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Zika fever in Southeast Asia and islands of the Pacific Ocean

Background

Zika virus was first isolated in 1947 from a sentinel rhesus monkey stationed on a tree platform in the Zika forest, Uganda. The virus is a member of the *Flaviviridae* family and is a single-stranded RNA virus with a positive-polarity RNA genome.[1,2] It is transmitted to humans by mosquitoes, and thus far, only *Aedes* mosquitoes are known to be the vector of Zika virus. The *Aedes* species that can carry the virus includes *Aedes africanus, Aedes apicocoargenteus, Aedes luteocephalus, Aedes furcifer, Aedes vitattus* and *Aedes aegypti*.[3] Other pathogenic vector-borne flaviviruses include dengue, West Nile, Japanese encephalitis and yellow fever.

The first human case of Zika virus infection was reported in Nigeria in 1954. Subsequently between 1968 and 2002, 606 strains of Zika virus including 10 human strains were isolated in Central and West Africa.[2] Other than being endemic in Africa, Zika virus is also present in Asia. In 1966, Zika virus was isolated from a pool of *Aedes aegypti* mosquitoes caught from shop houses in Pahang state of Malaysia.[2] Seven patients in Central Java of Indonesia were reported to be infected with Zika virus in 1977.[4]

Epidemiology

Islands in Pacific Ocean

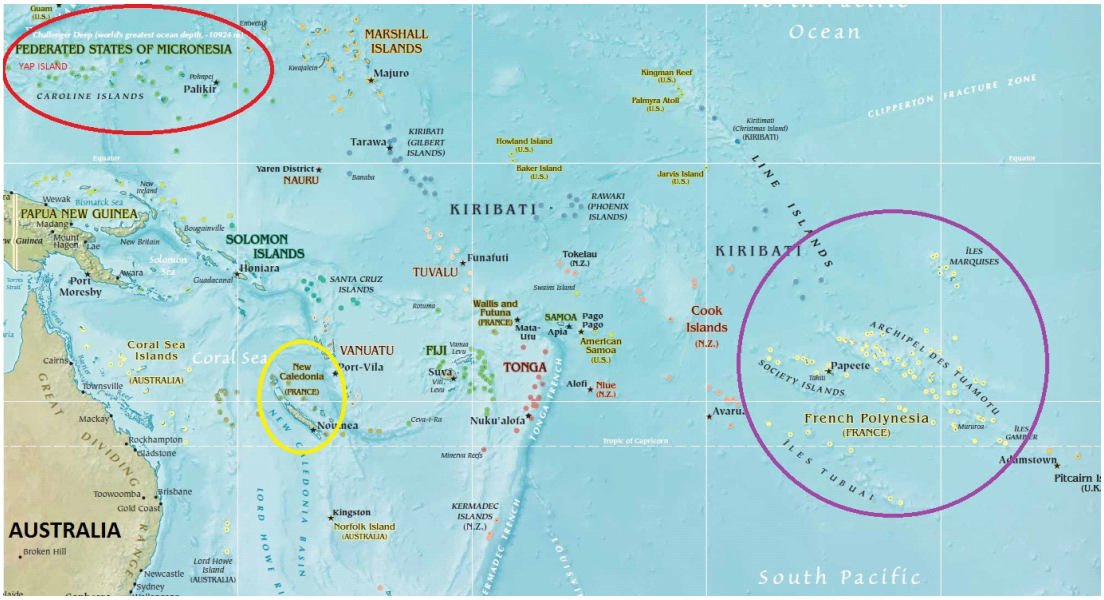
The first outbreak of Zika fever in the Pacific Ocean was reported in **Yap Island** in 2007 (*Fig. 1*). Yap Island is an archipelago of the western Caroline Islands, Federated States of Micronesia, about 850

miles east of Mindanao in the Philippines. Of the 185 suspected cases of Zika fever notified, 49 were confirmed and 59 were classified as probable.[5] Zika virus RNA was detected in 15 cases, and no dengue virus RNA was found among the tested samples. The confirmed and probable cases were aged between one and 76 years, and 66 (61%) of the cases were female. None of the cases required hospitalisation, or had hemorrhagic complications, and no death was reported. Of the 6,892 surveyed residents who were three years old and above, an estimated 5,005 (73%) persons were infected with Zika virus. Among these cases, 919 (18%) reported symptoms attributable to Zika fever. *Aedes hensilli* was the predominant species captured during the outbreak, but none of the tested mosquitoes was positive for Zika virus

Recently, an outbreak of Zika fever was reported in **French Polynesia** since October 2013 (*Fig. 1*). This was the second reported outbreak of Zika virus in the Pacific Ocean region. French Polynesia is an overseas country of France and consists of over a hundred islands and atolls. It is grouped into five archipelagoes: the Society Islands, the Austral Archipelago, the Tuamotu Archipelago, the Marquesas Archipelago and the Gambier Archipelago. The population of French Polynesia was estimated at 294,935 in 2011. French Polynesia has a tropical climate.

As of 28 February 2014, a total of 8,583 suspected cases of Zika fever were reported from most islands. Of the suspected cases, 388 were laboratory confirmed by reverse transcription polymerase chain reaction (RT-PCR).[6] The weekly number of cases had been on a decreasing trend since the peak in early December of 2013. By extrapolating the figures from the sentinel surveillance network, the number of patients with Zika virus infection that had sought

Figure 1
Map of Yap Island, French Polynesia and New Caledonia



Source of map: Central Intelligence Agency

medical attention during this period was estimated to be more than 30,000.[6] However, the total number of infections was believed to be higher as it was likely that many patients did not seek medical attention. Most of the cases did not required hospitalisation. French Polynesia is concurrently experiencing a dengue outbreak where a total of 1,741 cases were reported since February 2013. The circulating strain was DEN-1 and DEN-3.[6] Similar to Zika fever cases, the monthly number of dengue cases has been on a decreasing trend since December 2013.

