Early findings in outbreak of haemolytic uraemic syndrome among young children caused by Shiga toxin-producing *Escherichia coli*, Romania, January to February 2016

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As at 29 February 2016, 15 cases of haemolytic uraemic syndrome with onset between 25 January and 22 February were reported among children between five and 38 months in Romania, and three of them died. Cases were mostly from southern Romania. Six cases tested positive for *Escherichia coli* O26 by serology. Fruits, vegetables, meat and dairy products were among the possible common food exposures. Investigations are ongoing in Romania to control the outbreak.

On 9 and 10 February 2016, the National Institute of Public Health of Romania (NIPH) was alerted about 12 paediatric cases of haemolytic uraemic syndrome (HUS). The children, most of them under two years of age, were from Arges, Bucharest and Dolj districts and were all admitted to the Children's Emergency Hospital 'M.S. Curie', in Bucharest. All had initially presented with diarrhoea, some with bloody diarrhoea, with onset from 25 January to 9 February. The NIPH and the Romanian Ministry of Health formed an outbreak investigation team (OIT). On 18 February, experts from the European Centre for Disease Prevention and Control (ECDC) joined the OIT. On 20 and 26 February, three additional cases were reported, one from Bacau and two from Arges district. This communication describes the epidemiological and microbiological investigations as at 29 February 2016.

Outbreak investigation

In Romania, four regional hospitals (in Bucharest, Cluj, Iasi and Timisoara) are referral centres for management of HUS cases. To establish the baseline on number of HUS cases in Romania and to confirm the outbreak, we collected data on HUS admissions to these four hospitals between 2010 and 2015 in children under five years of age. We performed a descriptive time-series analysis by plotting the monthly number of HUS cases by date of diagnosis from 2010 to 2016, a 12–month moving average, and minimum, maximum and mean values observed by month between 2010 and 2015. We compared the number of diagnoses in January and February 2016 to the maximum monthly number of HUS diagnoses between 2010 and 2015.

We collected microbiological results of tests performed at district hospitals on stool samples from HUS cases diagnosed in January and February 2016.

The National Reference Laboratory (NRL) from Cantacuzino Institute in Bucharest tested for *stx* (*stx1* and *stx2*) and *eae* genes by PCR. Isolates were tested with O antisera against the main STEC serogroups by slide agglutination.

Serum samples from 12 cases were sent to the Istituto Superiore di Sanità (ISS) in Rome, Italy to be tested for

Distribution of haemolytic uraemic syndrome cases by month of diagnosis, Romania, January 2010 to February 2016

A.

B.

HUS: haemolytic uraemic syndrome.

Geographical distribution of haemolytic uraemic syndrome outbreak cases by place of residence, Romania, 25 January to 22 February 2016 (n=15)

Source: European Centre for Disease Prevention and Control (ECDC).

antibodies to the lipopolysaccharide (LPS) of six major STEC serogroups (O157, O26, O103, O111, O145, and O55) by ELISA [1].

For the purpose of this outbreak investigation, we formulated a case definition based on STEC laboratory confirmation, HUS diagnostic [2] and date of onset. A confirmed outbreak case was defined as any individual with onset of diarrhoea after 15 January 2016 in Romania and laboratory confirmation for STEC O26. A probable outbreak case was defined as any individual with onset of diarrhoea after 15 January 2016 in Romania, with clinical HUS but without laboratory confirmation for STEC O26 infection. Cases who travelled outside Romania within two weeks before symptom onset were excluded from the present investigation.

For active case-finding, a national HUS and severe diarrhoea surveillance system was set up on 15 February

2016. In addition, stool samples from 15 family members, all asymptomatic, of four confirmed and two probable cases were tested for *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter* and *E. coli* (enteropathogenic *E. coli*, Shiga toxin-producing *E. coli*, enterotoxigenic *E. coli* and *enteroinvasive E. coli*).

To generate a hypothesis on the cause of the outbreak, between 24 and 28 February, parents or guardians of the cases were interviewed with a questionnaire that comprised questions on food and water exposures and activities during the 10 days before the symptom onset. The questionnaire also comprised questions regarding episodes of diarrhoea among family members.

Findings

Between 2010 and 2015, 101 HUS cases were diagnosed in Romania, with an average number of 16 cases per year, a minimum of five cases in 2010 and

Distribution of haemolytic uraemic syndrome cases by date of diarrhoea onset, Romania, 25 January to 22 February 2016 (n=15)

All the stool samples from family members tested negative.
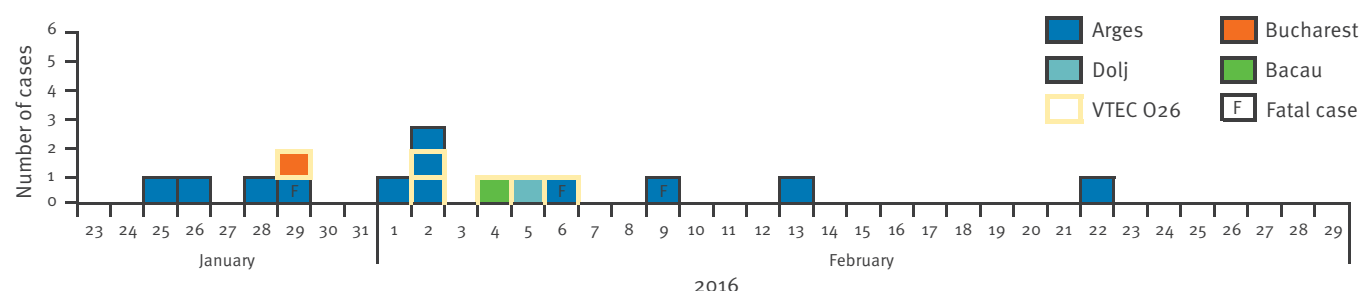
a maximum of 25 cases in 2015. The descriptive time-series analysis showed neither trend nor seasonality in the incidence of HUS from 2010 to 2016 (Figure 1a). As at 29 February 2016, 15 HUS cases were diagnosed in 2016: two in January and 13 in February. The number of cases diagnosed in February represented a 2.5-fold increase compared with the monthly maximum of five HUS diagnoses observed during the six previous years (Figure 1b).

Among the 15 cases identified in 2016, six were confirmed and nine were probable. They were aged between five and 38 months with a median age of 11 months. All but one were under two years of age. Nine children were female. Three cases died during this outbreak on 4, 15 and 16 February respectively.

Twelve cases (three confirmed, nine probable) resided in Arges district. The remaining three confirmed cases resided in Bucharest, Bacau and Dolj districts (Figure 2). The child from Bucharest spent the five days before onset in Bran (Brasov district, north-west of Arges) and this was considered the likely place of infection.

The date of onset of diarrhoea ranged from 25 January to 22 February (Figure 3).

Stool samples from three cases tested positive for enteropathogenic *E. coli* (EPEC) by agglutination using a polyvalent antisera at one district hospital laboratory. Because of poor quality, these specimens were not confirmed at the NRL.

Serum samples from 12 cases were sent to the ISS to be tested for *E. coli* O26 LPS antibodies. Six sera were positive: three cases were from Arges, the most affected district, and the remaining three cases were from Bucharest, Bacau and Dolj districts. One of these also tested positive for *E. coli* O157 LPS antibodies. NRL isolated *E. coli* from one of the serologically confirmed case and identified the *stx2* toxin and the *eae* genes. Furthermore, two additional stool samples from cases in Arges were tested for *stx1* and 2 and *eae*, and both were positive; serogroup results are still pending.

We interviewed parents or guardians of five confirmed and three probable cases and none of them mentioned episodes of diarrhoea among family members within the ten days before the disease onset of the child.

The food section of the interview showed that during the 10 days before their symptoms onset, the cases consumed: fresh fruits (apples (8/8), pears (6/8), oranges (7/8), bananas (7/8)), vegetables (roots (8/8), pepper (8/8), zucchini (7/8)), meat (chicken (7/8), beef (4/8)), cow milk (4/8, unpasteurized for one of them), cow soft cheese (7/8; homemade for four of them) and yoghurt (6/8). All the other food items were consumed by less than four cases.

Fruits and vegetables were bought either from small local food producers or supermarkets with larger distribution, meat was supplied by family's own production or bought from supermarkets and dairy products were bought from several shops and were sometimes homemade. Two food chains were mentioned by parents/guardians during the interviews: one that distributed food only in Arges district (mentioned in relation to the consumption of soft cheese and yoghurt); and a second one that distributed food in the whole country, mentioned in relation to the consumption of oranges.

No other common exposure related to living conditions and outdoor activities could be identified.

Discussion

We describe a HUS outbreak with 15 cases, all among young children, of whom three died. The last case was reported on 26 February and had onset of symptoms four days before. Sera from six cases were positive for *E. coli* O26 antibodies, among which one was confirmed by slide agglutination from culture.

Since HUS usually occurs as a complication of STEC infection [3] in a small proportion of patients, we cannot exclude that further milder cases of STEC infection may have occurred in association with this outbreak

even though no possible cases were identified in the investigation. Active case finding of HUS and severe diarrhoea is ongoing nationwide in Romania. As at 16 March, three additional confirmed and one probable case have been reported. These cases are from two districts previously not affected: Ialomita (two confirmed and one probable case) and Sibiu (one confirmed case) and had onset between 3 February and 4 March.

We hypothesise that this is a continuous common source food-borne outbreak, where most cases were infected by the same source over a four-week period. Different fresh food items may have been contaminated by the same source early in the food production chain and then distributed on the market over a prolonged period of more than a month.

Our study has several limitations. One is that we interviewed parents/guardians under a high emotional stress because this outbreak affected very young children and this may have led to lack of completeness of the clinical history. Moreover, due to incomplete characterisation of the strains, we may have included cases unrelated to this outbreak. Finally, for a number of cases infection may have occurred through person-to-person transmission from a close contact, without being exposed to a contaminated food item.

Large STEC outbreaks, often associated with HUS cases, have been reported in several countries in the last decades [4-10]. Since 2010, the number of cases reported annually in The European Surveillance System (TESSy) [11] has steadily increased. The most frequent serotype is O157 and STEC O26 is the second most commonly reported serotype in the European Union (EU) (>400 cases per year in TESSy). STEC O26 cases are generally younger than O157 cases, have more severe diarrhoea, with more aggressive forms and higher proportion of the infected individuals develop HUS or other severe outcomes [12].

Outbreaks caused by STEC O26 have been associated in the past with unpasteurised milk and dairy products in Austria, Belgium and Italy [13-15].

A number of investigations are still ongoing in Romania to control this outbreak: environmental i.e. sampling food products in shops and from food producers, reviewing food production procedures of foods highlighted during the interviews; epidemiological i.e. collecting information through interviews with parents and or guardians of confirmed and probable cases but too few cases are currently available for an analytical study; active case finding i.e. surveillance for HUS in children (with reminders to front-line healthcare workers) and testing suspected cases for pathogenic *E. coli*; microbiological i.e. PFGE and whole genome sequencing to confirm outbreak cases and compare with isolates from foods (when available), and with previously reported O26 strains in TESSy.

On 4 March, during an environmental investigation undertaken following the information collected from the exploratory questionnaires, different cheese samples from a milk processing establishment in Arges district tested positive for *stx* genes. Isolates of *E. coli* O26 were identified in a soft cheese from the same establishment. On 5 March, this establishment voluntarily stopped production and closed the factory; the batches of suspected products were withdrawn from the Romanian market. The Romanian Food Safety Authority started an investigation tracing the distribution of the products from this milk processing establishment. On 7 March a Rapid Alert System for Food and Feed (RASFF) news (reference 16-811) was issued by the Romanian health authorities indicating that in 2016 products from this establishment were sold in other EU countries as well: Belgium, Germany, Italy and Spain. Furthermore, individual importations of products from the implicated establishment to other countries by individuals returning from Romania cannot be excluded.

This is a stark reminder that STEC can cause infections with severe complications, particularly among young children. Detection of outbreaks in the absence of sensitive and timely surveillance systems can be challenging, particularly if local laboratory capacities are not optimal. Improving local laboratories diagnostic capacities and performing unspecific HUS surveillance in children should be considered as a public health priority to avoid this from happening again. It is not yet clear when this outbreak started, and neither the vehicles nor the source of contamination were identified. However, after initial suspicion, the investigations confirmed the outbreak and its causative agent. This is an example of good collaboration between EU countries in terms of provision of laboratory services support and expert advice, and ECDC, in providing expert deployment and support.

Conflict of interest

None declared.

Authors' contributions

Emilie Peron, Alina Zaharia and Lavinia Cipriana Zota investigated the outbreak, collected and analysed the data. Emilie Peron spearheaded writing of the manuscript. Codruta Usein, Alexandru Rafila and Gaia Scavia performed all the microbiological tests. Mihaela Bălgrădean provided clinical data. Josep Jansa and Laura Espinosa supervised the investigation and provided remote support. Ettore Severi, Otilia

Mårdh, Amalia Serban and Adriana Pistol coordinated the outbreak investigation.

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Measles outbreak in a refugee settlement in Calais, France: January to February 2016

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We report a measles outbreak in a refugee settlement in Calais, France, between 5 January and 11 February 2016. In total, 13 confirmed measles cases were identified among migrants, healthcare workers in hospital and volunteers working on site. A large scale vaccination campaign was carried out in the settlement within two weeks of outbreak notification. In total, 60% of the estimated target population of 3,500 refugees was vaccinated during the week-long campaign.

A measles outbreak occurred in a refugee camp in Calais, France, from January to February 2016 that affected both refugees and staff.

Outbreak setting

The current movement of refugees into European countries has challenged national public health systems not only with respect to ensuring adequate access to medical care, but also to implementing communicable disease surveillance and prevention in refugee populations. In France, several refugee settlements comparable to shanty towns have been established in the Northern Region (Nord-Pas-de-Calais-Picardie), notably around Calais. In January 2016 the population in the Calais settlement was estimated at 3,500 refugees whose objective is to transfer to the United Kingdom (UK). The stable part of the population in the two settlements is therefore a minority, which makes healthcare and monitoring a complex task. Refugees and volunteers move freely between these two main settlements [1].The population consists primarily of men (96%) with a median age of 25 years (interquartile range (IQR): 21–30) [2]. The proportion of children younger than 15 years is estimated at 3.9% of the 5–9 year-olds at 1.1% and of the 10–14 year-olds at 1.7%. A second, smaller settlement of 2,500 refugees was established at the end of 2015 at Grande-Synthe 30 km from Calais.

Medical centres run by non-governmental organisations (NGOs) including Médecins sans Frontières (MSF) and Médecins du Monde (MDM) are in operation in both settlements during the week. Specific consultation centres for vulnerable populations are also present in the Calais settlement and in Calais and Dunkerque hospitals. In addition, refugees have access to three local hospitals in the vicinity of the settlements (Calais, Dunkerque and Grande Synthe).

In France, disease surveillance relies mainly on mandatory notification of specific infectious diseases including measles and on reporting of health events to the regional health agencies (ARS).

Outbreak description

While no case of measles had been identified around Calais since September 2013, the ARS was notified on 15 January 2016 of two measles cases related to the Calais refugee settlement. The index case was a refugee in their 30s who had been living in the settlement for a month before symptom onset on 5 January. The second case was a volunteer in their 20s living and working in the settlement.

