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Ongoing hepatitis A outbreak in Europe 2013 to 2014: imported berry mix cake suspected to be the source of infection in Norway

B Guzman-Herrador (BernardoRafael.Guzman.Herrador@fhi.no)[1], L Jensvoll[2], M Einöder-Moreno[1,3], H Lange[1,3], S Myking[1,4], K Nygård[1], K Stene-Johansen[4], L Vold[1]

1. Department of Infectious Disease Epidemiology, Norwegian Institute of Public Health, Oslo, Norway
2. Norwegian Food Safety Authority, Head Office, Ås, Norway
3. European Program for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
4. Department of Virology, Norwegian Institute of Public Health, Oslo, Norway

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On 7 March 2014, an increase in hepatitis A virus (HAV) infections was identified in Norway. As of 12 April, 19 cases of HAV infection with a virus strain identical to an ongoing European outbreak have been identified. Six probable cases are currently under investigation. On 11 April, a frozen berry mix cake imported from another European country was found as the likely source of the outbreak; the importer has withdrawn the product in Norway.

On 7 March 2014, the Department of Infectious Disease Epidemiology at the Norwegian Institute of Public Health (NIPH) identified an increase in domestic cases of hepatitis A without travel history within the previous six weeks that were notified to the Norwegian Surveillance System for Communicable Diseases (MSIS). Over the past 10 years we have seen one to two domestic cases of hepatitis A notified monthly in Norway [1], with the exception of 2013 when a Nordic outbreak led to increase in notified cases. Between February and March 2014, more than 20 cases of hepatitis A were notified, most of them with no travel history. The patients identified until the beginning of March 2014 were mostly males living in Oslo. An alert was sent on 9 March to the municipal health authorities and to the Norwegian Food Safety Authority (NFSA). On 10 March, in order to inform other European countries about the increase, an urgent enquiry was posted on the European Epidemic Intelligence Information System platform (EPIS) run by the European Centre for Disease Prevention and Control (ECDC).

Initial epidemiological investigation

Hepatitis A is mainly transmitted by the faecal-oral route from person to person or contaminated food or water. It has an incubation period of approximately 30 days [2]. NIPH initiated exploratory interviews by telephone to obtain information on clinical symptoms and symptom onset, the mode of transmission and possible common exposures among the patients. The structured questionnaire included questions on consumption of food and drinks as well as other information (i.e. household composition, drug use, occupation) in the period from two to six weeks before symptom onset. The results of these interviews suggested a food-borne route of transmission.

In Norway, hepatitis A virus (HAV) is genotyped only in outbreak situations. As soon as the increase in cases was identified, the reference laboratory at the NIPH started to collect all anti-HAV IgM-positive blood samples diagnosed in the local and regional laboratories in 2014.

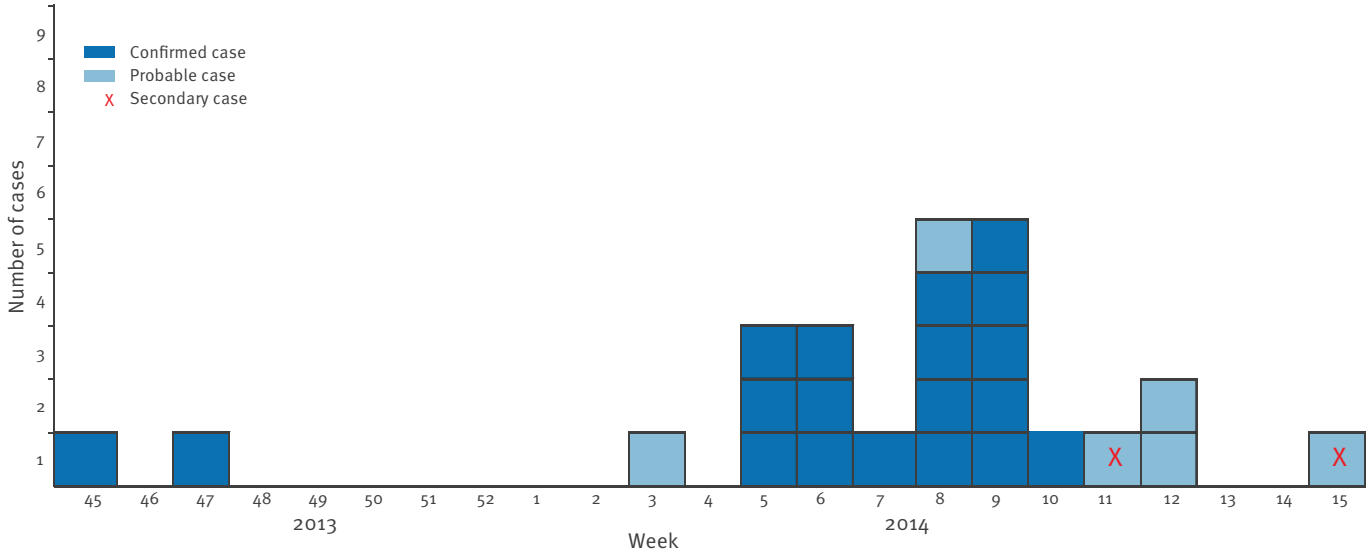
On 21 March, the first available typing results showed that 16 domestic cases out of 21 had an identical outbreak strain (NOR-2014-V1) of genotype IA based on a 466 bp sequence in the VP3-VP1 region of the HAV genome. To confirm the association with the European Hepatitis A outbreak ongoing since January 2013 that was associated with consumption of frozen berries [3,4], the reference laboratory repeated the sequencing using the region used in the case definition for that outbreak (VP1-2a region, 460bp) [5]. Results from 1 April confirmed that the Norwegian isolates were identical to those in the European outbreak (KF182323) and a message was posted in the European Early Warning and Response System platform (EWRS) on the same day.

Outbreak description

As HAV is generally only typed during outbreak investigations in Norway, the onset of the outbreak is difficult to determine because HAV samples are only stored for a limited time. As of 12 April, all samples collected

Figure

Cases of hepatitis A infection by week of symptom onset, Norway, November 2013–April 2014 (n=25)



since November 2013 for which serum was still available at local and regional laboratories have been typed and the following outbreak case definition has been developed:

- *Probable case*: a person living in Norway with clinical illness compatible with HAV infection and serum-positive for HAV IgM antibodies, with onset of symptoms since November 2013 and no travel history to endemic areas two to six weeks before onset of symptoms.
- *Confirmed case*: a probable case from whom the HAV outbreak strain is identified.

As of 12 April, 25 cases (19 confirmed and six probable) have been identified in Norway. Fifteen cases were men and all were adults (age range: 24–71 years; median: 43 years). The patients lived in 15 different municipalities, mainly in the south-east region of the country. Onset of symptoms ranged from 7 November 2013 to 9 April 2014. Most of the cases (n=21) had disease onset between Week five and Week 12 (28 January to 21 March). Two of the cases were close contacts of two previously diagnosed cases and were considered secondary cases (Figure). In addition, we were informed, through selective exchange on the EWRS, about a foreign tourist diagnosed with hepatitis A in March, after travelling on a cruise ship along the Norwegian coast in February.

Outbreak investigation

All primary cases were interviewed. We used trawling questionnaires for the first 13 cases. Different types of berries, salads, vegetables and fruits were the most commonly mentioned food items by the cases. A matched (1:3) case–control study is currently underway to test the hypotheses generated from the trawling questionnaires. A preliminary analysis has been performed including six of the 13 patients already interviewed as well as 10 additional primary cases that had not been interviewed with the trawling questionnaires. No exposure was significantly associated with the disease.

Since the incubation period of hepatitis A is relatively long and recall bias regarding food consumption in the interview results is likely, primary cases were also asked to provide their bank records on food purchases six weeks before disease onset. This was done to support the traceback of food items under suspicion, it allowed shops to provide information on brands and batches of the foods that were being sold at a given point. We were focussing specifically on berries and products containing berries because of the 100% match with the European outbreak strain identified in the laboratory investigation.

So far, five cases reported to have bought bags of frozen berries during the incubation period. Three of them had berries left in the freezer at the time of the investigation and allowed the NFSA to take samples to test for HAV. Results from samples from two of the patients were negative. Results from samples from the third patient are still pending.

When interviewed, several cases reported that they may have eaten different kinds of cake containing berries during the incubation period. Two cases stated that they had eaten a specific type of berry mix buttermilk cake from the same shopping centre in Oslo. On 9 April, when performing the traceback investigation, the NFSA discovered that the same type of berry cake had been consumed by a third case at a hotel in northern Norway. Company X had supplied the cake to both locations. On 10 April, NIPH sent an email with

the photograph of the berry mix buttermilk cake to the remaining cases, asking specifically if they had eaten that cake, and if yes, where they had eaten it. As of 14 April, we have information from 16 cases, of whom 11 confirmed eating the cake. Four could not remember, but said they may have eaten that cake and only one responded that they had not eaten the cake. The traceback investigation indicated that the berry mix buttermilk cake was imported frozen from Germany and distributed to several locations in Norway. Samples have been taken and results are pending.

Public health measures

On 1 April, as soon as the 100% match with the HAV strain in the ongoing European outbreak was confirmed, the NFSA and the NIPH informed the public that frozen imported berries should be boiled for one minute before consumption. The ECDC published a rapid outbreak assessment on 11 April, with updated information on the European outbreak, including the cases from Norway [5].

On 10 April, the NFSA alerted the importer of the berry mix buttermilk cake of the suspicion concerning the product. The importer immediately blocked the product in storage and notified their customers to do the same. On 11 April, once the investigation revealed that several more cases had eaten the berry mix buttermilk cake, the NIPH and the NFSA informed the importer and the public that the cake was the likely source of the outbreak. The Norwegian importer immediately withdrew the product from the market in Norway. The cake had been distributed to different restaurants, canteens and cafés in Norway and had been sold in a small number of shops. It had also been distributed to cruise ships sailing along the Norwegian coast. The same day, the NFSA posted a Rapid Alert System for Food and Feed (RASFF) notification about this product and the NIPH updated the EWRS message.

Traceback investigations are still ongoing to determine whether contaminated berries could have been distributed to Norway through other channels. An international traceback investigation is ongoing to find the origin of the berries used in the cake.