Other than French Polynesia, **New Caledonia** in the Pacific Ocean also reported an ongoing outbreak of Zika fever since end of November 2013. New Caledonia is a special collective of France located in the southwest Pacific Ocean, 1,210 km east of Australia (*Fig. 1*). As of 6 March 2014, New Caledonia recorded a total of 180 confirmed cases of Zika virus infection between 2013 and 2014.[7] Of the confirmed cases, 32 were reported to be imported cases from French Polynesia and 148 were indigenous cases. Coincidentally, New Caledonia is experiencing an increased number of dengue cases since September 2013 when a total of 107 cases were reported as of 7 March 2014.[8]

On 6 March 2014, **Chile** reported one confirmed case and 40 suspected cases of Zika fever in **Easter Island**, a special territory of Chile located at south-eastern Pacific Ocean[9] (*Fig. 2*). The cases had onset of symptoms related to Zika fever mainly in February.

In the midst of the on-going outbreaks in the South Pacific region, **Japan** reported two imported cases of Zika fever in citizens returning from Bora Bora in **French Polynesia** in December 2013 and January 2014, respectively.[10] The first case involved a Japanese man in his mid-twenties who was previously healthy. He presented symptoms of fever, headache, arthralgia and rash at a hospital in Japan. His blood sample was tested positive for Zika virus RNA by RT-PCR. The second case involved a previously healthy Japanese woman in her early thirties who presented symptoms of retro-orbital pain, fever, rash, and itches. Although her blood sample was tested negative for Zika virus by RT-PCR, Zika virus -specific IgM antibodies were detected by serology. Zika virus RNA was also detected in the urine sample from the case. Prior to this finding, there had been no report of Zika virus detection in the urine of an infected case. Genetic analysis of the partial Zika virus (E-protein genome sequence, 470 base pairs) isolated from the second case revealed that its sequence was 99.1% identical to the strain isolated from Cambodia in 2010, and 97.9% identical to the strain from Yap islands in 2007. In February 2014, **Norway** reported a case of

Figure 2

Map of Easter Island

Source of map: Central Intelligence Agency

Zika fever in a Norwegian who had travelled to Tahiti, another island in French Polynesia.[10]

Southeast Asia

In 2013, two tourists from **Canada** and **Germany** were diagnosed with Zika fever after returning from **Thailand** in May and December, respectively. The 45-year-old Canadian woman visited Bangkok and Phuket Island during her trip, [11] and developed symptoms including headache, fever, chills, backache, papular rash and inflammation at the sites of mosquito bites. The 53-year-old German man visited Phuket, Krabi, Ko Jum, and Ko Lanta, and also suffered several mosquito bites.[12] He developed maculopapular rash, malaise, fever, and shivering which lasted for four days. Initial serological tests for both cases were positive for dengue IgM antibody but negative for IgG antibody. Gene sequencing of the amplicon (780 base pairs) isolated from the first case showed 99% identity to the Zika virus strain from the Yap Island outbreak. Zika virus specific neutralizing antibodies were detected by neutralization assay in both cases.

Last year, **Australia** also reported a case of Zika fever in one returning citizen from **Indonesia** who complained of fever and rash.[13] Reports of cases of Zika virus infection in travellers to Thailand and Indonesia may indicate the presence of an undetected outbreak or endemicity of the virus in these countries.

The United States (US) Naval Medical Research Unit No. 2 (NAMRU-2) had been conducting surveillance for acute fever in **Cambodia** since 2006. Through this surveillance system, a case of Zika fever was detected in a three-year-old boy from Kampong Speu province in 2010.[14] The boy developed fever, sore throat, cough and headache which lasted for four days. His blood samples were tested negative for chikungunya and dengue virus IgM and IgG antibodies on acute- and convalescent-phase serum by ELISA. Although his blood sample was tested positive for flavivirus by universal flavivirus RT-PCR, species-specific PCR was negative for dengue and Japanese encephalitis, which are endemic in Cambodia. Nucleic acid sequencing of the isolated amplicon (RNA fragment) produced a 100 base pairs fragment with 100% sequence identity to the Zika virus strain from the Yap Island outbreak. In addition, antibody was detected by hemagglutination-inhibition tests on paired serum samples. This was the first reported case of Zika fever in Cambodia.

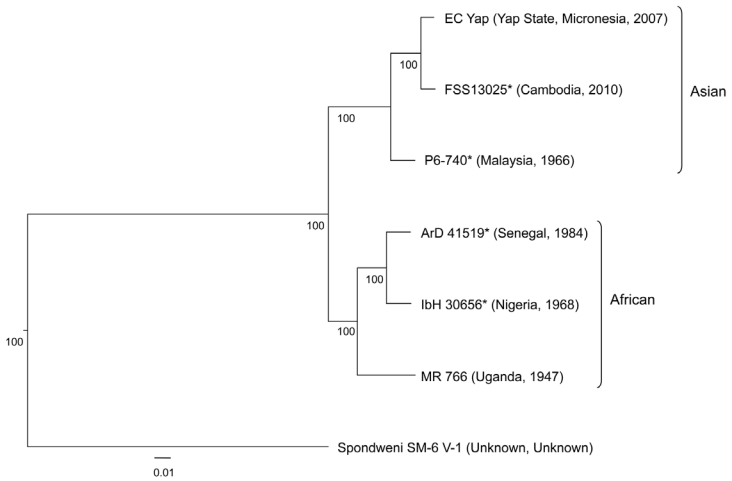
Genetic analysis

Phylogentic analyses of six Zika virus strains collected between 1947 and 2010 indentified two major lineages.[15] Three strains isolated from Yap Island, Cambodia and Malaysia were classified as the Asian lineage, while the remaining strains from Nigeria, Uganda, Senegal were classified under the African lineage (*Fig. 3*). The analysis suggested that the common ancestor of the Cambodian strain had been circulating in Southeast Asia (SEA) since at least the mid-1900s. Therefore, the virus was either introduced into Cambodia in 2010 or had been circulating unnoticeably until it was detected in 2010. The strain that caused the outbreak in Yap Island was most likely introduced into the island through travel or trade activities by an infected person, enzootic host species, and/or an infected and subsequently infective mosquito from Southeast Asia.[15]