The French Institute for Public Health Surveillance (InVS) regional office and the ARS immediately informed all volunteers and healthcare workers in the settlements, as well as the staff of the hospital emergency wards in Calais, Dunkerque and Grande-Synthe, about these measles cases in order to increase awareness, vigilance and rapid notification of any new suspected cases. In France a clinical case of measles is defined by the occurrence of fever ≥ 38,5 °C, a maculopapular

Confirmed measles cases, refugee settlement, Calais, 5 January–11 February 2016 (n = 13)
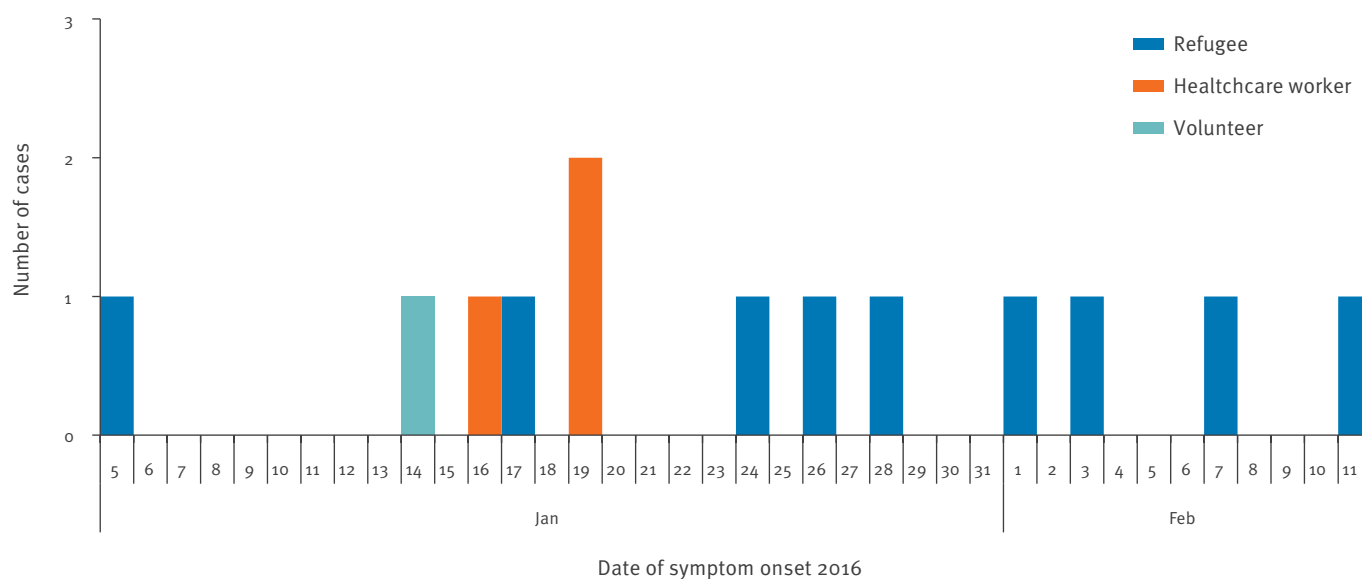
rash and at least one of the following: conjunctivitis, coryza, cough, Koplik's spots. Between 5 January and 11 February, a total of 13 clinical measles cases, all confirmed by positive salivary test for measles-specific IgM antibody, were reported in Calais among people who consulted a doctor in the settlement's medical centres or in local hospitals. Four more suspected cases concerning three refugee children in Grande-Synthe settlement and one Calais hospital staff were finally excluded.

Of the 13 cases, nine were refugees living in the settlement, three were healthcare workers from a local hospital, all in contact with the index case, and one case was a volunteer working in the Calais settlement. Date of symptom onset of the confirmed cases ranged from 5 January to 11 February (Figure 1), and the index case was a refugee who had been living in the settlement for more than a month.

The cases were predominately male (9/13 cases) and the age ranged from nine to 46 years (mean age: 25 years). Ten cases were hospitalised. The immunisation status of the nine refugee cases and the volunteer was not available. Of the three healthcare workers, one was unvaccinated and two had been vaccinated with two doses in the 1990s. All cases were confirmed to be genotype B3 by the national reference centre (NRC) for measles.

The Calais settlement is divided into de facto neighbourhoods based on ethnic group or country of origin. The first two refugee cases and the volunteer case lived in the same area of the settlement.

Control measures
On 19 January, because of the impossibility to implement contact tracing in the settlements among refugees and volunteers, the regional and national public health authorities agreed on a mass measles vaccination campaign in the Calais and Grande-Synthe settlements targeting refugees aged between one and 35 years. Thirty-five years of age was taken as an upper threshold following the French and international recommendations, assuming that the majority of people born before the 1980s have a high level of acquired immunity.

This immunisation campaign was carried out in the Calais settlement from 28 January to 5 February, and from 15 to 19 February in the Grande-Synthe settlement by teams of the French Ministry of Health (EPRUS) with the support of NGOs (MSF, MDM, Hands) in charge of primary healthcare in the settlement. Trivalent measles-mumps-rubella vaccines were mainly used, while measles monovalent vaccine was targeted to children between six months and one year of age and to women of child-bearing age, taking into account some of them may have been pregnant. A vaccination card as well as recommendations for the second dose were given to the people inoculated. In total, 2,051 refugees living in the Calais settlement and 466 in the Grande-Synthe settlement voluntarily accepted measles vaccination. No additional cases have been notified in either settlement since 11 February.

Discussion
The occurrence of a measles outbreak in the refugee settlement in Calais was not unexpected. A rapid risk assessment published by the European Centre for Disease Prevention and Control (ECDC) on 10 November 2015 concluded that while the risk of introduction of communicable diseases into Europe from the refugee population is extremely low, the living conditions of refugees (overcrowding, poor hygiene and sanitation, lack of adequate shelter in settlements) make this

population particularly vulnerable [3]. Furthermore, refugee populations may be at risk for certain communicable diseases as a result of disorganised health systems and low vaccine coverage in their countries of origin.

To date, reported communicable disease outbreaks in refugee populations in various European countries have included shigellosis, louse-borne relapsing fever and diphtheria [4-7]. In the settlement in Calais, an influenza A(H1N1) outbreak occurred in November 2015 during which 75 cases consulted the emergency room of the local area hospital, of whom 25 were confirmed for influenza (data not shown). This episode gave rise to the immediate implementation of an influenza vaccination campaign.

There are two possible sources for the introduction of measles into the refugee settlement in Calais: either through a refugee recently arrived in the settlement (given the long travel times for most refugees, the hypothesis of introduction directly from the country of origin into France is not likely) or through one of the numerous volunteers with unknown and possibly inadequate vaccination status working in the settlement. The latter hypothesis is supported by the fact that the index case arrived in Calais more than a month before symptom onset and had therefore been contaminated on the settlement. Furthermore, the genotype B3 measles virus identified during this outbreak is together with D8 the main genotype recently circulating in several European countries from which many volunteers originate, such as the UK or Spain [8,9]. In France, among all the positive specimens genotyped by the NRC, the genotype D8 was always nearly exclusive in 2015 [10], which supports the hypothesis that the B3 virus in the Calais outbreak was imported; a case who had not sought medical consultation in France would not have been identified. Finally, improved access to medical care in on-site consultation centres as well as an epidemiological surveillance system have been in place in the settlement since early December, which makes it unlikely that earlier measles cases would not have been detected.

This outbreak confirms the epidemic risk in refugee populations susceptible to communicable diseases circulating in Europe, such as measles [4]. In addition, the fact that several volunteers and healthcare workers were among the cases testifies to insufficient measles vaccine coverage in the European population, making them susceptible to contracting and also transmitting the virus. In March 2015, the ECDC indicated that measles cases in Europe had increased dramatically since 2010 and that in the past 10 years, on average 40% of cases were over 14 years-old [11]. In these circumstances, it is important that individuals in contact with refugee populations in settlements or in medical settings adhere to infection control measures and also verify that their vaccinations are up to date. This protects not only the volunteers and healthcare workers,

but also limits the risk of introducing communicable diseases into refugee populations that are highly susceptible for outbreaks.

The positive uptake of the vaccination campaign among refugees (60% of the estimated target population of 3,500 refugees in a one week campaign in the Calais settlement, and 40% of eligible individuals in the Grande-Synthe settlement) demonstrates that implementing vaccine strategies in these populations is feasible and should be undertaken before outbreak events. Immunisation efforts were aided by an awareness campaign organised by the NGOs present in the settlement and carried out before and during the immunisation campaign. It relied on representatives from different communities in the settlement informing residents of the upcoming immunisation campaign and of the benefits of vaccination.

In addition to the vaccination campaign, the limited size of this outbreak could also be attributed to a substantial herd immunity level in part of the refugees who originated from Middle-Eastern countries and had received vaccinations in their country of origin (status was verified on children's immunisation cards during the immunisation campaign) or had natural immunity following previous measles infection. It is unlikely that additional cases occurred in the settlements that were not identified, because access to medical care is sufficient, and because the implementation of the surveillance system is likely to have increased awareness and reactivity for the notification of disease events.

Given that the number of migrants is unlikely to diminish in the near future, European countries will have to continue to adapt medical and public health services in response to the needs of the refugee populations. The potential for introduction of communicable diseases by volunteers, including in this measles outbreak, cannot be excluded. Therefore, response strategies should ensure that volunteers and medical professionals involved in those settings have an updated vaccine status and target vaccination strategies for refugee populations when possible. In addition to public health measures and improved access to curative and preventive care, improving the living conditions of refugees by reducing overcrowding and providing shelter with adequate sanitation and hygiene will reduce the risk of communicable disease outbreaks.

Conflict of interest

None declared.

Authors' contributions

G. Jones drafted the manuscript; S. Haeghebaert, K. Wyndels, P. Chaud and B. Merlin were in charge of data collection epidemiological surveillance and investigation; M. Janssens is MSF medical coordinator; N. Simon is medical coordinator for refugees in Nord-Pas de Calais-Picardie district; M. Elmouden, F. Battist were responsible for management of patients; S. Haeghebaert, K. Wyndels, P. Chaud, D. Antona revised the manuscript.

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Steady increase of lymphogranuloma venereum cases, Czech Republic, 2010 to 2015

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Since the notification of the first case of lymphogranuloma venereum (LGV) in the Czech Republic in 2010, the numbers of LGV cases have steadily increased in the country. In 2015, 40 LGV cases were diagnosed, bringing the total for 2010–2015, to 88 cases. The profile of the most affected group, HIV-positive men who have sex with men with a previous sexually transmitted infection, matches that of those described in LGV outbreaks in western Europe.

In this report we present data on 83 lymphogranuloma venereum (LGV) cases from a total number of 88 LGV cases reported in the Czech Republic between 2010 and 2015, with 40 cases reported in 2015 alone.

Background

LGV is a sexually transmitted infection (STI) caused by the L1, L2 and L3 serovars of *Chlamydia trachomatis* [1]. Since 2003, LGV has been endemic in western European men who have sex with men (MSM) [1]. Recently, the number of reported cases has been increasing in countries like the United Kingdom (UK), the Netherlands and Spain [2-5]. The first case who presented with typical clinical manifestations compatible with LGV in eastern and central Europe was detected in 2010 in the Czech Republic at the Dermatovenerology department, Na Bulovce Hospital, Prague, in cooperation with the associated AIDS-Centre and the National Reference Laboratory for Chlamydia (NRL) in Prague [6]. Over the last five years, we have noticed an increasing number of patients with LGV in the Czech Republic. Additionally, four cases of LGV in HIV-positive Hungarian men were diagnosed between November 2012 and February 2014 in Hungary [7]. Data from other countries in eastern and central Europe are not publically available.

Study design and population

The data for this report were collected between February 2010 (when the first case of LGV was detected in the Czech Republic) and December 2015. All the patients described in this report were examined at the Department of Dermatovenerology, Na Bulovce Hospital, Second Medical Faculty, Charles University, Prague, in the Czech Republic. The department is a specialised secondary care centre and annually examines between 2,000 and 2,500 patients for STIs (of these, about a quarter are MSM or men who have sex with women and men (MSWM)). It also cooperates with the Department of Infectious Diseases at Na Bulovce Hospital to provide care for more than 1,200 HIV-positive patients. *C. trachomatis* nucleic acid amplification tests (NAATs) were performed on all patients with symptoms of urethritis or proctitis, all contacts reported by patients as sexual partners with confirmed chlamydial infection, on all patients with newly diagnosed syphilis, and on patients with a history of risky sexual behaviour (recent unprotected intercourse with unknown partners or foreigners or with multiple partners). This testing scheme was the same throughout the whole period reported here.

Laboratory methods

Standard validated NAATs for *C. trachomatis* were performed on samples taken from the rectum, urethra, pharynx and ulcers. All rectal samples that tested positive for *C. trachomatis,* as well as urethral and pharyngeal samples in MSM with risky sexual behaviour, were further tested for the LGV genotype in the NRL. The LGV genotype was identified by PCR amplification of a 262 bp fragment of target DNA using the dual-priming oligonucleotide (DPO) Seeplex STI Master Panel 5 test developed by Seegene Inc. (Korea). This method

Number of lymphogranuloma venereum cases and *Chlamydia trachomatis* D-K infections in MSM and MSWM, Na Bulovce Hospital, Prague, Czech Republic, 2010 to 2015 (n=83)
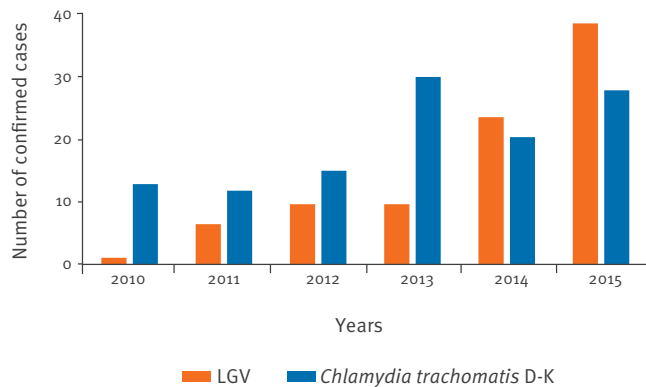
LGV: lymphogranuloma venereum; MSM: men who have sex with men; MSWM: men who have sex with women and men.

targets the *pmp-H* gene and enables the simultaneous detection of LGV-serovars and the differentiation of L1–3 from other serovars [8].

Data from confirmed cases

From a total number of 193 MSM and MSWM with a positive *C. trachomatis* test, 83 cases (43.0%) were further confirmed to have LGV by our department. While there was only one case of LGV diagnosed in 2010, we diagnosed 35 cases of LGV in 2015. In 2014 and 2015, the number of LGV cases was higher than the number of non-LGV chlamydial infections in MSM and MSWM (Figure).

Of the 83 patients with LGV, 76 (91.6%) were MSM, whereas only 7 (8.4%) were MSWM. In total, 70 (84.3%) of the patients were HIV-positive and 13 (15.7%) were HIV-negative at the time of diagnosis and in repeated tests three months later. The most common symptoms were rectal infections 72 (86.7%), whereas urethral 7 (8.4%), pharyngeal 2 (2.4%) and extra genital ulcers 2 (2.4%) were rare. In 24 (28.9%) patients the infections were asymptomatic. In six (7.2%) cases, patients were treated for inflammatory bowel disease (IBD) before the correct diagnosis of LGV was made. Risky sexual behaviour was reported by 64 (77.1%) patients. Co-infections were present in 40 (48.2%) cases, whereas syphilis 22 (26.5%) and gonorrhoea 24 (28.9%) were the most common. Hepatitis C was detected in only one case (Table).