Conclusion

Hepatitis A is a re-emerging foodborne health threat in Europe, illustrated by several multinational outbreaks over the last couple of years [4,6-8]. At least three of these outbreaks have been linked to berries [4,7,8]. Consumption of berries has increased from 1.25 to 3.81 kg per capita per year in Norway from 2003 until 2012 [9]. Berries are increasingly imported into Europe, and Norway has seen an increase in import of this product over the last five years. A substantial amount of imported berries are from countries with high endemic levels of hepatitis A [10]. In outbreaks, traceback of these products, especially frozen berries, has proven to be challenging and is illustrated in the current European outbreak in which the origin of the berries has not yet been found. This highlights the need to improve traceback systems for berries imported into Europe. Currently, several countries, including Norway, are participating in a working group, HAVTrace, which is being arranged by the European Commission in collaboration with the European Food Safety Authority (EFSA) and ECDC. The goal is to collect existing evidence and coordinate the traceback activity between the affected countries.

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Conflict of interest

None declared.

Authors' contributions

LV, KN, BGH, MEM and HL conducted the epidemiological investigation. LJ led the traceback investigation. KSJ and SM conducted the laboratory investigation. BGH drafted the manuscript. All authors have reviewed and agreed on the content of the manuscript.

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Infectious diseases prioritisation for event-based surveillance at the European Union level for the 2012 Olympic and Paralympic Games

A Economopoulou[1], P Kinross (Pete.Kinross@ecdc.europa.eu)[1], D Domanovic[1], D Coulombier[1]
1. European Centre for Disease Prevention and Control, (ECDC), Stockholm, Sweden

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In 2012, London hosted the Olympic and Paralympic Games (the Games), with events occurring throughout the United Kingdom (UK) between 27 July and 9 September 2012. Public health surveillance was performed by the Health Protection Agency (HPA). Collaboration between the HPA and the European Centre for Disease Prevention and Control (ECDC) was established for the detection and assessment of significant infectious disease events (SIDEs) occurring outside the UK during the time of the Games. Additionally, ECDC undertook an internal prioritisation exercise to facilitate ECDC's decisions on which SIDEs should have preferentially enhanced monitoring through epidemic intelligence activities for detection and reporting in daily surveillance in the European Union (EU). A team of ECDC experts evaluated potential public health risks to the Games, selecting and prioritising SIDEs for event-based surveillance with regard to their potential for importation to the Games, occurrence during the Games or export to the EU/European Economic Area from the Games. The team opted for a multilevel approach including comprehensive disease selection, development and use of a qualitative matrix scoring system and a Delphi method for disease prioritisation. The experts selected 71 infectious diseases to enter the prioritisation exercise of which 27 were considered as priority for epidemic intelligence activities by ECDC for the EU for the Games.

Introduction

A mass gathering (MG) has been defined as a gathering of more than 1,000 persons at a specific location for a specific purpose and for a defined duration [1,2]. As MGs can represent a burden for public health systems, some preparedness planning should be considered in advance to mitigate the unusual pressures. Adverse health events at MGs are relatively rare, but have been described in the literature [3,4].

In 2012, London hosted the Olympic and Paralympic Games (the Games), with events occurring throughout the United Kingdom (UK) between 27 July and 9 September 2012, with the majority in London and the south of England. The organisers expected approximately nine million spectators and 300,000 participants, including athletes, officials, media and workforce [5-7].

The European Centre for Disease Prevention and Control (ECDC) is mandated to identify, assess and communicate current and emerging risks to human health from communicable diseases [8]. Information regarding infectious and non-infectious events is collected by the epidemic intelligence team at ECDC in a database for event-based surveillance named the Threat Tracking Tool. A dedicated indicator-based surveillance database, The European Surveillance System (TESSy), collects data on mandatorily notifiable diseases sent by Member States of the European Union (EU) and European Economic Area (EEA) under Decision 2119/98/EC [9,10]: at the time of the Games, this included 27 EU countries plus Norway, Iceland and Liechtenstein.

Public health surveillance in the UK for London 2012 was coordinated by the Health Protection Agency (HPA), now part of Public Health England. Close collaboration between the HPA's international team and ECDC was established for detection and assessment of infectious and non-infectious events occurring worldwide during the Games. ECDC reinforced its event–based surveillance activities to enhance detection and assessment of these events relevant to the Games in a timely manner. For this purpose, keywords for tools such as software that aggregates a specific type of information from multiple online sources (media aggregator) had to be selected, to be used for threat detection. Special attention was paid to infectious disease events that are more common than non-infectious environmental events for a MG setting [11]. As the list of infectious diseases representing a risk for public health is long, financial and human resources limited and adverse health events rare, a priority-setting exercise was deemed necessary to facilitate ECDC's decisions on which infectious diseases should have preferentially

enhanced detection and monitoring, independently of the criteria set by HPA [12]. Non-infectious environmental events were not prioritised. In the absence of a standard method, prioritisation of infectious diseases for event-based surveillance during each MG is usually achieved empirically [11-15].

We aimed to employ a reproducible, transparent, qualitative method to prioritise infectious diseases occurring worldwide and representing a risk for public health during MGs, in order to develop a list of significant infectious disease events (SIDEs) that would enhance event-based surveillance at ECDC for the Games. The use of two independent approaches, one by ECDC and the other by HPA, for the prioritisation of infectious disease events is likely to have increased the overall sensitivity of event-based surveillance during the Games.

Methods

A team composed of three ECDC experts (generic expert team) was assigned to evaluate potential public health threats to the Games, as well as to select and prioritise SIDEs for the event-based surveillance system. To this end, the generic expert team opted for a multilevel approach including the selection of infectious diseases for prioritisation, qualitative scoring of diseases using a consensus-building Delphi method and a risk matrix [16-24].

A total of 56 ECDC experts from seven diseases programmes (disease expert teams) participated in the scoring and Delphi method. The disease programmes covered the following topics: food- and waterborne and zoonoses, vaccine-preventable diseases, emerging and vector-borne diseases, tuberculosis, airborne diseases, human immunodeficiency virus (HIV) and other sexually transmitted infections and antimicrobial resistance and healthcare-associated infections.

Selection of infectious diseases for surveillance

A list of infectious diseases to consider for prioritisation was compiled using the following criteria: (i) mandatorily notifiable infectious diseases that were reported to TESSy in 2010; (ii) potential infectious threats to Europe that had been identified and monitored in the Threat Tracking Tool in June to September of 2005 to 2011 inclusive, i.e. the months surrounding the 2012 Olympic and Paralympic Games; (iii) events reported in the HPA's weekly epidemiological reports from May to September 2011; (iv) diseases reportable to the World Health Organization according to the International Health Regulations, e.g. poliomyelitis due to wild type poliovirus; and (v) infectious agents with deliberate release potential [25-30].

Supportive information, including disease severity, incubation periods, transmissibility, routes of infection, geographical distribution, seasonality and distribution of vectors, was collated using sources such as clinical manuals and dedicated web pages [31-35]. The supportive information was used by both the generic expert team as well as the disease expert teams as reference material.

Scoring system and Delphi method

The generic team scored each disease individually for two parameters: public health impact and likelihood of occurrence. The public health impact was scored from one point (lowest impact) to five points (highest impact) by taking into account the following assigned criteria: morbidity, case- fatality rate, potential of sequelae, the existence of disease-specific treatments, the potential to provoke outbreaks and potential media interest.

The score for likelihood of occurrence ranged from one point (least likely) to five points (most likely). The assigned criteria used to score likelihood of occurrence were the incidence, geographical distribution, seasonal trends, mode of transmission and incubation period. Likelihood of occurrence was scored by the generic team according to three categories in the context of the Games: (i) being imported into the Games; (ii) occurring at the Games; and (iii) being exported from the Games to rest of the EU/EEA. 'Occurring at the Games' meant disease transmission during the Games. The disease expert teams were asked to assess only the likelihood of occurrence of diseases for two categories only: those occurring at the Games and being exported from the Games. The disease expert teams received a list of diseases within their field of expertise, the corresponding data from TESSy and the Threat Tracking Tool, a summary of threats monitored in the HPA weekly epidemiological reports and the collated supportive information from the generic expert team. The experts in each disease expert team discussed the scores to be assigned to each disease. Each team was requested to send one response per team to the generic team, indicating the attributed score for the public health impact of each disease within their field of expertise, in the context of the Games at UK and EU/EEA level.

A qualitative risk matrix was used by the generic team to assign for every disease a public health risk score of low, medium, high or highest by taking into account the scores for the public health impact and likelihood of occurrence (Table 1) [16]. A table with the scoring results attributed by the generic expert team and by the disease expert teams was then compiled. When there was a divergence in the scores of the teams, the scores were revised. A consensus was achieved according to Delphi method, through discussions between the generic team and each disease expert team separately.

Diseases with an overall public health risk score of high or highest, whether for diseases being imported to the Games, occurring at or being exported from the Games were then included in the final priority list.

Table 1

Risk matrix used by generic expert team to calculate public health risks for the 2012 Olympic and Paralympic Games

Likelihood of occurrence		Public health impact				
		1	2	3	4	5
		Lowest impact				Highest impact
1	Least likely	Lowest	Low	Medium	Medium	High
2		Low	Medium	Medium	Medium	High
3		Medium	Medium	Medium	High	High
4		Medium	Medium	High	High	Highest
5	Most likely	Medium	High	High	Highest	Highest

For diseases with the highest impact (a score of 5) and least likely occurrence (a score of 1), the public health impact was considered 'high' and for those with the lowest impact (a score of 1) and the most likely occurrence (a score of 5), the public health impact was considered 'medium'. This arose from the assessment that the risk from a disease with the highest impact on public health but least likely occurrence is considered greater than the risk from the disease with the lowest impact but most likely occurrence.

Results

Selection of diseases for surveillance

A list of 71 infectious diseases (including infectious agents that could be deliberately released) resulted from the selection of diseases to be included in the prioritisation exercise (Table 2).

In 2010, data in TESSy showed that in the EU/EEA, food- and waterborne diseases were the diseases reported most frequently, followed by sexually transmitted and airborne diseases. Measles, pertussis and infections due to *Haemophilus influenzae* were the most commonly reported vaccine-preventable diseases. Travel-related malaria was the predominating vector-borne disease.

According to the 2010 TESSy data for the UK, sexually transmitted diseases predominated, followed by food- and waterborne diseases. With regard to airborne diseases, tuberculosis predominated followed by Legionnaire's disease. The most commonly reported vaccine-preventable disease was meningococcal disease followed by infections due to *Haemophilus influenzae*. Finally, among vector-borne diseases, reports of imported malaria predominated.