Clinical presentation

The symptoms of Zika virus infection include fever, headache, red eyes, rash, muscle aches, and joint pain. The symptoms are usually milder compared

Figure 3
Lineages of Zika virus



Source: Haddow AD, Schub AJ, Yasuda CY et al. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. PLoS Negl Trop Dis 2010; 6(2): e1477. Available at: http://www.plosntds.org/article/info%3Adoi%2F10.1371%2Fjournal.pntd.0001477

to dengue and chikungunya and last for four to seven days.[16] None of the cases in the Yap Island's outbreak required hospitalisation. However, the health authority of French Polynesia reported a significant concurrent increase in neurological syndromes [including Guillain-Barré syndrome (GBS), and other neurological complications] and autoimmune illnesses (including immune thrombocytopenic purpura, ophthalmic and cardiac complications) with the Zika fever outbreak. As there is a concurrent dengue outbreak in the region, the cause of the complications and their possible links with Zika virus or dengue virus infections are being investigated.[6] No neurological complications have been reported to date in New Caledonia.

Investigations in French Polynesia identified one case of GBS that was associated with Zika fever.[17] The case was a woman in her early forties with acute articular rheumatism who had been hospitalised for neurological deficits. She developed tetraparesis predominantly in the lower limbs, diffuse myalgia, asymmetrical bilateral peripheral facial palsy, chest pain related to ventricular tachycardia, and orthostatic hypotension. Seven days prior to onset of paresis, the case had developed influenza-like illness, and was suspected to have Zika fever in view of the ongoing outbreak. She was eventually discharged after 13 days of hospitalisation, and had gradual recovery of the muscle strength of her lower limbs and face. Blood samples of the case tested positive for both Zika and dengue virus specific antibodies by ELISA. Further serological results indicated recent Zika infection and previous dengue infection. Although little is known on the underlying mechanism between Zika infection and GBS, the researchers suggested that dengue infection could have been a predisposing factor for developing GBS during Zika fever outbreak, as the latter had been associated with GBS.[17]

Treatment and prevention

Currently, there is no vaccine to prevent Zika virus infection. Non-steroidal anti-inflammatory and non-salicylate analgesics are usually prescribed to manage the patient's symptoms such as fever and joint pain.[18] Travellers going to countries where human cases of Zika fever have been reported are advised to protect themselves to minimise mosquito bites. The usual preventive measures such as use of insect repellent during outdoor activities, wearing of long-sleeved shirts and long pants are recommended.

Diagnosis

Currently, the main diagnostic tools available for Zika virus are serology and RT-PCR. The use of serology to diagnose Zika fever is challenging as the level of antibodies in serum may be low if tested during the initial phase of infection.[18] Serology is indicated when blood samples are collected after acute infection as the antibodies level is higher. However, serology is not highly specific as there is cross-reactivity between Zika virus and other flaviviruses.[4] This was demonstrated in the Zika fever outbreak in Yap Island where all of the sampled patients showed some limited degree of cross-reactivity with heterologous flaviviruses.[4] RT-PCR is commonly used during acute infection period when there is a high viral load in the blood. Presently, there are no commercial standardized PCR and serology test kit for Zika virus available in the market.

Local situation

Human cases

In Singapore, Zika virus is not a legally notifiable disease under the Infectious Disease Act (IDA). To date, no cases of human infection with Zika virus have been reported locally, although the vector for Zika virus, *Aedes* mosquitoes is present in Singapore. In 2012, a study conducted on hospital patients with dengue-like illness revealed that none of the 88 samples tested were positive for Zika virus.[19] The researchers had designed a specific and sensitive one-step PCR with internal control to test on the samples which were previously negative for dengue and chikungunya.

Vector

Studies conducted by the Environmental Health Institute (EHI) of National Environment Agency (NEA) reported that *Aedes albopictus* and *Aedes aegypti* were able to spread Zika virus under laboratory condition.[3] The Zika virus RNA was isolated from the mosquitoes' saliva and detected using a one-step real-time RT-PCR as previously described in the investigation of Zika virus outbreak in Yap Island.

Risk assessment

Although Zika virus has been detected in several Southeast Asian countries, the true prevalence of the virus in its vector and human populations in this region is not well understood. Thus far, there have not been reports of outbreaks of human infection with Zika virus in Southeast Asia, and none of the local dengue-like patients were tested positive. Nonetheless, in the presence of travel and trade, the importation of Zika virus is possible. In addition, with the presence of the vector - the *Aedes* mosquitoes in Singapore, there is a possibility of onward transmission of Zika virus in the community in the event of an imported case as seen in the case of New Caledonia.

Since cases of Zika fever have been reported in neighbouring countries in the region, the occurrence of sporadic importation of cases of Zika fever into

Singapore cannot be excluded. Such cases might have gone undetected as the symptoms of Zika fever are similar to that of dengue and chikungunya infection.

In view of the mild symptoms associated with Zika virus infection, the risk of serious public health impact to Singapore in the event of a community outbreak is assessed to be low. Vector control remains the cornerstone for controlling mosquito-borne diseases, including Zika fever. Ongoing vector control programmes by NEA against dengue and chikungunya will also control transmission of Zika virus.