Discussion

In this report we provide data on LGV diagnoses from one reference centre to understand the trends and epidemiology of this infection in a country in eastern/central Europe. The characteristics of the patients in our centre are very similar to those in the Netherlands and

the UK [3,9]. The diagnosis of LGV is predominantly in HIV-positive MSM with a previous history of STIs. Given that our centre is the referral centre for the whole country, patients from other parts of the Czech Republic and not only from Prague were diagnosed here. Thus, our data suggest that LGV infection is spreading in the Czech Republic. Only one report from Hungary published in 2015 described four cases of LGV [7]. The lack of data from eastern and central Europe contrasts sharply with the situation in western Europe, where several outbreaks have been reported recently [2,4]. This may be due to lower awareness among clinicians in these countries, or less testing and reporting, or less transmission.

The increasing number of LGV cases in the Czech Republic is probably due to several interconnected reasons. LGV is an infection predominantly found in HIV-positive MSM. The number of HIV-positive patients in the Czech Republic has been steadily growing by 10-15% every year over the last two decades [10]. More than 80% of these newly diagnosed HIV patients in the Czech Republic are MSM. According to the anecdotal evidence, sexual tourism in the Czech HIV-positive MSM community is also quite widespread. Several patients with confirmed infections in our study reported having had sexual contacts with foreigners from western Europe (Germany, Spain, UK) where LGV outbreaks have been described recently [1,2,4]. These imported infections may be further spreading in some subgroups of the local Czech MSM community with high-risk sexual behaviour. This conclusion is supported by the increasing number of gonococcal, non-LGV chlamydial and syphilis cases within this subgroup.

LGV is mandatorily notifiable in the Czech Republic [11]. The very small number of cases notified by other departments may be explained by the low level of awareness among dermatovenerologists, proctologists and urologists in the region. This fact is reflected in the cases with rectal symptoms. These patients were not requested to disclose information about having had receptive anal sex or about having been tested for *C. trachomatis* infection. Six patients with rectal LGV infection in our group were treated for IBD over the course of several months prior to the correct diagnosis. Four patients received immunosuppressive treatment (azathioprine, systemic corticosteroids), which aggravated their problems. It is therefore also necessary to educate gastroenterologists to check the patient´s clinical history for receptive anal intercourse and to perform tests for *C. trachomatis* in men with IBD symptoms [12].

Another problem is the very limited number of laboratories which are able to perform confirmatory tests. In the Czech Republic, this confirmatory test can only be performed by one laboratory (NRL). We are not aware of any other laboratory in the region that does routinely this form of testing.

Confirmed cases of lymphogranuloma venereum by selected characteristics, Na Bulovce Hospital, Prague, Czech Republic, 2010 to 2015 (N=83)

Characteristics	Number (percentage)
Overall number of confirmed cases	83 (100.0%)
Age group (years)	
15–24	5(6.0%)
25–34	33 (39.8%)
35–44	35 (42.2%)
45–54	9 (10.8%)
55–64	1 (1.2%)
Sexual orientation	
MSM	76 (91.6%)
MSWM	7 (8.4%)
HIV status	
Positive	70 (84.3%)
Negative	13 (15.7%)
Localisation	
Rectum	72 (86.7%)
Urethra	7 (8.4%)
Pharynx	2 (2.4%)
Ulcer in other location	2 (2.4%)
Symptoms	
Yes	59 (71.1%)
No	24 (28.9%)
Co-infection	
Syphilis	22 (26.5%)
Gonorrhea	24 (28.9%)
Chlamydia trachomatis (D-K)	6 (7.2%)
Hepatitis C	1 (1.2%)
Reinfection	
Yes	7 (8.4%)
No	76 (91.6%)
Clinical history of risky sexual behaviour[a]	
Yes	64 (77.1%)
No	19 (22.9%)

MSM: men who have sex with men; MSWM: men who have sex with women and men.

[a] Recent unprotected intercourse with unknown partners, foreigners or with multiple partners.

The question also remains as to the level of awareness that exists in the other countries of the eastern and central European region because the patients with the LGV infection in our cohort also reported having had sexual contacts with individuals from other countries in the region (Hungary, Poland and Slovakia). It is therefore possible that there is a similar trend in these countries as well.

Nearly half of the patients in our group had another previously undiagnosed sexually transmitted co-infection at the time of the LGV diagnosis. This reinforces the need for testing for HIV, syphilis, hepatitis C and also for gonococcal infections in these patients. In our study, 24 (28.4%) of the LGV infections were asymptomatic. These asymptomatic LGV cases were only diagnosed through NAATs screening of chlamydial infections in patients with newly diagnosed syphilis. Our department introduced this form of screening in 2009 because of the high number of asymptomatic gonococcal and chlamydial predominantly rectal infections. The percentage of asymptomatic infections in our group was almost identical to a recently published study from the UK [13]. Since nine patients had pharyngeal and urethral LGV, we recommend testing not only rectal samples, but also samples from the urethra and pharynx in high-risk patients. Asymptomatic or undiagnosed patients can quickly spread the infection within the MSM community because of frequent change of sexual partners and because having concurrent relationships are common in some parts of the MSM community [14,15]. The early diagnosis of symptomatic patients, tracing the sexual contacts of patients with confirmed LGV, and screening of asymptomatic high-risk patients (HIV-positive MSM with risky sexual behaviour) may help to control the spread of LGV. It is also necessary to educate patients about the risks of the disease, symptoms, protection, and points of contact in case of symptoms.

The main limitation of this report is that we are only presenting data from one centre; however, this is because we are the only centre that systematically focuses on LGV infections in our region. The number of reported asymptomatic LGV cases at our clinic may be higher compared to other centres due to the routine *C. trachomatis* screening we carry out on patients with newly diagnosed syphilis. We are not able to report the proportion of LGV positive tests from all tests performed for *C. trachomatis* because positive tests in heterosexual patients were not further tested for LGV.

Conclusion

The increasing number of patients with LGV in our department suggests that the LGV infection is already established and spreading in the Czech Republic and may be present in other countries in the region. Dermatologists, proctologists and gastroenterologists must be more aware of this condition, particularly in patients with rectal symptoms. To keep the situation under control, it is necessary to intensify testing and screening for chlamydial infections and LGV confirmation in eastern and central Europe.

Conflict of interest

None declared.

Authors' contributions

FR: prepared the manuscript, collected and analysed data;

KJ: prepared the manuscript, collected data;

HK: performed LGV genotyping, interpretation of data, critically revised the manuscript;

HZ: performed LGV genotyping, interpretation of data, critically revised the manuscript;

DVa: critically revised the manuscript, collected and analysed the data;

ZK: collected data, interpretation of data, critically revised the manuscript;

LM: collected data, critically revised the manuscript;

DVe: collected data, critically revised the manuscript;

DJ: collected data, critically revised the manuscript;

JH: interpretation of data, critically revised the manuscript;

All authors give final approval of the version to be submitted.

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Risk assessment, risk management and risk-based monitoring following a reported accidental release of poliovirus in Belgium, September to November 2014

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On 6 September 2014, the accidental release of 10^{13} infectious wild poliovirus type 3 (WPV3) particles by a vaccine production plant in Belgium was reported. WPV3 was released into the sewage system and discharged directly to a wastewater treatment plant (WWTP) and subsequently into rivers that flowed to the Western Scheldt and the North Sea. No poliovirus was detected in samples from the WWTP, surface waters, mussels or sewage from the Netherlands. Quantitative microbial risk assessment (QMRA) showed that the infection risks resulting from swimming in Belgium waters were above 50% for several days and that the infection risk by consuming shellfish harvested in the eastern part of the Western Scheldt warranted a shellfish cooking advice. We conclude that the reported release of WPV3 has neither resulted in detectable levels of poliovirus in any of the samples nor in poliovirus circulation in the Netherlands. This QMRA showed that relevant data on water flows were not readily available and that prior assumptions on dilution factors were overestimated. A QMRA should have been performed by all vaccine production facilities before starting up large-scale culture of WPV to be able to implement effective interventions when an accident happens.

Introduction

On 6 September 2014, the Belgium authorities reported to the European Commission, the World Health Organization (WHO), the European Centre for Disease Prevention and Control (ECDC) and the Dutch focal point for the International Health Regulations IHR the accidental release of 45 L of concentrated live poliovirus solution on 2 September at Rixensart, Belgium by a vaccine producing company [1]. The concentrated suspension was estimated to contain 10^{13} infectious wild poliovirus type 3 (WPV3) particles (Saukett strain)

for production of inactivated polio vaccine (IPV). The suspension was released into the sewage system, discharged directly to a wastewater treatment plant (WWTP) in Rosières and subsequently, following treatment, into the river Lasne. The river Lasne is an affluent of the river Dyle which is an affluent of the Schelde river which flows into the Western Scheldt (the Netherlands) and subsequently into the North Sea.

On 6 September, Belgium's High Council of Public Health stated that the risk of infection for the population exposed to the contaminated water was extremely low due to the high level of dilution and the high polio vaccination coverage (95%) in Belgium. Nevertheless, as a precautionary measure, Belgium's High Council of Public Health advised to avoid water activities in the Lasne downstream of the WWTP and a booster dose of IPV was offered to persons who had been in contact with the water of river Lasne from 2 September until the date when the precautionary measures were to be lifted. The vaccination coverage in some Dutch orthodox-reformed communities along the Western Scheldt is less than 90%. In addition, the IPV offered in Belgium (since 2001) and the Netherlands (since 1957) protects against disease but not against infection. Therefore, it does not efficiently interrupt (faecal-oral) transmission as was shown in Israel: Israel implemented IPV exclusively in 2005 and introduction of WPV type 1 in 2013 resulted in silent transmission that continued for almost a year [2]. Consequently, silent transmission of WPV3 after introduction in the Belgium or Dutch population cannot be excluded, while only unvaccinated persons are at risk for disease.

Polioviruses are non-enveloped picornaviruses and stay infectious for several weeks in freshwater and

Timeline showing sampling dates and the risk assessment and risk management process performed in the Netherlands following an accidental release of poliovirus in Belgium, September–November 2014
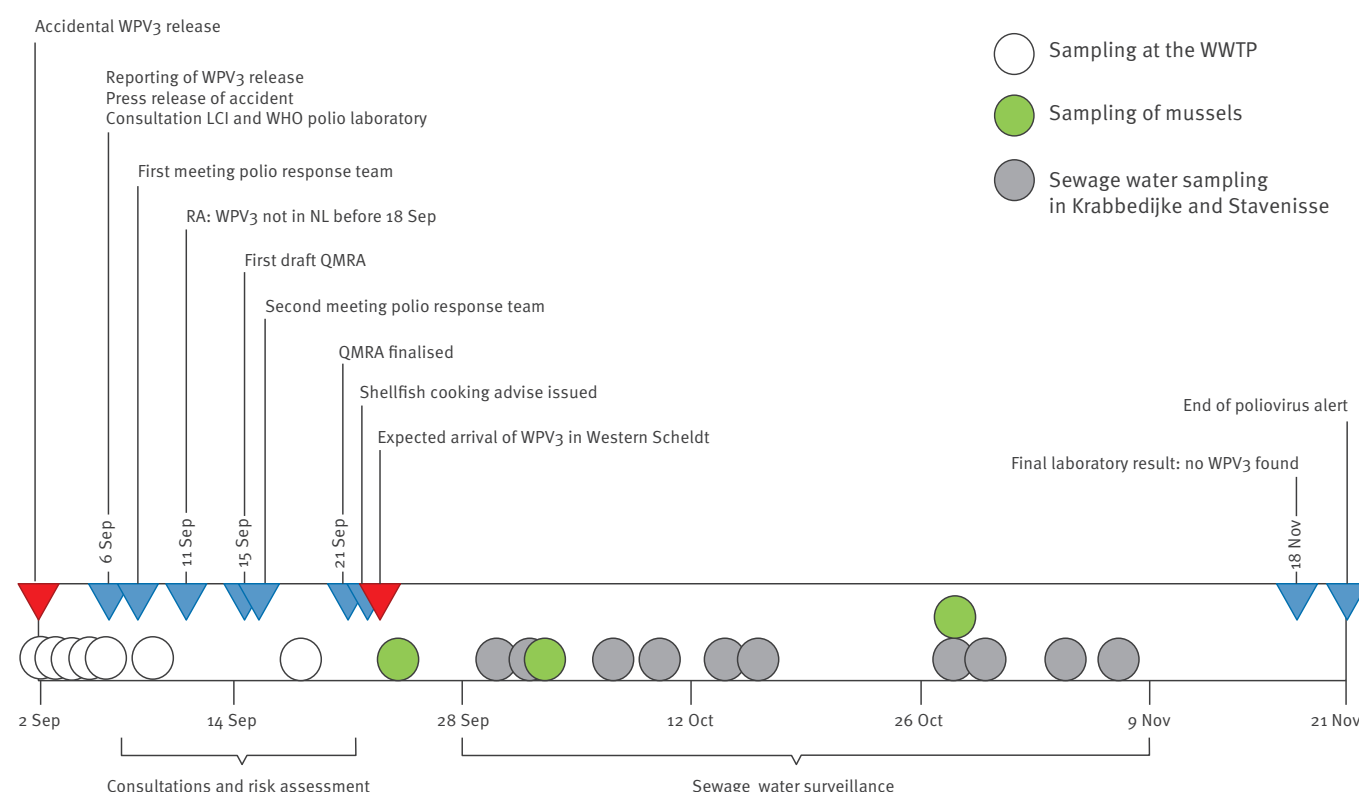
LCI: National Coordination Centre for Communicable Disease Control, the Netherlands; RA: risk assessment; QMRA: quantitative microbial risk assessment; WHO: World Health Organization; WPV3: wild poliovirus type 3; WWTP: wastewater treatment plant.

slightly shorter in seawater [3]. Accidental release may introduce WPV3 into the human population via different routes. Contaminated water may be ingested during swimming. Filter-feeding shellfish, which can concentrate virus particles in their digestive tissue [4,5], may be consumed raw and the shellfish harvesting season was about to start in the Netherlands, in the first week of October 2014.

Because of the risks of circulating wild-type polioviruses and coinciding risks of acute flaccid paralysis (AFP) patients, accidental release of live poliovirus by a vaccine producer may have consequences for the worldwide polio eradication [6]. The European region was certified polio-free in 2002 and has successfully maintained its polio-free status despite numerous challenges. Recently, Ketsuriani et al. concluded that "National polio outbreak preparedness plans need strengthening" within the European region [7]. The authors referred mostly to plans to be executed when an AFP case or poliovirus circulation is already confirmed. In addition, "strategies must be designed to guard against the risk of polio reemergence due to long-term vaccine-derived polio viruses (VDPV) excretors, bioterrorism and accidental release of wild or live vaccine viruses" [6].

This paper describes the actions that were undertaken in the Netherlands following the reporting of the accidental release of WVP3 in Belgium. Based on the infection risks that were assessed by quantitative microbial risk assessment (QMRA), measures were implemented to prevent introduction of WPV3 in the Dutch population and risk-based monitoring was implemented for early detection of poliovirus circulation. Moreover, the paper focuses on the critical control points where the risk assessment and response process can be improved.

