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Fireworks-related injury surveillance in the Philippines: trends in 2010–2014

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Analysis of the annual fireworks-related injury surveillance data collected by the Philippines Department of Health (DOH) in 2010–2014 was conducted to describe the profile of such injuries in the Philippines.

Surveillance data were collected from DOH's Online National Electronic Injury Surveillance System and analysed. A case was defined as any person who had sustained injury from fireworks in any form within the 16-day surveillance period (21 December to 5 January) and had presented to any of the 50 sentinel hospitals.

Of the 4649 cases, there were 4706 fireworks-related injuries involving 5076 anatomic sites in 2010–2014. A significant decrease of cases in 2014 was observed when compared with the previous study years ($P = 0.02$). The number of cases peaked at public holidays. Males (80%) were more commonly injured, and children aged 5 to 14 years were primarily affected (47%). Ignition of illegal fireworks accounted for half (50%) of the injuries; most injuries (68%) occurred in street settings. The majority of injuries (57%) were sustained by fireworks igniters. The most common anatomic injury sites were hands (44%), legs (21%) and eyes (14%). Illegal fireworks were related to 100% (4/4) of the deaths and 49% (105/214) of the cases who needed amputations.

Fireworks-related injuries declined significantly in 2014. Public awareness campaigns may have contributed to reducing the injury occurrences. As illegal fireworks accounted for all deaths and more than half of the amputations, law enforcement should be directed toward preventing importing, distributing and using illegal fireworks.

Fireworks usage at New Year's festivities is a tradition in the Philippines. It is believed that fireworks attract good fortune and drive away evil spirits; however, fireworks also result in thousands of injuries every year.¹

The establishment of annual fireworks-related injury surveillance in the Philippines started in 1991 involving three sentinel hospitals.² In 2010, the online National Electronic Injury Surveillance System (ONEISS) was set up³ and hospital staff from 50 selected sentinel hospitals were trained to report fireworks-injury cases upon visit to emergency room.

Despite a national law that bans the private use of fireworks, there are still several fireworks-related injuries across 81 provinces in the country. The purpose of this study is to describe the profile of fireworks-related injuries in the Philippines using the ONEISS surveillance data from 2010 to 2014.

METHODS

This is a descriptive study investigating fireworks-related injuries using ONEISS surveillance data from 50 sentinel hospitals in the Philippines between December 2010 and January 2015. This includes 33 hospitals of the Philippines Department of Health, four local government hospitals and 13 private hospitals (**Figure 1**).

For our study, a case of fireworks-related injury was defined as any person who sustained injury from fireworks in any form in the 16-day surveillance period (21 December to 5 January of the next year) and presented to any one of the sentinel hospitals. Recorded case data included demographics (e.g. age and sex); injured body part(s); location of incident; date of injury; and type of fireworks used.

Two-sided *t*-test with a significance level of 0.05 was used to compare the surveillance data trends over

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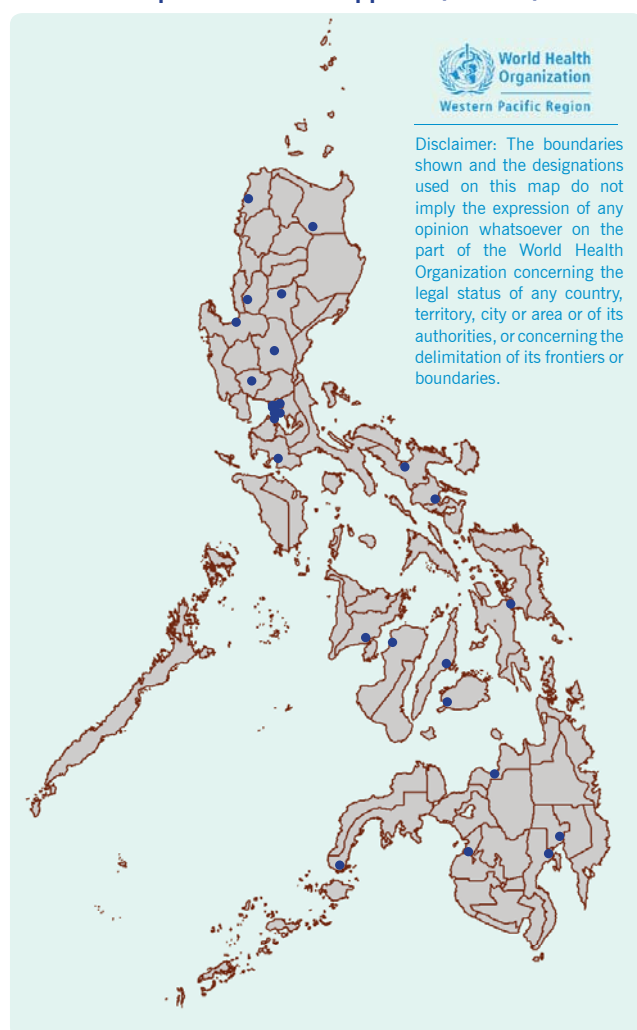
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Figure 1. **Spot map of fireworks-related injury sentinel hospitals in the Philippines ($n = 50$)**



time. Notification rate by city/municipality was computed based on the 2010 population census data from the Philippine Statistics Authority.⁴ Analysis was performed using Stata/SE 12.0 for Windows (StataCorp LP, Lake Drive, TX, USA).

RESULTS

There were 4706 fireworks-related injuries in 4649 cases, involving 5076 anatomic sites in total. The number of fireworks-related injuries in 2014 ($n = 840$) was 12% less than the four-year mean ($n = 953$) of the period 2010–2013. This decrease was statistically significant ($P = 0.02$). A bi-modal peak in injury cases was shown during the 16-day annual surveillance periods. A small peak on 25 December and a sharp peak over a two-day period between 31 December and 1 January of the next year were observed. This trend was consistent for all five study years (Figure 2).

During the study period, blast injuries not requiring amputation accounted for 80.6% (3792/4706) of the total injuries. A total of 696 (13.7%) eye injuries were also reported. Amputation was required for 214 (4.5%) of the injuries. Four fireworks-related deaths were reported (case fatality ratio: 4/4649, 0.086%) (Table 1). Ignition of illegal fireworks accounted for 50.2% (2363/4706) of injuries. Most of the severe injuries (amputations and eye injuries) were due to illegal

Figure 2. **Distribution of fireworks-related injury cases during the 16-day surveillance period from 21 December to 5 January, the Philippines, 2010–2014**

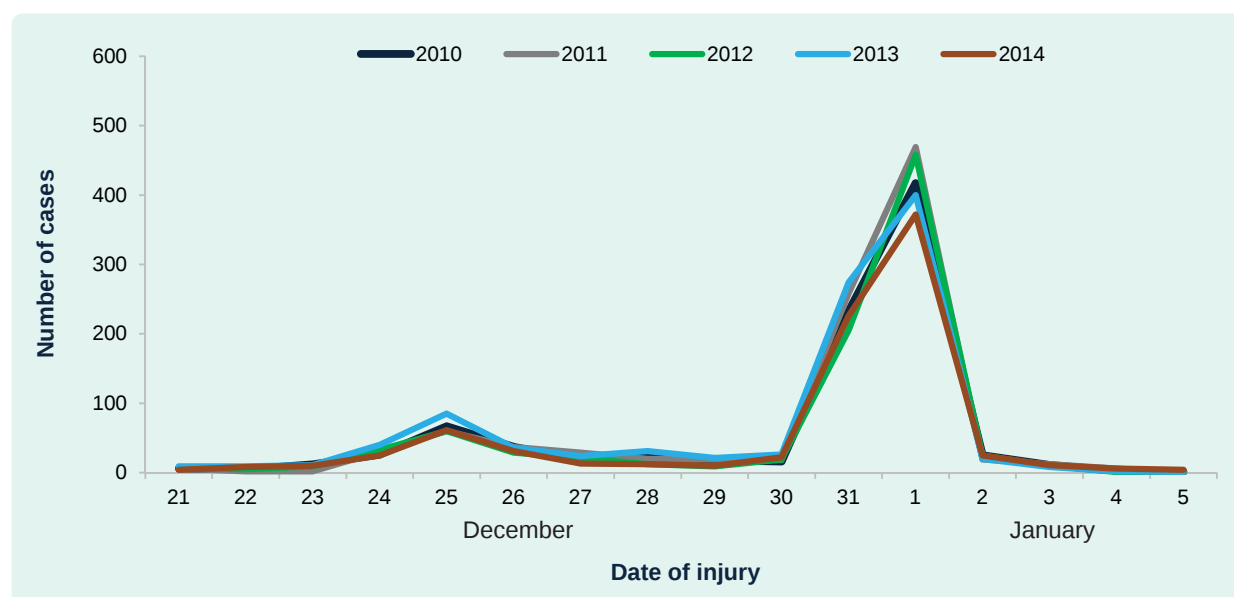


Table 1. **Types of fireworks-related injuries, the Philippines, 2010–2014 (*n* = 4706)***

Injury type	2010	2011	2012	2013	2014	Total (%)
Blast injury not requiring amputation	730	795	753	843	671	3792 (80.6)
Eye injury	135	131	137	147	146	696 (14.8)
Blast injury requiring amputation	56	58	34	25	41	214 (4.5)
Death	0	3	0	1	0	4 (0.1)
Total	921	987	924	1016	858	4706 (100)

* Cases were classified in one or more injuries types. There were 4649 injury cases with 4706 injury types in total. Of these, 4593 cases had one injury type, 55 had two types and 1 had three types.

Table 2. **Types of fireworks-related injury by firework types, the Philippines, 2010–2014 (*n* = 4706)***

Injury type	Type of fireworks			Total
	Illegal (%)	Legal (%)	Unknown (%)	
Blast injury with no complication	1948 (51.4)	1374 (36.2)	470 (12.4)	3792
Eye injury	306 (44.0)	288 (41.4)	102 (14.7)	696
Blast injury requiring amputation	105 (49.1)	82 (38.3)	27 (12.6)	214
Death	4 (100)	0 (0)	0 (0)	4
Total	2363 (50.2)	1744 (37.1)	599 (12.7)	4706

* Cases were classified in one or more injuries types. There were 4649 injury cases with 4706 injury types in total. Of these, 4593 cases had one injury type, 55 had two types and 1 had three types.

fireworks ignition. This included 100% (4/4) of deaths and 49.1% (105/214) of amputations. (Table 2).

The number of fireworks-related injury cases was higher in males than females (80.0% in males versus 20.0% in females). Children aged 10–14 years old (24.5%) and 5–9 years old (22.0%) accounted for almost half of the cases (Table 3).

Most of the reported injuries (68.3%) occurred on streets and (57.0%) were sustained by fireworks igniters (Table 3). The most common anatomic sites of injury were hands (43.7%), legs (21.0%) and eyes (13.7%) (Table 4).

The notification rate of fireworks-related injuries was highest in the Dagupan City (7.03 per 10 000 individuals) in Pangasinan province, followed by Mandaluyong City (5.48 per 10 000 individuals) of Metro Manila and the municipality of Bayumbong (5.40 per 10 000 individuals) in Nueva Vizcaya province (Table 5).

DISCUSSION

The results showed a significant decrease in the overall number of fireworks-related injuries reported in

2014 compared with 2010–2013 in the Philippines. However, the number of more severe injuries that may lead to life-long disabilities⁵ did not decline (Table 1). This may be due to consistent usage of illegal fireworks that accounted for most of the severe injuries. The results also revealed that death from fireworks-related injury is a rare event in the Philippines. Risk of death by road traffic injury is much higher than that of fireworks in the Philippines.⁶ Fireworks injuries did not generally cause death in another study.⁷

The observed sharp peak of injury cases during the New Year's holiday period were similar to that reported from the United States of America,⁷ where the celebration of Independence Day accounted for 95% of such injuries. A high percentage of injuries have also been reported elsewhere in association with national holidays such as Charshanbeh Soori in the Islamic Republic of Iran,⁵ Diwali Festival in India,⁸ Greek Orthodox Easter in Greece⁹ and New Year's celebration in France.¹⁰

More cases were observed among males than females. This observation was similar to several previous studies.^{5,7,8,10,11} More injuries happened on the street than at home, similar to a study from the Islamic Republic of Iran.⁵ Also, our findings indicate that almost

Table 3. Demographic characteristics of the fireworks-related injury cases, the Philippines, 2010–2014 (*n* = 4649)

Characteristics	2010	2011	2012	2013	2014	Total (%)
Sex						
Male	732	832	700	885	621	3770 (80.0)
Age group (years)						
0–4	46	51	41	35	43	216 (4.6)
5–9	201	224	205	215	180	1025 (22.0)
10–14	209	223	201	284	220	1137 (24.5)
15–19	89	92	102	103	81	467 (10.0)
20–24	75	92	85	85	72	409 (8.8)
25–29	72	73	65	59	56	325 (7.0)
30–34	63	60	57	55	49	284 (6.1)
35–39	54	52	32	41	38	217 (4.7)
40–44	45	31	28	29	28	161 (3.5)
45–49	23	31	31	24	25	134 (2.9)
50 or above	44	58	57	67	48	274 (5.9)
Place of occurrence						
Street	634	691	661	652	536	3174 (68.3)
Home	275	287	237	306	286	1391 (29.9)
Other	12	9	6	39	18	84 (1.8)
Involvement						
Igniter	541	520	450	634	503	2648 (57.0)
Bystander	380	467	454	363	337	2001 (43.0)
Total	921	987	904	997	840	4649 (100)

Table 4. Anatomic sites of the fireworks-related injury cases, the Philippines, 2010–2014 (*n* = 5076)*

Anatomic site	2010	2011	2012	2013	2014	Total (%)
Hands	411	446	376	548	435	2216 (43.7)
Legs	200	240	242	212	172	1066 (21.0)
Eye	135	131	137	147	146	696 (13.7)
Head and neck	89	75	133	103	88	488 (9.6)
Arms	70	87	65	65	70	357 (7.0)
Anterior torso	21	22	32	25	20	120 (2.4)
Abdomen	13	26	17	19	12	87 (1.7)
Posterior torso	8	8	14	7	9	46 (0.9)
Total	947	1035	1016	1126	952	5076 (100)

* Case may sustain injury in one or more anatomical sites. There were 4649 injury cases with 5076 anatomical sites in total (4460 cases had one anatomical site; 70 had two sites; 51 had three sites; 30 had four sites; 25 had five sites; and 13 had six sites).