(Contributed by Public Health Intelligence Unit, Epidemiology & Disease Control Division, and Communicable Diseases Division, Ministry of Health)

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An outbreak of microsporidial keratoconjunctivitis following exposure to mud in a rugby tournament

Introduction

Microsporidia are spore-forming unicellular intracellular parasites which have recently been shown to be fungi based on phylogenetic analyses [1]. They are ubiquitous in the environment and at least 14 species have been implicated in human infections [2]. Human ocular microsporidiosis first came into prominence as an opportunistic infection in patients with acquired immune deficiency syndrome in the 1980s and, subsequently, in other immunocompromised patients [3,4]. In the 1990s, *Vittaforma corneae* (formerly known as *Nosema corneum*) [5] was first described as the cause of corneal infection in an immunocompetent person [6] and disseminated infection in an immunocompromised patient [7]. Since the early 2000s, microsporidial keratoconjunctivitis has been increasingly reported, mostly in Singapore [8-10] and India, [11] among healthy, immunecompetent persons. The infections result predominantly from eye contact with soil or mud in outdoor activities.

Notification

On 18 May 2012, the Ministry of Health (MOH) received a notification from the Centre for Health Protection (CHP), Hong Kong, of a suspected outbreak of microsporidial keratoconjunctivitis affecting 18 boys of a rugby club who had participated in an international rugby tournament in Singapore on 21-22 April 2012. We report the epidemiology, clinical features and laboratory findings of the outbreak.

Methods and materials

After the notification, epidemiological investigations were undertaken immediately. A medical

alert of the outbreak was circulated to all registered medical practitioners. Local cases identified by clubs and medical practitioners (including ophthalmologists) were interviewed by telephone or email using a set of questionnaires to obtain relevant clinical and epidemiological data such as age, gender, nationality, clinical signs and symptoms, date of onset of illness, medical treatment sought, and details of activities at the tournament.

Corneal scrapings collected by ophthalmologists were tested by microscopy; modified trichrome staining was used to detect microsporidial spore-like structures. Samples demonstrating these structures were then subjected to DNA extraction and microsporidia-specific PCR sequencing following previously described protocols [12]. Soil water samples collected from the tournament venue on 22 May 2012 were tested for microsporidia by the Department of Pathology, Singapore General Hospital. The strategy of serial centrifugation [13] was adopted, followed by modified trichome staining to detect the presence of microsporidia spore-like structures. Samples demonstrating these structures also underwent microsporidia-specific PCR sequencing for species identification [14].

To investigate possible infections in participants from outside Singapore, the International Health Regulations (IHR) National Focal Points (NFPs) of the countries involved and team representatives of foreign rugby clubs were contacted for information on participants in whom symptoms of eye infection developed after the tournament.

The rugby tournament involved 1,594 players in 107 teams from rugby clubs from Singapore, Hong Kong, Malaysia, Australia and the United Arab Emirates. There were 1,511 boys and 83 girls; Singapore clubs were represented by 1,122 male players and 69 female players. Two types of rugby were played: 'touch' rugby and 'full-contact' rugby.

A probable case of microsporidial keratoconjunctivitis was defined as follows: two or more of the following eye signs or symptoms - redness, pain/foreign body sensation, itch, blurred vision, photosensitivity and/or epiphora – developing from two to 30 days after the person participated in the rugby tournament plus a clinical diagnosis by an ophthalmologist using slit lamp biomicroscopy. Biomicroscopy typically revealed the classic coarse, multifocal, granular punctuate epithelial keratitis, along with mild follicular or papillary conjunctivitis. The case was classified as confirmed if corneal scraping were collected and microsporidia spores were shown by microscopy and modified trichrome staining.

Results

Of the 72 local players traced and interviewed, 48 cases (46 probable and two confirmed) among the boys and one probable case among the girls were identified. Among foreign participants, four confirmed cases were identified (*Table 1*). Besides these affected players, five probable cases among Singapore residents, comprising two coaches, two spectators and one referee were identified. In addition, six probable sporadic cases not linked to the tournament but had participated in other outdoor activities with exposure to mud were also notified during the outbreak period.

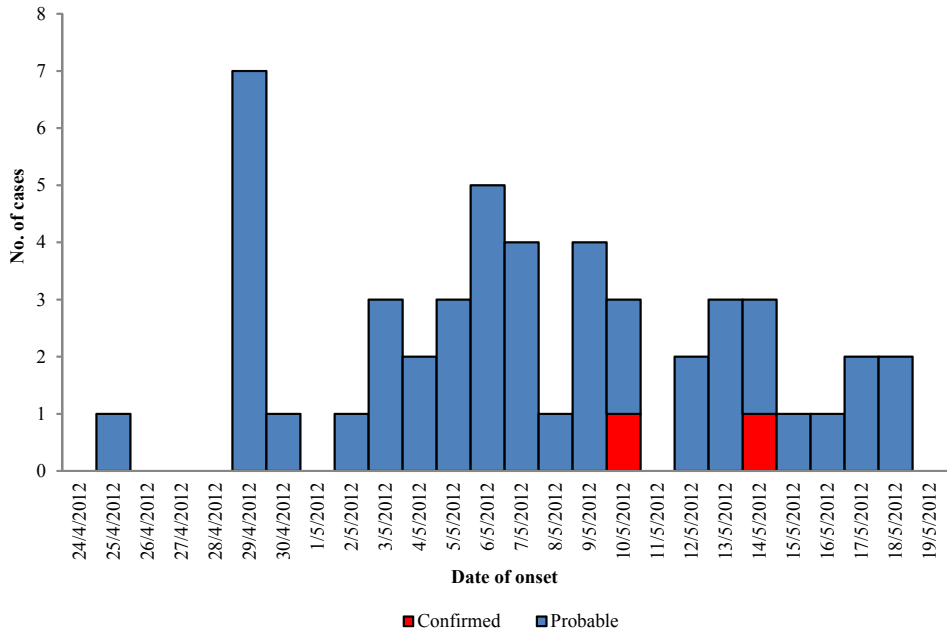
Forty -six (93.9%) of the 49 affected Singapore players interviewed were children of expatriates. Symptoms developed from 25 Apr to 18 May 2012 (*Fig. 4*). Their ages ranged from six years to 17 years (median age, 12 years). The attack rate of