Methods

Consultations and qualitative risk assessment
On 8 September, a Dutch response team was convened consisting of representatives of the National Coordination Centre for Communicable Disease Control, the Centre for Zoonosis and Environmental Microbiology (WHO Collaborating Center for Risk Assessment of Food and Waterborne Pathogens) and the Centre for Infectious Diseases Research, Diagnostics and Screening (WHO Specialised Laboratory for Polio) from the RIVM, as well as the Dutch Food Safety Authority (NVWA), the Department of Waterways and Public Works and the Crisis Expert Team Environment and

Estimated inactivation of wild poliovirus type 3 at 18.5°C in water
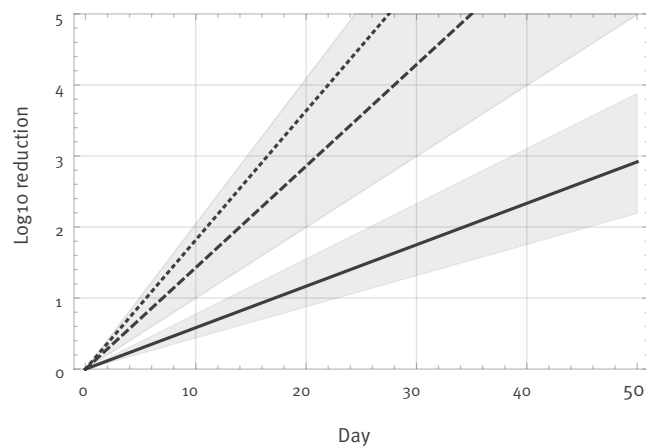
Solid line: freshwater (μ=0.13 day⁻¹); dashed line: seawater (μ=0.30 day⁻¹); dotted line: estimated inactivation of wild poliovirus type 3 according to the World Health Organization (μ=0.42 day⁻¹); grey shading: 95% confidence intervals.

Probability of exposure and dose-response curves for poliovirus type 1 strain SM for adults and wild poliovirus type 3 strain Fox for premature and newborn infants

Beta Poisson dose response model

Probability of exposure

PV1 SM; adults
α=3.8x10⁶, β=7.74x10⁶

PV3 Fox; newborn infants (1500-2200g)
α=0.299, β=0.552

PV3 Fox; premature infants (1740-216g)
α=0.533, β=2.064

Source: [14].

Drinking Water. In this meeting, the consequences for the Dutch population based on a qualitative risk assessment were discussed as well as possible measures. It was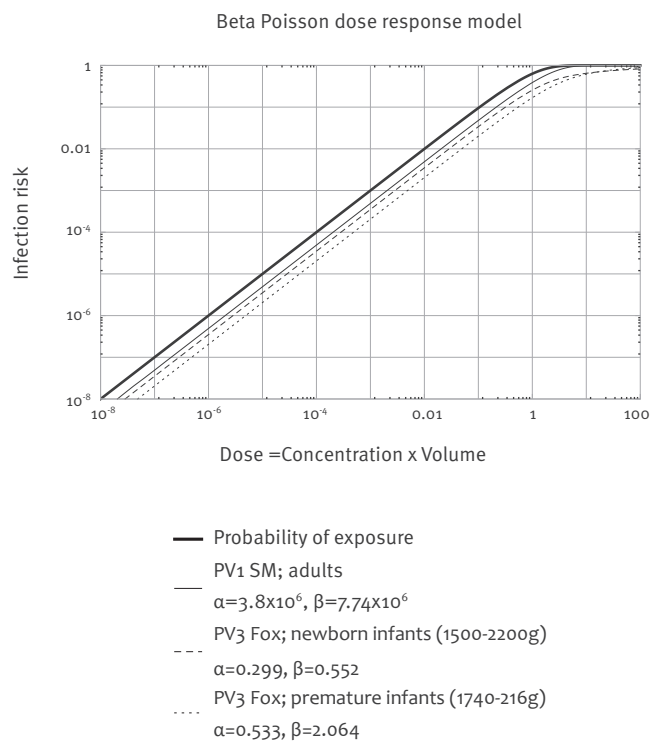 decided to perform a QMRA to support the decision making on public health measures. Between 8 and 21 September 2014 the response team communicated frequently via email, in meetings and teleconferences. Several experts outside the response team were consulted. Throughout the analysis period from 8 September to 18 November, the WHO Regional Office for Europe (WHO/Europe) and the Belgian Scientific Institute of Public Health were updated ad hoc on the laboratory results and progress in the risk assessment (Figure 1). Following a thorough risk assessment by a multidisciplinary group of experts in the response team, two possible routes of transmission that may pose a public health risk were identified: ingestion of contaminated seafood and ingestion of contaminated water during recreational activities. These two routes of transmission were further considered in the next step, the quantitative microbial risk assessment.

Quantitative microbial risk assessment

WPV3 concentration in wastewater effluent

The travel time of the wastewater from the pharmaceutical company to the wastewater treatment plant is three to four hours through a ca 3 km closed sewerage system. We assumed no longitudinal mixing had occurred and the virus load entered the WWTP in a short time interval.

WPV3 concentrations in wastewater were estimated using data provided by GlaxoSmithKline (GSK) (10^{13} WPV3 particles in 45 L) and data provided by the WWTP. Following primary treatment where particles larger than 6 mm are removed, treatment continues biologically in two different lines. The first biological line consists of extended aeration with biological nitrification/denitrification and simultaneous physicochemical precipitation of phosphates, in a dual-zone reactor with selector valve and post-anoxia and internal recirculation of nitrates. The second line is similar to the first line but extended with an ultrafiltration system. Under dry weather conditions, the residence time in the WWTP is 22 hours with a discharge rate of 5.3×10^5 L/h; assuming full mixing, the dilution factor is 2.6×10^5. Because of mostly conventional wastewater treatment, WPV3 concentrations were assumed to be reduced by 0.7–2 $\log_{10}$ (5 to 100 times) [8]. The worst case value of only 0.7 $\log_{10}$ reduction was applied in this QMRA.

WPV3 concentration in the surface waters

The poliovirus particles were assumed to be completely mixed in each water body they passed and subject to inactivation and dilution. Sedimentation (and resuspension) was not considered. Among enteric viruses, poliovirus is relatively stable. First order rate inactivation was calculated using data from a meta-analysis by Bertrand et al. [3] for a temperature of 18.5°C:

$C_t = C_o \, exp \, (-\mu \, t)$ (1)

where C_o is the initial concentration (particles/L), μ is the inactivation rate coefficient (day^{-1}) and t is the time (days). For poliovirus at 18.5 °C, $\mu = 0.13$ in freshwater and $\mu = 0.33$ in seawater.

Figure 2 shows reduction by inactivation of poliovirus for freshwater as well as seawater, including model and prediction uncertainty. For comparison, we included the inactivation rate of WPV3 according to Dowdle and Birmingham as used by WHO [9], showing that inactivation according to WHO data is within the prediction interval given by Bertrand et al. [3]. The prediction uncertainty is huge, ranging from ca 0.1 log$_{10}$ in 50 days to ca 5 log$_{10}$ in eight days. For the QMRA, we applied the mean inactivation rate according to Bertrand et al. [3].

Travel times in the various water bodies as well as dilution factors representing dry weather conditions, were obtained from Rijkswaterstaat (RWS, Department of Waterways and Public Works, the Netherlands).

Exposure and infection risk from swimming in the Western Scheldt and oyster consumption

Because no health-based targets are set for polioviruses in surface water or shellfish, the infection risk that is included in the Dutch drinking water directive for tapwater was taken as a reference for an acceptable risk level [10]. The acceptable infection risk in that directive is set at less than one infection in 10,000 persons that consume unboiled drinking water per year. In this study we set the acceptable risk of infection level at $< 1 \times 10^{-4}$ per swimming episode or portion of shellfish consumed raw.

Exposure to WPV3 by swimming in the Western Scheldt during WPV3 contamination was defined as the ingested number of virus particles of dose D. D was calculated from the WPV3 concentration and the gamma-distributed volume of water (mL) that was swallowed per swimmer per swimming event [11]. The gamma distribution parameter values are $r = 0.45$ and $\lambda = 60$ (mean: 27 mL) for men, $r = 0.51$ and $\lambda = 35$ (mean: 18 mL) for women and $r = 0.64$ and $\lambda = 58$ (mean: 37 mL) for children [12]. In the exposure and risk calculations, 10,000 Monte Carlo samples were generated. Exposure to WPV3 by consuming raw shellfish included the consumed amount of raw shellfish per meal and the assumption that shellfish had concentrated WPV3 100 or 1,000 times by filtering the water [13]. To estimate the infection risk, the beta-Poisson dose response model for WPV3 (strain Fox) in newborns was used [14]:

$P_{inf,person,day} = 1 - {}_1F_1(a, a + \beta; -D)$ (2)

where a and β are infectivity parameters that are pathogen-specific and ${}_1F_1$ is the confluent hypergeometric function (Figure 3).

Samples and sampling sites

Samples were collected by GSK or staff from the Catholic University of Leuven and sent to the National Institute for Public Health and the Environment in the Netherlands (RIVM) for analysis after storage at 2–6 °C for variable times (one night to six weeks). RIVM houses the closest WHO Specialised Laboratory for polio. Sample shipment was facilitated by Belgium's Scientific Institute of Public Health (WIV-ISP) and WHO/Europe. The following samples were analysed: water from the sewage system at GSK, and influent, effluent, sludge and sediment from the WWTP in Rosières. Sludge was removed daily from the WWTP and mixed with high lime doses (40%). Treated sludge was subsequently incinerated. During the first days after the incident (2–5 September), only water samples were collected, sludge samples from the first days were not available. Mussels were collected in the Western Scheldt east of Kruiningen (Kloosterzande) on 24 September and on 3 and 28 October. Between 30 September and 10 November, 19 sewage samples (1 L grab samples) were collected in the villages of Krabbendijke (sampling a secondary school with ca 500 students aged 11–19 years and staff) and Stavenisse (sampling ca 1,800 individuals of all ages). The vaccination coverage for poliovirus in both communities is less than 80% [15].

Sample processing

The water samples were concentrated 50 to 300 times to a volume of 2–3 mL by ultrafiltration using Amicon ultrafiltration membranes PM10 in Amicon stirred ultrafiltration cells at 50–75 psi pressure, at 4 °C. When the target volume of 2–3 mL was reached, the pressure was released and the membrane was rinsed to resuspend the viruses. The concentrated fraction was collected and stored until processing at −20 °C. Approximately 20 g sediment and sludge samples were treated as described [16]. Mussel samples were processed on the day of collection and viruses were extracted from 3 × 10 batches of five pooled digestive tracts [17].

Virus culture for detection of infectious polioviruses

The concentrated and pretreated samples were extracted with chloroform (30% v/v, to remove bacteria, fungi and enveloped viruses) and subsequently inoculated (3 × 100 µL) on 3–7 day-old L20b cells in tubes for detection of infectious polioviruses. L20b is a mouse cell line expressing the gene for the human cellular receptor for poliovirus. These cells support isolation of polioviruses 1, 2 and 3 and only a limited number of other human viruses [18]. The inoculated L20b cells were incubated at 37 °C. The majority of water and sludge samples and the sewage samples from Krabbendijke and Stavennisse were also inoculated on Rd and Ht-29 cells (3 × 100 µL for each cell type). These cell lines support isolation of a wide range of human enteric viruses including most enteroviruses [19,20]. The inoculated RD and Ht-29 cells were incubated at 37 °C and 3 rpm. Cytopathic effect (CPE) was monitored

FIGURE 4

Part of Belgium and the Netherlands showing the estimated concentrations of wild poliovirus type 3 in water bodies and the DKTP vaccination rates in the Netherlands, September–November 2014
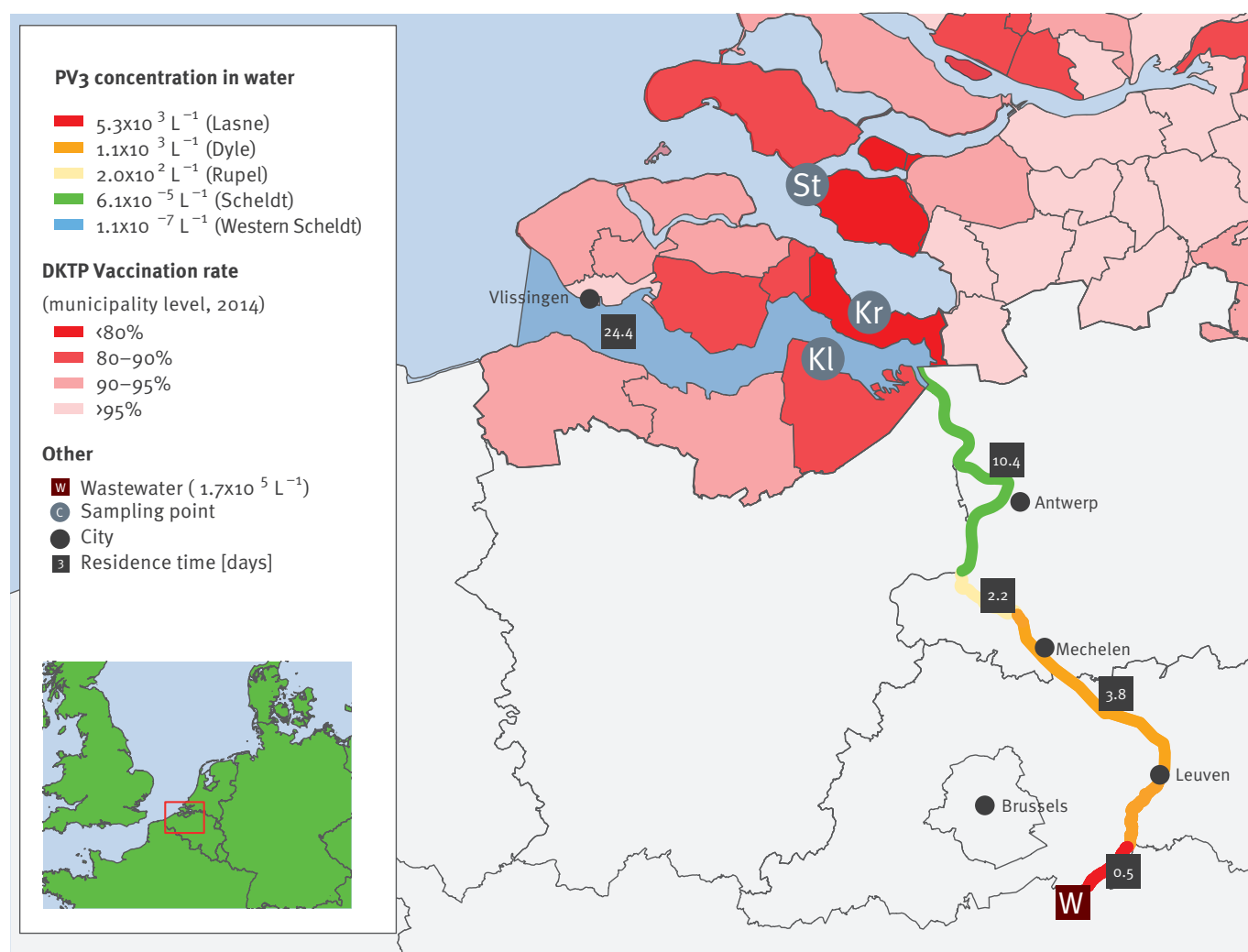
DKTP: diphtheria, pertussis (Dutch: kinkhoest), tetanus, and polio; Kl: mussel sampling site, Kloosterzande, the Netherlands; Kr: sewage sampling site Krabbendijke, the Netherlands; PV3: poliovirus type 3; St: sewage sampling site Stavenisse, the Netherlands; W: wastewater treatment plant, Rosières, Belgium.

