Impact factor 5·7

Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 20 | Weekly issue 43 | 29 October 2015

RAPID COMMUNICATIONS

RESEARCH ARTICLES

NEWS

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

Cyclospora infection linked to travel to Mexico, June to September 2015

GL Nichols [1], J Freedman [1], KG Pollock [2], C Rumble [1], RM Chalmers [3], P Chiodini [4], G Hawkins [2], CL Alexander [5], G Godbole [1,4], C Williams [6], HA Kirkbride [1], M Hamel [7], JI Hawker [1]

1. National Infection Service, Public Health England, London, United Kingdom
2. Health Protection Scotland, Glasgow, United Kingdom
3. Cryptosporidium Reference Unit, Public Health Wales, Swansea, United Kingdom
4. PHE National Parasitology Reference Laboratory, Hospital for Tropical Diseases, London, United Kingdom
5. Scottish Parasite Diagnostic and Reference Laboratory, Glasgow, United Kingdom
6. Public Health Wales, Cardiff, United Kingdom
7. Public Health Agency of Canada, Ottawa, Canada

Correspondence: Gordon Nichols (gordon.nichols@phe.gov.uk)

Citation style for this article:
Nichols GL, Freedman J, Pollock KG, Rumble C, Chalmers RM, Chiodini P, Hawkins G, Alexander CL, Godbole G, Williams C, Kirkbride HA, Hamel M, Hawker JI. Cyclospora infection linked to travel to Mexico, June to September 2015. Euro Surveill. 2015;20(43):pii=30048. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2015.20.43.30048

Article submitted on 12 October 2015 / accepted on 28 October 2015 / published on 29 October 2015

***Cyclospora cayetanensis* was identified in 176 returned travellers from the Riviera Maya region of Mexico between 1 June and 22 September 2015; 79 in the United Kingdom (UK) and 97 in Canada. UK cases completed a food exposure questionnaire. This increase in reported *Cyclospora* cases highlights risks of gastrointestinal infections through travelling, limitations in *Cyclospora* surveillance and the need for improved hygiene in the production of food consumed in holiday resorts.**

On 14 July 2015, Health Protection Scotland (HPS) identified an unusual increase in *Cyclospora* infections in travellers to Mexico. National and international partners were informed and upon further investigation, a total of 176 cases have been identified in England, Scotland, Wales and Canada. An outbreak control team managed the investigation in the United Kingdom (UK). UK patients were interviewed about travel history, food consumption, clinical symptoms and demography using a questionnaire. The majority of cases had travelled to the Riviera Maya region of Mexico.

Investigation of UK cases

Cyclospora cases were identified in primary clinical diagnostic and commercial laboratories by microscopy or molecular testing. Cases were confirmed in reference laboratories using microscopic methods (e.g. examination of a wet preparation by bright field microscopy and, if structures resembling *Cyclospora* were observed, viewing under UV light for autofluorescence). In addition, smears were permanently stained using modified Ziehl Neelsen and examined.

In the UK, probable cases were defined as individuals with onset of gastrointestinal (GI) symptoms or a specimen date on or after 1 June 2015, travel to Mexico in the previous 14 days and *C. cayetanensis* oocysts identified in stool specimens by a local diagnostic laboratory. Confirmed UK cases were probable cases confirmed microscopically by national reference laboratories. Cases without either local or national reference laboratory confirmation were excluded from this analysis. No cases associated with travel to Mexico were identified in the UK in 2015 before 1 June.

Outbreak description

Between 1 June and 22 September 2015, 79 probable or confirmed case-patients (hereafter called cases) were reported in England (n = 55), Scotland (n = 21) and Wales (n = 3). No further cases linked to Mexico have been identified in the period since that date (as at 28 October 2015). Symptom onset dates were available for 62 confirmed or probable cases and ranged from 8 June to 19 August 2015 (Figure 1). Travel information was available for 60 cases; the earliest departure date from the UK to Mexico was 22 May and the latest date of return was 30 August 2015. The median age of cases was 44 years (range: 15–66) with 46 of 79 cases 40 years and older; 43 of 79 were female. Only 43 of the 79 cases diagnosed in local laboratories were confirmed by a reference laboratory.