From June to September, between 2005 and 2011, ECDC's Threat Tracking Tool had monitored 435 threats: among those, 128 were due to food- and waterborne diseases and 100 were related to Legionnaire's disease.

From June to September 2011, 371 health events were documented in the HPA weekly epidemiological reports: among those, 71 were mentioned as gastroenteritis.

Scoring system and Delphi method

The scores attributed by the generic expert team and by the disease expert teams differed for some diseases while it was similar for others (Table 2). The main differences in the scoring were for food-and waterborne diseases and antimicrobial resistance and healthcare-associated infections. The likelihood of infections due to food- and waterborne diseases within the UK was scored higher by the disease expert team. The antimicrobial resistance and healthcare-associated infections expert team added seven groups of pathogens for surveillance. They considered that carriage of the more common nosocomial infections was likely, although the likelihood of infection would be very low in non-hospitalised attendees at the Games. These included community-acquired and hospital-acquired meticillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and extended-spectrum beta-lactamase-producing *Enterobacteriaceae*.

During consultations, the generic expert team and disease expert teams discussed differences and found consensus scores. The Delphi method resulted in the inclusion of influenza, influenza-like illness and diphtheria, which were not considered relevant in the first round, and also to the modification of the ranking position for some diseases (Table 3).

Compiling list of surveillance priorities

After the application of the risk matrix and Delphi method, 27 diseases were considered as priorities for epidemic intelligence activities (Table 3). Food- and waterborne accounted for eight: *Escherichia coli* infections, campylobacteriosis, typhoid fever, salmonellosis, shigellosis, cholera, hepatitis A and viral gastroenteritis (including norovirus, rotavirus and adenovirus). Zoonoses accounted for four: leptospirosis, rabies, anthrax and arenavirus diseases. Four airborne diseases were selected: influenza, Legionnaires' disease, tuberculosis and 'other acute respiratory infections'. Four vaccine-preventable diseases were included – meningococcal disease, measles, pertussis and diphtheria – and three emerging diseases – smallpox, Ebola or Marburg viruses and severe acute respiratory syndrome (SARS). Infections due to invasive group A streptococcal infections and invasive pneumococcal disease were considered as a priority; among sexually transmitted infections, syphilis and HIV infection were included.

Reported events

From all infectious disease signals detected during the Games, 49 SIDEs were selected by ECDC's epidemiologic intelligence using the priority list (Table 3) and presented to ECDC's 'round table' (a daily expert meeting for monitoring and assessment of threats within ECDC's mandate, identified though epidemic intelligence) as relevant for the Games. Of the 49 SIDEs selected, 11 were reported to HPA by ECDC.

Table 2a

Generic expert and disease expert team scores for public health risk of infectious diseases (n=71) prioritised for epidemic intelligence screening activity for the 2012 Olympic and Paralympic Games by the European Centre for Disease Prevention and Control

Disease category	Pathogen/disease/syndrome	Overall public health risk of infection/outbreak				
		Generic expert team's assessment			Disease expert teams' assessments	
		Imported to the Games	Occurring at the Games	Exported from the Games	Occurring at the Games	Exported from the Games
Airborne diseases	Avian Influenza A(H5N1) in humans	NA3	NA3	NA3	Lowest	Low
	Influenza	Medium	Low	Medium	High	Highest
	Tuberculosis	High	High	High	High	Medium
	Other acute respiratory illness	Low	Low	Medium	Highest	Highest
Emerging and vector-borne diseases	Arenavirus diseases (e.g. Lassa, or New world arenaviruses)	High	NA1	NA1	Highest	Highest
	Chikungunya	Medium	NA1	NA1	High	High
	Crimean-Congo haemorrhagic fever	Medium	NA1	NA1	High	Medium
	Dengue	Medium	NA1	NA1	High	High
	Ebola or Marburg diseases (filoviruses)	High	High	High	Highest	Highest
	Hantaviral infections (Old and New world)	Medium	NA1	NA1	Low	Low
	Invasive group A streptococcal (iGAS) infections	High	High	High	ND	ND
	Leishmaniasis/Chagas disease	Medium	NA1	NA1	Low	Lowest
	Louse-borne typhus	Medium	NA1	NA1	Medium	Low
	Lyme disease	Medium	NA1	NA1	Low	Low
	Malaria	Medium	NA1	NA1	High	Medium
	Pneumonic plague	Medium	Medium	Medium	Highest	Highest
	Q-fever	Medium	Medium	Medium	Medium	Low
	Rabies	High	NA4	NA4	Medium	Medium
	Rift Valley fever	Medium	NA1	NA1	Low	Low
	Severe acute respiratory syndrome (SARS)	High	High	High	Highest	Highest
	Smallpox	High	High	High	Highest	Highest
	Tick-borne encephalitis	Medium	NA1	NA1	Low	Lowest
	West Nile fever	Medium	NA1	NA1	Medium	Low
	Yellow fever	Medium	NA1	NA1	High	High
Food- and waterborne diseases	Botulism (in food brought by visitors)	Medium	Medium	Medium	High	Medium
	Brucellosis	Medium	Medium	Medium	Medium	Low
	Campylobacteriosis	High	High	Medium	Medium	Low
	Cholera	High	High	Medium	High	High
	Cryptosporidiosis	Medium	Medium	Medium	Medium	Low
	Escherichia coli infections (including enterohaemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC), verocytotoxin-producing *E. coli* (VTEC)	Highest	Highest	Highest	High	High
	Viral gastroenteritis (including norovirus, rotavirus, adenovirus)	High	High	High	Medium	Medium
	Giardiasis	Medium	Medium	Medium	Medium	Low
	Hepatitis A	High	High	High	High	High
	Hepatitis E	Medium	Medium	Medium	Low	Lowest
	Legionellosis	NA3	High	High	Low	Lowest
	Listeriosis	Medium	Medium	Medium	Medium	Low
	Salmonellosis	High	High	High	High	Medium
	Shigellosis	High	High	High	Medium	Low
	Trichinosis	Medium	Medium	Medium	Low	Lowest
	Typhoid fever	High	High	High	Medium	Medium
	Yersiniosis	Medium	Medium	Medium	Medium	Low

NA1: not applicable due to absence of the pathogen, vector or conditions for transmission; NA2: not applicable because persons infected with such pathogens were not likely to visit or participate in the Games; NA3: not applicable because human-to-human disease transmission is either not possible or very limited; NA4: not applicable because of long incubation period; ND, not determined.

Discrepancies in generic expert team and the disease expert team scores were discussed between these groups through the Delphi method to find the consensus.

Table 2b

Generic expert and disease expert team scores for public health risk of infectious diseases (n=71) prioritised for epidemic intelligence screening activity for the 2012 Olympic and Paralympic Games by the European Centre for Disease Prevention and Control

Disease category	Pathogen/disease/syndrome	Overall public health risk of infection/outbreak				
		Generic expert team's assessment			Disease expert teams' assessments	
		Imported to the Games	Occurring at the Games	Exported from the Games	Occurring at the Games	Exported from the Games
Zoonoses	Anthrax	High	High	High	Medium	Low
	Echinococcosis	Low	Low	Low	Lowest	Lowest
	Leptospirosis	High	High	Medium	Medium	Low
	Toxoplasmosis	Low	Low	Low	Lowest	Lowest
	Tularaemia	Medium	Medium	Medium	Low	Low
Sexually transmitted infections	*Chlamydia* infections	Medium	High	Medium	Medium	Medium
	Gonorrhoea	Low	Medium	Medium	Medium	Medium
	Hepatitis B	Medium	Medium	Medium	Medium	Medium
	Hepatitis C	Medium	Medium	Medium	Medium	Medium
	Human immunodeficiency virus (HIV) infection	High	High	NA4	High	NA4
	Lymphogranuloma venereum (LGV) infection	Medium	Medium	Medium	Medium	Medium
	Syphilis	High	High	High	High	High
Vaccine-preventable diseases	Diphtheria	Medium	Medium	Medium	Highest	Highest
	Invasive *Haemophilus* influenza	Medium	Medium	Medium	Lowest	Lowest
	Measles	High	High	High	Medium	Medium
	Invasive meningococcal disease	Highest	Highest	Highest	Highest	Highest
	Mumps	Medium	Medium	Medium	Low	Low
	Pertussis	Medium	High	Medium	Low	Low
	Invasive pneumococcal disease	NA2	NA2	NA2	Lowest	Lowest
	Poliomyelitis	Medium	Medium	Medium	Highest	Highest
	Rubella	Medium	Medium	Medium	Medium	Medium
	Tetanus	Medium	Medium	Medium	Lowest	Lowest
	Varicella	Medium	Medium	Medium	Low	Low
Antimicrobial resistance and healthcare-associated infections	Community-associated meticillin-resistant *Staphylococcus aureus* (CA-MRSA)	NA2	NA2	NA2	Low	Low
	Healthcare-associated meticillin-resistant *Staphylococcus aureus* (HA-MRSA)	NA2	NA2	NA2	Low	Low
	Vancomycin-resistant enterococci (VRE)	NA2	NA2	NA2	Low	Low
	Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*	NA2	NA2	NA2	Low	Low
	Carbapenemase-producing *Enterobacteriaceae*	NA2	NA2	NA2	Low	Low
	Nosocomial transmission of transmissible spongiform encephalopathies variant (Creutzfeldt–Jakob disease)	NA4	NA4	NA4	NA4	NA4
	Carbapenem-resistant *Pseudomonas aeruginosa*	NA2	NA2	NA2	Low	Low
	Healthcare-associated infections (in general)	NA2	NA2	NA2	NA2	NA2

NA1: not applicable due to absence of the pathogen, vector or conditions for transmission; NA2: not applicable because persons infected with such pathogens were not likely to visit or participate in the Games; NA3: not applicable because human-to-human disease transmission is either not possible or very limited; NA4: not applicable because of long incubation period; ND, not determined.

Discrepancies in generic expert team and the disease expert team scores were discussed between these groups through the Delphi method to find the consensus.