Table 5. Fireworks-related injury incidence by city and municipality, the Philippines, 2010–2014

Region	Province	City/municipality	Incidence (per 10 000 population)
1	Pangasinan	Dagupan City	7.03
NCR	Metro Manila	Mandaluyong City	5.48
2	Nueva Vizcaya	Bayombong (Capital)	5.40
NCR	Metro Manila	Manila City	5.08
1	Ilocos Norte	Paoay	3.76
1	Ilocos Norte	Batac	3.36
1	La Union	San Fernando City	3.13
NCR	Metro Manila	Marikina City	2.90
NCR	Metro Manila	Las Piñas City	2.86
2	Nueva Vizcaya	Solano	2.85
1	Pangasinan	Mangaldan	2.73
1	Pangasinan	San Jacinto	2.65
3	Pampanga	Santo Tomas	2.63
NCR	Metro Manila	Navotas	2.61
1	Pangasinan	Calasiao	2.52
3	Pampanga	San Fernando City	2.45
NCR	Metro Manila	Quezon City	2.45
NCR	Metro Manila	Pasig City	2.39
3	Nueva Vizcaya	Villaverde	2.26
NCR	Metro Manila	Pateros	2.18
6	Iloilo	Oton	2.06
NCR	Metro Manila	Valenzuela City	2.02
4A	Rizal	San Mateo	2.00
6	Negros Occidental	Bacolod City	1.99

NCR, National Capital Region.

half of fireworks injuries occurs in the group aged 5–14 years, echoing findings in some previous studies.^{7,11} The number of injury cases was found to be higher in urban than rural areas. More cases in urban areas could be attributed to the higher population density, although we cannot find substantiating evidence in the current published literature. We found the most affected anatomical site of fireworks injuries was hands, which was consistent with previous studies,^{5,12} although one study showed that eyes followed by hands was most common.¹³

Despite legislation and awareness campaigns conducted by various government agencies in the Philippines, the main cause of firework-related death and severe injuries is illegal fireworks. This is similar to some previous studies.^{5,10} In a previous study in the United States of America, stricter law enforcement for restricting firework usage led to a sevenfold decrease in injury rates.¹³ Legislation enforcement to

restrict the distribution and use of fireworks should be considered.

There were limitations in this study. Only hospitalized patients were captured by the sentinel surveillance system. Mild cases who did not require hospitalization were missed. Also, the sentinel sites cover only 24 out of 81 provinces. The notification rate estimates do not represent the national fireworks-related injury burden; they only reflect the situation within these hospital catchment areas. As this study focused more on the surveillance data analysis, evaluation for the surveillance system was not included. Future studies are needed to reveal the system's performance.

CONCLUSION

The overall number of fireworks-related injuries declined in 2014. However, the number of severe injuries did not decline. Public awareness campaigns should target

preventing the use of illegal fireworks since they account for the majority of fireworks-related deaths and severe injuries. Law enforcement efforts should be directed toward eliminating importing, distributing and use of illegal fireworks.

Conflicts of interests

None declared.

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Descriptive epidemiology of infectious gastrointestinal illnesses in Sydney, Australia, 2007–2010

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Objective: There is a lack of information about the prevalence of gastrointestinal illnesses in Australia. Current disease surveillance systems capture only a few pathogens. The aim of this study is to describe the epidemiology of infectious gastrointestinal illnesses in Sydney, Australia.

Methods: A retrospective cross-sectional study of patients with gastrointestinal symptoms who visited tertiary public hospitals in Sydney was conducted between 2007 and 2010. Patients with diarrhoea or loose stools with an enteric pathogen detected were identified. Demographic, clinical and potential risk factor data were collected from their medical records. Measures of association, descriptive and inferential statistics were analysed.

Results: In total, 1722 patients were included in this study. *Campylobacter* (22.0%) and *Clostridium difficile* (19.2%) were the most frequently detected pathogens. Stratified analysis showed that rotavirus (22.4%), norovirus (20.7%) and adenovirus (18.1%) mainly affected children under 5 years; older children (5–12 years) were frequently infected with *Campylobacter* spp. (29.8%) and non-typhoid *Salmonella* spp. (24.4%); infections with *C. difficile* increased with age. *Campylobacter* and non-typhoid *Salmonella* spp. showed increased incidence in summer months (December to February), while rotavirus infections peaked in the cooler months (June to November).

Discussion: This study revealed that gastrointestinal illness remains a major public health issue in Sydney. Improvement of current disease surveillance and prevention and control measures are required. This study emphasizes the importance of laboratory diagnosis of enteric infections and the need for better clinical data collection to improve management of disease risk factors in the community.

Gastrointestinal (GI) illnesses are a significant public health problem, resulting in one third of working Australians missing on average one day of work each year.¹ GI illnesses are a burden to the health-care system, costing approximately 1.2 billion Australian dollars annually.^{2,3} In Australia, the national disease surveillance system captures only campylobacteriosis, typhoid fever, giardiasis and salmonellosis; however, campylobacteriosis is not reportable in New South Wales (NSW), the largest state. In NSW, medical practitioners and hospitals are required to report notifiable conditions to the local public health units (PHU) on the basis of reasonable clinical suspicion. Pathology laboratories are required to notify a positive result for specified infectious

diseases and medical conditions. Primary schools and childcare centres are encouraged to seek advice from their local PHU when they suspect an infectious disease outbreak in their centres using standard reporting forms from the Australian Department of Health.^{4–7}

The PHU in the state of NSW are responsible for investigating reports of enteric disease based on established reporting requirements, they then enter the data into the state-wide NSW Notifiable Conditions Information Management System. Outbreaks are detected through a variety of sources including notifiable diseases surveillance data, reports from general practitioners, institutions or laboratories and the public.^{4–6}

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The surveillance data reveal that enteric viruses, mainly norovirus and rotavirus, are the most common causes of non-food GI illness, accounting for approximately 15–18% of all GI illness cases in NSW.^{4,5} One study showed that approximately 25% of all cases of gastroenteritis are foodborne with an estimated 4.1 million foodborne gastroenteritis cases occurring in 2010. Pathogenic *Escherichia coli*, norovirus, *Campylobacter* and non-typhoid (N-T) *Salmonella* were responsible for over 93% of foodborne illness from known pathogens. However, the majority of cases (80%) did not have a known pathogen identified.^{8,9}

Previous studies revealed that approximately 30% of people will seek medical attention for GI illness;^{10,11} among this group, only about 20% (range: 14–27%) will have confirmatory tests with stool specimens.¹² In addition, only a few selected pathogens are reportable to the infectious disease surveillance system. Therefore, several emerging and re-emerging pathogens cannot be captured.¹³ Previous reports indicated that a significant proportion of illnesses were not reported in the surveillance system and that the majority of pathogens causing illness remain unknown.^{8,9} This creates a paucity of information about the prevalence of GI illnesses in Australia. This study described the clinical and epidemiological characteristics and the common pathogens associated with GI illnesses in Sydney, Australia in 2007–2010.

METHODS

Study design and data collection

A retrospective cross-sectional study was conducted on patients who presented to the four public referral hospitals or affiliated clinics in Sydney with GI symptoms and had an enteric organism detected in their stool from January 2007 to December 2010. Hospitals in this study were selected by convenience sampling. Cases were randomly selected using an online random number generator (StatTrek, Atlanta).

Demographic details (age, gender, post code, country of birth, relationship status and language spoken); clinical data (vital signs, date of onset, date of hospitalization, date of hospital separation, symptoms, diagnosis, organism[s] detected, treatment received and co-morbidities [surgery, HIV/AIDS,

cancer, transplant]); and potential risk factor data (antibiotic use/chemotherapy, chronic GI illness, consumption of suspect food, men who have sex with men [MSM] status and travel history) were collected from the patients' medical records.

Laboratory results for all stool specimens that tested positive for an enteric organism were collected from 2007 to 2010 except for one hospital that only included data from 2008 to 2010. Patients with diarrhoea (liquid or watery stools taking the shape of the container) or loose (unformed) stools were identified from the laboratory records provided by the hospitals.

Laboratory methods

The laboratory methods for the diagnosis of enteric organisms have been previously described.^{14,15} Tests for fungi or other pathogens were conducted only by special requests from clinicians.

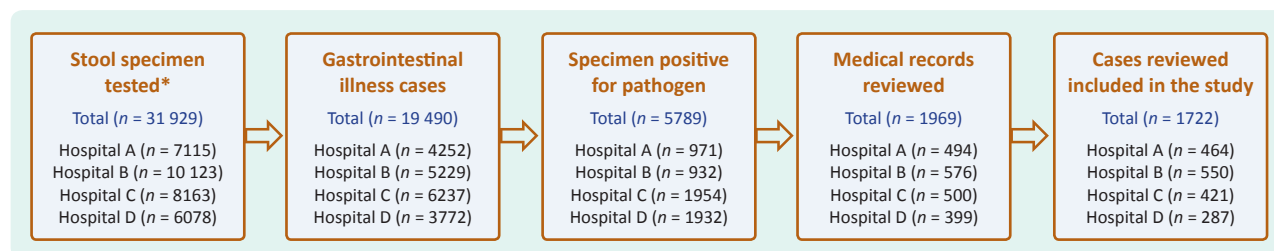
Virology

Briefly, all laboratories conducted testing for adenovirus and rotavirus routinely in all children aged 5 or younger unless otherwise indicated or requested by the clinician. Rotavirus, adenovirus serotypes 40 and 41 and norovirus were detected by either an enzyme immunoassay (EIA), or the RIDA® Quick Rotavirus/Adenovirus Combi immunochromatographic test and the RIDASCREEN® norovirus test (R-Biopharm Inc., Darmstadt, Germany). All tests were conducted following the manufacturer's recommendations.

Bacteriology

Bacteria identification was routinely performed in all laboratories using standard culture methods. In summary, selective media were used: Xylose Lysine Deoxycholate agar was inoculated for the detection of *Salmonella*, *Shigella* and *Yersinia*; *Aeromonas*, *Plesiomonas* and *Vibrio* spp. on Horse Blood Agar; *Campylobacter* spp., *Campylobacter* agar and *Clostridium difficile* on *C. difficile* agar, Oxoid Australia. *C. difficile* was detected using EIA for hospitalizations greater than three days or if otherwise indicated (e.g. history of antibiotic use, chemotherapy or immuno-suppressed patients).

Figure 1. Flow diagram for participant selection from the four referral hospitals, Sydney, Australia, 2007–2010



* Multiple stool specimen counted.

Parasitology

All hospitals processed stools by a wet preparation in saline and examined for white blood cells, red blood cells and cysts, ova and parasites (COP). Direct microscopy was routinely performed on all stool specimens for the detection of COP and concentration techniques were performed on request at some hospitals. Techniques included a modified iron haematoxylin stain incorporating carbol fuchsin to enhance the detection of acid-fast *Isospora*, *Cryptosporidium*, *Cyclospora*, and direct DNA extraction using a QIAamp DNA stool minikit (Qiagen, Hilden, Germany) for the identification of *Entamoeba* spp, as previously described.¹⁶ EIA was performed as a screening test for *Giardia intestinalis*, *Cryptosporidium parvum* and *Entamoeba histolytica*. All positive findings from the EIA were confirmed by microscopy.

Sample size

Based on previous literature,¹⁷ we estimated that each laboratory receives approximately 10 000 specimens per year over the study period and the prevalence of uncommon microbes is approximately 5% for diarrhoeal cases. A sample size of 436 was required for each study site at a 95% confidence level with 80% power and 2% margin of error. Oversampling of cases was performed to avoid any shortfalls in missing medical records.