Cases occurred over an extended period and in people who stayed at 32 different hotels on the Riviera Maya coast of Mexico, from Cancun to Tulum. A formal epidemiological study was not therefore possible. Questionnaires were completed for 46 of 79 cases, with 43 reporting all-inclusive catering, of whom 24 (56%) reported also eating outside their hotel.

Figure 1

Epidemic curve of *Cyclospora* cases by onset date, United Kingdom, 1 June–24 August 2015 (n = 62)

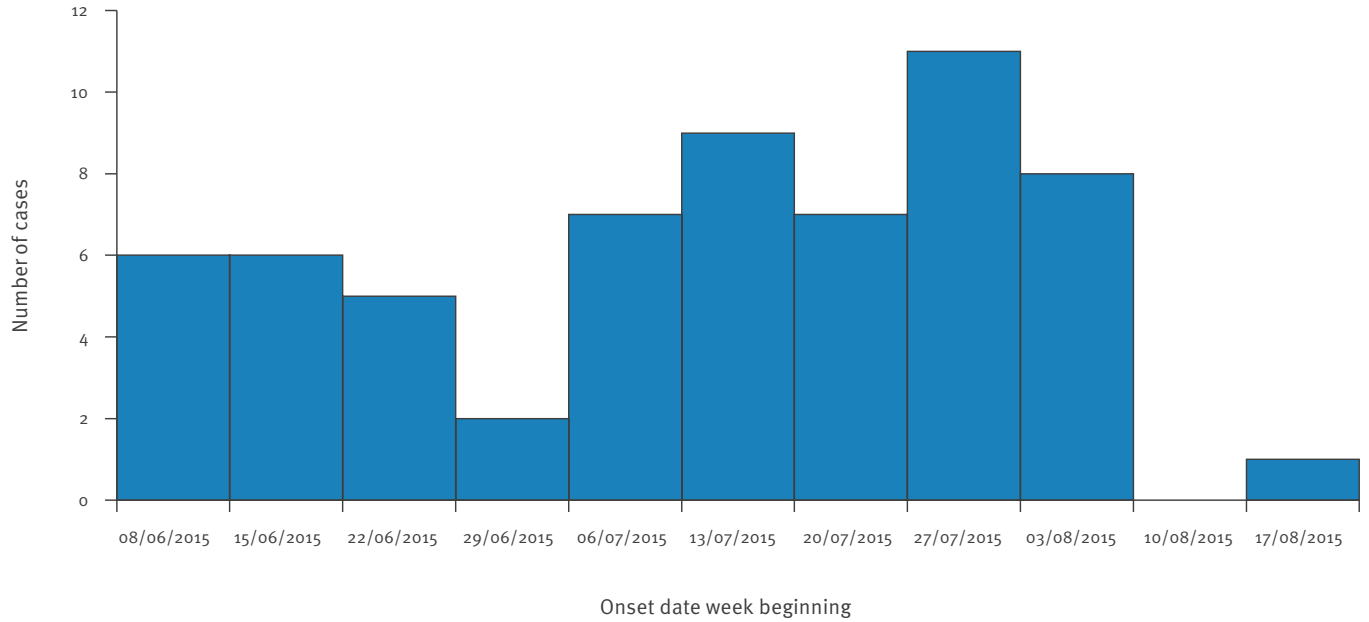
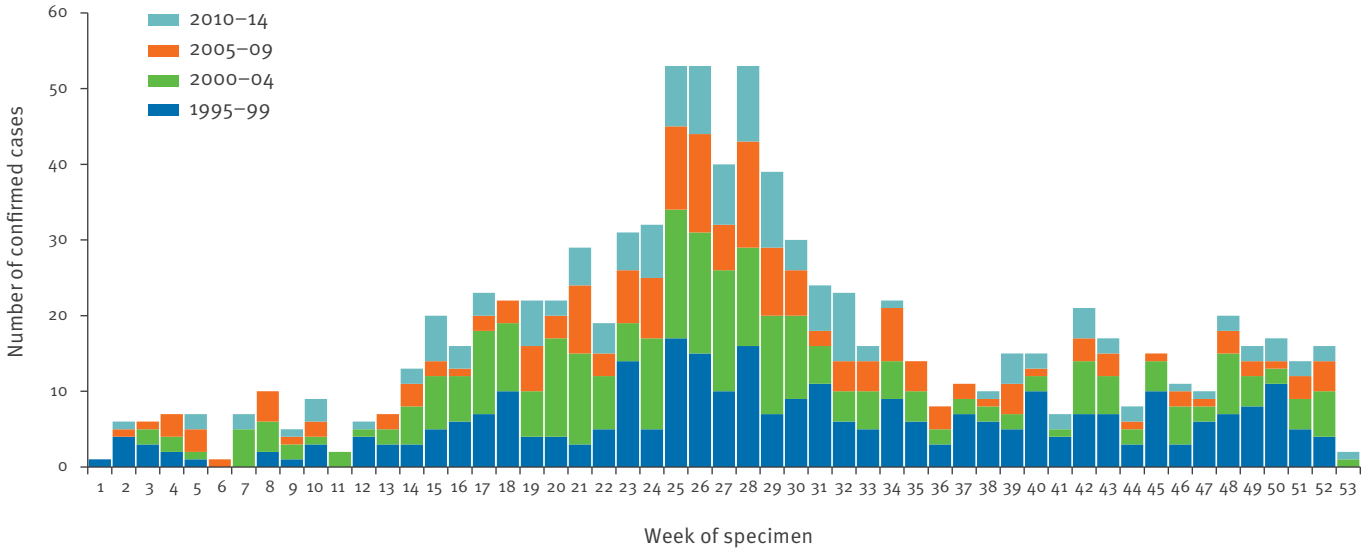