Source for vaccination coverage data: [15].

by light microscopy every working day following inoculation for at least seven days.

RNA extraction and RT-PCR for detection of poliovirus RNA

Viral RNA was extracted from 200 µL concentrated water samples, extracted sludge or sediment, mussel homogenate or cell cultures using the MagNAPure LC total nucleic acid isolation kit with a MagNAPure LC instrument as described [21]. Enterovirus RNA was amplified by semi-nested enterovirus RT-PCR (snEV-RT-PCR, PCR1) as described by Nix et al. [22]. If samples were negative, they were retested after 10 times dilution to reduce inhibition. In addition, a subset of samples, including all samples positive in PCR 1, was analysed by Intravacc (biopharmaceutical company

developing vaccines, formerly part of RIVM) using an RT-PCR specific for poliovirus type 3 Saukett strains G/H (PCR 2) according to Nijst et al. [23].

Results

Consultation and qualitative risk assessment

On 8 September, the consequences for the Dutch population based on a qualitative risk assessment were discussed by the Dutch response team (Figure 1). It was concluded that given the release of 10^{13} infectious WPV3 particles, significant numbers of infectious polioviruses were likely to be passing through the Belgium rivers, that infectious poliovirus could enter the Dutch waters, that contact with contaminated water could not be excluded and that silent transmission in the Belgian

Estimated particle travel times, dilution factors concentrations and risk of infection by swimming in different water bodies in Belgium and the Netherlands following an accidental release of poliovirus in Belgium, September–October 2014

Water body	Travel or residence time (days)	Dates of WPV3 arrival	Dilution factor	WPV3 concentration n/L	Risk of infection per child[a] per event
Wastewater treatment plant	0.92	2–3 Sep	2.6×10^5	1.7×10^5	NR
Lasne	0.5	3 Sep	4	5.3×10^3	0.86
Dyle	3.8	10 Sep	3	1.1×10^3	0.77
Dyle at Rumst	0	10 Sep	2	5.3×10^2	0.72
Rupel	2.2	12 Sep	2	2.0×10^2	0.61
Western Scheldt at Belgian-Dutch border	10.4	23 Sep	8×10^5	6.1×10^{-5}	8.0×10^{-7}
Western Scheldt near Vlissingen	14	6 Oct	4.4×10^6	1.1×10^{-7}	1.4×10^{-9}

NR: not relevant.

[a] Per child was chosen because children are more likely to swim and more likely to be infected with poliovirus after exposure.

Risk of infection with wild poliovirus type 3 per person per event in the Netherlands (shellfish consumption or swimming) following an accidental release of poliovirus in Belgium, September–November 2014

	Western Scheldt at Belgian–Dutch border		Western Scheldt near Vlissingen	
	Risk of infection			
Consumption of raw shellfish				
Shellfish consumption	100 × concentrated[a]	1,000 × concentrated[a]	100 × concentrated[a]	1,000 × concentrated[a]
10 g	2.2×10^{-5}	2.2×10^{-4}	3.9×10^{-8}	3.9×10^{-7}
15 g	3.2×10^{-5}	3.2×10^{-4}	5.9×10^{-8}	5.9×10^{-7}
150 g	3.2×10^{-4}	3.2×10^{-3}	5.9×10^{-7}	5.9×10^{-6}
350 g	7.5×10^{-4}	7.5×10^{-3}	1.4×10^{-6}	1.4×10^{-5}
Swimming event				
Man	5.8×10^{-7}		1.1×10^{-9}	
Woman	3.9×10^{-7}		7.0×10^{-10}	
Child	8.0×10^{-7}		1.4×10^{-9}	

[a] 100× and 1,000× concentrated represent two different scenarios, in which the shellfish concentrated the virus particles from the surrounding waters by a factor of 100 or 1,000.

population could not be excluded. Furthermore, a QMRA was needed to support decision making on preventive measures for the Western Scheldt area. Along the Western Scheldt coast are some designated swimming areas. During swimming, water may be swallowed and oral ingestion is an efficient infection route for poliovirus. Therefore, a QMRA for poliovirus infection by swimming was included. Oysters and mussels are not harvested commercially in the Western Scheldt but harvesting for private use is permitted. Because bivalve molluscs concentrate enteric viruses from their growing waters, it was decided to include poliovirus in shellfish in the QMRA.

Contamination of commercial oyster growing areas in the nearby Eastern Scheldt was highly unlikely, because the net water flow is small and mostly from the Eastern Scheldt to the Western Scheldt (Scheldt–Rhine Canal). It was concluded that commercial shellfish harvesting was not affected. No commercial harvesting of samphire and aster occurs in the Western Scheldt, and the samphire harvesting season was ending. Uptake and possible concentration of infectious poliovirus by the plants is not described in the literature. Therefore, samphire and aster consumption was not considered as a risk. Shrimps harvested from the Western Scheldt are boiled immediately on the boat and cooled down using Western Scheldt water. Because there is no concentration of virus particles in the boiled shrimps from this cooling water, they were not considered relevant. The involved surface waters are not used for irrigation or drinking water production.

Quantitative microbial risk assessment

Estimated WPV3 concentrations in water bodies
The limits of detection for the water samples at the time of sampling were between 2×10^2 and 1×10^3 infectious polioviruses per litre. The limit of detection in mussels was one infectious poliovirus per mussel.

Estimated WPV3 concentrations in the water bodies are listed in Table 1 and shown in Figure 4. Dilution factors in the first four water bodies after the WWTP are small; hence, estimated concentrations are high and easily detectable when sampled at the appropriate moments. Even if removal by sewage treatment had been 2 $\log_{10}$ instead of 0.7 $\log_{10}$, WPV3 would have been detectable. In the large saline water bodies with tidal effects near Antwerp, the dilution factors are large, dropping estimated WPV3 concentrations many orders in magnitude. The longer residence times in the large water bodies account for additional inactivation.

Estimated poliovirus concentration in the WWTP effluent and sediment relative to the limits of detection
Parameters used included the concentration factors of our method, volumes /quantity of sludge/sediment/ number of mussels analysed, the detection limit of the culture method (one infectious poliovirus per 100 μL concentrated and pretreated sample per culture tube) and the inactivation rates for storage time–temperature combinations to estimate the limit of detection in the original samples. The sediment samples should have contained more than 2–20 infectious polioviruses per gram sediment/sludge at the time of sampling to be detected with the methods used. The limit of detection in mussels was one infectious poliovirus per mussel.

The estimated WPV3 concentration in the WWTP effluent on 3 September 2014 was 1.7×10^5 WPV3/L for the low treatment scenario (0.7 $\log_{10}$) (Table 1) and 8×10^3 WPV3/L in the case of 2 $\log_{10}$ reduction by the treatment. Based on mixing in the WWTP, WPV3 concentrations in the effluent were expected to decline at a rate of 4 $\log_{10}$ per seven days. The poliovirus concentration on 3 September in the quiescent basin was estimated to be from 8×10^3 to 170×10^3 per litre water, well above the detection limit of 700 polioviruses per litre on the date of analysis. Samples collected on 6 September were estimated to contain more than 10^2 infectious polioviruses per gram sediment at the time of sampling and consequently, more than 10 poliovirus particles per gram sediment at the time of analysis.

Estimation of infection risks, intervention measures and communication
As indicated in Table 1, the estimated infection risk from swimming in the Belgian rivers was high (>50%). However, these results only became available when the estimated virus concentrations had decreased considerably. The counter measures implemented by the Belgian authorities were focused at the river Lasne

only. None of the waters downstream of the WWTP were used for irrigation or drinking water production.

It was calculated that the polioviruses would not reach the Dutch waters before 18 September (Figure 1). Swimming in the Western Scheldt was estimated not to be a high risk activity. The estimated infection risk from consuming poliovirus-contaminated raw shellfish from the eastern part of the Western Scheldt corresponded to 3.2 infections per 10,000 people (Table 2). The estimated infection risk from shellfish consumption harvested in the Western part of the Western Scheldt was low ($<1.5 \times 10^{-5}$). Based on these estimates, the Dutch response team advised on 21 September "to heat shellfish harvested in the western part of the Western Scheldt in boiling water for at least 90 seconds" from 22 September onwards. The cooking advice was published on the website of the RIVM and the Dutch Food safety authority and sent to the local public health services in Zeeland. At several locations along the Western Scheldt, signs were placed informing about possible poliovirus contamination and the cooking advice. It was noticed and covered online by several national and local newspapers on the same day (21 September), including the *Reformatorisch Dagblad*, the daily newspaper for the orthodox reformed community in the Netherlands. The advice was also communicated to WHO/Europe and ECDC.

Virus detection
In the samples collected in or close to the WWTP in Rosières, Belgium, no infectious poliovirus was detected using culture on L20b cells and no poliovirus RNA was detected using the snEV-RT-PCR or the poliovirus type 3 Saukett strain-specific RT-PCR (Table 3). Several RD and Ht-29 cell cultures showed CPE after inoculation, and different echoviruses and a human coxsackie A virus were detected. Several of the cultured samples yielded mixed sequences. Because poliovirus exclusion was the goal of these experiments, no further attempts were made to obtain additional sequencing information. In the schedule applied, it took seven days after arrival of a sample at the RIVM to obtain snEV-RT-PCR results and 10 days to obtain the L20b culture results.

No infectious poliovirus and no poliovirus RNA were detected in the mussels and 77% of the samples were negative for enterovirus, but twice an echovirus type 25 was detected, once an echovirus type 11 and four times a non-polio enterovirus.

All 19 sewage samples collected in Stavenisse and Krabbendijke were negative for poliovirus but positive for other enteroviruses. Echovirus type 18 was found in four of nine samples from Krabbendijke and echovirus type 20 was found in six of 10 samples from Stavenisse. Coxsackievirus types A2 and B5 and ECHO-virus types 3, 6 and 11 were also detected. No polioviruses were detected in sewage samples taken in the same period for the regular surveillance programme for exclusion of

TABLE 3

Characteristics and laboratory analysis of samples collected at the wastewater treatment plant, Rosières, Belgium, September 2014 (n = 18)

Sampling site	Type of sample	Sampling date (dd/mm/yyyy)	Start of processing (dd/mm/yyyy)	Volume (mL)	Treatment	Culture on L20B cells	Culture on RD/Ht-29 cells	PCR 1[a]	PCR 2	EV typing
GSK plant	Water[b]	2/9/2014	13/10/2014	500	Conc to <3 mL	Neg	Neg	**Pos**[c]	Neg	NPEV[d]
WWTP entrance	Water[b]	2/9/2014	11/10/2014	900	Conc to <3 mL	Neg	**Pos**	**Pos**	Neg	Echovirus type 11
WWTP exit	Water[b]	2/9/2014	11/10/2014	900	Conc to <3 mL	Neg	Neg	**Pos**	Neg	NPEV[d]
WWTP entrance	Water	3/9/2014	16/9/2014	100	Conc to <2 mL	Neg	ND	**Pos**	Neg	Echovirus type 9
WWTP basin	Water	3/9/2014	11/10/2014	500	Conc to <3 mL	Neg	Neg	**Pos**	Neg	NPEV[d]
WWTP basin	Water	3/9/2014	11/10/2014	500	Conc to <3 mL	Neg	Pos	**Pos**	Neg	NPEV[d]
WWTP exit	Water	3/9/2014	16/9/2014	100	Conc to <2 mL	Neg	Neg	Neg	Neg	ND
WWTP entrance	Water	4/9/2014	11/10/2014	500	Conc to <3 mL	Neg	Neg	**Pos**	Neg	Echovirus type 3
WWTP exit	Water	4/9/2014	11/10/2014	500	Conc to <3 mL	Neg	Neg	**Pos**	Neg	NPEV[d]
WWTP entrance	Water	5/9/2014	16/9/2014	100	Conc to <2 mL	Neg	ND	Neg	Neg	ND
WWTP exit	Water	5/9/2014	16/9/2014	100	Conc to <2 mL	Neg	ND	Neg	Neg	ND
WWTP entrance	Water	5/9/2014	13/10/2014	400	Conc to <3 mL	Neg	**Pos**	**Pos**	Neg	Echovirus type 11
WWTP exit	Water	5/9/2014	13/10/2014	400	Conc to <3 mL	Neg	Neg	**Pos**	Neg	NPEV[d]
WWTP basin	Water	6/9/2014	16/9/2014	500	Conc to <5 mL	Neg	Neg	Neg	Neg	ND
WWTP basin	Sludge	6/9/2014	16/9/2014	NA	Extracted	Neg	ND	Neg	Neg	ND
WWTP entrance	Sediment	9/9/2014	10/9/2014	NA	Extracted	Neg	ND	**Pos**	Neg	NPEV[d]
WWTP exit	Sediment	9/9/2014	10/9/2014	NA	Extracted	Neg	ND	**Pos**	Neg	Coxsackievirus A type 9
WWTP basin	Sludge	18/9/2014	19/9/2014	NA	Extracted	Neg	ND	**Pos**	ND	NPEV[d]

Conc: concentrated; GSK: GlaxoSmithKline; EV: enterovirus; NA: not applicable; ND: not determined; Neg: negative; NPEV: non-polio enterovirus; Pos: positive; WWTP: wastewater treatment plant.

[a] PCR1 was performed on RNA extracted from the concentrated samples or form the RD/Ht-29 cultures.

[b] Pooled samples, collected over a 24 hour period.

[c] Cultures of RD/Ht29 were analysed by PCR, independent of cytopathic effect.

[d] No growth on L20B cells, positive for enterovirus RNA by PCR1, but sequencing did not yield a typable sequence because of insufficient RNA (weak band on blot) or (most often) mixed infection.

poliovirus circulation in the Dutch Bible belt (data not shown).

On 21 November, it was made public that no poliovirus had been found in the water, sludge and sediment samples, nor in the mussels harvested in the eastern part of the Western Scheldt, and that no circulation of poliovirus had been found in the two communities in Zeeland. On the same day, the warning signs along the Western Scheldt were removed.

Discussion

No infectious poliovirus was detected using culture methods and no poliovirus RNA was detected using molecular methods in any of the samples. Based on calculations presented in this paper, at least the WWTP basin and the effluent samples from 3, 4 and 5 September were expected to contain detectable numbers of viruses after the release of 10^{13} infectious polioviruses into the WWTP. The discrepancy between the laboratory results and the reported release does, however, not diminish the relevance of this risk assessment exercise.

Complete mixing of the WPV3 suspension in the WWTP and only a 0.7 $\log_{10}$ reduction by treatment were assumed. Higher reductions by the WWTP can occur when viruses attach well to solid surfaces. Generally, polioviruses have an isoelectric point near neutral pH and attach well to solid surfaces [24,25]. However, WPV3 Saukett strain has an isoelectric point of 5.8 [26] and may therefore remain in suspension in wastewater and during its transport in river water. Estimates for poliovirus concentrations in the wastewater effluent were made assuming primary and secondary sewage treatment only. If membrane ultrafiltration had been performed on 100% of the wastewater, an additional reduction of more than 4 $\log_{10}$ could have been achieved by the WWTP [27] and consequently, poliovirus concentrations in the effluent would have peaked on 3 September at six WPV3 per litre and decreased after that. A more likely scenario with ca 50% of the water treated by membrane ultrafiltration would result in an additional reduction of only 0.3 $\log_{10}$. To conclude, the estimated numbers of WPV3 particles that were discharged by the WWTP may have been overestimated.

