Road | Rural | 21 | 0 | 45 | 0:1 | (0:3465) |

[a] The numbers given are preliminary data from press releases.

[b] Species of adult mosquitoes collected in each location where entomological surveillance was conducted. The numbers do not necessarily represent adult mosquito density in each area, as the numbers of traps and man-hour committed were not consistent.

[c] Ratio between the number of *Ae. aegypti* : *Ae. albopictus* larvae collected through routine surveillance at respective locations. Number of larvae (*Ae. aegypti : Ae. albopictus*) collected in each cluster is shown in parentheses.

the known evidence may therefore explain why the mutant virus with A226V caused outbreaks in less urbanized areas in Singapore where *Ae. albopictus* dominates, but had little impact on *Ae. aegypti* dominant urbanized areas and vice versa. A similar observation has also been made in India, where the emergence of CHIKV with A226V was first reported in rural areas of Kerala region that are predominantly inhabited by *Ae. albopictus* mosquitoes. The low transmission rate of the mutant virus in urban and suburban Singapore could also be due to the aggressive dengue control programme, which targets mainly *Ae. aegypti* mosquitoes.

Based on these observations, National Environment Agency's *Aedes spp.* control strategy was revised and operations were expanded, especially into areas where *Ae. albopictus* mosquitoes are present . Because *Ae. albopictus* mosquitoes generally prefer to dwell outdoors, in contrast to *Ae. aegypti*, measures such as outdoor fogging and residual spraying of external walls were conducted in chikungunya outbreak areas. The longitudinal monitoring of *E1* gene sequences of CHIKV is in progress to monitor the possible origins of viral strains causing the local chikungunya episodes in Singapore. Our results showed that Singapore, being a travel hub and a cosmopolitan city, is vulnerable to multiple importations of CHIKV. The aggressive A226V variant of the ECSA genotype that has established itself in the region is posing a challenge to Singapore. Because *Ae. albopictus* is a common vector species in the region, the establishment of the A266V CHIKV variant in the region may continue to pose challenge in the years to come.

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