Statistical analysis

Descriptive analysis was done for demographic characteristics. The association between demographic characteristics, clinical symptoms, pathogens detected and potential risk factors was examined using the Pearson's chi-squared test. Associations between potential risk factors (age group, surgery, transplant, HIV/AIDS, cancer, chronic GI illness, antibiotic use, travel history, consumption of suspect food and MSM status) and selected pathogens (*Blastocystis* spp, *Dientamoeba*

fragilis, *Campylobacter*, *C. difficile*, N-T *Salmonella* and *Shigella*) were placed into a binary logistic regression model. A backward stepwise multiple logistic regression was conducted for those selected pathogens with variables having a P-value lower than 0.25 in the univariate analysis.¹⁸ Subjects with missing variables were excluded from the regression model. Odds ratios (OR) and 95% confidence intervals (95% CI) for the association were reported. Statistical analyses were performed using SPSS version 18.0 (International Business Machines Corp, New York, USA).

Ethics

This study received ethical approval from the Human Research Ethics Committees for each of the four hospitals and the University of Technology, Sydney and was guided by the Australian National Statement on Ethical Conduct of Research involving humans.

RESULTS

Study population

Four public referral hospitals were included in the analysis.

Of the 19 490 patients with diarrhoea or loose stools at the four selected hospitals, 1722 cases were included in this study (Figure 1). The recruitment of cases at Hospital D was lower than expected due to administrative issues. For Hospital C, only the medical records between January 2008 and December 2009 period were reviewed and the laboratory results between January 2008 and December 2010 were included, whereas the other hospitals covered the period between January 2007 and December 2010.

Participants were aged between 25 days and 99 years (mean: 28.3 years, standard deviation

Table 1. Distribution of cases from the four referral hospitals, Sydney, Australia, 2007–2010 ($n = 1722$)

Characteristics	Hospital A <i>n</i> (%)	Hospital B <i>n</i> (%)	Hospital C <i>n</i> (%)	Hospital D <i>n</i> (%)	Total <i>n</i> (%)
Age group (years)					
< 5	120 (25.9)	396 (72.0)	0 (0.0)	121 (42.2)	637 (37.1)
5–12	32 (6.9)	111 (20.2)	0 (0.0)	25 (8.7)	168 (9.8)
13–24	34 (7.3)	43 (7.8)	61 (14.6)	26 (9.1)	164 (9.6)
25–49	82 (17.7)	0 (0.0)	156 (37.4)	49 (17.1)	287 (16.7)
50–75	123 (26.5)	0 (0.0)	119 (28.5)	28 (9.8)	270 (15.7)
> 75	72 (15.6)	0 (0.0)	81 (19.7)	38 (13.2)	191 (11.1)
Unknown	1 (0.1)	0 (0.0)	4 (0.3)	0 (0.0)	5 (0.3)
Sex					
Male	241 (52.2)	313 (56.9)	258 (61.7)	143 (50.5)	955 (55.5)
Female	221 (47.8)	237 (43.1)	160 (38.3)	140 (49.5)	758 (44.0)
Unknown	2 (0.1)	0 (0.0)	3 (0.2)	4 (0.3)	9 (0.5)
Total	464 (27.0)	550 (31.9)	421 (24.3)	287 (16.8)	1722 (100.0)

[SD]: 29.5 years). The majority of the participants at Hospitals A and C were in the age groups older than 12 years (67%), while children under 5 years were predominantly seen at Hospitals B (72%) and D (42.2%) (Table 1). The overall mean length of stay in hospital was 8.9 days (SD: 21.4 days) and this increased with age. Patients aged 50–75 years (mean: 20.3 days, SD: 30.4 days) and those 75 years and older (mean: 18.2 days, SD: 18.5 days) had a longer length of stay compared with children under 5 years (mean: 4.3 days, SD: 16.7 days) and 5–12 years (mean: 4.3 days, SD: 10.3 days).

Pathogens associated with GI illness

Campylobacter spp. (22.0%), *C. difficile* (19.2%) and N-T *Salmonella* (14.0%) were the most frequently detected bacteria. The most frequently detected viruses were norovirus (10.7%), rotavirus (9.4%) and adenovirus (7.6%); *Blastocystis* spp. (7.2%), *G. intestinalis* (2.9%) and *D. fragilis* (1.8%) were the most common enteric protozoa detected. Other organisms were detected in less than 3% of cases.

Except for *Campylobacter* and N-T *Salmonella* spp., which increased in summer months (December to February), no seasonal patterns were found for infections, with bacterial pathogens (Figure 2, Panel A). In contrast, viral infections, which predominantly affected children under 5 years, showed clearer seasonal patterns (Figure 2, Panel B). Rotavirus and norovirus

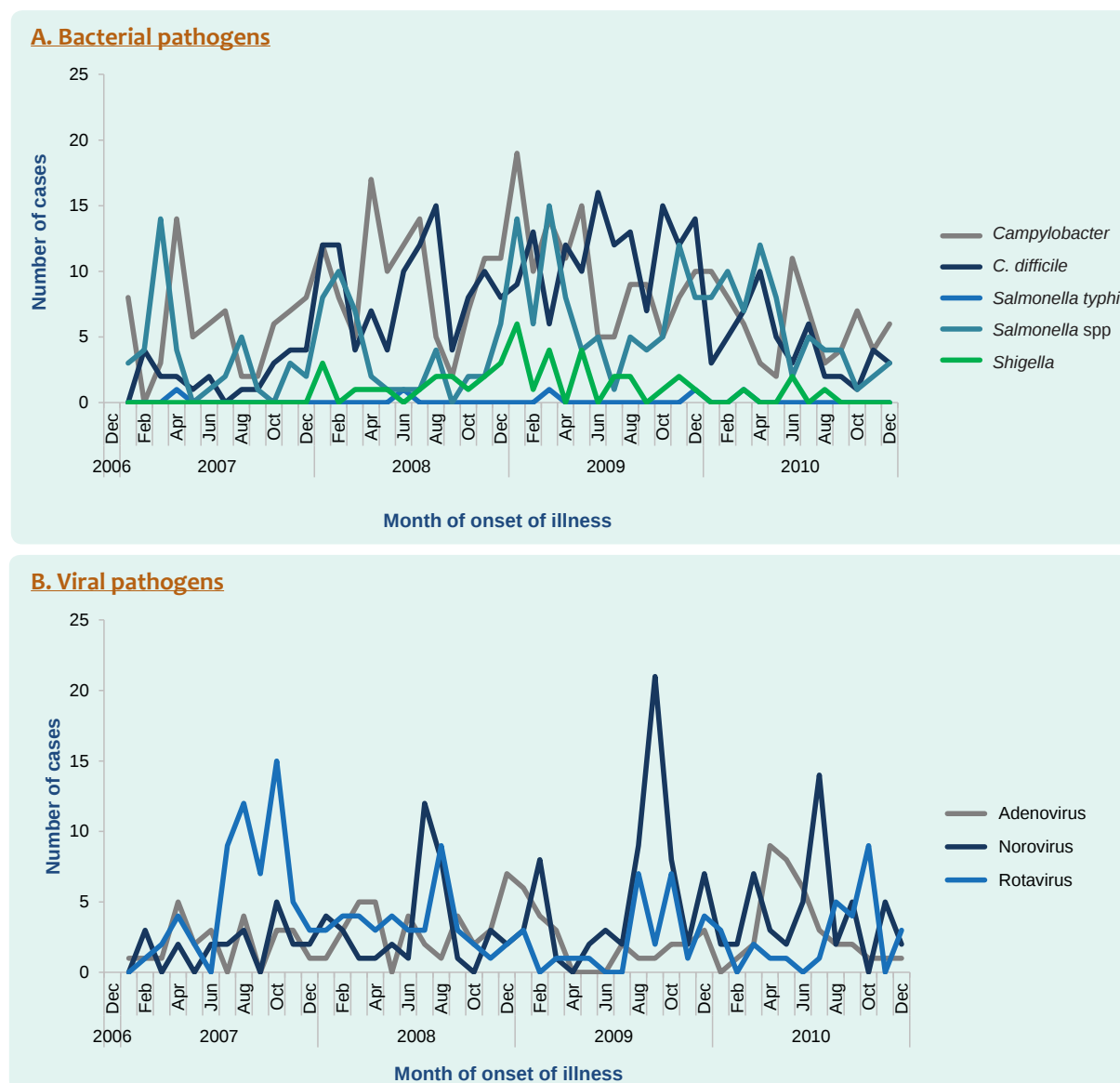
activities peaked in the cooler months (June to October and July to September, respectively); adenovirus showed a less consistent trend.

Demographic and clinical characteristics

Overall, *Campylobacter* infection was associated with watery diarrhoea (49.1%), abdominal pain (63.1%) and fever (56.5%; $P < 0.01$). N-T *Salmonella* infection was significantly associated with vomiting (60.8%), abdominal pain (59.2%) and fever (66.3%; $P < 0.01$). The majority of people infected with norovirus, rotavirus and adenovirus infections reported vomiting and lethargy ($P < 0.01$) (Table 2).

Children under 5 years ($n = 637$) presented with diarrhoea (97.8%; $P > 0.05$), vomiting (66.7%; $P = 0.001$), lethargy (56.5%; $P < 0.001$) and fever (body temperature > 37.8 °C; 56.0%; $P < 0.05$). Older children aged 5–12 years ($n = 168$) had similar clinical symptoms except that they reported higher rates of abdominal pain (72.0%) and fever (64.9%); however, these symptoms were not statistically significant ($P > 0.05$) when compared with other age groups. The most frequently detected GI pathogens in the less than 5 years age group were rotavirus (22.4%), norovirus (20.7%), adenovirus (18.1%), *Campylobacter* spp. (15.1%) and N-T *Salmonella* spp. (12.3%). Children aged 5–12 years were also frequently infected with *Campylobacter* spp. (29.8%) and N-T *Salmonella* spp. (24.4%) (Table 2).

Figure 2. Distribution of selected pathogens associated with diarrhoea at four referral hospitals by month, Sydney, Australia, 2007–2010



Patients older than 12 years mainly presented with diarrhoea (range: 99–100%) and abdominal pain (range 27–76%). Common pathogens infecting people in the 13–24 years and 25–49 years age groups were *Campylobacter* spp. (42.7% and 30.9%, respectively) and N-T *Salmonella* spp. (21.3% and 16.3%, respectively); those 50 years or older were predominantly infected with *C. difficile* (50–75 years, 46.3% and > 75 years, 53.9%). Sixty-nine per cent of people infected with *C. difficile* were 50 years or older ($P < 0.001$). Infections with *Blastocystis* spp. also increased with increasing age ($P < 0.01$). Overall, infections with enteric viruses decreased with increasing

age ($P < 0.01$), although *G. intestinalis* was highest in children aged 12 years or younger ($P < 0.01$) (Table 2).

Multivariate analysis

In multivariate analyses, MSM status was associated with *Shigella* spp., (OR = 5.0; 95% CI: 1.6–16.0), *Blastocystis* (OR = 2.3; 95% CI: 1.0–5.4) and *D. fragilis* (OR = 12.8; 95% CI: 1.3–130.7). Infection with *C. difficile* was associated with prolonged antibiotic therapy (OR = 6.3; 95% CI: 3.2–12.2), recent surgery (OR = 2.2; 95% CI: 1.1–4.6) and chronic GI illness (OR = 2.4; 95% CI: 1.1–5.3) (Table 3).