Table 3

Highest priorities for epidemic intelligence for the 2012 Olympic and Paralympic Games by the European Centre for Disease Prevention and Control (n=27)

Pathogen/disease/syndrome	Imported to the Games		Occurring at the Games		Exported from the Games	
	Risk[a]	Likelihood / public health impact	Risk[a]	Likelihood / public health impact	Risk[a]	Likelihood / public health impact
Meningococcal disease	Highest	5 / 5	Highest	5 / 5	Highest	3 / 5
Escherichia coli infections (including enterohaemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC), verocytotoxin-producing *E. coli* (VTEC)	Highest	5 / 4	Highest	5 / 4	Highest	5 / 4
Cholera	High	5 / 3	High	5 / 3	Medium	2 / 3
Salmonellosis	High	5 / 2	High	5 / 2	High	5 / 2
Viral gastroenteritis (including norovirus, rotavirus, adenovirus)	High	5 / 2	High	5 / 2	High	5 / 2
Measles	High	4 / 4	High	4 / 4	High	4 / 4
Typhoid fever	High	4 / 4	High	4 / 4	High	4 / 4
Campylobacteriosis	High	4 / 3	High	5 / 3	Medium	3 / 3
Shigellosis	High	4 / 3	High	4 / 3	High	4 / 3
Influenza	High	4 / 2	Medium	2 / 2	Medium	2 / 2
Other acute respiratory infections	High	4 / 2	Medium	1 / 2	Medium	2 / 2
Invasive group A streptococcal (iGAS) infections	High	3 / 4	High	4 / 4	High	3 / 4
Leptospirosis	High	3 / 4	High	3 / 4	Medium	1 / 4
Syphilis	High	3 / 4	High	3 / 4	High	3 / 4
Tuberculosis	High	3 / 4	High	3 / 4	High	3 / 4
Hepatitis A	High	3 / 2	High	4 / 2	High	4 / 2
Anthrax	High	2 / 5	High	1 / 5	High	1 / 5
Human immunodeficiency virus (HIV) infection	High	2 / 5	High	2 / 5	NA4	NA4
Arenavirus diseases (Lassa, Junin, Machupo, Guanarito, Sabiá)	High	1 / 5	NA1	NA1	NA1	NA1
Ebola or Marburg viruses (filoviruses)	High	1 / 5	High	1 / 5	High	1 / 5
Rabies	High	1 / 5	NA4	NA4	NA4	NA4
Severe acute respiratory syndrome (SARS)	High	1 / 5	High	1 / 5	High	1 / 5
Smallpox	High	1 / 5	High	1 / 5	High	1 / 5
Diphtheria	Medium	5 / 5	Low	4 / 5	Low	2 / 5
Pneumococcal disease	Medium	2 / 4	High	3 / 4	Medium	1 / 4
Pertussis	Medium	2 / 3	High	4 / 3	Medium	2 / 3
Legionnaires' disease	NA3	NA3	High	4 / 3	High	4 / 3

NA1: not applicable due to the absence of the pathogen, vector or conditions for transmission; NA2: not applicable because persons infected with such pathogen were unlikely to visit or participate in London 2012; NA3: not applicable because human-to-human disease transmission is either not possible or very limited; NA4: not applicable because of long incubation period.

[a] Risk: the public health risk of infection/outbreak for each pathogen/disease/syndrome, calculated from its likelihood and public health impact scores using the risk matrix (Table 1).

Discussion

Although epidemiological surveillance during MGs is an important activity of several public health institutions worldwide, few articles provide methodological guidance for event-based surveillance and prioritisation of diseases in this context. Therefore, in preparation for the 2012 Games, we reviewed projects executed for different public health topics but using similar prioritisation methodologies [17-24].

The methodology used to compile a list of SIDEs for the Games was designed to be comprehensive, pragmatic and reproducible. Its representativeness was promoted by considering threats monitored in previous summers in the UK, the EU/EEA and globally. Considering that most events recorded for previous MGs were related to infectious disease outbreaks and very few to environmental hazards, the latter were not included in the prioritisation exercise. The literature confirms that infectious diseases are in fact more common than environmental hazards for the MG setting [11].

The SIDEs that were considered had all already been described in the literature or had been captured by one

of the aforementioned surveillance systems. Therefore, those that were not recorded in these outputs by definition could not be included in the priority list for monitoring, due to this limitation of our methodology. Undoubtedly, unexpected events should always be considered as a potential eventuality during MGs, e.g. the emergence of a worldwide event such as the SARS outbreak in 2003. Therefore, when ensuring preparedness for MGs, surveillance for the unexpected should always be included, e.g. by including syndromic surveillance or by recording numbers of hospitalisations due to unexplained illness.

There were no surprises regarding the prioritisation results, which were similar to those from other MGs [2,11]. Food- and waterborne diseases were considered the most probable to occur followed by airborne. The normal seasonal trend of increased bacterial gastroenteritis during warmer months in the northern hemisphere can explain this ranking. An increase in the number of cholera cases has been reported by infectious disease surveillance systems globally in recent years; the HPA monitored imported cholera in the UK in the summer months of 2011. This fact and possible high media attention contributed to the inclusion of imported cholera among diseases for prioritised surveillance even though it is unlikely that isolated cases of cholera could give rise to outbreaks in the UK or be spread from the UK to other countries. The influenza pandemic of 2009 contributed to influenza's ranking as a high likelihood of occurrence in the context of a summer Olympics and Paralympics, combined with the expected visitors from southern-hemisphere countries during their influenza season, and the possibility of summer outbreaks of influenza in the UK [36,37]. Indeed, the HPA's weekly epidemiological reports for summer 2011 included the monitoring of an outbreak of imported influenza A(H1N1)pdm09 virus infections. The high score for meningococcal diseases is also not surprising given their high infectiousness and case-fatality rate. The long incubation period of infectious diseases such as HIV infection and tuberculosis (TB) meant that this was not a priority for the Games; however, given that single cases would attract media and public attention, HIV and TB were considered as a priority for surveillance.

The criteria used to define the likelihood of disease occurrence were the incubation period, incidence, geographical distribution, seasonal trends and mode of transmission, i.e. guided by scientific evidence. The independent scoring of the public health impact and the likelihood of occurrence of a disease, and the use of risk matrix as part of the scoring system maximised the achievable objectivity and added credence to the prioritisation method.

Assessment of public health risk, especially in the context of MGs, presents some difficulties. Ranking of the public health impact of an infectious disease – characterised by its frequency, severity of the outcomes and risk of secondary transmission – can be performed in more or less quantitative terms. Public reaction to infectious disease threats cannot be quantified as easily, however, as it is driven by cultural and emotional conditions. Therefore, diseases such as malaria or hantavirus infection – for which secondary transmission was almost impossible and consequently there was no risk of outbreak – were considered for prioritisation because of the media attention that such a disease could had have. However, both aspects must be considered when assessing the public health impact.

The Delphi method assured a high level of consensus and promoted objectivity in disease prioritisation. Assessment by multidisciplinary disease expert teams allowed the prioritisation to benefit from experts' specific knowledge, team work and grouping of similar diseases to obtain more comparable scoring.

Expert opinion inherently carries some degree of subjectivity, and experts were asked to provide a semi-qualitative score based on expert judgement and background data. Subjective judgments can be influenced by topical or newsworthy disease trends. The compiled background data, which included descriptors of current trends, were therefore an indispensable tool to reduce subjectivity in the scoring process. Similarly, discussions between the generic team that acquired and collated the data and disease expert teams in a Delphi process ensured that ranking was reconsidered. Although this method demanded time for preparation and committed resources, it strengthens the validity of the prioritisation process.

During the Games, no major SIDEs were detected (data not shown). This may be explained by effectiveness of preparedness measures before the Games, particularly by the UK, aided by an appraisal of the global epidemiological situation.

The priority list of diseases was given to epidemic intelligence tool developers, who, considering HPA criteria [12], ensured that SIDEs keywords were incorporated in different languages into news aggregator and Internet-trawling software used by the epidemic intelligence teams at ECDC for threat detection. There is a scientific prerequisite to have a reliable, transparent and evidence-based method to rely on when setting priorities. To our knowledge, the combination of a risk matrix and Delphi method has not been used yet elsewhere to develop a priority list of diseases for monitoring during MGs. Being completed relatively quickly (one week) with minimal resources (approximately half a working day per expert), this approach provides a scientific tool for a review of diseases when preparing for a MG. Besides its role during these Games, this tool could provide a permanent legacy, as the protocols can be adapted and the methodology repeated with amendments by ECDC or other institutions.

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Conflict of interest

None declared.

Authors' contributions

AE and PK: generated study design and methodology; collected data; applied methodology; members of generic team; coordinated Delphi process. DD: writing and editorial contribution to article. DC: initiated study; contributed to study design and methodology; member of generic team. All authors have critically reviewed and approved the final article.

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Comparison of diagnostic clinical samples and environmental sampling for enterovirus and parechovirus surveillance in Scotland, 2010 to 2012

H Harvala (heli.simmonds@hotmail.com)[1,2], J Calvert[1], D Van Nguyen[1], L Clasper[1], N Gadsby[2], P Molyneaux[3], K Templeton[1,2], C McWilliams Leitch[1], P Simmonds[1,2]

1. Infection and Immunity, Roslin Institute, University of Edinburgh, Edinburgh United Kingdom
2. Specialist Virology Centre, Royal Infirmary Edinburgh, Edinburgh United Kingdom
3. Department of Medical Microbiology, Aberdeen Royal Infirmary, Aberdeen United Kingdom

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Human enteroviruses (EV) and parechoviruses (HPeV) within the family *Picornaviridae* are the most common causes of viral central nervous system (CNS)-associated infections including meningitis and neonatal sepsis-like disease. The frequencies of EV and HPeV types identified in clinical specimens collected in Scotland over an eight-year period were compared to those identified in sewage surveillance established in Edinburgh. Of the 35 different EV types belonging to four EV species (A to D) and the four HPeV types detected in this study, HPeV3 was identified as the most prevalent picornavirus in cerebrospinal fluid samples, followed by species B EV. Interestingly, over half of EV and all HPeV CNS-associated infections were observed in young infants (younger than three months). Detection of species A EV including coxsackievirus A6 and EV71 in clinical samples and sewage indicates that these viruses are already widely circulating in Scotland. Furthermore, species C EV were frequently identified EV in sewage screening but they were not present in any of 606 EV-positive clinical samples studied, indicating their likely lower pathogenicity. Picornavirus surveillance is important not only for monitoring the changing epidemiology of these infections but also for the rapid identification of spread of emerging EV and/or HPeV types.

Introduction

While poliovirus (PV) eradication is approaching its goal, other picornaviruses, including enteroviruses (EV) and parechoviruses (HPeV) are growing in clinical importance. In Europe, EV are the most common cause of viral meningitis in children and young adults, and the newly emerging HPeV type 3 is proving to be an important cause of central nervous system (CNS) infection in neonates [1]. At the same time, the incidence of hand, foot and mouth disease (HFMD) and severe EV71 infections in children is increasing in Asia, and the virus strain(s) responsible could potentially be imported to Europe [2].