Table 1

No. of cases and attack rates among players from 5 participating countries in an international rugby tournament, Singapore, 21-22 April 2012

Country	Total no. of players	Probable cases (%)	Confirmed cases (%)	Overall attack rate (%)
Singapore	1191	47 (3.9)	2 (0.2)	49 (4.1)
Hong Kong	82	0	3 (3.7)	3 (3.7)
Malaysia	281	0	1 (0.4)	1 (0.4)
Australia	19	0	0	0
United Arab Emirates	21	0	0	0
Total	**1594**	**47 (2.9)**	**6 (0.4)**	**53 (3.3)**

Fig ure 4
Onset of eye symptoms of 49 affected local participants after an international rugby tournament in Singapore, 21 - 22 Apr 2012



'full-contact' rugby players (5.7%) was significantly higher than that among 'touch rugby' players (0.5%) (p <0.0001) (*Table 2*).

The main presenting ocular symptoms were redness (100 %), pain/foreign body sensation (83.7 %), photosensitivity (77.6 %), blurred vision (75.5 %), itching (69.4 %), and epiphora (46.9 %). Twelve cases (24.5 %) had bilateral infection. The median incubation period, based on the interval between date of last exposure and onset of illness, was 15 days (range 3 – 26 days). None of the cases were hospitalised. All cases responded well to treatment.

Forty-six (93.9%) of the affected players reported having had mud enter their eyes while playing in the tournament. Of these, 87.8 % did not share any personal articles such as towels and handkerchiefs with other players, 24.5 % used the available shower facilities, and 83.7 % indicated that they had washed their faces with water from water hoses or mineral water bottles after each match.

Laboratory analysis of three corneal scrapings collected from two affected local players and one Malaysian player who sought treatment at a private eye centre in Singapore revealed microsporidia spore-like structures by microscopy with modified trichrome staining. One corneal scraping was confirmed as *V. corneae* by PCR sequencing with 97% sequence homology to at least two published *V. corneae* sequences. The same species was also identified in three affected players from Hong Kong rugby clubs [15]. Spore-like structures consistent with microsporidia were also detected in 12 out of 21 soil water samples (average dimensions 2.87 µm by 1.68 µm). *V. corneae* was detected in one soil water sample.

Discussion

This is a single-source microsporidial keratoconjunctivitis outbreak, to which several factors contributed. First, extreme weather (two days of heavy rain preceding the tournament and on its first day) resulted in the muddy condition of the field. Second, in 'full contact' rugby, the risk for exposure of the face and eyes to mud and ground water is high because the defensive players would have to stop the player with the ball by tackling him or her to the ground. Third, the limited washing facilities at the tournament venue resulted in many players having to wash up at home many hours after exposure to mud.

Table 2

No of cases and attack rates by type of rugby contact among local players in an international rugby tournament, Singapore, 21-22 April 2012

Category	No. of players	No of teams	Probable cases (%)	Confirmed cases (%)	Overall attack rate (%)
Touch rugby*	369	33	2 (0.5)	0	2 (0.5)
Full-contact Rugby#	822	48	45 (5.5)	2 (0.2)	47 (5.7)
Total	**1191**	**81**	**47 (3.9)**	**2 (0.2)**	**49 (4.1)**

***for boys between 6 and 8 years and all girls' teams**
 # for boys between 9 and 18 years

The main limitation of the study is that the majority of the reported cases were not confirmed by laboratory identification of microsporidia. We could not justify obtaining corneal scrapings from the affected players because the participating ophthalmologists became extremely aware of the characteristic signs and symptoms of microsporidial keratoconjunctivitis . In addition, because of limited amount of clinical materials available for testing of *V. corneae,* no further genetic studies were undertaken to establish their relatedness.

Microsporidial keratoconjunctivitis is an emerging eye infection in Singapore. Public health professionals should be aware that it may be prevalent in other countries when keratoconjunctivitis is considered as a diagnostic possibility.

(Based on Tan J, Lee P, Lai Y et al. Microsporidial keratoconjunctivitis after rugby tournament, Singapore. Emerg Infect Dis 2013; 19:1484-6)

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Outbreak of gastroenteritis caused by *Salmonella* Enteritidis associated with the consumption of food from a factory canteen

Introduction

Salmonellosis is one of the most common food-borne diseases in the world. An estimated 93.8 million cases of *Salmonella* gastroenteritis with 155,000 deaths occur annually throughout the world[1]. In 2012, Singapore reported 1,499 cases of salmonellosis of which 357 cases were caused by *Salmonella* Enteritidis. Consumption of contaminated poultry meat and eggs remains the chief source of human salmonellosis. In particular, studies have also identified poultry and eggs to be the most common sources of serotype Enteritidis infection[3,4].

On 21 Sep 2013 and 22 Sep 2013, the Ministry of Health (MOH) received two separate notifications of an outbreak of gastroenteritis involving 15 individuals who had consumed dinner provided by an in-house factory canteen on 20 Sep 2013.

This report describes the epidemiological, microbiological and environmental investigations of this outbreak.

Epidemiological investigations

The cases were identified and their personal particulars including age, gender and ethnicity were recorded. Clinical signs and symptoms, date of onset of symptoms, food items consumed and type of medical treatment sought were obtained. The canteen was inspected for hygiene irregularities.

The laboratory findings of stool samples of cases hospitalised were requested from the attending physicians. Stool samples were also requested from other cases and food and environmental samples were taken for microbiological analyses. The common enteropathogens tested included *Campylobacter, Salmonella, Staphylococcus aureus, Clostridium perfringens , Escherichia coli*, rotavirus and norovirus. All three food handlers from the canteen were referred to the Communicable Disease Centre, Tan Tock Seng Hospital, for medical screening and stool testing. The food handlers were also interviewed about food preparation processes.