Table 2. Distribution of selected bacterial and viral pathogens based on clinical and risk factors in diarrhoea cases at four referral hospitals, Sydney, Australia, 2007–2010

Characteristics	<i>Campylobacter</i> n (%)	<i>C. difficile</i> n (%)	<i>Salmonella</i> spp n (%)	<i>Shigella</i> spp n (%)	<i>Salmonella</i> <i>typhi</i> A n (%)	Norovirus n (%)	Rotavirus n (%)	Adenovirus n (%)
Total	377 (22.0)	330 (19.2)	238 (13.9)	45 (2.6)	12 (0.7)	183 (10.7)	161 (9.4)	130 (7.6)
Age group (years)								
< 5	96 (25.5)	29 (8.8)	78 (32.8)	5 (11.1)	2 (16.7)	131 (71.6)	142 (88.2)	115 (88.5)
5–12	50 (13.3)	7 (2.1)	41 (17.2)	3 (6.7)	4 (33.3)	9 (4.9)	15 (9.3)	8 (6.2)
13–24	70 (18.6)	17 (5.2)	35 (14.7)	4 (8.9)	1 (8.3)	6 (3.3)	1 (0.6)	4 (3.1)
25–49	89 (23.6)	49 (14.8)	47 (19.7)	28 (62.2)	4 (33.3)	4 (2.2)	0 (0)	0 (0)
50–75	44 (11.7)	125 (37.9)	26 (10.9)	4 (8.9)	1 (8.3)	10 (5.5)	3 (1.9)	1 (0.8)
> 75	28 (7.4)	103 (31.2)	11 (4.6)	1 (2.2)	0 (0)	23 (12.6)	0 (0)	2 (1.5)
Signs and symptoms								
Diarrhoea	376 (99.7)	325 (98.2)	238 (100.0)	45 (100.0)	11 (91.7)	179 (97.8)	159 (98.8)	127 (97.7)
Vomiting	167 (44.1)	99 (29.9)	146 (60.8)	24 (53.3)	7 (58.3)	116 (63.4)	138 (85.7)	80 (61.5)
Abdominal pain	239 (63.1)	94 (28.4)	142 (59.2)	34 (75.6)	7 (58.3)	32 (17.5)	27 (16.8)	16 (12.3)
Fever	214 (56.5)	92 (27.8)	159 (66.3)	31 (68.9)	10 (83.3)	76 (41.5)	110 (68.3)	58 (44.6)
Dehydration	90 (23.7)	45 (13.6)	75 (31.3)	20 (44.4)	3 (25.0)	35 (19.1)	82 (50.9)	39 (30.0)
Anorexia/loss of appetite	81 (34.2)	45 (27.4)	73 (45.1)	6 (16.7)	6 (54.5)	37 (31.9)	67 (48.6)	38 (43.7)
Lethargy	62 (29.0)	37 (25.0)	57 (38.5)	5 (15.6)	6 (60.0)	56 (51.4)	89 (66.4)	45 (54.2)
Respiratory symptoms	31 (14.5)	16 (10.7)	17 (11.5)	0 (0)	6 (60.0)	36 (33.0)	33 (24.6)	34 (41)
Stool description								
Severe/explosive	39 (10.3)	5 (1.5)	31 (12.9)	12 (26.7)	3 (25.0)	12 (6.6)	16 (9.9)	9 (6.9)
Watery	186 (49.1)	208 (62.8)	118 (49.2)	20 (44.4)	2 (16.7)	131 (71.6)	118 (73.3)	96 (73.8)
Loose-unformed	11 (2.9)	46 (13.9)	7 (2.9)	0 (0)	4 (33.3)	21 (11.5)	9 (5.6)	14 (10.8)
Bloody/mucous	128 (33.8)	31 (9.4)	74 (30.8)	13 (28.9)	1 (8.3)	8 (4.4)	10 (6.2)	9 (6.9)
Persistent >14 days	11 (2.9)	29 (8.8)	10 (4.2)	0 (0)	2 (16.7)	9 (4.9)	7 (4.3)	2 (1.5)
Co-morbidities								
Surgery	15 (154.0)	112 (34.0)	7 (2.9)	1 (2.2)	0 (0)	15 (8.2)	4 (2.5)	8 (6.2)
HIV/AIDS	3 (0.8)	8 (2.4)	2 (0.8)	17 (37.8)	0 (0)	0 (0)	0 (0)	0 (0)
Cancer	19 (5.0)	51 (15.5)	9 (3.8)	1 (2.2)	1 (8.3)	13 (7.1)	1 (0.6)	5 (3.8)
Transplant (organ, bone marrow)	7 (1.9)	31 (9.4)	2 (0.8)	0 (0)	0 (0)	5 (2.7)	1 (0.6)	4 (3.1)
Other potential risk factors								
Antibiotic use/chemotherapy	28 (7.4)	216 (65.9)	34 (14.2)	11 (25.0)	2 (16.7)	40 (22.0)	19 (11.9)	20 (15.4)
Chronic gastrointestinal illness	24 (6.4)	57 (17.3)	11 (4.6)	3 (6.8)	0 (0)	9 (4.9)	5 (3.1)	3 (2.3)
Consumption of suspect food	54 (14.4)	2 (0.6)	37 (15.5)	9 (20.5)	0 (0)	2 (1.1)	4 (2.5)	1 (0.8)
Involved in FBI outbreak	3 (0.8)	1 (0.3)	7 (2.9)	0 (0)	0 (0)	1 (0.5)	1 (0.6)	2 (1.5)
MSM status*	3 (4.3)	4 (3.6)	1 (4.0)	18 (58.1)	0 (0)	0 (0)	0 (0)	0 (0)
Travel within six weeks	26 (6.9)	8 (2.4)	31 (13.0)	12 (27.3)	9 (75.0)	7 (3.8)	15 (9.3)	1 (0.8)

FBI, foodborne illness; MSM, men who have sex with men; and N-T, non-typhoid.

* Information available for cases from Hospital C only (n = 313).

Table 3. Multiple logistic regression of diarrhea cases from four referral hospitals, by selected pathogens, Sydney, Australia, 2007–2010 (*n* = 301)

Characteristics	Odds ratio	95% confidence interval	P-value
<i>Blastocystis</i> spp.			
MSM status	2.3	1.0–5.4	0.055
Co-morbidity surgery	2.1	0.9–4.7	0.068
Age group (years) (Ref > 75)			
< 5	0.0	Undefined	Undefined
5–12	0.0	Undefined	Undefined
13–24	0.5	0.1–5.1	0.582
25–49	3.0	0.9–9.9	0.070
50–75	3.1	1.0–9.8	0.050
<i>Dientamoeba fragilis</i>			
MSM status	12.8	1.3–130.7	0.031
Co-morbidity transplant	10.1	0.7–146.2	0.089
Co-morbidity cancer	13.8	1.6–122.1	0.018
<i>Clostridium difficile</i>			
Age group years (Ref > 75)			
< 5	0.0	Undefined	Undefined
5–12	0.0	Undefined	Undefined
13–24	0.0	Undefined	Undefined
25–49	0.0	Undefined	Undefined
50–75	0.8	0.4–1.8	0.611
Gender (Ref = Male)	0.4	0.2–0.9	0.031
Co-morbidity surgery	2.2	1.1–4.6	0.030
Co-morbidity HIV	0.4	0.1–1.1	0.070
Co-morbidity cancer	0.3	0.1–0.9	0.030
MSM status	0.3	0.1–1.0	0.051
Antibiotic use/chemotherapy	6.3	3.2–12.2	< 0.001
Chronic GI	2.4	1.1–5.3	0.035
Suspect food	0.2	0.0–1.5	0.113
<i>Campylobacter</i> spp.			
MSM status	0.2	0.0–0.7	0.011
Co-morbidity transplant	7.0	1.6–30.6	0.010
Co-morbidity cancer	7.5	2.1–26.6	0.002
Antibiotic use/chemotherapy	0.1	0.0–0.2	< 0.001
Age group (years) (Ref > 75)			
< 5	0.0	Undefined	Undefined
5–12	0.0	Undefined	Undefined
13–24	2.5	0.8–7.4	0.101
25–49	1.8	0.7–4.4	0.206
50–75	0.3	0.1–0.9	0.038
<i>N-T Salmonella</i> spp.			
MSM status	0.2	0.0–1.8	0.154
Co-morbidity surgery	0.2	0.0–1.4	0.094
Age group (years) (Ref > 75)			
< 5	0.0	Undefined	Undefined
5–12	0.0	Undefined	Undefined
13–24	14.4	2.4–85.8	0.003
25–49	3.6	0.8–16.9	0.102
50–75	0.7	0.1–4.6	0.693
Gender (Ref = Male)	7.4	2.2–24.8	0.001
<i>Shigella</i> spp.			
MSM status	5.0	1.6–16.0	0.007
Co-morbidity HIV	3.3	1.0–10.9	0.055
Suspect food	3.5	1.1–11.2	0.037

GI, gastrointestinal illness; MSM, men who have sex with men; and Ref, reference group.

DISCUSSION

To our knowledge, this is the largest multihospital study to describe the epidemiology of infectious GI illnesses in NSW, Australia. We provided an overview of GI illnesses associated with GI pathogens among people seeking care in Sydney across four major public hospitals.

There are 53 public hospitals in the eight local health districts in the Sydney Metropolitan Area, and four (8%) were included in this study to represent high density population centres. Clinical laboratories within the selected hospitals provide laboratory services for smaller hospitals in their local health districts and for some rural health services in the Newcastle, Illawarra and Hunter regions. This captures a wide population of NSW.

Viral gastroenteritis had a distinct seasonal pattern with rotavirus and norovirus infections peaking in the cooler months; adenovirus showed a less consistent monthly trend. These seasonal trends have been previously described in Sydney¹⁴ and other settings^{19,20} and is useful for public health planning and resource allocation. Whereas infections with *Campylobacter* and N-T *Salmonella* spp. were mainly foodborne, both appeared to have occurred more frequently in warmer months in the study. However, the seasonal difference was not statistically significant, probably due to small sample size. Increased incidence of viral gastroenteritis in cooler months and bacterial illnesses in warmer months implies that health promotional messages should be developed to target the respective high risk groups in each season. The relatively high prevalence of antibiotic-associated *C. difficile* infections suggests that existing protocols and practices for the control of *C. difficile* should be carefully reviewed and modified where necessary.

For parasites, *Blastocystis* was the most common parasite detected in symptomatic patients in this study; in contrast, a previous study found *Giardia* and *Cryptosporidium* to be the main intestinal parasites associated with enteric infections in Australia.²¹ This study only detected *Giardia* and *Cryptosporidium* in only 3% and 1% of cases, respectively. Previous literature revealed that *Blastocystis* spp. have emerged as the most commonly detected enteric protozoa in developed settings.²² Despite much controversy about

the pathogenicity of *Blastocystis* spp., several reports have described their association with abdominal pain, persistent diarrhoea and irritable bowel syndrome-like symptoms,^{23–25} and other reports postulate that pathogenicity may be subtype dependent.²⁶ *D. fragilis*, an emerging protozoan pathogen, was found in 3% of cases. The combination of conventional and molecular diagnostics has led to the increased detection of *D. fragilis* in Australia with its prevalence rivalling *Giardia* in developed settings.^{24,27,28}

This study found that GI illnesses affected people of all ages; however, the clinical symptoms and the prevalence of GI pathogens varied across different age groups. There were slightly more males than females in this study, which is in contrast to Australian national data which suggest an overall higher rate of GI illness in females, especially in the 20–40 years age group.⁹ The reason for these differences is not clear, but it may be related to differences in exposure between males and females at different stages of the lifespan. For example, a study from the United States of America found that more males than females will seek medical attention for severe GI symptoms.¹²

Children were more likely to be infected with enteric viruses, especially rotavirus, norovirus and adenovirus, as previously described in NSW.^{2,14,15} However, older patients were more likely to be infected with *C. difficile* as also described in Australia²⁹ and elsewhere.^{30,31} In this study, older patients (aged 50 years or above) had longer lengths of stay in hospital compared with younger children. Dysfunction of the immune system with aging and co-morbidities may increase the length of stay.^{32,33} The increased risk of *C. difficile* infection associated with prolonged antibiotic use and particularly among people with extended length of stay indicates a need for good antibiotic stewardship. Existing protocols should be carefully reviewed and modified where necessary.³⁴

There was a significant association between infection with *Shigella* spp., HIV/AIDS and MSM, which warrants further investigation. *Shigella* spp. are easily transmitted via faecal-oral sexual contact,³⁵ and outbreaks linked to unsafe sexual practices have been described among MSM,³⁶ a high-risk group for HIV/AIDS in Australia.³⁷ Public health education and promotion could be targeted toward high risk groups.

This study, like most retrospective studies, has some limitations. Only symptomatic cases that had a positive laboratory test were included in this study which may bias the results because for asymptomatic cases, the likelihood of patients reporting to hospitals is low. Obtaining clinical information from asymptomatic cases is difficult. Also, reporting to hospital for a microbiological test would be strongly influenced by the location of the hospitals and whether or not testing facilities are conveniently located in relation to their routine activities. Current clinical guidelines for the management of acute gastroenteritis do not recommend routine collection and testing of stools; hence, the results cannot represent the full spectrum of community acquired gastroenteritis.

The hospital data were reviewed retrospectively. Incompatible data records among hospitals prevented analysis of some risk factors. Also, information on some potential risk factors (e.g. MSM status, HIV/AIDS diagnosis and diarrhoea) may have been incomplete and may have affected the results.

Only some enteric pathogens are included in testing protocols. As a result, some known pathogens such as *Staphylococcus aureus* and *Bacillus cereus*, which are likely to cause foodborne outbreaks,⁶ were not tested in most stool specimens. Sensitivity of some of the tests such as microscopy and EIA^{15,28,38} are limited and some cases may be missed.^{14,36,38} Also, stool testing protocols differ among hospitals. The immunochromatographic test used by one hospital detected all adenovirus serotypes, not just the enteric serotypes 40 and 41; hence, a positive result does not necessarily mean the serotype found was the cause of the GI illness. In addition, testing for norovirus at some hospitals mainly occurred when outbreaks were suspected, which may have resulted in selection bias.

CONCLUSION

This study has revealed that GI illness is a major public health issue in Sydney, Australia with implications for resource management and disease surveillance and control. The study has identified various risk factors that can be addressed by public health interventions. Information on disease risk factors is essential for the control of infectious diarrhoea and should be routinely collected in a systematic way across hospitals. The consistent use of well-organized electronic medical records is recommended.