Enteroviruses were traditionally divided into polioviruses (PV, three serotypes), coxsackie A viruses (CAV, 23 serotypes), coxsackie B viruses (CBV, six serotypes) and echoviruses (E, 28 serotypes) based on their antigenic and pathogenic properties in humans and laboratory animals [3]. However, this biological division has been replaced by a molecular classification based on VP1 sequencing, and more recently discovered EV types have been numbered in the order of their identification (50 numbered EV types by 1 April 2014) [4]. Sequence analysis of enteroviruses furthermore showed evidence for a deeper grouping into four species A–D (EV-A to D) that cuts across these previous biologically defined categories. EV within species B (all echoviruses, CBV1-6 and CAV9) are the most commonly identified cause of viral meningitis in Europe, whereas viruses causing HFMD generally fall within species A.

The first two serologically distinct HPeV types, originally described as echoviruses (E22 and E23) in the *Enterovirus* genus, were discovered over 50 years ago (reviewed in [5]). However, they were renamed as HPeV and reclassified into their own *Parechovirus* genus in 1999 based on their molecular and biological properties [6]. Since then, a further 14 HPeV types (HPeV3 to 16) have been discovered, with HPeV type 3 specifically associated with severe neonatal CNS-infections [5].

Laboratory detection of EV or HPeV generally uses molecular methods such as reverse transcription PCR (RT-PCR), which are faster and more sensitive than viral cell culture. The 5'non-coding region (NCR) is the most conserved region among EV and HPeV, and is therefore targeted in many diagnostic screening procedures. Unfortunately, sequences from this region provide little or no information on the (sero)type of the infecting

virus, and sequencing of a structural gene region such as VP1 is required to enable type identification for EV and HPeV [5,7]. Molecular typing is important for ensuring that PVs are not re-introduced into the countries where they have already been eradicated and for more general surveillance and epidemiological purposes. However, as a surveillance method, such screening has severe limitations given that only a very small subset of EV infections are diagnosed through referral of clinical samples for virological testing. In the United Kingdom (UK), EV detection is clinically focussed on neurological disease including viral meningitis and done very rarely in cases of, for example, HFMD since these patients are not normally hospitalised. Analysis of sewage for the presence of EV and HPeV provides an alternative and additional surveillance method without referral bias, and complements the clinical data with a potentially more accurate representation of virus types circulating in the community.

In the current study we have performed comprehensive typing of EV and HPeV detected in diagnostic clinical samples from the east of Scotland over a three-year period. Frequencies of EV and HPeV types identified in 2010 to 2012 were compared with those identified in previous years (2005 to 2010; [1]) to provide a longer term indication of their incidences and age distributions. They were also compared with types identified as circulating in the community through sewage surveillance in Edinburgh and surrounding areas to provide a more complete description of the clinical epidemiology of EV and HPeV infections.

Methods

Cerebrospinal fluid samples

A total of 3,415 cerebrospinal fluid (CSF) samples referred to the Specialist Virology Centre in Edinburgh for virology testing during the three-year study period from 2010 to 2012 were included in this study; these were compared to previous results from 3,957 CSF samples obtained between 2005 and 2009 [1]. EV screening was done by separate RT and PCR reactions [8] until real-time EV RT-PCR (modified from [9]) was introduced into routine use in the beginning of 2009 and combined further with real-time human parechovirus (HPeV) RT-PCR in 2011 [10]. CSF samples collected before 2011 were tested retrospectively for HPeV by RT-PCR [1,11]. In addition, 82 EV-positive and 10 HPeV-positive CSF samples were referred for typing from elsewhere in Scotland.

Other clinical samples

Eleven of 19 EV-positive vesicular swab samples obtained from individuals with HFMD (11 of them were hospitalised) in Edinburgh between 2010 and 2011 as well as 185 EV-positive clinical specimens (64 faecal samples, 12 vesicle swabs and 109 respiratory samples) and seven HPeV-positive samples (four faecal and three respiratory samples) submitted for typing from elsewhere in Scotland between 2010 and 2012 have been included in this study. Samples submitted for typing from elsewhere in Scotland were screened using EV primers, which are also known to detect human rhinovirus (HRV).

Clinical samples positive for enterovirus and parechovirus

Samples were anonymised and archived according to the protocol approved by the Lothian Regional Ethics Committee (08-S11/02/2). Extracted RNA was amplified by a combined RT- and first-round PCR using the High Fidelity Superscript III Platinum Taq (Invitrogen, UK) followed by a second amplification reaction with nested primers specific for species B VP1 sequences [12]. If negative, the PCR was repeated with species A VP1 primers and with general VP4 primers; these also amplify HRV sequences and are also used for HRV typing. Positive HPeV samples were amplified in the VP3/VP1 region [11].

Sewage specimens

A total of 40 waste water samples were collected approximately a week apart in twelve consecutive months (June 2009–May 2010) from the Veolia Wastewater Treatment works, which processes all waste water from the sewage system and road run-off in the urban and surrounding areas of Edinburgh (population size approximately 650,000 people). A total of 100 g of solid waste (50 g ± 2.5 g from two sampling sites) was resuspended in 200 mL sterile phosphate buffered saline by vortexing for at least one minute to remove any particulate matter from the sample. Centrifugation and size fractionation using filters (Millipore, Pall, UK) of different pore sizes were used to enrich samples as previously described [13]. RNA was extracted from the filtrate using the Qiagen extraction kit (Qiagen, UK) and then reverse-transcribed using the Reverse Transcription System (Promega, UK). Amplification of cDNA was performed by nested PCRs using different primers for each EV species and HPeV [11,12].

Sequencing

Amplified VP3/VP1 and VP4 regions from clinical and sewage specimens were directly sequenced using the BigDye Terminator kit (Applied Biosystems, Warrington, UK) using inner primers [11,12]. Sequences were aligned using SSE version 1.1 (http://www.virus-evolution.org/Downloads/Software/).

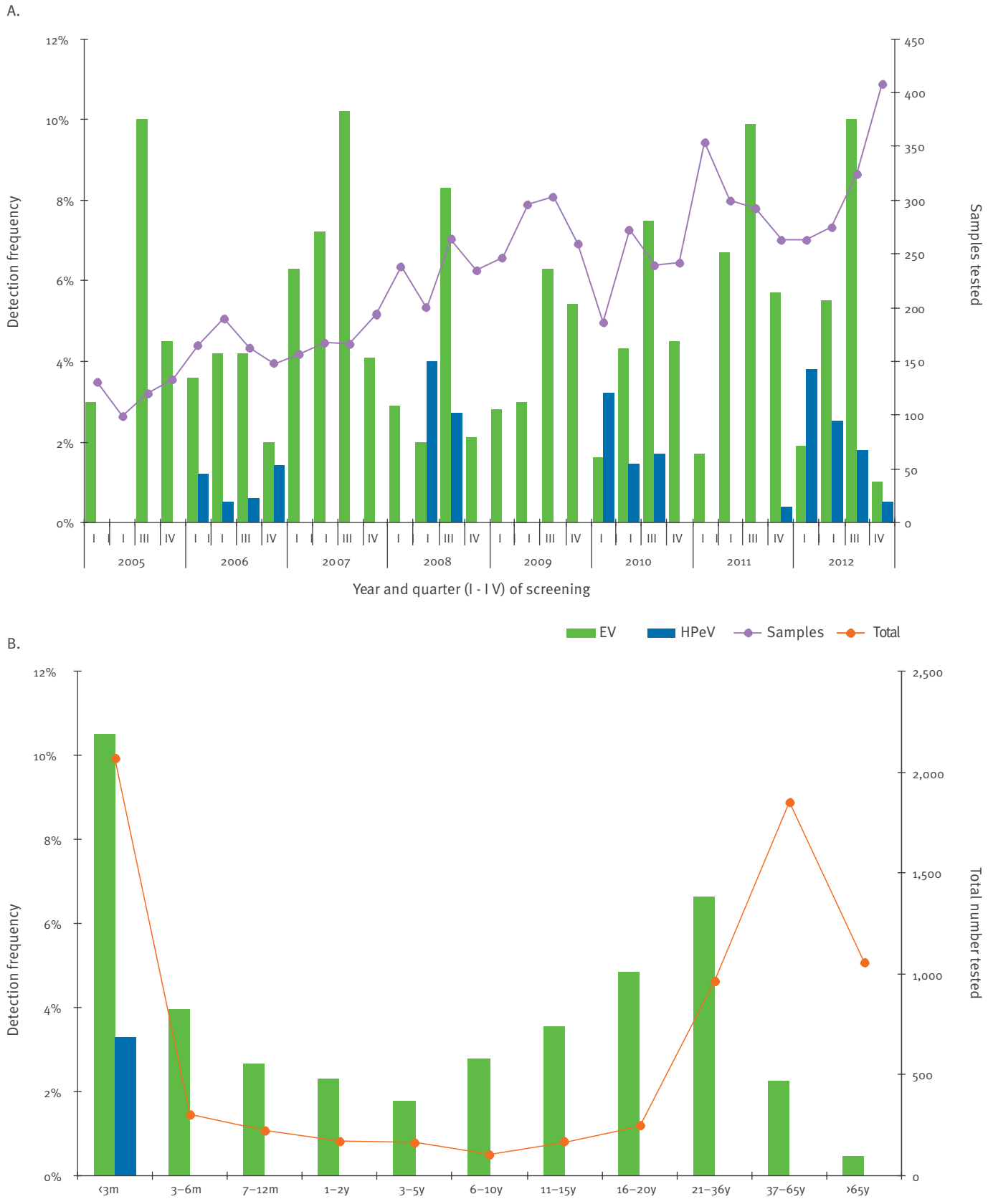
Results

Enterovirus and parechovirus infections

Screening of the 3,415 archived CSF samples identified EV RNA in a total of 150 individual specimens obtained from 150 different individuals and HPeV RNA in a total of 35 specimens obtained from 35 different individuals. EV were detected throughout the three-year study period: 41 of 1,043 (4%) in 2010, 69 of 1,172 (6%) in 2011 and 40 of 1,200 (3%) in 2012 (Figure 1A). Marked annual changes in the incidence of HPeV infections were in keeping with our previous five-year report [5];