For estimating infection risks, the beta-Poisson dose response model of WPV3 Fox in newborn infants was used [14]. Dose response data also exist for WPV3 Fox in premature infants and for poliovirus type 1 SM in adults. In Figure 2, these dose response curves are compared with the probability of exposure. All dose response curves were very close to each other and not far below the exposure probability line (indicating that every exposure leads to infection). This demonstrates that polioviruses are highly infectious: exposure to only a few WPV3 particles may suffice to cause an infection and consequently virus multiplication, shedding and spreading [28,29]. Given this knowledge, choosing the dose response data of WPV3 Fox in newborn infants was justified.

We cannot explain the lack of poliovirus detections: in several samples, the estimated WPV3 concentrations were well above the limit of detection for poliovirus enteroviruses were detected and in these samples. At several steps, the sensitivity for detection of infectious poliovirus could have been increased. The samples taken directly following the release were sent to the WHO Specialised Laboratory for Polio in the Netherlands for analysis after 10 to 40 days of storage at 2–8 °C. Even though poliovirus is a stable non-enveloped virus, this will have resulted in some loss of infectivity. In addition, larger volumes of water and sludge from the first days could have been collected.

The QMRA concluded that shellfish consumption could lead to infection of more than one in 10,000 persons consuming raw shellfish. Even though the Western Scheldt is not a commercial shellfish harvesting area, this was considered an unacceptable risk and consequently, a shellfish cooking advice was issued. Not detecting a poliovirus in the 150 mussels we tested was expected at these low levels of contamination.

The risk of infection (>50%) estimated for swimming in the Belgian rivers from 3 to 12 September was considered high. Nevertheless, the statement by the Belgium's High Council of Public Health that the risk of a person developing polio after contact with the contaminated waters was "extremely small" was true because less than 1% of non-vaccinated persons will develop polio after infection, and this percentage is even lower for vaccinated persons. However, an accidental release of this magnitude may be considered a real threat for poliovirus eradication. It is important to realise that enormous quantities of water are required to dilute a release of 10^{13} infectious wild poliovirus to negligible poliovirus concentrations and an acceptable risk of infection, quantities of water that are not readily available in small rivers.

The Belgian authorities cooperated well, and information requests to WIV-ISP were dealt with appropriately. There was no legal obligation for the Belgian authorities to report the release of poliovirus to the Dutch authorities since the accident happened at a location from which it takes more than two days for the contamination to reach the country's borders (Convention of Helsinki, 1992 [30]). In addition, as no infectious poliovirus was found there was no obligation to report to WHO. Nevertheless, the current paper describes a risk assessment that ideally should have been performed by all vaccine production facilities before starting up large-scale culture of WPV, to evaluate consequences of accidental poliovirus release into the environment (see also GAPIII [6]). Such a risk assessment may be used immediately in case an accident occurs and provide the basis for immediate actions such as risk communication, preventive measures and risk-based monitoring involving independent experts.

Appropriate data on water quantities and dilutions on the whole trajectory were difficult to obtain because only average values were available, while it was unusually dry during the weeks following the accident. In addition, different models used by different institutes resulted in a broad range of dilution factors in the tidal area. Therefore, input data for the QMRA changed several times. In fact, a risk assessment based on data available on 15 September was issued on 18 September and did not result in implementation of any measures because the infection risk in Dutch waters or via shell fish consumption never exceeded 1×10^{-4}. On 21 September, new data, supported by a wider consensus, were provided and the QMRA was conducted again on that day, which resulted in the risk estimates presented in this paper. In a period with average or high rainfall, the virus would be diluted more in the Belgium rivers and the risk of infection would be lower. Extreme rainfall causing sewage overflow at the time of an accidental release could result in a higher number of viruses released into the river Lasne. We assumed a worst-case scenario with only 80% of the viruses removed by the WWTP, and even in a scenario with sewage overflow, the extra dilution in the river Lasne

would have reduced the infection risk (by the transmission routes studied).

Preventive measures were implemented just before the front of the poliovirus contamination was estimated to reach the Belgian–Dutch border (on 23 September) and warning signs could be placed in time. Because the messages on the RIVM website were noticed and published by local and national news sites within a day, it was concluded that the communication concerning this accident was proportional and the population at risk had been reached.

Based on our experience presented here and the time needed for detection of infectious poliovirus, surveillance for live poliovirus in surface waters downstream of polio vaccine production plants is unlikely to be timely or efficient. Adequate safeguards in the production process minimising the risk of infectious virus release and adequate wastewater treatment on site, are more likely to be a safe strategy. In addition, this specific WWTP is equipped for tertiary water treatment by ultrafiltration with a high capacity. Applying the ultrafiltration to all wastewater taken on 2 and 3 September would have reduced the estimated infection risks for swimming in the Lasne from 86% to less than 5%.

We conclude that the reported release of 10^{13} infectious poliovirus particles has not resulted in the expected detectable levels of poliovirus in any of the samples from Belgium and the Netherlands taken after the incident. No signs for poliovirus circulation in the two Zeelandic communities sampled or in the Dutch Bible belt were found. The reported release of poliovirus type 3 Saukett strain by the vaccine production plant did not result in poliovirus circulation in the Netherlands in the period from 2 September to 7 November 2014. This risk assessment following the accidental release of WPV by the poliovirus vaccine production plant showed that relevant data on water flows were not readily available and that prior assumptions of dilution factors were highly overestimated. A QMRA should have been performed by all vaccine production facilities before starting up large-scale culture of WPV in order to be able to implement effective interventions when an accident happens.

Acknowledgements

Conflict of interest

None declared.

Authors' contributions

Erwin Duizer conceived the study, analysed the data and wrote the manuscript. Saskia Rutjes contributed to the risk assessment, organized the mussel sample analysis and provided comments on the manuscript. Ana Maria de Roda Husman participated in data analysis and interpretation and provided comments on the manuscript. Jack Schijven performed the QMRA and provided comments on the manuscript.

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SURVEILLANCE AND OUTBREAK REPORT

Interim estimates of 2015/16 vaccine effectiveness against influenza A(H1N1)pdm09, Canada, February 2016

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Using a test-negative design, the Canadian Sentinel Practitioner Surveillance Network (SPSN) assessed interim 2015/16 vaccine effectiveness (VE) against influenza A(H1N1)pdm09 viruses. Adjusted VE showed significant protection of 64% (95% confidence interval (CI): 44–77%) overall and 56% (95% CI: 26–73%) for adults between 20 and 64 years-old against medically attended, laboratory-confirmed A(H1N1)pdm09 illness. Among the 67 A(H1N1)pdm09-positive specimens that were successfully sequenced, 62 (>90%) belonged to the emerging genetic 6B.1 subclade, defined by S162N (potential gain of glycosylation) and I216T mutations in the haemagglutinin protein. Findings from the Canadian SPSN indicate that the 2015/16 northern hemisphere vaccine provided significant protection against A(H1N1)pdm09 illness despite genetic evolution in circulating viruses.

Introduction

In contrast to the early and intense 2014/15 influenza season dominated by A(H3N2) viruses that were mismatched to vaccine [1,2], the beginning of the 2015/16 northern hemisphere season had low-level, mixed circulation of influenza A and B viruses. Notable influenza activity in North America and some European countries did not start until December 2015 and A(H1N1)pdm09 viruses predominated among influenza A detections, with some regional variation observed [3-5]. An increasing proportion of A(H1N1)pdm09 viruses belonging to the newly emerging 6B.1 subclade, defined by S162N

(conferring a potential gain of glycosylation) and I216T mutations in the haemagglutinin (HA) protein, has been identified since October 2015 [5-7].

In February 2016, the Influenza – Monitoring Vaccine Effectiveness in Europe (I-MOVE) multicentre case–control study was published reporting early estimates of 2015/16 vaccine effectiveness (VE) against A(H1N1)pdm09 of <50% based on a test-negative study design [8]. This finding raised possible concerns about reduced protection conferred by the A/California/07/2009(H1N1)pdm09 vaccine component that has been recommended for the northern hemisphere seasonal influenza vaccine since the 2009 pandemic, including for the forthcoming 2016/17 season [7,9,10]. Here we present interim VE findings for A(H1N1)pdm09 viruses collected through the Canadian Sentinel Practitioner Surveillance Network (SPSN) also using a test-negative study design. Detailed genetic characterisation of sentinel viruses was undertaken to assess the contribution of the emerging 6B.1 subclade in Canada and its potential impact on measured VE.

Methods

Patients ≥1-year-old presenting within seven days of influenza-like illness (ILI) onset to community-based sentinel sites in four provinces (Alberta, British Columbia, Ontario, and Quebec) were eligible for study inclusion. ILI was defined as acute onset of respiratory illness with fever (based on physician's assessment

Influenza detections by type/subtype and week of specimen collection, Canadian Sentinel Practitioner Surveillance Network (SPSN), 1 November 2015–27 February 2016 (n = 1,375)[a]
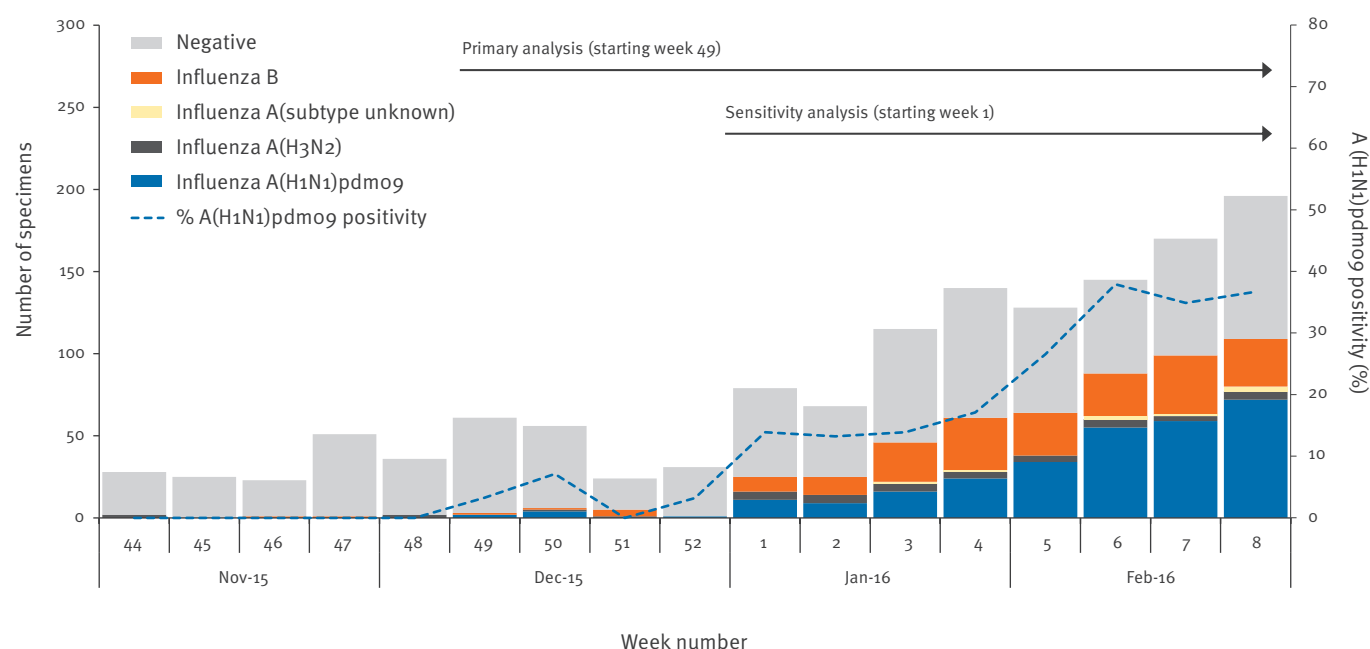
[a] Includes specimens collected from week 44 2015 (starting 1 November) to week 8 2016 (ending 27 February). Specimens were included in the epidemic curve if the patient met the influenza-like illness case definition, had specimen collection within 7 days of illness onset, was ≥1 year-old at time of illness onset, had valid laboratory results, and had known information for all covariates assessed in vaccine effectiveness analysis (age, comorbidity, influenza-like illness onset date, province, and specimen collection date); specimens were included regardless of the patient's vaccination status or timing of vaccination. Missing collection dates were imputed as the laboratory accession date minus two days.

or self reported by the patient) and cough and one or more of the following symptoms: arthralgia, myalgia, prostration or sore throat. Fever was not required for patients ≥ 65-years-old. Epidemiological information was collected from consenting patients/guardians using a standard questionnaire at the time of specimen collection. Ethics review boards in each participating province provided study approval.

Nasal/nasopharyngeal specimens were tested for influenza viruses by real-time, reverse-transcription polymerase chain reaction (RT-PCR) at provincial reference laboratories.

Sequencing of the HA1 region was attempted on a subset of original patient specimens that tested RT-PCR-positive for A(H1N1)pdm09 and contributed to VE analysis to identify mutations in established antigenic sites (Sa, Sb, Ca1, Ca2, and Cb) [11,12].
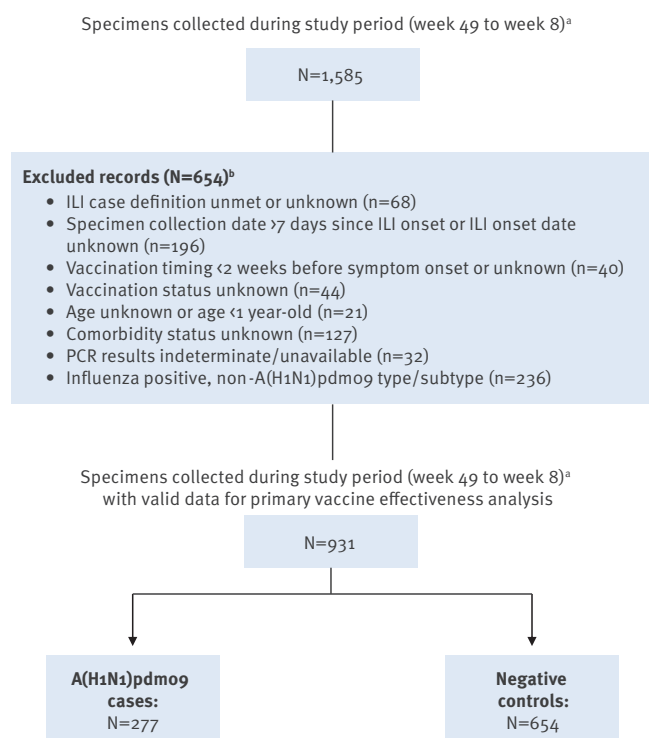
A subset of A(H1N1)pdm09-positive specimens were cultured in Madin-Darby canine kidney (MDCK) or rhesus monkey kidney cells and submitted to Canada's National Microbiology Laboratory for antigenic characterisation by haemagglutination inhibition (HI) assay using turkey erythrocytes, as previously described [12-14].

Specimens collected from week 49 2015 (starting 6 December), corresponding to the first week of A(H1N1)pdm09 detection (Figure 1), to week 8 2016 (ending 27 February) were included in the primary VE analysis. In sensitivity analyses, the study period was restricted to specimens collected from week 1 2016 (starting 3 January) onwards, corresponding to the first week when A(H1N1)pdm09 positivity exceeded 10% (Figure 1).