Conflicts of interest

None declared.

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Circulation of influenza B lineages in northern Viet Nam, 2007–2014

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Introduction: Influenza B viruses circulate throughout Viet Nam, and their activities vary by region. There have been two antigenically distinct lineages of influenza B viruses co-circulating in the past 20 years; however, only one lineage is selected as a component of contemporary trivalent seasonal influenza vaccines. To improve the understanding of circulating influenza B lineages and influenza vaccine mismatches, we report the virus lineages circulating in northern Viet Nam over an eight-year period (2007–2014).

Methods: Lineages of 331 influenza B viruses were characterized by haemagglutination inhibition assay against standard reference ferret (Yamagata) and sheep (Victoria) antisera. Sequence analysis of the haemagglutinin gene was performed in 64 selected influenza B isolates.

Results: The proportion of influenza B lineages changed by year. The Yamagata lineage predominated in 2007, 2008 and 2012; the Victoria lineage predominated in 2009–2014 except 2012. The two lineages showed continuous evolution over time. The Northern Hemisphere's influenza vaccine components were mismatched with the predominant circulating viruses in 2007, 2009 and 2014.

Discussion: The seasonality of influenza B activity is more variable in tropical and subtropical regions than in temperate zones. Our data showed a common co-circulation of both influenza B lineages in northern Viet Nam, and it was difficult to predict which one was the predominant lineage. Quadrivalent influenza vaccines containing both lineages may improve the effectiveness of influenza vaccine programmes in the future.

Influenza infection occurs as an annual seasonal epidemic in winter or early spring in countries with temperate climates.¹ Currently, four antigenically distinct groups of influenza viruses have been identified as the cause of human infection, including two subtypes of influenza A (A/H1N1 and A/H3N2) and two lineages of influenza B. The two influenza B lineages are represented by the reference strains B/Victoria/2/87 and B/Yamagata/16/88. They have co-circulated with influenza A viruses since 1983.² The proportion of the two B lineages varies by year and country; however, current seasonal influenza vaccine only includes one influenza B strain. As the two lineages have no cross-reactivity, the decision for vaccine lineage selection can be difficult in years when both influenza B lineages are circulating.³ Furthermore, differences in evolutionary and epidemiological dynamics between the Victoria and Yamagata lineages can confound the selection.⁴

In Viet Nam, influenza constitutes an important cause of influenza-like illness (ILI) among outpatients seeking clinical care.⁵ Influenza viruses circulate year-round with two distinct peaks in virus circulation⁶ unlike

in temperate climates where a single peak in the winter season is typical. Moreover, the climates of southern and northern Viet Nam differ remarkably. The climate in northern Viet Nam is humid and subtropical, while southern Viet Nam has a tropical climate all year round. Transmission patterns of influenza vary considerably in the two regions.⁷ The patterns of influenza B virus in Viet Nam did not appear synchronous with seasonal influenza A viruses. Influenza A viruses peak in the spring usually in February and March. Influenza B viruses peak from November to March in the north, are detected at similar levels throughout the year in the southern region and are at much higher levels in November to May in the central region.⁶

The Viet Nam National Influenza Surveillance System (NISS) was established in 2005 based on sentinel sites in four regions (northern, southern, highlands and central). The National Influenza Center (NIC) at the National Institute of Hygiene and Epidemiology, Ha Noi (NIHE) conducts influenza virological surveillance in northern Viet Nam. The surveillance data provides information on the effect and seasonality of influenza in

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Viet Nam and monitors influenza virus strains circulating throughout the country.^{5,6}

The two influenza B lineages have co-circulated and caused seasonal outbreaks in Viet Nam and the Asia-Pacific region since 1987;⁸ however, laboratory-based surveillance and detailed analyses of viral transmission patterns have not been conducted previously. In this study, we report the circulating lineages of influenza B in Viet Nam in the years 2007 to 2014 to improve the knowledge about this circulating virus.

METHODS

Study population

Subjects of all ages presenting to one of the seven sentinel sites in northern Viet Nam (two central hospitals in Ha Noi, three district hospitals and two outpatient clinics)⁵ with ILI using the World Health Organization (WHO) definition (body temperature $\geq 38^{\circ}\text{C}$ plus cough and/or sore throat) within three days of onset were included in the study.⁸

Sample collection

Nasopharyngeal swabs (NPS) or throat swabs (TS) were collected by trained nurses using cotton swabs (Hanacomedical Co., Ltd., Saitama, Japan). Samples were collected from the first two ILI patients per day on weekdays. Swabs were stored in in-house viral transport media.^{3,8} Samples were transferred on Friday or Monday of the following week on ice to the NIC for virological testing.⁶

Viral culture and antigenic characterization

The NPS and TS influenza B positive samples by reverse transcription polymerase chain reaction were selected for viral isolation according to NISS protocols. Viruses were harvested and stored at -80°C . Influenza B isolates were subtyped using the haemagglutination inhibition assay (HI) with reference antigens and antiserum of B/Victoria and B/Yamagata lineages using the WHO reagent kit. Ferret or sheep sera (pre-treated with receptor-destroying enzymes [Denka Seikan Co., Ltd., Tokyo, Japan]) were raised against reference strains representing the B/Victoria lineage (B/Brisbane/32/2002) and B/Yamagata (B/Shanghai/361/2002). HI assays were

performed in 96-well micro-titre plates with 0.5% chicken erythrocytes cells. Reference antiserum was diluted from 1:10 to 1:1280. Influenza B viruses were diluted to 4 haemagglutinin (HA) units/25 μl and tested following WHO guidelines.⁹ The lineages of influenza B isolates were identified by comparing them with both reference antisera; the higher titre is assumed to be homologous to Victoria or Yamagata lineage.

Vaccine strain comparison

The characterized influenza B strains were compared with the strains of WHO-recommended vaccine components for the Northern and/or Southern Hemispheres to check if the influenza B lineage was matched each year from 2007 to 2014. Mismatches between influenza B strains and vaccine strains of the same lineages were noted when their HI titre differences were more than twofold.

Molecular characterization

The influenza B isolates with sufficient 8 HA units were selected for HA genetic analysis by sequencing at NIC-NIHE. RNA extraction was conducted on 140 μl aliquot of each isolate using the viral RNA extraction kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The RNA was transcribed to cDNA using the influenza A virus universal primer (Uni 12) AGC AAA AGC AGG as described.¹⁰ The HA gene was amplified with segment-specific primers for influenza B with primers HA-25F:ATC CAC AAA ATG AAG GCA and HA1140R: ACC AGA ATA GCT CCGA. The PCR products were purified with PCR purification kit (Qiagen, Valencia, CA, USA) and labelled with Big Dye Terminator v3.1 cycle sequencing kits (Applied Biosystems, Waltham, MA, USA) according to manufacturer's instruction and then analysed by an ABI 3100 automatic DNA sequencer.

Sequences were assembled using Lasergene analysis software, version 8.0 (DNASTAR, Inc., Madison, WI, USA). Multiple sequences alignment was conducted with CLUSTAL-X (Conway Institute University College Dublin, Dublin 4, Ireland) for the major coding regions of HA segments.¹¹ Phylogenetic trees of the HA sequences were constructed by the maximum likelihood (ML) method with bootstrapping (1000 replicates), referencing the HA genes of strains B/Brisbane/32/2002 (B/Victoria), B/Jiangsu/10/2003 (B/Yamagata) and strains from the National Center for Biotechnology

Table 1. Number of influenza B lineage isolates included for phylogenetic analysis, northern Viet Nam, 2007–2014 (*n* = 64)

Year	Number of influenza B isolates selected for haemagglutinin gene analysis		
	Victoria lineage	Yamagata lineage	Total
2010	3	2	5
2011	14	8	22
2012	7	7	14
2013	4	4	8
2014	11	4	15
Total	39	25	64

Information (NCBI) influenza virus resource website.¹² ML trees were estimated using the best fit nucleotide substitution model.¹³ To quantify amino acid sequence diversity, Basic Local Alignment Search Tool (BLAST) in Molecular Evolutionary Genetics Analysis (MEGA) 5 was used to search within NCBI to find the closest sequence available for representative strains of each lineage.¹¹

All sequences reported in this study have been deposited in the GenBank database under accession numbers from KT359277 to KT359340.¹⁴

Ethical approval

The National Institute of Hygiene and Epidemiology, Viet Nam and Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America provided ethical committee approval for the study. All participants provided written informed consent.

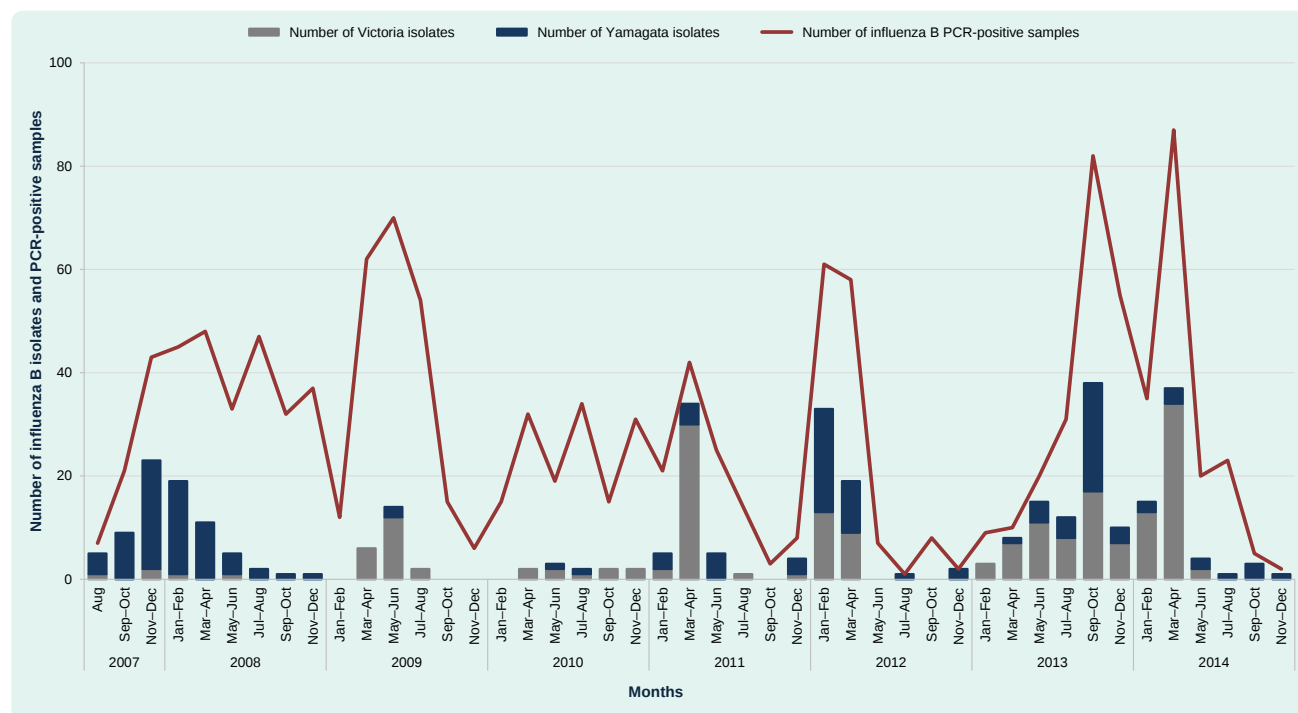
RESULTS

In total, 331 influenza B isolates were collected from NISS and we selected 64 virus isolates that had HI titres higher than 8 HA units for sequence analysis (Table 1).

Influenza circulating patterns

Data from NISS in the years 2007 to 2012 indicated that influenza B circulated throughout the year with activities primarily peaking in March and April (Figure 1). For the 331 influenza B isolates, we found 195 isolates belong to the Victoria lineage and 136 isolates belong to the Yamagata lineage. They were detected in all years of the study. The Yamagata lineage was predominant in 2007, 2008 and 2012. The Victoria lineage was

Figure 1. Number of influenza B isolates and PCR-positive samples by bi-monthly, northern Viet Nam, 2007–2014



PCR, polymerase chain reaction.