Figure 1

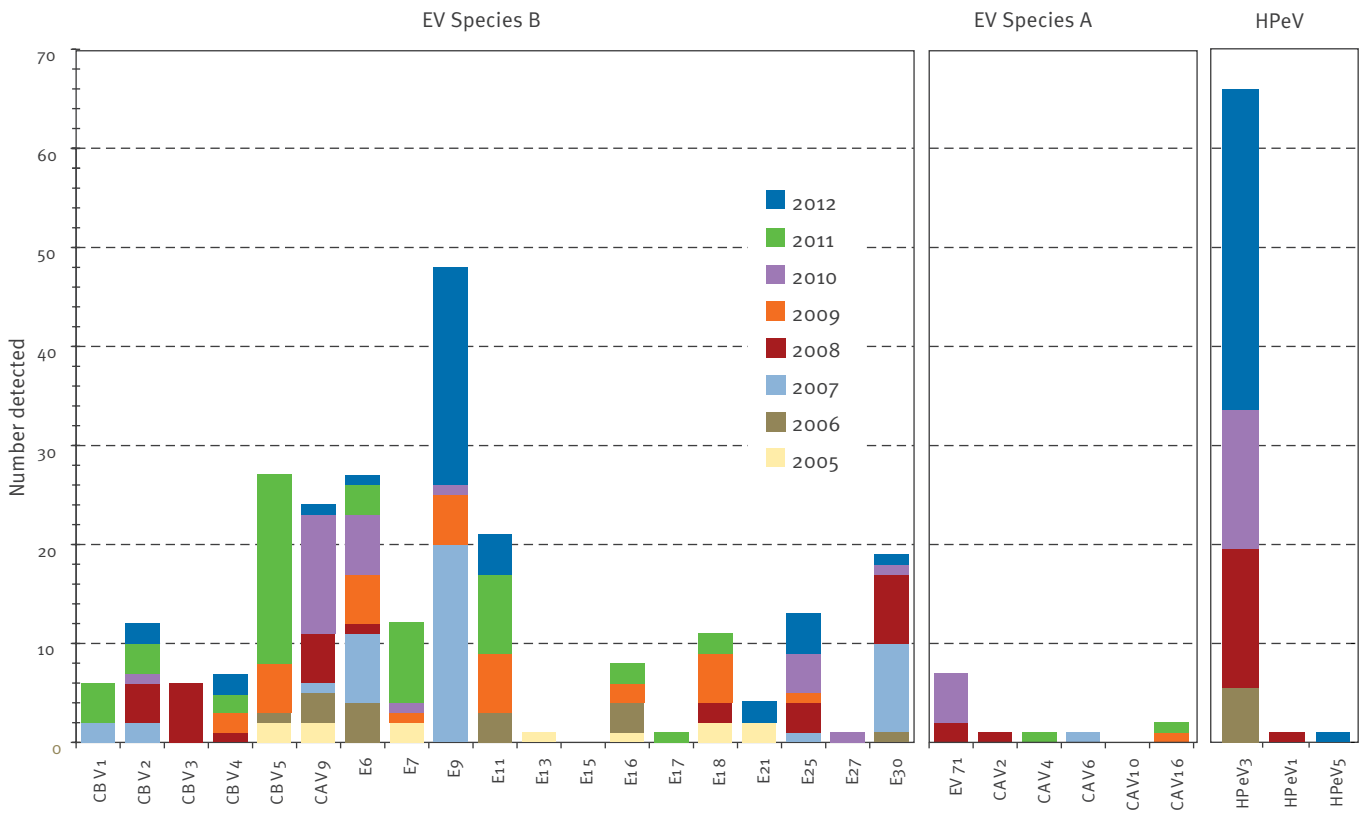
Detection frequency of enterovirus and human parechovirus in cerebrospinal fluid samples, by time (A) and by age (B), Edinburgh, 2005–2012 (n=7,372)



EV: enterovirus; HPeV: human parechovirus; m: months; y: years.
I: January, February, March; II: April, May, June; III: July, August, September; IV: October, November, December.

Figure 2

Distribution of enterovirus and human parechovirus types in cerebrospinal fluid samples, Scotland, 2005–2012 (n=404)



CAV: coxsackie A virus; EV: enterovirus; HPeV: human parechovirus.

most HPeV infections were recorded in even years, 14 in 2010 (1.3%) and 23 in 2012 (1.8%), whereas screening of the total 3,513 CSF samples collected during the odd years (2,342 in 2005, 2007 and 2009 as previously reported [1] and 1,171 in 2011 [this study]) resulted in only a single HPeV detection (Figure 1A). The highest frequency of EV infections was seen in young children under the age of three months (217/2,066, 11%), whereas individuals infected with HPeV were exclusively infants under the age of three months (58/2,066, 3%; Figure 1B).

Enterovirus and parechovirus type identification

All 606 EV-positive clinical samples and 75 HPeV-positive samples were subjected to genotyping, including the sequences obtained and typed in 2005 to 2009 in our previous study [1]. As a result, a high proportion of these (uncultured) clinical samples could be directly typed for EV (498/606; 82%), specifically 82% of CSF samples (336/410), 78% of faecal samples (50/64), 83% of vesicle samples (19/23) and 85% of respiratory samples (93/109). EV-positivity of untypeable samples was confirmed by 5'UTR PCR. For HPeV, all 75 5'UTR screening-positive samples could be amplified and typed in the VP3/VP1 region.

Cerebrospinal fluid samples

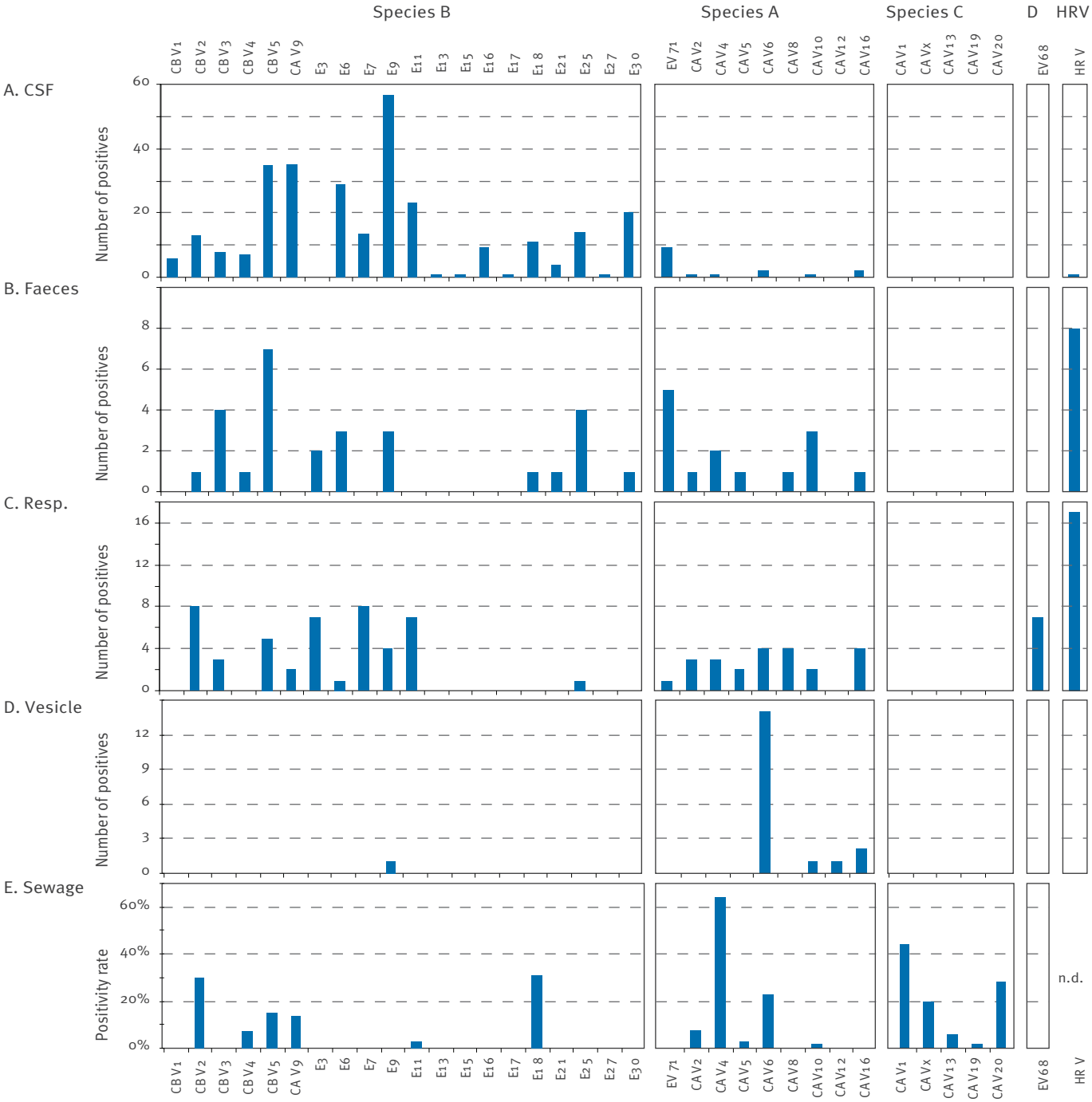
A total of 19 different species B and six species A EV serotypes were detected in CSF samples in this study. The six most frequently detected EV types were E9 (57/336; 17%), CAV9 (35/336; 10%), CBV5 (35/336; 10%), E6 (29/336; 9%), E11 (23/336; 7%) and E30 (20/336; 6%) corresponding to 59% of CNS-associated EV infections, along with occasional detections of species A serotypes CAV2, CAV4, CAV6, CAV10, CAV16 and EV71 (Figures 2 and 3). Rapid changes in serotype frequencies were observed. Almost all EV were typed as E9 in 2007, CAV9 in 2010, CBV5 in 2011 and E9 in 2012. No virus predominated in 2005, 2006, 2008 and 2009. All except two of the HPeV strains identified in CSF samples were HPeV3 (66/68), whereas the remaining ones were HPeV1 and HPeV5. Over the eight-year study period, HPeV3 remained the most prevalent picornavirus in CNS-related infections.

Other clinical samples

A total of 13 different species B and six different species A EV were identified in the 50 successfully typed faecal samples, with CBV5 and EV71 being the most common types (Figure 3). In addition, a high proportion of EV-positive faecal samples (8/50) were identified as HRV as reported previously [14]. It is known that

Figure 3

Comparison of enterovirus types identified in clinical and sewage samples obtained in Scotland, 2010–2012



CAV: coxsackie A virus; CSF: cerebrospinal fluid; EV: enterovirus; ND: not determined; HPeV: human parechovirus; HRV: human rhinovirus.