A case was defined as a previously well individual who developed diarrhoea (two or more episodes within 24 hours) with or without accompanying fever, abdominal pain or vomiting, after consuming dinner from the factory canteen on 20 Sep 2013.

Findings

A total of 85 cases out of the 170 people who consumed dinner from the in-house canteen on 20 Sep 2013 were identified, giving an attack rate of 50%. All cases were male foreign workers who worked in an engineering project where the canteen was located. The mean age of the cases was 30 years. Their presenting symptoms were watery diarrhoea (100%), abdominal pain (95.3%), fever (85.9%), headache (77.6%), nausea (51.8%) and vomiting (24.7%). Of the 85 cases, 12 were hospitalised (14.1%) while the

rest sought outpatient treatment (85.9%). All have since recovered.

The only common meal that the cases had reportedly consumed prior to their onset of symptoms was the dinner provided by the canteen on 20 Sep 2013. The food items of the dinner which included rice, hard-boiled egg, stir-fried vegetables and fried chicken, were packed into individual food packets. The onset of illness was from 0000 hours on 21 Sep 2013 to 1700 hours on 24 Sep 2013 (*Fig. 5*). The incubation period ranged from 5 to 96 hours and the mean and median incubation periods were 29 and 24 hours, respectively.

Of the stool samples obtained from 57 cases, 32 (56%) tested positive for *Salmonella* Enteritidis. Genotyping of *Salmonella* Enteritidis cultured from stool samples (determined by multiple-locus variable number of tandem repeats analysis, MLVA), performed by the National Public Health Laboratory (NPHL), showed that all isolates were of *Salmonella* Enteritidis MLVA type F (*Fig. 6*). Out of three food handlers sent for screening, one was positive for the same strain of *Salmonella* Enteritidis. All three food handlers were asymptomatic before and during the incident, and none had any recent travel history.

Hygiene lapses were observed during the field inspection at the canteen. These included dirty cutlery and serving utensils, dirty receptacle for storing utensils and a dirty chopping board for cutting raw food.

Two food samples (raw eggs and ice) and four environmental swabs collected for microbial analyses were negative for all the food borne pathogens tested. However, two environmental swabs were found to have high total plate count (curry puff maker-3,200 MPN/swab; container for tongs-830,000 MPN/swab), total coliforms (container for tongs->1,100 MPN/swab) and faecal coliforms (container for tongs->1,100 MPN/swab).

Figure 5
Onset of illness of 85 reported cases of gastroenteritis who consumed dinner from a factory canteen on 20 Sep 2013

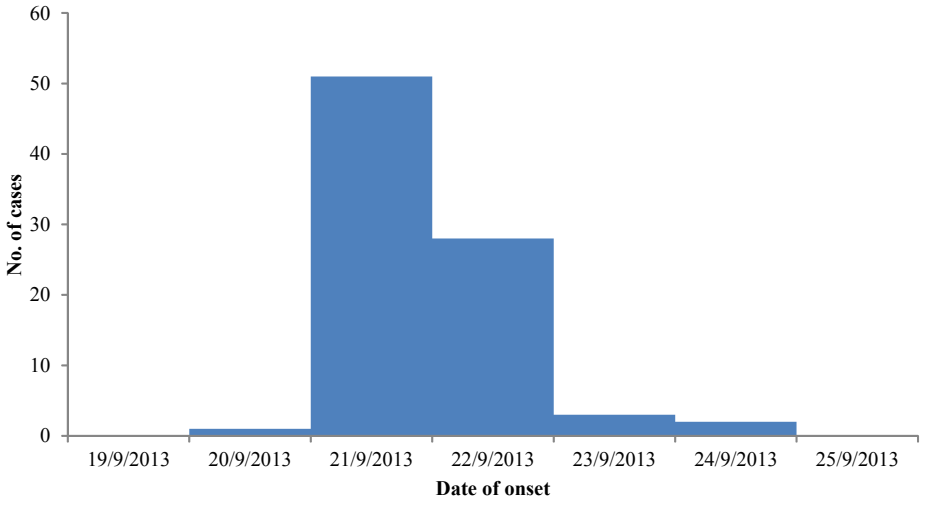
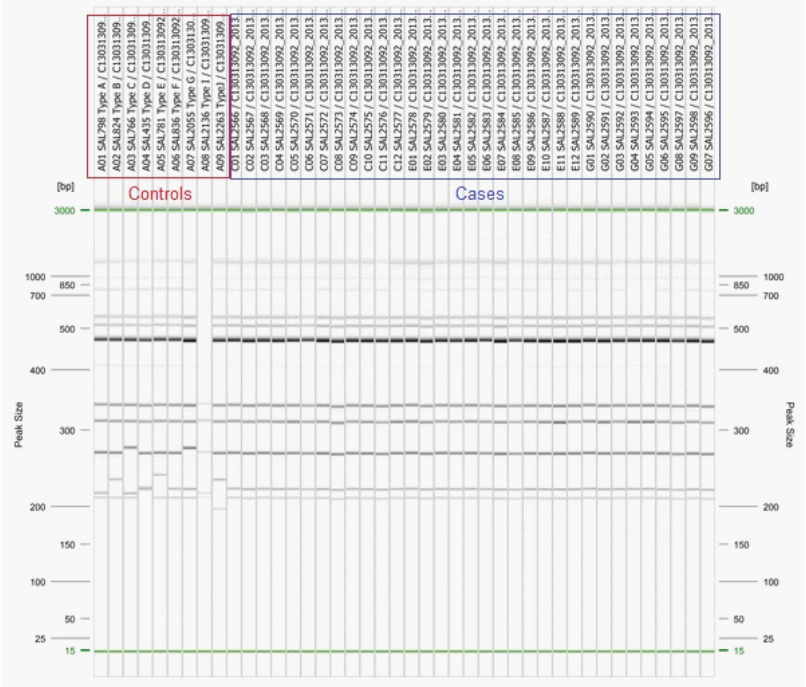


Figure 6
MLVA typing of Salmonella Enteritidis isolated from 32 cases in a factory outbreak of gastroenteritis, 20-24 Sept 2012



Discussion

This was a point-source outbreak of salmonellosis caused by *Salmonella* Enteritidis. The reported symptoms (diarrhoea, abdominal pain, fever, nausea and vomiting) and the mean incubation period of 29 hours fit the clinical and epidemiological description of *Salmonella* Enteritidis infection (12-36hrs). This was further supported by the isolation of a similar strain of *Salmonella* Enteriditis MLVA type F from all 33 stool samples taken from the cases and one implicated food handler.