Table 2. Influenza B vaccine components and distribution of influenza B lineages in northern Viet Nam, 2007–2014

Northern hemisphere		Southern hemisphere		Number of isolates in Viet Nam included in the study		
Influenza seasons	Vaccine candidate strains	Influenza seasons	Vaccine candidate strains	Years	Victoria lineage (n = 195)	Yamagata lineage (n = 136)
2007–2008	B/Malaysia/2506/2004-like virus	2007	B/Malaysia/2506/2004-like virus	2007	2	24
2008–2009	B/Florida/04/2006-like virus	2008	B/Florida/04/2006-like virus	2008	2	29
2009–2010	B/ Brisbane/60/2008 -like virus	2009	B/ Brisbane/60/2008 -like virus	2009	21	3
2010–2011	B/Brisbane/60/2008-like virus	2010	B/Brisbane/60/2008-like virus	2010	12	1
2011–2012	B/Brisbane/60/2008-like virus	2011	B/Brisbane/60/2008-like virus	2011	37	4
2012–2013	B/Brisbane/60/2008-like virus.	2012	B/Brisbane/60/2008-like virus.	2012	21	31
2013–2014	B/Wisconsin/1/2010-like virus.	2013	B/Wisconsin/1/2010-like virus.	2013	50	32
2014–2015	B/Massachusetts/2/2012-like virus	2014	B/Massachusetts/2/2012-like virus	2014	50	12

Note: B/Malaysia/2506/2004-like virus and B/Brisbane/60/2008-like virus belong to the B/Victoria /7/87 lineage. B/Florida/04/2006-like virus, B/Wisconsin/1/2010-like virus and B/Massachusetts/2/2012-like virus belong to the B/Yamagata/16/88 lineage.

predominant in 2009 through 2011 and 2013 through 2014 (Table 2).

Antigenic analysis

The HI titres of the reference antisera against most of the influenza B/Victoria lineage study isolates (190/195, 97.3%) were less than twofold different from the WHO-recommended vaccine strain (B/Brisbane/60/2008). Similarly, almost all influenza B/Yamagata isolates (124/136, 91.2%) reacted well with the reference antisera raised against the recommended vaccine strains (B/Florida/04/2006; B/Wisconsin/01/2010 or B/Massachusetts/2/2012).

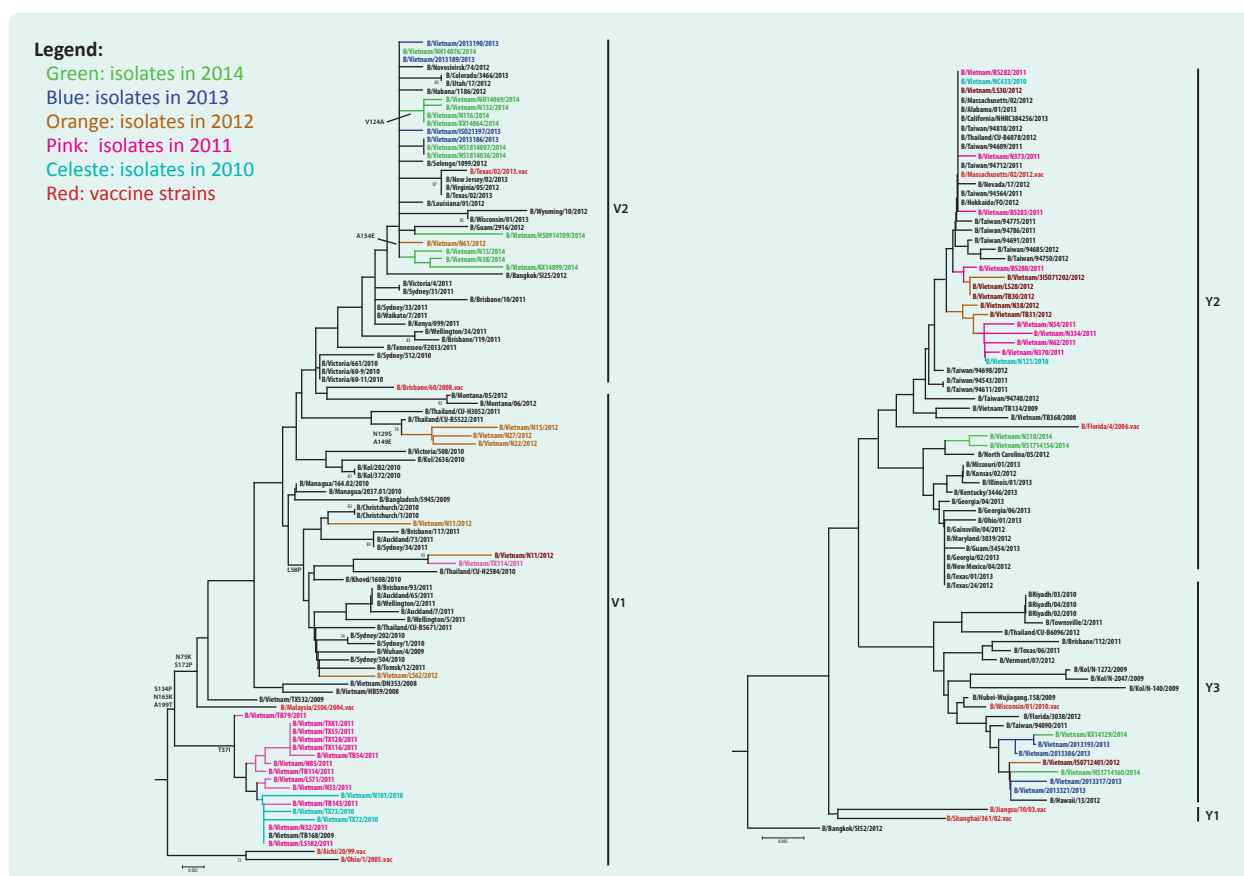
Phylogenetic analysis

The HA gene phylogenetic analysis of the 64 virus isolates revealed that 39 isolates belonged to the Victoria lineage and 25 isolates to the Yamagata lineage. The phylogenetic trees showed different genetic diversities of Victoria and Yamagata lineages based on nucleotide differences in the HA1 region. The influenza B/Victoria phylogenetic tree can be diversified into two clades (V1 and V2). Clade V1 contained all of the isolates in 2010 (3 isolates), 2011 (14 isolates) and almost all isolates in 2012 (6/7 isolates). Clade V1 was clustered

with isolates from Australia, Bangladesh and Thailand. They all shared amino acid changes at S134P, N165K and A199T compared to the recommended vaccine strain (B/Malaysia/2506/2004). In total, 16 isolates collected in 2012 (1 isolate), 2013 (4 isolates) and 2014 (11 isolates) were grouped into Clade V2. They were highly homologous with viruses from Australia, Thailand, the United States of America and the reference B/Brisbane/32/2002 strain (Figure 2).

The Yamagata lineage was split into three clades as Y1, Y2 and Y3. Clade Y1 did not contain any of the 25 influenza B/Yamagata isolates in our study. Clade Y2 was grouped by 17 isolates in 2010–2012, two isolates in 2014 and others from China, the United States of America and Japan. Clade Y2 was closely related to the recommended vaccine B/Florida/4/2006 strain. The common amino acids different from Clade Y2 to reference strain B/Jiangsu/10/2003 are at R48K, P108A, I150S, I166N, T182A, S203N and D230G. Clade Y3 had four isolates in 2013 and two isolates in 2014 together with circulating viruses in China, Thailand and the United States of America. In 2009–2012; this clade showed amino acid differences at N116K, K299E and E313K when compared to the B/Jiangsu/10/2003 stain (Figure 2).

Figure 2. Phylogenetic analysis of the haemagglutinin gene of the circulating influenza B lineages in northern Viet Nam, 2010–2014 ($n = 64$)



Circulating and vaccine strains

For most of the study years of 2007 through 2014 (6/8, 75.0%), the same influenza B vaccine candidate strains were recommended for both the Northern and Southern Hemispheres, except 2009 and 2012. Vaccine formulation in the Northern Hemisphere was updated in 2009 from B/Malaysia/2506/2004 to B/Brisbane/60/2008 (Victoria lineage). The Yamagata lineage was also updated in 2012 from B/Florida/04/2006-like to B/Wisconsin/1/2010 and B/Massachusetts/02/2012 in 2014.

Possible mismatches were found between the Vietnamese predominant influenza B circulating strains and the WHO-recommended influenza B vaccine components (Northern Hemisphere) in 2007 (B/Yamagata lineage), 2009 and 2014 (B/Victoria lineage) (Table 2).

Furthermore, the WHO-recommended strains only matched about half of the contemporary circulating

influenza B strains in 2008–2012. For example in 2012 (B/Yamagata lineage), only 31/52 (59.9%) isolates in this study were grouped as Yamagata lineage (Table 2).

DISCUSSION

Co-circulation of both influenza B lineages with different proportions by year is common not only in subtropical countries such as Viet Nam and Hong Kong Special Administrative Region (SAR), China, but also in temperate countries such as the United States of America and European countries.^{1,3,15,16} This is the first report about circulation of influenza B lineages in Viet Nam.

Our data indicated that the pattern of circulating influenza B lineages varies, similar to the findings in Australia, Brazil, Hong Kong SAR, China and Scotland.^{17–20} Mismatches were found between the vaccines and circulating strains throughout the study years, suggesting the vaccines may not have been effective for northern Viet Nam in those years.

Our study found no differences in titres in most of the circulating viruses against the reference, indicating that these viruses were not antigenically distinguishable from reference and vaccine candidate strains. There was no evidence of major antigenic drift of the influenza B viruses during the study period. Therefore, the influenza B isolates in this study still shared most of their antigenic properties with the vaccine-candidate strains.

Phylogenetic analysis showed both Victoria and Yamagata lineages have high similarity to viruses circulating in Viet Nam and neighbouring countries. The genetic diversity of the HA gene indicated at least two subclades within each lineage, and the presence of amino acid substitutions in HA epitopes of isolates indicated antigenic drift is ongoing (Figure 2).

Influenza B viruses, unlike influenza A viruses, have multiple evolutionary lineages which can co-exist for considerable periods of time.²¹ This has occurred since the early 1980s when a new lineage (B/Yamanashi/16/88-like) appeared to evolve from B/USSR/100/83-like viruses. Since then it has co-circulated with the existing virus lineage (B/Victoria/2/87-like).² According to data from the WHO Global Influenza Surveillance and Response System, both lineages of influenza B viruses have co-circulated in different countries concurrently,^{3,22} making the selection of annual influenza vaccine components for influenza B difficult.

Although influenza B viruses are less likely to trigger widespread epidemics than influenza A, influenza B viruses co-circulate with influenza A and sometimes are predominant in Viet Nam. The prevention of influenza infection remains a public health priority in Viet Nam; vaccination is the main tool for prevention. Trivalent vaccine has been used worldwide, but it may not be effective if the influenza B vaccine components are mismatched. Recently, the quadrivalent influenza vaccine that contains two influenza B strains was used in some countries such as Canada, United States of America and several European countries.²³ The efficacy of quadrivalent vaccines was found to be higher than that of the trivalent ones,²⁴ although its effectiveness is yet to be determined in Viet Nam. Quadrivalent influenza vaccines may be one solution to help improve the efficacy of influenza vaccine programmes in the future. Meanwhile, changes of influenza B strains in the upcoming influenza seasons remain unpredictable. It is recommended that influenza surveillance be continued year-round for monitoring the

disease trends to help inform influenza vaccine policies for disease prevention and control.

This study has several limitations. As the sample size is small, the overall trends of influenza B circulation may be difficult to discern without further analysis. Circulating patterns of the two influenza B lineages may not be representative of all of Viet Nam as the study only included samples from northern Viet Nam. Future studies that include variables from different geographical regions as well as climate, social conditions and other factors are encouraged. Information for other genes such as neuraminidase (NA) and internal gene segments is lacking. Nevertheless, results from another study has shown that the evolutionary and epidemiology dynamics observed in NA and internal gene segments were similar to those observed in the HA genes in both the Victoria and Yamagata lineages.⁴

Our results provide additional information about virological characteristics of seasonal influenza B viruses in northern Viet Nam, which may lead to new influenza vaccine policies in the future.

Conflicts of interest

None declared.

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Non-tuberculous mycobacteria: baseline data from three sites in Papua New Guinea, 2010–2012

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Objective: To determine the proportion of non-tuberculous mycobacteria (NTM) in samples of pulmonary tuberculosis (TB) cases from Papua New Guinea who were diagnosed using acid-fast microscopy.

Methods: As part of a case detection study for TB, conducted in three provincial hospitals in Papua New Guinea, sputum samples of suspected tuberculous cases aged 15 years or older were collected from November 2010 to July 2012. Mycobacterial species isolated from sputum and grown in culture were examined to distinguish between NTM and the *Mycobacterium tuberculosis* complex (MTBC).

Results: NTM were detected in 4% (9/225) of sputum samples grown in culture. Five (2.2%) of them were identified as NTM only and four (1.8%) were identified as mixed cultures containing both MTBC and NTM. Four different NTM species were identified; *M. fortuitum*, *M. intracellulare*, *M. terrae* and *M. avium*.

Discussion: This is the first report from Papua New Guinea identifying NTM in three different locations. As NTM cannot be distinguished from *M. tuberculosis* through smear microscopy, the presence of NTM can lead to a false-positive diagnosis of tuberculosis. The prevalence of NTM should be determined and a diagnostic algorithm developed to confirm acid-fast bacilli in a smear as *M. tuberculosis*.