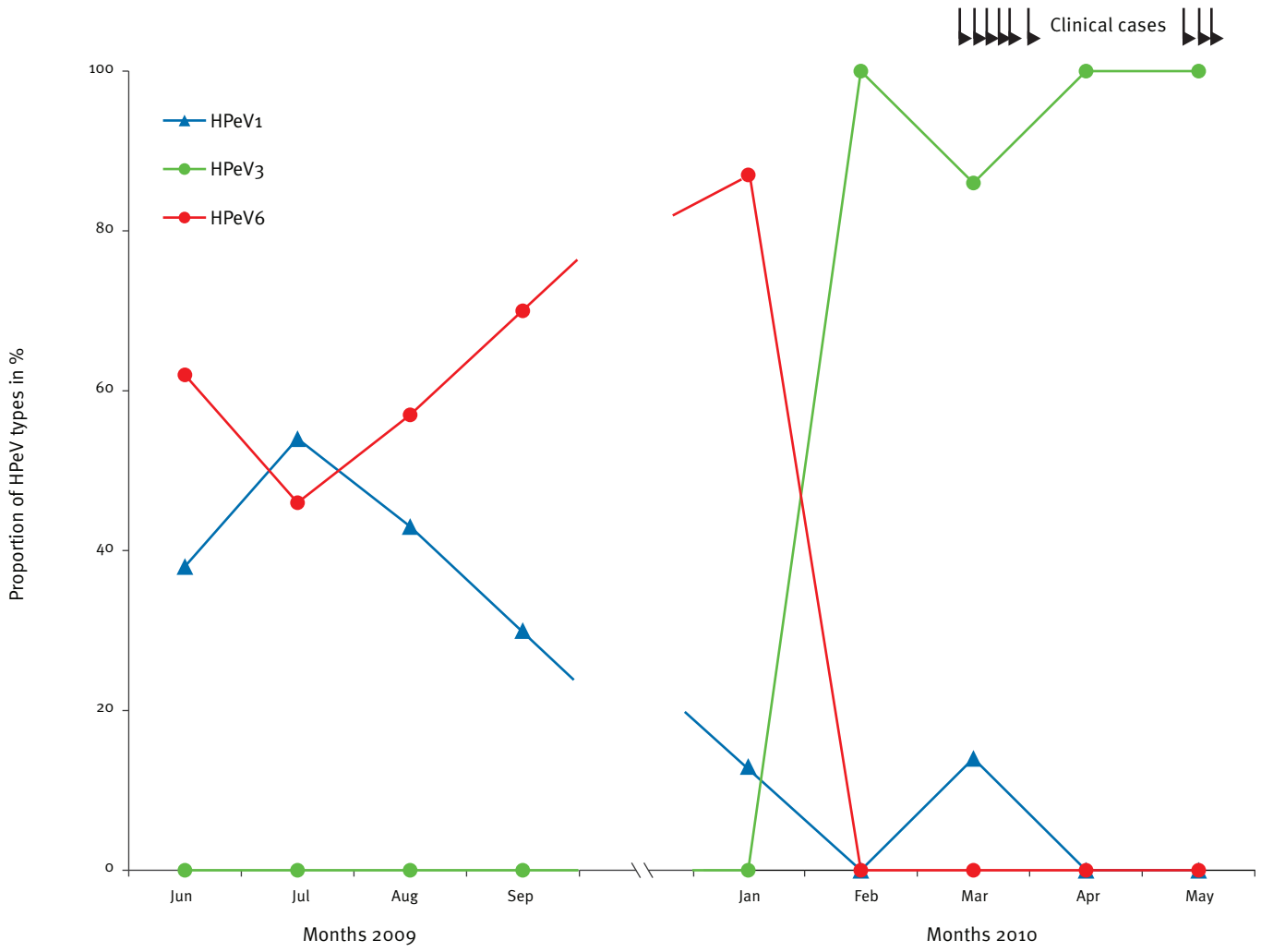
EV primers will detect some HRV [14]. The species A serotype, CAV6 was the most frequently identified variant among the vesicular swabs (14/19). Furthermore, a total of eight individual species A and 10 species B EV types were found in the 93 typed throat swabs, along with EV68 (species D) and several HRV strains. All four HPeV-positive faecal samples were typed as HPeV3, whereas the two HPeV-positive respiratory samples were identified as HPeV5 and one as HPeV3.

Sewage specimens

During a one-year period from June 2009 to May 2010, EV was identified in 37 of 40 sewage samples, and HPeV in 31 of 40. Samples were screened for EV using four species-specific VP1 primer sets, allowing EV from all species to be identified. However, species D EV were not detected in this study. From 353 cloned sequences obtained, a total of 95 amplicons were identified as species C and by phylogenetic analysis could be identified as CAV1 (42/95), CAV22 (27/95), CAV13

Figure 4

Human parechovirus typing of sewage samples obtained in Edinburgh, June 2009–May 2010.



HPeV: human parechovirus.

The appearance of HPeV3 in clinical CSF samples is indicated by the vertical arrows.

(6/95) and CAV19 (2/95) (Figure 3). The remaining 18 sequences clustered together but separately from all known species C types, including the newly identified EV109, EV113 and EV116-118. Their assignment as a new species C type will require complete VP1 sequences, which will be the subject of a further study. No PV was detected in sewage.

The 73 species B variants identified from sewage were most closely related to E18 (23/73), CBV2 (22/73), CBV5 (11/73), CAV9 (10/73), CBV4 (5/73) and E11 (2/73), whereas 61 species A strains clustered closely with CAV4 (39/61), CAV6 (14/61), CAV2 (5/61), CAV5 (2/61) and CAV10 (1/61). Interestingly, CAV9 and E18 were also among the most common EV detected in clinical specimens during the same time. On the other hand, although CAV4 was commonly identified in sewage, it was an example of EV-A, which has very rarely been identified in clinical specimens.

The presence of HPeV was determined using primers which amplify the VP3/1 region, enabling genotype identification. In total, 124 HPeV sequences were found; HPeV3 being the most common type (62/124), followed by HPeV6 (36/124) and HPeV1 (26/124). HPeV3 appeared in sewage one week before the first clinical case was diagnosed with HPeV3 infection (Figure 4).

Discussion

This study describes the epidemiology of EV and HPeV infections based on CSF screening of hospitalised individuals over an eight-year period in Edinburgh, combines these data with local sewage surveillance, and samples submitted for typing from elsewhere in eastern Scotland. Because of the similar sensitivity of PCR for all EV species (A to D) [10], our rates of detection are not influenced by variability and insensitivity of viral cell culture, an important drawback of previous EV

surveillance reports that relied mainly on virus isolation. Similarly, the use of PCR provides a better reflection of the relative importance of HPeV in CNS-related disease.

Diversity of enteroviruses

Over the eight-year study period, 326 individuals presented with CNS-associated EV infection with the diagnosis established by virus detection in CSF. Interestingly, more than half of these EV infections (58%) were observed in infants under the age of three months, a higher proportion than we found in our surveillance study covering only the first five-year period [1]. This increasing percentage may reflect decreasing seroprevalence against EV infections in Europe [15]. Surveillance data from several countries have shown that approximately 29% to 44% of CNS-associated EV infections occur in young children under the age of one year [16,17], but specific data on young infants are rare.

The six most common EV serotypes (all from species B) identified in CSF samples collected in Edinburgh were E9, CAV9, CBV5, E6, E11 and E30. Four of these (E6, E9, E30 and CAV9) have been among the most abundant serotypes isolated from clinical specimens in the UK and elsewhere previously [16,17]. Although these EV types have often been associated with large outbreaks due to appearance of new recombinant forms, no outbreaks occurred over the study period in Scotland.

In addition, six different species A EV serotypes (CAV2, CAV4, CAV6, CAV10, CAV16 and EV71) were detected in CSF samples obtained from young children with sepsis-like illness, of which CAV6 was the most common type identified from subjects with HFMD. The number of clinical specimens obtained from individuals with vesicular rash (usually vesicular fluid samples obtained from individuals with likely HFMD) was approximately 10-fold less (approximately 100 samples per year) than the number of CSF samples obtained from individuals with suspected meningitis. Typically, surveillance data from the UK and elsewhere in Europe are restricted to EV-infected individuals admitted to hospital; those presenting with HFMD are often diagnosed by their general practitioner without laboratory testing. Information on the epidemiology of HFMD in Europe is therefore very limited. However, detection of species A EV including EV71 in CSF samples (nine cases) and CAV6 in CSF (one case), vesicular swabs (12 cases) and sewage (14/61) indicates that these viruses are circulating in Scotland, and could lead to outbreaks as previously reported in Finland and France [18,19].

Further evidence for the widespread circulation of EV species A variants is provided by the frequent detection of CAV2, CAV4, CAV5, CAV6 and CAV10 in waste water collected in Edinburgh. However, as shown previously, some of these viruses may not always cause symptomatic infections leading to hospitalisation [3]. For example, CAV4 and CAV10 were among the EV types frequently detected in faecal samples collected from healthy Norwegian infants [20]. On the other hand, both HFMD and severe EV71 infections are spreading in Asia, as exemplified by a recent outbreak in Cambodia associated with 95% mortality. To identify potential global spread, surveillance in the UK and elsewhere in Europe is essential, including testing of community-collected samples as recently carried out in France as part of large prospective observational study [19]. Detection of EV in sewage could provide the earliest indications of such spread, although the sheer diversity of circulating EV types at any one time prevented identification of a clear temporal match between types identified in clinical and sewage surveillance specimens despite the large number of sequences obtained from the two sample sets.

Despite the adoption of PCR-based methods for virus detection, no species C EV were identified in any of the clinical specimens. However, species C EV serotypes were abundant in sewage in the Edinburgh, including CAV1, CAV13, CAV19, CAV22 and a possible new EV type. Species C enteroviruses were also the most commonly detected viruses in sewage screening on the Philippines [21]. This dichotomy is best explained by a specifically lower pathogenicity of non-polio EV-C variants [3]. Furthermore, all circulating vaccine-derived PV (cVDPV) have been shown to be recombinants of oral polio vaccine (OPV) strains and co-circulating species C EV [22,23]. This is the first report of the existence of these less pathogenic species C non-PV EV in sewage in Europe. The authors speculate that they may serve as a reservoir for recombination and thus drive the emergence of recombinant cVDPVs in areas where OPV is still used.