Patients received 2015/16 influenza vaccine as part of the seasonal vaccination campaign, typically commencing in October in each province. Patients who self-reported receiving at least one dose of influenza vaccine ≥ 2 weeks before ILI onset were considered vaccinated; those vaccinated < 2 weeks before ILI onset were excluded. Odds ratios (OR) for laboratory-confirmed, medically attended A(H1N1)pdm09 illness in vaccinated compared to unvaccinated participants were derived using logisitic regression. VE (expressed as a percentage) was calculated as 1 − OR. ORs were adjusted for age group, comorbidity, province, interval from specimen collection to ILI onset, and calendar time (based on 2-week interval for specimen collection). All analyses were conducted using SAS version 9.3 (SAS Inc., Cary, NC).

Study exclusions, interim influenza A(H1N1)pdm09 vaccine effectiveness (VE) evaluation, Canadian Sentinel Practitioner Surveillance Network (SPSN), 6 December 2015–27 February 2016 (n = 1,585)

Specimens collected during study period (week 49 to week 8)[a]

N=1,585

Excluded records (N=654)[b]
- ILI case definition unmet or unknown (n=68)
- Specimen collection date >7 days since ILI onset or ILI onset date unknown (n=196)
- Vaccination timing <2 weeks before symptom onset or unknown (n=40)
- Vaccination status unknown (n=44)
- Age unknown or age <1 year-old (n=21)
- Comorbidity status unknown (n=127)
- PCR results indeterminate/unavailable (n=32)
- Influenza positive, non-A(H1N1)pdm09 type/subtype (n=236)

Specimens collected during study period (week 49 to week 8)[a]
with valid data for primary vaccine effectiveness analysis

N=931

A(H1N1)pdm09 cases:
N=277

Negative controls:
N=654

ILI: influenza-like illness; PCR: polymerase chain reaction.

[a] Includes specimens collected from week 49 2015 (starting 6 December) to week 8 2016 (ending 27 February).

[b] Exclusions are not mutually exclusive; specimens may have >1 exclusion criterion that applies. Counts for each criterion will sum to more than the total number of specimens excluded.

Results

From 6 December 2015 to 27 February 2016, 1,585 specimens were collected, of which 1,167 (74%) met study inclusion criteria (Figure 2). Influenza viruses were detected in 513 (44%) specimens, including 321 (63%) influenza A, 191 (37%) influenza B, and one influenza A/B co-infection. Of the 314 of 322 (98%) influenza A viruses with known subtype, 277 (88%) were A(H1N1) pdm09.

Overall 14% (n=40) of cases and 31% (n=200) of controls were considered vaccinated (p<0.01) (Table 1).

Among vaccinated participants who had available data for prior vaccination history, 89% (198/222) of participants ≥2 years-old had also received the prior season's 2014/15 vaccine, 83% (172/207) ≥3 years-old had received both the 2014/15 and 2013/14 seasonal vaccines, and 79% (132/168) ≥7 years-old had received the 2009 monovalent A(H1N1)pdm09 pandemic vaccine, for which ca 95% of the product distributed in

Canada was AS03-adjuvanted [15]. Among the 38 vaccinated cases with available data, 37 (97%) had received prior 2014/15 vaccine, 95% (35/37) had received both 2014/15 and 2013/14 vaccines, and 81% (22/27) had received 2009 monovalent A(H1N1)pdm09 vaccine.

After adjustment for relevant covariates, VE against A(H1N1)pdm09 was 64% (95% confidence interval (CI): 44–77%) for the primary analysis and 62% (95%CI: 41–76%) when restricted to specimens collected from week 1 2016 onwards (Table 2). Adjusted VE was 56% (95%CI: 26–73%) and 59% (95%CI: 21–79%) among adults between 20 and 64 years-old, and 20 and 49 years-old, respectively.

Sequencing was attempted on 102 A(H1N1)pdm09-positive specimens collected up to 15 February 2016. Amplification was successful for 67 (66%) of these viruses. All 67 sequenced viruses (100%) had the antigenic site mutation K163Q (Sa) and the non-antigenic site mutations A256T and K283E in HA1 associated with clade 6B, along with antigenic site mutations S185T (Sb) and S203T (Ca1) present in all clade 6 viruses [6]. Sixty-two (93%) viruses had the additional mutations S162N (Sa), conferring a potential gain of glycosylation at residues 162–164, and I216T (non-antigenic) defining the emerging 6B.1 subclade. Two (3%) viruses had the additional mutation V152T within the receptor binding site (RBS) associated with the emerging 6B.2 subclade. One 6B.1 subclade virus had a V152I mutation in addition to S162N and I216T mutations.

Of the 30 sentinel viruses collected in December and January characterised by HI assay, all were considered antigenically similar to the A/California/07/2009(H1N1) pdm09 reference strain.

Discussion

In this interim analysis, we measured statistically significant VE of 64% (95%CI: 44–77%) against circulating A(H1N1)pdm09 viruses largely belonging to the emerging 6B.1 subclade. This point estimate is slightly lower than but comparable to the significant VE measured by our network in 2013/14 mid-season (74%; 95%CI: 58–83%) [13] and end-of-season (71%; 95%CI: 58–80%) [12] analyses against dominant clade 6B A(H1N1)pdm09 viruses. In 2013/14, clade 6B viruses had the antigenic site K163Q mutation but had not yet acquired the adjacent S162N mutation associated with the newly emerging 6B.1 subclade. Despite some genetic evolution in A(H1N1)pdm09 viruses, our 2015/16 VE estimate remains closely aligned with a recent meta-analysis of test-negative studies globally for which pooled VE for seasonal vaccine against A(H1N1)pdm09 since 2010 was 61% (95%CI: 57–65%) [16].

Our point estimates of VE against A(H1N1)pdm09 are higher (but with overlapping confidence intervals) compared with those reported in similar mid-season analysis from the European I-MOVE multicentre case–control

Table 1

Characteristics of participants included in interim influenza A(H1N1)pdm09 vaccine effectiveness (VE) evaluation, Canadian Sentinel Practitioner Surveillance Network (SPSN), 6 December 2015–27 February 2016 (n = 931)

Characteristic	Overall n (column %)[a]	Distribution by case status n (column %)[a]			Vaccination coverage n (row %)	
		A(H1N1)pdm09 cases	Negative controls	P value[b]	Vaccinated	P value[b]
N (row %)	931 (100)	277 (30)	654 (70)	–	240 (26)	–
Age group in years						
1–8	132 (14)	35 (13)	97 (15)		23 (17)	
9–19	113 (12)	25 (9)	88 (13)		14 (12)	
20–49	411 (44)	142 (51)	269 (41)	<0.01	74 (18)	<0.01
50–64	179 (19)	57 (21)	122 (19)		64 (36)	
≥65	96 (10)	18 (7)	78 (12)		65 (68)	
Median (range)	36 (1–92)	37 (1–83)	35 (1–92)	0.62	53 (1–92)	<0.01
Sex[c]						
Female	571 (62)	164 (60)	407 (63)		156 (27)	
Male	346 (38)	109 (40)	237 (37)	0.37	81 (23)	0.19
Unknown	14	4	10		3	
Comorbidity[d]						
No	746 (80)	239 (86)	507 (78)	<0.01	152 (20)	<0.01
Yes	185 (20)	38 (14)	147 (22)		88 (48)	
Province						
Alberta	243 (26)	84 (30)	159 (24)		70 (29)	
British Columbia	241 (26)	47 (17)	194 (30)	<0.01	65 (27)	0.14
Ontario	323 (35)	95 (34)	228 (35)		83 (26)	
Quebec	124 (13)	51 (18)	73 (11)		22 (18)	
Collection interval in days						
≤4	697 (75)	229 (83)	468 (72)	<0.01	169 (24)	0.07
5–7	234 (25)	48 (17)	186 (28)		71 (30)	
Median (range)	3 (0–7)	3 (0–7)	3 (0–7)	<0.01	3 (0–7)	0.01
Month of specimen collection[e]						
December	152 (16)	7 (3)	145 (22)		38 (25)	
January	298 (32)	56 (20)	242 (37)	<0.01	78 (26)	0.96
February	481 (52)	214 (77)	267 (41)		124 (26)	
Vaccination status						
Any vaccination[f]	261/952 (27)	43/280 (15)	218/672 (32)	<0.01	NE	–
≥2 weeks before ILI onset	240 (26)	40 (14)	200 (31)	<0.01	NE	–
LAIV[g]	11/128 (9)	1/22 (5)	10/106 (9)	0.69	NE	–
QIV[h]	33/140 (24)	5/22 (23)	28/118 (24)	0.92	NE	–
Adjuvanted[i]	16/35 (46)	4/5 (80)	12/30 (40)	0.16	NE	–
Prior vaccination history						
2014/15 vaccine[j]	308/858 (36)	68/252 (27)	240/606 (40)	<0.01	198/308 (64)	<0.01
2013/14 vaccine[k]	301/811 (37)	74/240 (31)	227/571 (40)	0.02	185/301 (61)	<0.01
2009 monovalent vaccine[l]	296/673 (44)	79/199 (40)	217/474 (46)	0.15	132/296 (45)	<0.01

ILI: influenza-like illness; LAIV: live attenuated influenza vaccine; NE: not estimated; QIV: quadrivalent influenza vaccine.

[a] Unless otherwise specified, the values presented in this column are the number of specimens per category and percentage relative to the total. Where the denominator for the percentages differs from the total, fractions supporting the calculation of percentages are shown.

[b] Differences between cases and controls and vaccinated and unvaccinated participants were compared using the chi-squared test, Fisher's exact test or Wilcoxon rank-sum test.

[c] The percentage was only calculated among the total patients whose sex was known.

[d] Includes chronic comorbidities that place individuals at higher risk of serious complications from influenza as defined by Canada's National Advisory Committee on Immunization (NACI) including: heart, pulmonary (including asthma), renal, metabolic (such as diabetes), blood, cancer, or immune comprising conditions; conditions that compromise management of respiratory secretions and increase risk of aspiration; or morbid obesity (body mass index ≥40) [29].

[e] Missing collection dates were imputed as the laboratory accession date minus two days.

[f] Participants who received seasonal 2015/16 influenza vaccine <2 weeks before ILI onset or for whom vaccination timing was unknown were excluded from the primary analysis. They were included for assessing 'any' vaccination, regardless of timing, for comparison with other sources of vaccination coverage.

[g] Among participants between two and 59 years-old who received 2015/16 influenza vaccine ≥2 weeks before ILI onset and had known information for type of vaccine. Among participants between two and 17 years-old for whom LAIV is recommended by NACI [29], 44% (11/25, including one case) with known information had received LAIV. Among participants between two and five years-old for whom LAIV is preferentially recommended by NACI [29], 36% (5/14, including one case) with known information had received LAIV.

[h] Among participants who had known information for trivalent vs. quadrivalent vaccine. QIV includes both inactivated influenza vaccine (IIV4) and live-attenuated influenza vaccine (LAIV4) products.

[i] Among participants ≥65 years-old who received 2015/16 influenza vaccine ≥2 weeks before ILI onset and had known information for adjuvanted vaccine receipt.

[j] Children <2 years-old in 2015/16 were excluded from 2014/15 vaccine uptake analysis as they may not have been eligible for vaccination during the autumn 2014 vaccination campaign.

[k] Children <3 years-old in 2015/16 were excluded from 2013/14 vaccine uptake analysis as they may not have been eligible for vaccination during the autumn 2013 vaccination campaign.

[l] Children <7 years-old in 2015/16 were excluded from 2009 monovalent A(H1N1)pdm09 vaccine uptake analysis as they may not have been eligible for vaccination during the autumn 2009 vaccination campaign.

TABLE 2

Interim vaccine effectiveness (VE) estimates against influenza A(H1N1)pdm09, Canadian Sentinel Practitioner Surveillance Network (SPSN), 6 December 2015–27 February 2016 (n = 931)

Covariates	VE % (95%CI)	N total Cases: n (n vac, % vac); Controls: n (n vac, % vac)
Primary analysis[a,b]		
Unadjusted	62 (44–74)	
Age group (1–8, 9–19, 20–49, 50–64, ≥65 years)	62 (43–74)	
Comorbidity (no, yes)	58 (39–72)	**Total: 931**
Province (AB, BC, ON, QC)	62 (44–74)	Cases: 277 (40, 14%);
Interval from specimen collection to ILI onset (≤4, 5–7 days)	61 (43–73)	Controls: 654 (200, 31%)
Calendar time (2-week interval)[c]	66 (49–77)	
Age group, comorbidity, province, interval, calendar time	64 (44–77)	
Restricted to specimens collected from week 1 to week 8, 2016[b]		
Unadjusted	63 (45–75)	
Age group (1–8, 9–19, 20–49, 50–64, ≥65 years)	63 (44–75)	
Comorbidity (no, yes)	60 (40–73)	**Total: 776**
Province (AB, BC, ON, QC)	62 (44–75)	Cases: 270 (40, 15%);
Interval from specimen collection to ILI onset (≤4, 5–7 days)	62 (44–74)	Controls: 506 (161, 32%)
Calendar time (2-week interval)[c]	65 (48–76)	
Age group, comorbidity, province, interval, calendar time	62 (41–76)	
Restricted to adults 20–64 years-old[a,b]		
Unadjusted	58 (34–73)	
Age group (20–49, 50–64 years)	58 (34–74)	
Comorbidity (no, yes)	56 (30–72)	**Total: 590**
Province (AB, BC, ON, QC)	58 (33–73)	Cases: 199 (28, 14%);
Interval from specimen collection to ILI onset (≤4, 5–7 days)	57 (33–73)	Controls: 391 (110, 28%)
Calendar time (2-week interval)[c]	56 (28–73)	
Age group, comorbidity, province, interval, calendar time	56 (26–73)	
Restricted to adults 20–49 years-old[a,b]		
Unadjusted	62 (29–80)	
Comorbidity (no, yes)	61 (28–79)	
Province (AB, BC, ON, QC)	63 (31–80)	**Total: 411**
Interval from specimen collection to ILI onset (≤4, 5–7 days)	61 (27–79)	Cases: 142 (14, 10%);
Calendar time (2-week interval)[c]	59 (23–79)	Controls: 269 (60, 22%)
Comorbidity, province, interval, calendar time	59 (21–79)	

AB: Alberta; BC: British Columbia; CI: confidence interval; ILI: influenza-like illness; ON: Ontario; QC: Quebec; vac: vaccinated; VE: vaccine effectiveness.

[a] Restricted to specimens collected from week 49 2015 (starting 6 December) to week 8 2016 (ending 27 February).

[b] Patient specimens were included in VE analysis if the patient met the ILI case definition, had specimen collection within 7 days of ILI onset, was ≥1 year-old at time of ILI onset (based on age eligibility of ≥6 months for influenza vaccine during the autumn 2015 vaccination campaign), received 2015/16 influenza vaccine ≥2 weeks before ILI onset, had valid laboratory results, and had known information for all covariates assessed in VE analysis (age, comorbidity, ILI onset date, province, and specimen collection date).