Figure 2

Cyclospora cases reported per week to national surveillance, England and Wales, 1995–2014 (n = 923)



Of 44 cases with symptom details recorded, all had diarrhoea (range: 5–62 days; mean: 16 days) which was rapid onset in 30. Other symptoms included abdominal pain (n = 35), fatigue (n = 31), nausea (n = 27), vomiting (n = 24), fever (n = 19), weight loss (n = 19) and headache (n = 17). There were no hospitalisations or deaths.

Of 45 cases for whom food histories were available, 43 consumed fruit or berries, 41 consumed salad or vegetables and 35 consumed fresh herbs. Specific items mentioned by cases included fresh mint in drinks (n = 15), strawberries or raspberries (n = 9) and coriander (n = 6). Most cases ate from all-inclusive buffets which also included a number of meat and fish products, cheese and desserts. Consumption of bottled water and ice was reported by 39 and 38 cases, respectively.

Awareness was raised among laboratories and public health professionals by circulating diagnostic aid sheets and travel advice and communicating with health authorities in Mexico, UK tour operators, the European Centre for Disease Prevention and Control, the Public Health Agency of Canada, the United States (US) Centers for Disease Control and Prevention (CDC) and European Union countries.

Figure 3

Region visited by travel-related *Cyclospora* cases, England and Wales, 1995–2014 (n = 923)