Furthermore, EV68 was the only species D virus identified in clinical specimens. It has recently been associated with cases of severe respiratory tract infections in Europe [24] and elsewhere, and thus this finding was not unexpected. However, EV68 infections are generally under-recognised and underreported because the virus has an HRV-like 5'NCR and will often be reported as HRV infections. No species D EV were identified in our sewage screening despite the use of a sensitive PCR with species D-specific VP1 primers [12]. Due to the potential recent emergence of new species D EV types including EV111 and EV120 from sub-Saharan Africa, EV surveillance targeting EV-D types is also relevant. For example, EV94 within species D was identified in the Democratic Republic of the Congo from an individual with acute flaccid paralysis in 2007 and has since been shown to circulate widely in northern Europe based on seroprevalence studies [25].

Diversity of parechoviruses

In contrast to the diversity of EV detected in CSF samples, all but two of the 68 HPeV-positive CSF samples detected over the eight-year period were HPeV3 (the exceptions being one type 1 and one type 5). HPeV3 was the most frequently identified picornavirus type in CNS-related infection in this study, and exclusively

seen in young children under the age of three months (Figure 1). The biannual cycle of HPeV3 infections observed in this study (Figure 1) is consistent with previous reports of much higher frequencies of HPeV3 infections occurring in even-numbered years in northern Europe [1,5,26,27]. In addition, HPeV3 was absent in the sewage water collected in Edinburgh in 2009 and only appeared in sewage one week before the first clinical case was diagnosed in early 2010 (Figure 4). Although more extensive sewage surveillance data is required, this striking correlation demonstrates the potential value of environmental surveillance in detecting changes in HPeV (and other virus) circulation and in anticipation of its subsequent clinical presentations. This is particularly relevant in the case of HPeV3 as, despite being now well recognised as a major cause of severe neonatal sepsis-like illness occasionally leading to fatality [26], routine screening of children with sepsis-like illness is infrequent in the UK and elsewhere. HPeV infections are still considerably under-diagnosed.

Acknowledgments

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Conflict of interest

None declared.

Authors' contributions

Heli Harvala and Peter Simmonds conceived the study. Heli Harvala analysed the data and wrote the manuscript. Naomi Gadsby, Pamela Moleneaux and Peter Simmonds provided contribution to the manuscript, and all the authors approved the final version. Joe Calvert, Carol McWilliams Leitch and Peter Simmonds organised and performed the sewage collection. All the authors assisted with enterovirus and parechovirus typing.

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Measles on a cruise ship: links with the outbreak in the Philippines

S Mandal (sema.mandal@phe.gov.uk)[1], M Ramsay[1], K Brown[2]
1. Immunisation, Hepatitis and Blood Safety Department, Centre for Infectious Disease Surveillance and Control, Public Health England, London, United Kingdom
2. Virus Reference Department, Public Health England, London, United Kingdom

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To the editor:

In their recent article, Lanini et al. describe an outbreak of measles on a cruise ship in the Mediterranean during February 2014 involving 27 cases (21 crew members, four passengers, two unknown) [1]. The article reported measles sequence data which appear to attribute the outbreak to measles cases in United Kingdom. We discuss this and several omissions from the article which are critical in our understanding of the source of transmission and the appropriateness of interventions on the cruise ship.

Firstly, as all reported cases clustered in time, the outbreak appears to have the characteristics of a point source from a single (unidentified) index case. The identical B3 strain sequences obtained from 10 cases support this hypothesis. Considering the incubation period of measles, the index case was likely symptomatic in week 6 (4–10 February 2014).

Secondly, the outbreak strain (MVs/Tonbridge.GBR/5.14) was identical to that identified by Public Health England (PHE) in an English resident who had clearly been infected in the Philippines. This individual was infectious during their return flight, but diagnosed only after arrival in England. In contrast to what might be inferred from Lanini et al., these epidemiological and microbiological findings are consistent with the cruise ship outbreak being linked to the ongoing outbreak in the Philippines [2] and not due to indigenous measles in the United Kingdom. Following a successful measles catch-up campaign in England in 2013 in response to high numbers of confirmed measles cases, measles activity in England has declined [3,4] and has remained low during 2014. From 1 December 2013 to the beginning of April 2014, however, PHE has received 13 reports of measles in persons returning from the Philippines, where there is a large outbreak affecting the National Capital Region (Manila) and other parts of the country [2]. Of these 13 reported cases in English residents, 12 have been confirmed and nine have been genotyped by the Virus Reference Department at PHE. The sequences comprise four closely related B3 strains. PHE is aware of several additional cases epidemiologically linked to cases who travelled to Philippines, in household contacts or acquired during air travel (on a flight or at an airport). We were surprised that the association between the cruise ship outbreak and the outbreak in the Philippines was not discussed by the authors, particularly considering that most cases were in crew members, 71% of the 968 crew members were from Asia, and three cases were reported in Filipino staff [1].

Thirdly, we expected the authors to discuss the likely susceptibility to measles of passengers based on their age, not only vaccination history. There were 3,352 passengers on board of whom 86% were nationals of the European Union; the median age of passengers was 41 years (range: six months to 93 years). The relative paucity of passenger cases (4/27) compared to crew cases (21/27) and the large passenger denominator most likely reflects the reduced susceptibility to measles of the passengers due to past immunisation or past infection, particularly if many were older adults. It would have been useful, therefore, to describe age-specific attack rates. An age-based risk assessment may have led to a more proportionate response in reassuring older passengers and avoiding unnecessary vaccination in those likely to be immune. Of course, intensity and frequency of contact is likely to be a factor in transmission and one could hypothesise that crew-to-crew and passenger-to-passenger contact were more likely than crew-to-passenger interactions; however, since measles is of such high infectivity, susceptibility to infection is likely to have been the major factor in transmission. Attack rates are not given by the authors, but with the information available we estimate this to be 0.6% overall, 2.2% in crew members and 0.1% in passengers, suggesting that overall susceptibility was low.

In summary, the rapid communication by Lanini et al. [1] illustrates the effectiveness of rapid cross-border coordination and control measures which are critical for highly infectious communicable diseases such as

measles. However, it did not sufficiently draw out the key epidemiological characteristics of the population at risk, taking into account historic and geographic variation in disease incidence and vaccination coverage. In the absence of this information readers are not able to understand the reason for the outbreak, the risk of such outbreaks recurring and the best ways to prevent them in the future.

Conflict of interest

None declared.

Authors' contributions

S Mandal contributed to the concept and drafted the letter. M Ramsay contributed to the concept and edited the letter. K Brown contributed to the concept, supervised the sequencing, and edited the letter.

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Author's reply: Measles on a cruise ship - links with the outbreak in the Philippines

S Lanini1, M R Capobianchi (maria.capobianchi@inmi.it)[1], T Derrough[2], E Severi[2], L Vellucci[3], M G Pompa[3]
1. National Institute for Infectious Diseases (INMI) "Lazzaro Spallanzani" Rome, Italy
2. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
3. Ministry of Health, Directorate General for Prevention, Rome, Italy

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To the editor:

We thank Mandal et al. for their letter in response to our paper. Firstly we would like to point out that most the concerns raised are due to the fact that our article was a preliminary report, which was also stated in the title. It was intended to rapidly inform about an outbreak of measles affecting European and non-European citizens that was ongoing at the time of publication and to alert public health, clinical and laboratory experts in various countries of the possibility of cases among people who had been on the cruise. In fact, most of those concerns are being addressed in the on-going investigation.

With regard to the potential source of the outbreak, we intentionally refrained from indicating that the ongoing measles cluster between 20 February 2014 and 1 March 2014 would unequivocally suggest a point-source outbreak with a unique primary case. Although this is a sensible hypothesis, we are currently analysing a large amount of data from all crew members employed on the ship between 2 January and 9 April 2014, to specifically address this question and other open issues. This also includes the identification of the primary case, the actual duration of the epidemic, the overall number of cases among the crew members and potential risk factors for infection. The long time period under investigation includes a long pre-epidemic period (about seven weeks before symptom onset of the earliest cases on 20 February) to identify any potentially unrecognised case, and a 32-day post-epidemic period (i.e. about twice the median incubation time after the last case recorded on board) to confirm the end of the outbreak. The results will be published once the data analysis has been completed.

In the microbiological results section we explained that "phylogenetic analysis demonstrated that identified sequences were 100% identical to each other, confirming a common origin, and to two British strains identified in February 2014 (MVs/Brighton.GBR/8.14/ and MVs/Tonbridge.GBR/7.14/, not shown)". In fact, we did not link the cruise outbreak ship to B3 strains indigenous from the United Kingdom (UK). In the letter Mandal et al. state that the outbreak strain (MVs/Tonbridge.GBR/5.14) is identical to that identified in an English resident who had clearly been infected in the Philippines, and that our epidemiological and microbiological findings are consistent with the cruise ship outbreak being linked to the ongoing outbreak in the Philippines rather than to indigenous measles in the UK. This hypothesis is not contradicted by our preliminary analysis. However, at the time of our analysis, neither the measles nucleotide database (MeaNS) nor Blast showed sequences from the Philippines belonging to B3 genotype. We did not have any evidence to conclude where the strain responsible for the cruise ship outbreak was initially acquired. In fact, the primary case could have been a passenger as well as a crew member who may have been infected anywhere (including the Philippines, the UK or Italy). The evidence is not convincing enough to draw a conclusion that the crew members from the Philippines were the source of the outbreak following direct importation from their home country.

Finally, concerning the attack rate among passengers, we should stress that the inference of 0.1% made by the authors of the letter is not supported by the available data, since we do not have information on the exact number of cases among passengers. It is worth noting that we will hardly be able to obtain the precise number of cases among passengers. In fact, as the duration of the cruise (seven days) is shorter than the incubation time of measles (7–18 days), no passenger is expected to develop symptoms while on board. Therefore passengers are not likely to take part in the diseases propagation within the ship, although this hypothesis could not be definitely ruled out. In addition, these cases will only occasionally be brought to our attention by local health authorities throughout the world. All these issues have been clearly discussed in our paper.

At present, activities are underway to better describe the epidemiological features of this outbreak and to provide final conclusions on the event.

Conflict of interest

None declared.

Authors' contributions

All authors are part of the outbreak investigation team and reviewed and approved the manuscript.

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