A part from the *Mycobacterium tuberculosis* complex (MTBC), the genus *Mycobacterium* includes over 120 species of non-tuberculous mycobacteria (NTM).¹ NTM can be found in the environment, including water and soil, which is the suspected source of occasional infection of humans. Asymptomatic colonization as well as symptomatic disease can be caused by NTM,² including, among others, chronic pulmonary disease with symptoms similar to tuberculosis (TB) such as chronic cough (with or without sputum production), chest pain and weight loss.^{3,4} Different NTM have been associated with different disease presentations. The *M. avium* complex (including *M. avium* and *M. intracellulare*) is most often associated with pulmonary infection. *M. fortuitum* has been associated with pulmonary infection but more often affects the skin, soft tissue or bones. Immunocompromised cases (e.g. human immunodeficiency virus [HIV] positive cases) are susceptible to NTM infection, particularly disseminated *M. avium* disease.² However, immunocompetent cases with no predisposing conditions can also be affected.^{5–8}

Standard first-line anti-TB treatment drugs are less effective against NTM compared to *M. tuberculosis* (*Mtb*),^{2,9} and no single regimen for NTM exists to date. Depending on the NTM species, recommendations for treatment regimens include treatment with antibiotics and sometimes even surgical removal of infected tissue.^{2,10} The *M. avium* complex is treated with combination therapy consisting of Clarithromycin, Rifampicin and Ethambutol and should be continued for one year.¹¹ While the regimen includes Rifampicin and Ethambutol, two of the standard first-line anti-TB drugs, the length of the TB regimen is not sufficient to address *M. avium* complex infections. Additionally, Isoniazid (apart from Rifampicin the most potent first-line anti-TB drug) has only a limited effect on *M. avium*,⁹ and relapses are common.²

Little data are available on the prevalence of NTM infections in TB high-burden countries, but the incidence can nevertheless be substantial.¹² High TB-burden countries also tend to be resource-poor

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countries, and the diagnosis of pulmonary TB is based on the microscopic detection of acid-fast bacilli (AFB) in sputum samples. Smear microscopy cannot distinguish between NTM and *Mtb*. Mixed infections as well as false-positive TB diagnosis cannot be ruled out. Many diagnostic assays are not optimized to detect different NTM species; if NTM are present in conjunction with *Mtb*, the former might remain undetected or cause failure of drug susceptibility testing (DST).^{13–15} Exposure to NTM has been suggested to impact on the efficacy of the Bacille Calmette-Guérin vaccine¹⁶ and to exhibit cross-reactivity to the tuberculin skin test (TST), leading to increased difficulties in interpreting TST-positive results and evaluating the protection through the only available vaccine against TB.^{17,18}

Very little information is available on NTM in Papua New Guinea. Data from a leprosy trial conducted in Karimui (Eastern Highlands Province) in the 1960s^{19,20} as well as a TST sensitivity study conducted in the Marawaka area of the Eastern Highlands of Papua New Guinea²¹ found no evidence for environmental mycobacteria being present in this area. Therefore it was important to investigate the presence of NTM in sputum samples collected in Papua New Guinea. Here we describe the NTM detected and provide baseline information on these bacteria in Papua New Guinea.

METHODS

As part of a case detection study for TB, conducted between November 2010 and July 2012 in selected provincial hospitals in Papua New Guinea, sputum samples of suspected TB cases aged 15 years or older were collected for laboratory testing. The sampling procedure has been described previously.²²

Upon diagnosis of TB through AFB Ziehl-Neelson (ZN) microscopy or chest X-ray, sputum samples were decontaminated following Petroff's method;²³ inoculated into BD Bactec® Mycobacterial Growth Indicator Tube (MGIT) media (Becton, Dickinson and Co., Franklin Lakes, New Jersey, USA); and subsequently sent to the Queensland Mycobacterium Reference Laboratory in Brisbane, Australia for culture. The samples were incubated in the MGIT until they became culture positive (i.e. growth could be detected). A repeat ZN smear was prepared on all culture-positive isolates to confirm the presence of acid-fast organisms. A rapid immuno-

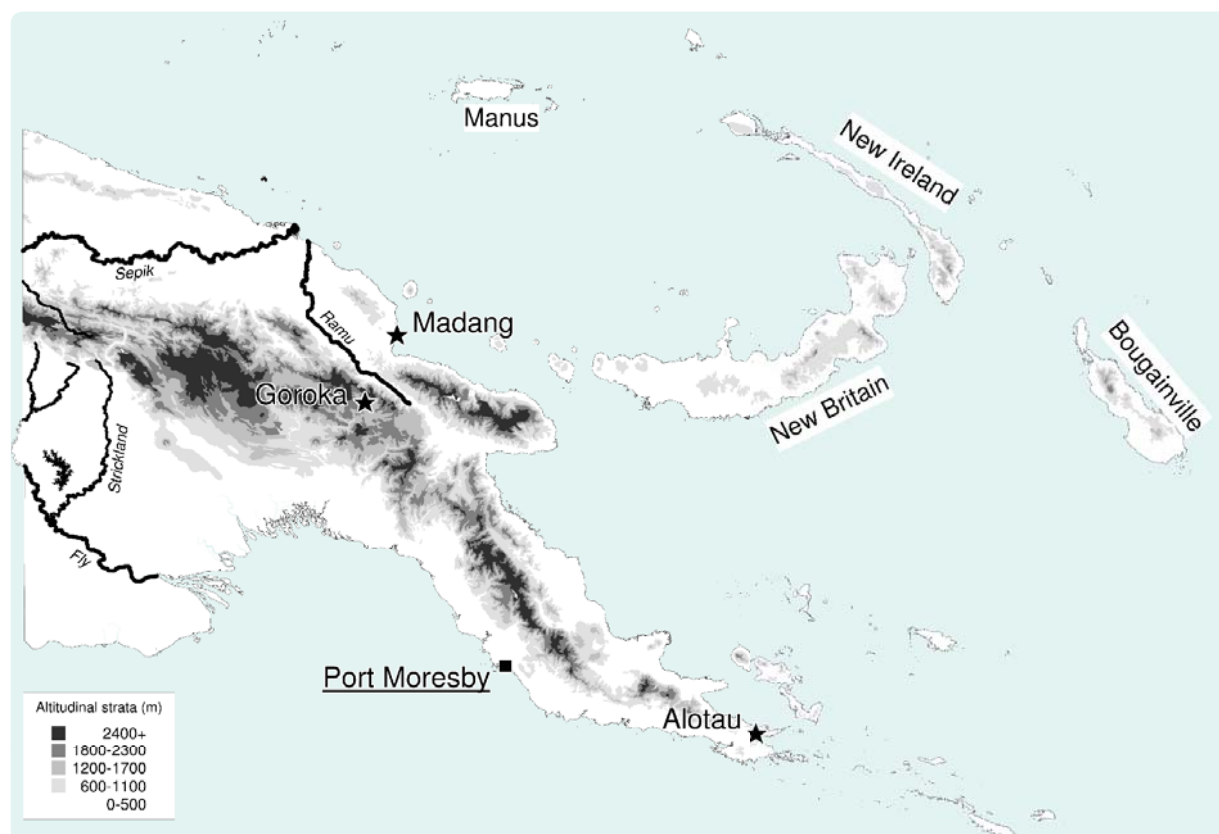
chromatographic identification test (SD BIOLINE/BD TB Ag MPT64 Rapid, Standard Diagnostics, Giheung-gu, Republic of Korea) was used to confirm the AFB as MTBC.

When the rapid test was negative or the microscopic morphology did not suggest the AFB were MTBC, further molecular analysis was conducted to identify the isolate as NTM or MTBC. In brief, DNA was extracted using crude boil method at 95 °C for 30 minutes, followed by sonication for 15 minutes. The extracted DNA was then used as a template for polymerase chain reaction (PCR) amplification either according to the GenoType® Mycobacterium Common Mycobacteria line probe kit (Hain LifeSciences, Nehren, Germany) according to the manufacturer's protocol for the GenoType 16S rRNA (Forward primer 5' AGAGTTGGATCCTGGCTCAG; Reverse primer 5' CCTACGAGCTCTTTACG). The amplified product was purified using 4ul EXOSAP-IT (Affymetrix, San Diego, California, USA) and 10ul of primary amplification product (37 °C 15 minutes, 80 °C 15 minutes, 40 °C soak). A repeat gel was run using Invitrogen Bufferless Gel system (ThermoFisher Scientific, Waltham, Massachusetts, USA). The sequencing reaction was performed using the Big Dye Terminator method on ABI3130 sequencer (Distribio, Dudelange, Luxembourg), and the resulting sequences were analysed by comparing them to the National Center for Biotechnology Information Genbank database. In case cultures were identified as MTBC, DST was subsequently performed by the proportion method,²⁴ as described previously.²⁵ However, if a culture turned out to be NTM, no DST was performed.

Demographic and clinical symptoms of the cases were also collected for analysis. Statistical analysis was carried out with Stata 12.1 (Stata-Corp, College Station, Texas, USA). Excel was used for basic calculations. Due to a small sample size, no statistical analysis for the NTM population was performed.

Ethical approval for this study was granted by the Papua New Guinea Institute of Medical Research Institutional Review Board (IRB No. 0913) and the Papua New Guinea Medical Research Advisory Council (MRAC No. 10.02). The Ethik-Kommission beider Basel has been informed and had approved the study. Written informed consent was obtained from all study participants.

Figure 1. Map of TB passive case detection study sites in Papua New Guinea, 2010–2012



Note: Map designed by authors using MapInfo Professional 7.0.

RESULTS

A total of 396 sputum samples were collected in three provincial hospitals in Papua New Guinea (Figure 1). Of the collected samples, 335 were sent to Australia for culture and 225 samples grew in culture. NTM were detected in 4% (9/225) of those samples. Five (2.2%) samples contained a NTM only, consisting of three isolates of *M. fortuitum*, one isolate of *M. terrae* and one isolate of *M. intracellulare*. Four (1.8%) isolates were identified as mixed cultures containing both bacteria of the MTBC and NTM. These included three cultures of MTBC and *M. avium* and one culture of MTBC and *M. intracellulare* (Table 1).

All but one of the NTM infections were detected in females. All the cases with either a mixed infection or a NTM infection only had reported productive coughs for at least two weeks. All the cases with a mixed infection of MTBC and NTM additionally reported weight loss and at least one other symptom, including breathing difficulties ($n = 3$), chest pain ($n = 3$) fever and night sweats ($n = 2$). Among the five cases with an NTM infection

only, four cases reported shortness of breath and fever. Three of those cases experienced weight loss and either chest pain or night sweats, or both. The case infected with *M. intracellulare* reported no other symptoms except for productive cough and headache. None of the cases had reported any previous TB episode (Table 1).

DISCUSSION

To our knowledge this is the first study describing the presence of NTM in Papua New Guinea. In five (2.2%) of the 225 cases, the isolate was identified as a NTM. Without culture results from at least one more follow-up sample, this may indicate several false-positive TB cases. General symptoms caused by NTM infections cannot be distinguished from symptoms observed in TB cases, and the appearances of the bacteria cannot be differentiated when examined by AFB ZN light microscopy.

It is interesting that in our case cohort all but one NTM isolates were found in females; the only isolate identified in a male was *M. terrae*. There are some NTM species which were more commonly isolated

Table 1. Characteristics and symptoms reported of the cases with NTM detected in their sputum samples, Papua New Guinea, 2010–2012 (*n* = 9)

Sex	Age (years)	Mycobacterial strain	Symptoms					Disease outcome
			Fever	Weight loss	Night sweat	Breathing difficulties	Chest pain	
Female	50	MTBC + <i>M. avium</i>	Yes	Yes	Yes	Yes	No	Defaulted
Female	28	MTBC + <i>M. avium</i>	No	Yes	No	Yes	No	Lost to follow-up
Female	19	MTBC + <i>M. avium</i>	Yes	Yes	Yes	No	No	Lost to follow-up
Female	32	MTBC + <i>M. intracellulare</i>	Yes	Yes	No	Yes	Yes	Treatment completed
Female	18	<i>M. fortuitum</i>	Yes	Yes	Yes	Yes	Yes	Cured
Female	28	<i>M. fortuitum</i>	Yes	No	Yes	Yes	No	Treatment completed
Female	50	<i>M. fortuitum</i>	Yes	Yes	No	Yes	Yes	Unknown
Female	60	<i>M. intracellulare</i>	No	No	No	No	No	Cured
Male	36	<i>M. terrae</i>	Yes	Yes	Yes	No	Yes	Unknown

MTBC, *Mycobacterium tuberculosis* complex; and NTM, non-tuberculous mycobacteria.

from females.^{2,7,26} Another study showed an increased prevalence of funnel chest (*pectus excavatum*) and abnormal narrowing of the thoracic dimension in female cases infected with NTM of the *M. avium* complex not seen in males.²⁶ Also, the so-called Lady Windermere syndrome, a specific pulmonary disorder caused by bacteria of the *M. avium* complex, was only found in women.²⁷

There are only a few reports on NTM from TB-endemic countries,³ and it is generally difficult to compare our findings with studies from other countries. In a recently published study from Nigeria, for example, 15% of culture-grown mycobacteria isolated from presumptively diagnosed pulmonary TB cases were NTM.²⁸ Compared to that study, a ratio of 2.2% in our study is relatively low. However, culture criteria of these two studies differed. Whereas in our study only smear-positive samples were cultured. A 2013 study also included smear-negative samples, which turned out to be more strongly associated with NTM infections than smear-positive samples.²⁸ It is likely that limiting culture to smear-positive isolates in our study has reduced the chances of detecting NTM in sputum. However, culturing smear-positive samples only is in accordance with the protocols of the National TB Programme of Papua New Guinea and a result of logistic challenges arising from the lack of an in-country culture facility.