As all the food items were mixed in one food packet and all the cases had consumed the same items, a case- control analysis to identify the specific vehicle of transmission could not be conducted. However, the food items consumed are commonly implicated in outbreaks of salmonellosis such as raw or under cooked poultry, meat, eggs and dairy products [5]. In Singapore, outbreaks of food borne *Salmonella* Enteritidis have been associated with the consumption of contaminated cakes, bread and egg-based dessert [6-8].

Our interview with the asymptomatic *Salmonella* Enteritidis-positive food handler revealed that she was the only person in charge of food preparation. Owing to an extra order of food placed late in the day, she had to prepare a second batch of food within a short period to meet the time for dinner. In light of this, it is possible that proper food preparation practices were not strictly adhered to and could have resulted

in under-cooked food or cross-contamination. Cross contamination through the use of previously contaminated surfaces or utensils and improper food handling techniques during the food preparation process are also well known risk factors for the transmission of *Salmonella* Enteritidis[5]. The relative ease for *Salmonella* to be transferred from contaminated chickens to utensils, kitchen surfaces, hands and other foods and to remain viable for up to six hours after transfer has been documented[9].

Our investigations had observed several environmental hygiene lapses including dirty utensils, chopping board and storage receptacle. In addition, the environmental swabs collected from the kitchen also indicated that environmental hygiene standards were not properly maintained. The management of the canteen was advised to ensure that proper personal and food hygiene practices and high standards of environmental hygiene are to be observed at all times,

(Contributed by Lin YJ, Fauzy M, Badaruddin H, Seet SK, Chew S, Zulaina S, La MV and Tay J. Communicable Diseases Division, Ministry of Health)

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An outbreak of *Vibrio parahaemolyticus* food poisoning

Introduction

On 30 April 2012 and 2 May 2012, the Ministry of Health (MOH) received two separate food poisoning notifications involving four persons who had developed symptoms of gastroenteritis after consuming food purchased from a food stall on 27 and 28 April 2012. On 4 May 2012, MOH was informed

by a hospital clinician of eight *Vibrio parahaemolyticus* gastroenteritis cases detected within two weeks. Further enquiries confirmed that all these eight cases had also consumed food purchased from the same implicated food stall. This report summarizes the findings of the outbreak investigation.

Methods

Through interviews of the 12 cases and active case detection, another 15 persons who developed symptoms of gastroenteritis after consuming food purchased from the implicated food stall were identified. Of these, 13 were friends, colleagues or relatives of the cases while the remaining two had initially reported consuming food from a different food establishment.

A case was defined as a previously well individual who developed watery diarrhoea (two or more episodes within 24 hours) with/without any other gastrointestinal symptoms such as vomiting, fever or abdominal cramps after consuming food from the implicated food stall between 20 and 28 April 2012.

Epidemiological and demographic data of the affected cases, such as gender, ethnicity, food history, date of onset of illness, signs and symptoms and medical treatment sought were obtained.

All food handlers working at the implicated food stall were referred to the Communicable Disease Centre and screened for enteropathogens (*Shigella, Campylobacter, Vibrio* and *Salmonella*). Food and environmental samples were also obtained from the food stall for microbial analysis.

Serotyping and genotyping of *V. parahaemolyticus* isolated from food and stool samples were performed by the National Public Health Laboratory (NPHL). The *V. parahaemolyticus* serotypes were determined by agglutination reaction with the panel of O and K antisera (Denka-Seiken). Genotyping was determined by multiple-locus variable number of tandem repeat analysis (MLVA). Eight variable-number tandem repeats (VNTR) loci were amplified in three multiplex PCR reactions[1]. The PCR products obtained were mixed together, then directly analysed using the DNA 1000 Kit on the Bioanalyzer (Agilent) to generate fingerprint patterns.

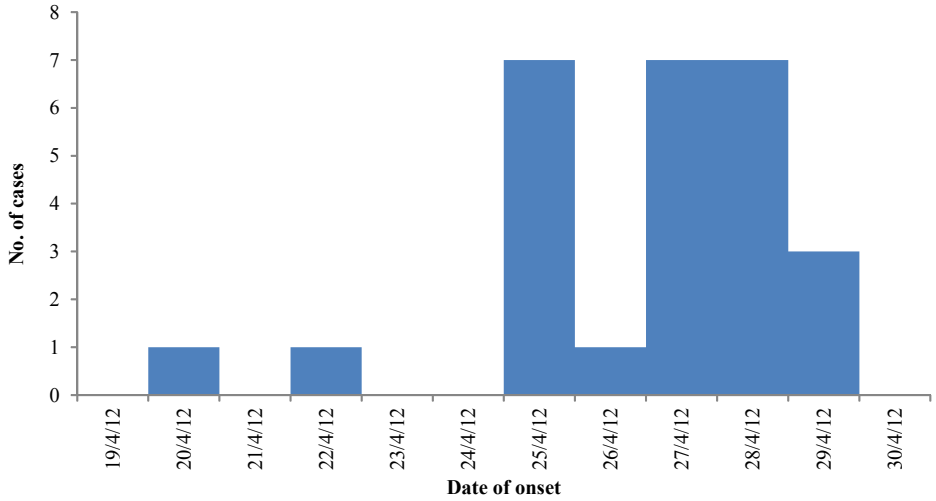
Clinical and epidemiological findings

Based on the case definition, a total of 27 cases were identified. The presenting symptoms reported in order of decreasing frequency were watery diarrhoea (100%), vomiting (82%), abdominal cramps (52%) and fever (30%). Eight (30%) cases were hospitalized, nine (33%) cases sought outpatient treatment while the rest (37%) self-medicated.