[c] Based on date of specimen collection; missing collection dates were imputed as the laboratory accession date minus two days.

study, which indicated VE against A(H1N1)pdm09 of 44% (95%CI: -3 to 70%) overall and 41% (95%CI: -25 to 72%) in adults between 18 and 64 years-old, although estimates were not statistically significant [8]. Because of the low vaccination coverage in Europe (<15% among controls) and late start to the 2015/16 influenza season, the I-MOVE study likely had limited statistical power to measure stable or significant VE in mid-season analysis [8]. Their findings are, however, comparable to their previously published estimates against A(H1N1)pdm09 from the 2013/14 and 2014/15 seasons (ranging from 48 to 54%) [17,18]. Our estimates

are also slightly higher than the point estimate of 51% reported for A(H1N1)pdm09 by the United States (US) Flu VE Network for the current 2015/16 season [19], although this US estimate is also not substantially different from their recently published estimate of 54% (95%CI: 46–61%) for the A(H1N1)pdm09-dominant 2013/14 season [20]. The lack of further epidemiological and genomic detail in interim findings from elsewhere prevents direct comparison to our Canadian SPSN results. In addition to possible virologic differences in the mix of circulating strains contributing to VE analysis, differences in study methods, patient

populations, and vaccination programmes, including the use of AS03-adjuvanted vaccine during the 2009 pandemic in Canada [15], should be taken into account in comparing VE estimates across settings or seasons [16].

As seen in prior SPSN analyses [12-14], the largest proportion of specimens in the current analysis was collected from younger, non-elderly adults between 20 and 49 years-old (44%), more notable among cases than controls (51% vs 41%) (Table 1). Adjusted VE estimates in age-stratified analyses were comparable to, but slightly lower than, our primary analysis at 59% (95%CI: 21–79%) when restricted to adults aged between 20 and 49 years-old, and 56% (95%CI: 26–73%) when broadened to include all adults between 20 and 64 years-old. This may reflect random variation owing to the smaller sample size in age-stratified analyses or unmeasured residual confounding across patient age groups. Variation by age could also reflect cohort effects resulting from different immunological priming/boosting as well as varying responses to vaccination by age or other patient factors. Over 80% of vaccinated participants in our study had received prior 2014/15 and 2013/14 seasonal vaccines; however, repeat vaccination effects could not be assessed in interim analyses because of the small number of participants who were vaccinated in the current, but not prior, season. These considerations warrant further evaluation in end-of-season VE or serological analyses and should also be taken into account in comparing VE estimates across studies or seasons with different participant age-distribution or immunological profiles.

Consistent with virus circulation globally [5,6], all sentinel A(H1N1)pdm09 viruses sequenced in our study belonged to clade 6B, with 62 of 67 (93%) more specifically falling within the emerging 6B.1 subclade. Information on genetic characterisation was not provided in the I-MOVE study [8], but separately published surveillance data for Europe report that about 80% of 6B viruses contain the S162N and I216T mutations [6]. The S162N mutation is located in antigenic site Sa close to the RBS and adjacent to the clade-defining K163Q mutation that other investigators have hypothesised to have facilitated resurgent A(H1N1)pdm09 activity disproportionately affecting middle-aged adults in 2013/14 [12,21]. The S162N mutation confers a potential gain of glycosylation at residues 162–164 that may mask K163Q and other epitopes relevant for neutralising antibody binding [6,22,23]. Despite genetic evolution, most circulating 6B viruses characterised globally, including the sentinel viruses assessed in this study, remain antigenically similar to the A/California/07/2009(H1N1)pdm09 reference strain (belonging to clade 1) based on HI and virus neutralisation assays [3-7]. Interim VE estimates from the Canadian SPSN were also not markedly affected by recent molecular changes in circulating A(H1N1)pdm09 viruses and are consistent with the recent World Health Organization (WHO) decision to retain the A/California/07/2009(H1N1)pdm09 vaccine strain for the forthcoming 2016/17 season [7]. Our interim VE estimates were submitted alongside other estimates from the Global Influenza Vaccine Effectiveness (GIVE) Collaboration and contributed to the February 2016 WHO consultation meeting on the composition of influenza vaccines for the 2016/17 northern hemisphere season [24].

Limitations of this analysis include the small number of cases available for interim analysis and resulting wide 95% CIs, particularly in stratified analyses. Although the validity of the test-negative design for deriving VE estimates has been demonstrated relative to randomised controlled trials and simulation studies [25-27], residual bias and confounding due to the observational study design cannot be ruled out. VE was measured against medically attended outpatient illness and may not be generalisable to more severe outcomes, although a recent meta-analysis suggests that VE estimates derived using the test-negative design do not substantially differ between outpatient and inpatient settings [28]. Interim estimates are only presented for A(H1N1)pdm09 viruses; where possible, VE for other types/subtypes, including clade- and lineage-specific estimates, will be explored in end-of-season analyses.

Interim VE analyses from the Canadian SPSN suggest that the 2015/16 northern hemisphere vaccine has provided significant protection against A(H1N1)pdm09 viruses belonging to the emerging 6B.1 subclade. Due to considerations such as the late start of the 2015/16 influenza season and smaller number of accrued cases, estimates may vary in end-of-season analyses and should be interpreted with caution. Further investigation into the impact of evolving antigenic site mutations, including the role of S162N and its potential glycosylation effects, on vaccine protection is required.

l'Institut national de santé publique du Québec, and the Public Health Agency of Canada.

Conflict of interest

Authors' contributions

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ESCAIDE 2015: an operational scientific conference on infectious diseases for professionals from Europe and beyond

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The ninth annual 'European Scientific Conference on Applied Infectious Disease Epidemiology' (ESCAIDE), organised by the European Centre for Disease Prevention and Control (ECDC), took place between 11 and 13 November 2015 in Stockholm. One aim of ESCAIDE is to share applied scientific knowledge on infectious diseases surveillance, prevention and control in Europe and internationally. Other aims are (i) to build a multidisciplinary network of independent health professionals, (ii) to strengthen and expand the international response capacity against communicable disease, and (iii) the sharing of experiences on translating evidence from epidemiological and microbiological investigations into actions leading to public health protection.

In 2015, more than 600 public health specialists from 55 countries participated to share their knowledge and experiences on current challenges in the field of infectious diseases. Scientific work was presented in five plenaries, 21 parallel sessions and three moderated poster sessions composed of 24 different tracks. Oral presentations were shared online [1].

The global public health threat of antimicrobial resistance
The keynote speech of the conference was given by Jan Kluytmans (University Medical Center Utrecht, the Netherlands) presenting 'Antibiotic resistance: a tragedy of the commons'. He described the extensive use of antimicrobial drugs in humans and animals and the consequences on antimicrobial resistance (AMR) development. The underlying drivers for AMR are the lack of basic hygiene, a high uncontrolled consumption of antibiotics, and transfer of resistance-conferring molecular elements between animal species, including humans [2]. In spite of this, actions against AMR linked to contaminated food consumption remain infrequent and uncoordinated. He concluded that prudent use of antimicrobials should be advocated and the use of important antibiotics, at least for livestock, should be more tightly controlled.

The Antimicrobial Resistance and Causes of Non-prudent Use of Antibiotics project was presented by John Paget (the Netherlands Institute for Health Services Research, the Netherlands) in a parallel session entitled 'Antimicrobial Resistance'. Research to assess and define the key factors explaining the non-prudent use of antibiotics across seven selected European Union (EU) countries will end in June 2016. Research findings will be translated into policy actions for the more cautious use of antibiotics.

Social media for public health purposes
In this plenary session, the usefulness of social media as tools in communicable disease surveillance and control was discussed. In the last fifteen years many health web-based informal channels have fundamentally changed access to, and dissemination of, medical information, in the field of public health surveillance and outbreak detection and intervention. The integration of health data from official sources with Internet-based data can be an added value to public health surveillance systems in providing information for better risk assessments of communicable diseases.

John Brownstein (Boston Children's Hospital, the United States of America) showed the current sources in the use of non-traditional data sources for the purposes of infectious disease surveillance and epidemic intelligence gathering. 'HealthMap' utilises online informal sources for disease outbreak monitoring and real-time surveillance of emerging public health

threats. Food-borne illness surveillance efforts can be supplemented by the business review site 'Yelp.com', as described by Nsoesie et al. [3]. Other examples presented included 'Thermia', which is a decision support framework based on current clinical guidelines for fevers and associated febrile illnesses, and 'Flu Near You', which is an anonymous community health project in North America that reports and maps influenza-like symptoms weekly. 'UberHEALTH' is a new model of healthcare delivery, which includes the option to have influenza vaccination delivered at home, active in over 70 cities around the world.

Ingemar Cox (University College London, UK and the University of Copenhagen, Denmark) discussed how Internet-based health data sources could facilitate medical research evaluating analysis methods used in recent literature. Digital data offers the potential to access a massive volume of patient-reported outcomes and unfiltered real time, multi-dimensional information on patients' experience. On the other hand, e-data have the limitations of a wide variation in availability and costs to researchers, and storage ability for researchers to utilise data are limited by access to funds and software developers. Moreover, ethical challenges and privacy issues are unclear, and rules are needed to opportunely treat and de-identify e-data.

Epidemiological investigations for public health protection

The plenary session on the occasion of the 20th EPIET anniversary was dedicated to the recurrent food-borne Hepatitis A virus (HAV) outbreak that occurred in 13 EU and European Economic Area countries between 2012 and 2014 involving 1,589 cases. During these investigations, an EPIET and EUPHEM network of experts gave valuable support. Jane Richardson of the European Food Safety Authority and Johanna Takkinen (ECDC) summarised the food tracing activities and recommendations that followed the consecutive multi-country outbreaks. They emphasised the multidisciplinary approach and the good EPIET and EUPHEM collaboration as key factors for controlling the outbreak. Gaia Scavia (Istituto Superiore di Sanità, Italy) gave a national perspective of the investigation. The HAV outbreaks were caused by exposure to contaminated lots of mixed berries from various origins. Sequencing and centralised collection of the viral strains in the Hepatitis A Laboratory-Network database were essential for hypothesis generation. Tracing data were exchanged via the European Rapid Alert System for Food and Feed. A common sequencing protocol was prepared by a EUPHEM fellow at the Dutch National Institute for Public Health and the Environment. Compliance with good hygiene, manufacturing and agricultural practices were recommended in order to focus on preventive measures rather than trying to remove or inactivate the virus from contaminated food.

Emerging challenges to vaccine programmes

Nicole Guiso (Institut Pasteur, France) presented the impact of human immunization with different vaccines against Bordetella pertussis on the selection of escape mutants and the possibly consequent reduction in vaccine effectiveness [4] in a further plenary session. With the aim to better understand the impact of vaccination on B. pertussis populations or the role of Bordetella species evolution on pertussis vaccines effectiveness, the speaker suggested to consider not only the vaccine composition and strategies used, but also the biological surveillance of disease, the vaccine coverage and the characteristics of the circulating B. pertussis and B. parapertussis populations.

Annette Mankertz (Robert Koch-Institute, Germany) pointed out the slight increase in secondary vaccination failure regarding measles [5] and the frequent secondary vaccine failure related to mumps [6] occurring worldwide in recent years. She discussed the underlying causes, including antigen escape and waning immunity due to a lack of natural booster.

Nonspecific side effects of children vaccines in the world's poorest countries were discussed by Christine Stabell Benn (Statens Serum Institut and University of Southern Denmark, Denmark). The Bandim Health Project is a health and demographic surveillance platform to test real-life effects of health interventions in Guinea-Bissau. It has shown that vaccines' nonspecific effects involve cross-reactivity of the immune system with unrelated pathogens. Live attenuated vaccines seem to improve the immune system's ability to fight other pathogens, while inactivated vaccines might reduce it. Moreover, both positive and negative nonspecific effects seem strongest for females [7].

Public health events in 2015: Ebola virus and Middle East respiratory syndrome coronavirus

In the last plenary, Pierre Formenty from the World Health Organization (WHO) discussed the Ebola crisis in West Africa, highlighting the lessons learnt for prevention of future crises. Molecular evidence for sexual transmission of Ebola virus (EBOV) in Liberia was recently described [8] and viral persistence in human body fluids was assessed. The post Ebola survivor programme combines health essential services as well as non-health services.

Results from efficacy testing of the recombinant, replication-competent vesicular stomatitis virus-based vaccine expressing a surface glycoprotein of Zaire Ebolavirus in a ring vaccination trial [9] in Guinea, West Africa, was presented by Gunnstein Norheim (Norwegian Institute of Public Health, Norway). The study was performed towards the end of the epidemic and succeeded due to a novel study design, multi-partner international team and close collaboration with the national Ebola response team.

Stephan Günther (Bernhard-Nocht-Institute for Tropical Medicine, Germany) described the European Mobile Laboratory Project (2012—2015). Over 10,000 samples were tested in Guinea, Liberia, Sierra Leone and Nigeria from March 2014 to February 2015 by mobile laboratories, reducing the need to transport samples over long distances. Moreover, a MinION nanopore sequencing, coupled to a newly developed web-based pipeline for real-time bioinformatics analysis on a laptop, allowed the first complete EBOV sequence in Guinea to be obtained.

Maria Van Kerkhove (Institut Pasteur, France) discussed the extent of Middle East respiratory syndrome coronavirus (MERS-CoV) infection and its transmission to humans. Since 2012 the WHO reported over 1,611 cases from 26 countries, with more than 575 deaths. Genetic data supported multiple sporadic introductions into human populations by contact with dromedary camels and possibly other not yet identified animals. Some 0.15% of the general population were found to be seropositive for anti-MERS-CoV antibodies in Saudi Arabia [10]. The author suggested active surveillance in both animals and humans to stop camel-to-human and human-to-human transmission, and to develop a clear guidance for at risk populations.

Parallel and poster sessions
The core content of the conference consisted of parallel and poster sessions with work presented by qualified professionals and training fellows working in the field of infectious disease prevention and control. A wide range of topics were discussed covering areas related to infectious diseases through multidisciplinary efforts in a 'one-health' approach. The experience of many outbreak investigations including food, water and vector-borne diseases and zoonoses were shared. AMR and healthcare- associated infections, HIV and sexually transmitted infections, vaccine-preventable diseases, vaccine coverage, safety and effectiveness, tuberculosis, as well as influenza and other respiratory viruses were also addressed. Moreover, intervention and surveillance studies on communicable diseases, international health, challenges due to mass gatherings, novel methodological approaches and modelling offered up-to-date knowledge and insights to the participants.

ESCAIDE side events
A number of side events complemented the conference programme. The 'BarCamp' was a dynamic assembly where the audience generated the content. Three very topical subjects (herd immunity, translating outbreak results into food regulation, lessons learnt from migrants' health) generated fruitful discussions. 'Meet the expert' sessions allowed for a deeper exchange with some of the plenary speakers and at the fourth Eurosurveillance scientific lunchtime seminar, Maria Zambon (Public Health England, UK) and Jacob Moran-Gilad (Ministry of Health and Ben-Gurion University,

Israel) elaborated on aspects of using new laboratory methods to support outbreak detection.

Conclusions
ESCAIDE is the leading conference on applied infectious disease epidemiology in Europe. Every year it connects hundreds of public health front-line professionals in the field of communicable diseases. This integrated laboratory-field epidemiology network for outbreak detection, investigation and response, strengthens Europe's defences against infectious disease threats by being open to multidisciplinary participants worldwide to foster knowledge exchange and professional discussions.

Early detection and response have proved to be key in preventing the spread of any communicable disease. Expertise diversity, pragmatism and close multidisciplinary collaborations as well as community engagement and local study teams were critical components in outbreak investigations. Moreover, a 'One Health' approach was also recommended as a successful strategy to fight against infectious diseases.

Conflict of interest
None

Authors' contributions
MS wrote the manuscript. AJ critically reviewed the paper and gave input to the content, which was incorporated in the report. Both authors read and approved the final manuscript.

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