Our study population was furthermore limited to suspected pulmonary TB cases aged 15 years or

above from three sites within Papua New Guinea, and it is unclear whether inferences can be made to the rest of Papua New Guinea. Nevertheless, compared to the few studies conducted in Papua New Guinea in the 1960s and 1980s,^{19–21} where tuberculin skin testing did not provide evidence for NTM, our results highlight the existence of NTM in the community and the potential impact on TB diagnosis in the country. While the possibility remains that the presence of NTM in sputum specimens is due to colonization with these environmental organisms, they can also lead to false-positive TB diagnosis when AFB smear microscopy is used alone. The standard anti-TB treatment is not ideal for NTM, as different antibiotics than the ones used against TB are required to treat NTM,^{2,10} leading to an additional burden for the case as well as the National TB Programme. With an increasing burden of HIV/AIDS, NTM may also become an increasing source of disease, requiring different approaches for case management and treatment.

In Papua New Guinea, the diagnosis of multidrug-resistant (MDR) TB was for a long time based on the observation of repeated treatment failure despite compliance with treatment.²⁹ Since 2012, TB drug resistance surveillance based on Xpert® MTB/RIF assay (Cepheid, Sunnyvale, California, USA) has started in a few major cities.³⁰ However, it probably remains difficult for many health facilities to obtain a culture/DST-confirmed diagnosis of MDR-TB. If the actual cause of treatment failure is not drug resistance, but an NTM infection,

this would have a major impact on individual case management, especially if the symptoms of the disease are similar to those of MDR-TB. This has been shown in a study from India, where 17.6% of the suspected MDR pulmonary TB cases were actually NTM infections.³ An additional challenge to the laboratory is the presence of mixed infections of NTM and MTBC; reliable DST for MTBC may be difficult if the strain cannot be isolated in pure culture, leading to false positivity including incorrect designation of MDR-TB and extensively drug-resistant TB.

As our sample size of detected NTM is small, further studies are required to obtain significant data to establish a valid diagnostic algorithm and treatment guidelines for pulmonary diseases caused by NTM. However, no NTM identification is yet performed in the framework of the National TB Programme in Papua New Guinea, and to date, no biosafety level 3 laboratory required for culturing mycobacteria is available in the country. Samples from cases suspected of having MDR-TB are shipped to a mycobacterium reference laboratory in Australia for culture. In-country mycobacterial culture would distinguish TB from NTM infections much more rapidly and at the same time improve the detection of drug-resistant TB.

It is recommended that NTM infection surveillance could be added to the TB drug resistance surveillance of the National TB Programme.³⁰ Data from NTM surveillance would determine NTM's role in pulmonary disease in Papua New Guinea and would inform health authorities to target interventions and response in the future. This would relieve both cases and the health system. As Xpert® MTB/RIF assay is not detecting NTM, smear-positive but Xpert® MTB/RIF-negative results could be used as an indicator for NTM infection and as a basis for further investigation. Until culture becomes available within the country, PCR-based assays amplifying the internal transcribed spacer region of 16–23S rRNA could be implemented at the country's Central Public Health Laboratory to distinguish NTM from MTBC directly from clinical samples.³¹

Conflicts of interest

None declared.

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The influence of the Great East Japan Earthquake on tuberculosis control in Japan

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The Great East Japan Earthquake and subsequent tsunami hit the Pacific Ocean side of north-eastern Japan on 11 March 2011,¹ resulting in more than 18 000 deaths and missing people in three prefectures: Iwate, Miyagi and Fukushima.² Of those deaths, 65% were aged 60 years and older, and more than 90% were caused by drowning.³ The earthquake also destroyed nuclear power plants in Fukushima, causing high levels of radioactive contamination.⁴ As a result, there were 386 739 evacuees staying in 2182 temporary shelters such as community centres, schools and gymnasiums one week after the disaster.⁵

In Japan, tuberculosis (TB) control activities are conducted by public health centres (PHCs) and treatment support is provided by public health nurses (PHNs). This study describes the TB situation in the affected areas and assesses the effectiveness of Japan's TB control efforts after the disaster.

METHODS

We obtained data on casualties of the disaster from the National Police Agency and Ministry of Internal Affairs and Communications.² From April 2011 to March 2014, teams of medical doctors and PHNs of the Japan Anti-Tuberculosis Association (JATA) visited eight PHCs and three hospitals for TB patient follow-up in the eight disaster-affected PHC areas where the mortality or missing rate was higher than 0.1%. Data for each TB patient, including bacteriological test results, regimen and treatment outcome were collected by the PHNs for analysis. Information on individual TB patient support and TB outbreaks in shelters were collected at consultation meetings with local staff during the JATA

team visits.⁶ TB outbreaks were confirmed by the interferon- γ release assay as reported elsewhere.^{7,8}

TB notification data at PHCs were obtained with permission of local governments. TB notification rates were compared between disaster-affected and non-affected areas using the chi-square test. Analysis was conducted using Microsoft Excel (Microsoft Excel 2010, Redmond, USA). A *P*-value < 0.05 was considered statistically significant. Ethical approval was obtained from the Research Institute of Tuberculosis, JATA.

RESULTS

There were 96 TB patients on treatment in the eight PHC areas at the time of the disaster. The consultation meetings revealed that no TB patients had defaulted from treatment in these areas.

Death of TB patients from disaster

Seven TB patients died during the disaster (five from PHC D, one from PHC G and one from PHC H). Mortality of TB patients (7.3%) was higher than that of the general population (1.3%) in these areas. In the PHC D area, mortality of TB patients was much higher than that of the general population (23.8% versus 2.7%) (**Table 1**). Mortality of TB patients aged 60 years or older (30.7%, 4/13) was higher than that of those younger than age 60 (12.5%, 1/8) in this area.

TB outbreak in shelters

Two TB outbreaks in different shelters were reported in the disaster-affected PHC areas in 2011. The first

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Table 1. **PHC areas with mortality or missing rates higher than 0.1% in three affected prefectures in Japan after the Great East Japan Earthquake, 2011**

PHC area	Number of TB patients on treatment	Number of TB patients dead or missing (%)	Death or missing rate of general population (%) ²
A (Iwate)	1	0 (0)	3.5
B (Iwate)	6	0 (0)	4.2
C (Iwate)	5	0 (0)	1.4
D (Miyagi)	21	5 (23.8)	2.7
E (Miyagi)	8	0 (0)	2.4
F (Miyagi)	18	0 (0)	0.7
G (Fukushima)	10	1 (10.0)	0.8
H (Fukushima)	27	1 (3.7)	0.1
Total	96	7 (7.3)	1.3

PHC, public health centre; and TB, tuberculosis.

outbreak involved an 80-year-old female staying in a 60 square-metre shelter with about 50 people. The ventilation was poor as windows were closed due to cold weather. Nine people were confirmed to have latent TB infection (LTBI). Another outbreak involved a 50-year-old male staying in a large shelter with about 2500 people. In the subdivision where the subject stayed, ventilation was poor due to low ceilings and the surrounding three walls. In this outbreak, two TB patients and 18 people with LTBI were identified.

TB notification trend

From 2010 to 2013, the annual TB notification rate did not change significantly in the eight disaster-affected PHC areas (11.4, 9.4, 11.2 and 9.9 per 100 000 individuals, $P = 0.262$) and in other PHC areas (12.0, 10.5, 10.3 and 11.1 per 100 000 individuals, $P = 0.096$) in the three prefectures. TB notification rates were also not significantly different between the disaster-affected areas and other areas in 2011–2013 ($P = 0.115$).

DISCUSSION

We found no TB patients had defaulted from treatment in disaster-affected areas. An increase in TB notifications was also not observed after this disaster, but TB outbreaks in shelters occurred.

Immediately after the disaster, 11.8% (45/380) of hospitals were damaged and could not receive TB patients.⁹ Nevertheless, the TB notification results indicated that epidemics did not occur after this disaster

probably because the majority of the health systems were still well-maintained and functioning.¹⁰ The consultation meetings revealed that in the week after the disaster in Fukushima, PHC staff engaged in specific post-disaster work such as surveys of casualties and damaged medical facilities, assisting evacuation of patients from hospitals, irradiation screening for evacuees and supervision of shelters. Nevertheless from the second week onward, TB control activities were gradually resumed.

PHNs' efforts on timely resumption of TB control activities contributed to no treatment defaults. For example, in Miyagi, the PHC D building was completely immersed by water. All paper records of TB patients were lost, and all computers with patients' electronic records were damaged. Despite this situation, the PHNs conducted active patient searches to locate all 21 registered TB patients. Treatment of surviving TB patients was resumed at the end of March 2011.

Various partners, including other PHCs, medical facilities and TB patients' family members provided information on the TB patients for reporting. For example, in PHC G, one patient was missing after the tsunami. However, information of the remaining nine evacuated patients was provided by the partners and treatment continued. The successful tracking of TB patients indicated that the partners understood the necessity of reporting. Good coordination among partners also contributed to no TB treatment defaults.

The disaster-related mortality of TB patients was found to be higher than that of the general population. Although there was no evidence that TB was directly

associated with the deaths in this disaster, co-morbidities of the TB patients might have led to inferior mobility and hindered their evacuation. Also, the mortality was found to be higher in the older age groups.² Older people have been considered less able to make a quick evacuation.¹ Special evacuation strategies should be formulated to reduce the mortality of these vulnerable groups.

To prevent TB outbreaks in shelters, information on TB prevention and diagnosis should be disseminated. In response to the first reported outbreak, JATA provided a two-page guideline for TB prevention and diagnosis at shelters in April 2011.⁶ Official letters were sent to the local governments to encourage its utilization.

As this study did not have comprehensive documentation for all TB patients except for treatment outcome and selected data, only some examples were reported. This may have affected the results' representativeness and accuracy.

To conclude, the results showed that post-disaster measures were effective in supporting the TB patients. TB should be included in the protocol for health care for evacuees in shelters.

Conflicts of interest

None declared.

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Elements of successful management of an imported Middle East respiratory syndrome case in Guangdong, China

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Recently, the Middle East respiratory syndrome (MERS) in the Republic of Korea was featured by the *Western Pacific Surveillance and Response Journal* (WPSAR) describing the key for controlling this epidemic as transparency and communication.¹ Since the discovery of MERS-coronavirus (MERS-CoV) in 2012, there have been several MERS-confirmed cases in the Western Pacific Region, including two from the Philippines.^{2,3} During the 2015 MERS epidemic in the Republic of Korea, one imported case was confirmed in Guangdong Province, China on 29 May 2015.⁴ Based on our experiences of combating severe acute respiratory syndrome, influenza A(H1N1) and A(H7N9) epidemics, we agree that communication is the key, and international information exchange plays a critical role in infectious disease risk communication.

While Fung et al.¹ emphasized transparency and communication between the local government and the public, here we focus more on the importance of coordination within the government and communication among international partners. For the imported MERS case, timely information of the situation was shared effectively among the World Health Organization, China and the Republic of Korea during the critical moments under the framework of the International Health Regulations (2005).⁵ An outbreak investigation team involving the local hospitals, Chinese Center for Disease Control and Prevention (China CDC) and other relevant parties was formed and coordinated by the Chinese government. The role and responsibility of each team member was clearly defined to ensure efficiency. Hospitals were responsible for case treatment and infection control;

China CDC was responsible for epidemiologic investigation, field disinfection, public risk communication and cooperating with the immigration and security department for close contacts tracing and quarantine. These minimized the probability of secondary transmission of MERS-CoV in hospitals as well as in the community.

With sufficient and accurate information, timely and suitable measures can be applied for effective infection control. Similar to the first imported MERS case in the Philippines in 2015,³ immediate responses such as identification of the case and close contacts were taken to control virus spread. The Chinese local health department was able to locate and transfer the case to a designated hospital within four hours after WHO notification. Laboratory results were also quickly confirmed by the Guangdong provincial CDC and China CDC. Efforts were made to trace every close contact (defined by National Health and Family Planning Commission of China)⁶ through a variety of approaches, including the use of social networks. In total, 86% (62/72) of close contacts were traced within one day after the notification, and all close contacts were traced within five days after the notification. These contacts were quarantined according to the national regulations on emergency public health events.⁶ We found none of the contacts had developed respiratory symptoms and none tested positive for MERS-CoV.

To conclude, the successful management of the imported MERS case in China echoed the merits of a rapid “information for action” response for emerging

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infectious diseases and should be promoted by countries with similar infection risk.

Conflicts of interest

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