All the cases had consumed food purchased from the implicated food stall between 20 and 28 April 2012 and developed onset of illness between 20 and 29 April 2012. The mean and median incubation period was calculated to be 9.1 hours and 8.0 hours, respectively, with a range of 2.0-17.5 hours. The shape of the epidemic curve shown (*Fig. 7*) was consistent with a common source outbreak.

Cases comprised 13 (56.6%) Malays and 12 (44.4%) Chinese. There were 22 females and 5 males, giving a female to male ratio of 4.4:1. Though the cases had consumed a variety of dishes from the implicated food stall, 25 out of 27 cases (93.0%) reported having consumed hard-boiled egg which was a common ingredient.

Figure 7
Onset of illness of 27 cases of gastroentritis who had consumed food from a food stall between 20 Apr and 28 Apr 2012



Food preparation

Routinely, food preparation began at 0500 hours in the stall. Dishes such as beef rendang (spicy meat dish), squid and several other items were prepared and displayed within the food showcase for sales at room temperature by 0600 hours. The food items were served upon receiving the orders without re-heating. Furthermore, certain ready-to-eat food items were kept at room temperature for more than 12 hours.

The hard-boiled eggs, a common ingredient among the dishes ordered, were prepared and left overnight to cool at room temperature for use the next day. Further processing such as peeling and washing were carried out the next morning after preparation of the dishes mentioned above. The washing of the hard-boiled eggs was done in the same sink where washing of raw squid was carried out. The sink was not cleaned between the preparation of the raw squid and peeling and washing of the hard-boiled eggs.

Hygiene lapses detected at the food stall were cockroach infestation, improper storage of a chopping board (chopping board was in contact with a trash bag) and dirty and unkempt premises.

Laboratory findings

V. parahaemolyticus was isolated from the stool samples obtained from the eight hospitalized cases and the hard-boiled eggs collected during the field inspection. *Escherichia coli* was isolated from hard-boiled eggs, rice and fried tauhu (tofu). Fecal coliforms were isolated from rice, chilli and fried coconut (240, 43 and 23 MPN/g, respectively). All six food handlers screened were negative for enteropathogens.

Six of the eight *V. parahaemolyticus* isolates from the hospitalized cases and the *V. parahaemolyticus* isolate from the hard-boiled eggs were further analyzed. A total of five distinguishable strains were identified (*Table 3*).

Table 3

MLVA and serotyping of *V. parahaemolyticus* isolates

Sample	Organism isolated	O group	K type	MLVA type
Case 1	*V. parahaemolyticus*	O3	K6	4
Case 2	*V. parahaemolyticus*	O3	K6	4
Case 3	*V. parahaemolyticus*	O3	K6	4
Case 4	*V. parahaemolyticus*	O3	K6	6
Case 5	*V. parahaemolyticus*	ungroupable	untypeable	5
Case 6	*V. parahaemolyticus*	O1	K32	7
Hard boiled eggs	*V. parahaemolyticus*	O2	untypeable	not done

Discussion

The clinical and epidemiological findings of this common-source outbreak are suggestive of *V. parahaemolyticus* infections associated with the consumption of hard-boiled eggs. The aetiology was confirmed when the causative agent was detected in the stool samples of eight hospitalized cases and a sample of hard-boiled eggs collected from the implicated food stall. However, the isolation of five different strains of *V. parahaemolyticus* suggests that food items prepared at the implicated food stall were contaminated by several different strains over a period of time. The poor food hygiene practices and poor environmental hygiene could have facilitated this outbreak.

V. parahaemolyticus is a bacterium that thrives in brackish saltwater and is a common cause of food-borne illness in Asia[2]. It causes watery diarrhoea, often with abdominal cramping, vomiting, nausea and fever. The mean incubation period is 17 hours with a range of 4-90 hours. Typically, the infection is of mild or moderate severity and lasts a median of 2 to 6 days[3]. The severity of illness and short incubation period reported by some of the cases suggest ingestion of high doses of *V. parahaemolyticus*.

V. parahaemolyticus is not native to eggs and poultry but is commonly found in seafood products such as oysters, squid and shrimp[3]. While the exact mechanism of contamination of the hard-boiled eggs is unknown, it is possible that cross-contamination might have occurred when the same sink was used to clean raw squid and then to peel and wash the hard-boiled eggs without any cleaning of the sink in between these processes. Storage of ready-to-eat food (including hard-boiled eggs) at ambient temperature for more than 12 hours could have facilitated the proliferation of *V. parahaemolyticus* to reach high infectious doses. Studies have shown that the optimum temperature for growth of *V. parahaemolyticus* is between 20°C and 35°C where under optimal conditions the organism can double in number in less than 20 minutes[3-5].

Because of the thriving business, the output demand was exceptionally high. Due to the space constraint of the stall, environmental, food and personal hygiene practices could have been compromised.

This is evident from the widespread contamination of ready-to-eat food with *V. parahaemolyticus*, *E. coli* and fecal coliforms. The chopping board was also stored in a manner which could lead to cross-contamination while the premises was dirty and unkempt. Such poor hygiene practices could have facilitated the sustenance of *V. parahaemolyticus* in the environment for the duration of the outbreak.

The food stall ceased operations from 7 May 2012 to 16 May 2012 for thorough cleaning and sprucing up. The licensee resumed business after the National Environment Agency was satisfied that the necessary improvements had been made.

(Contributed by Pream RS, Pang QY, Toh HY, Abdul RO, La MV, Siti Z, Chew S, Badaruddin H and Tay J, Communicable Diseases Division, Ministry of Health)

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