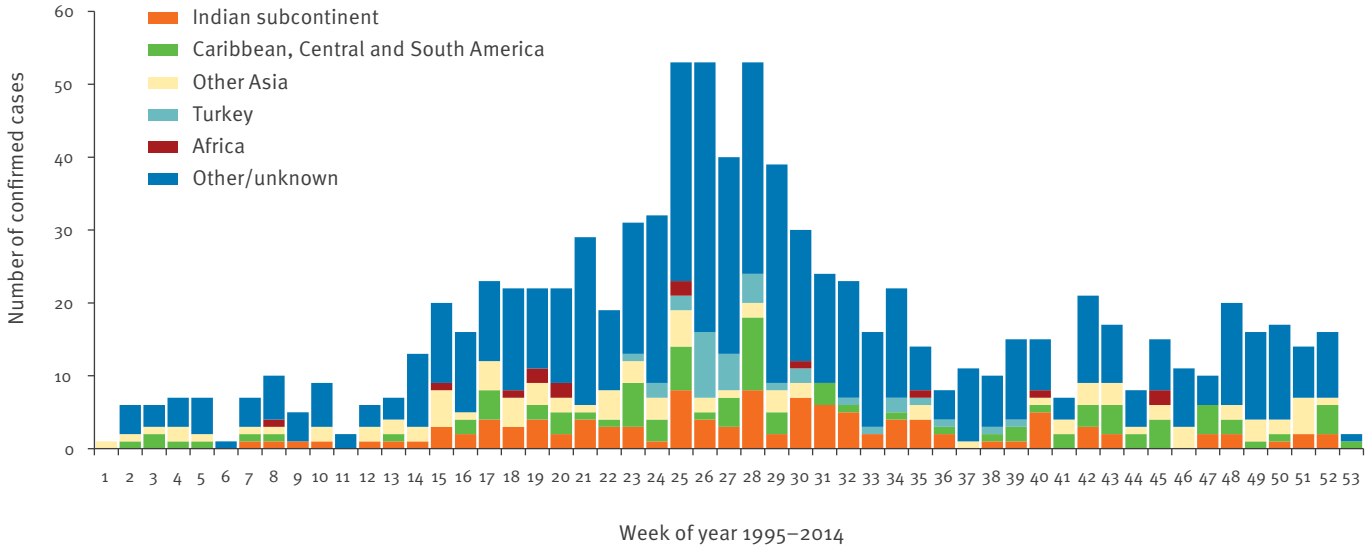
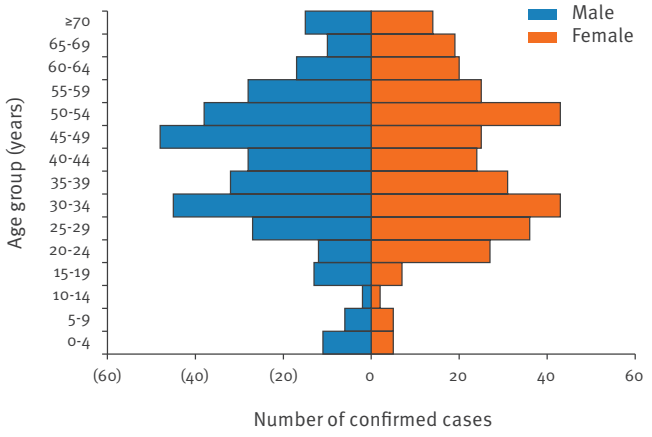


Figure 4

Age and sex distribution of *Cyclospora* cases reported to national surveillance, England and Wales, 1995–2014 (n = 658)



Discussion

Cyclospora cayetanensis is a protozoan parasite that causes treatable diarrhoea [1-3], and predominantly occurs in tropical and subtropical countries [4-6]. *Cyclospora* oocysts sporulate 10 days after being defecated and become infectious. Outbreaks of cyclosporiasis [3,7], have been linked to contaminated snow peas [8], basil [9], salad/herbs [10], raspberries and other berries [3,11,12], and drinking water [13,14]. Sporadic infections follow travel to endemic countries, including Mexico [15-17], and imported basil from Mexico was implicated in an outbreak in Canada [18].

An increase in cyclosporiasis has been observed in UK travellers to Mexico this summer. A similar increase has also been noted in Canada: while Canada has no routine travel surveillance, 97 cases of Cyclospora infection in travellers returning from Mexico were reported from May to August 2015; the cases reporting staying at various resorts in the same geographical area as the UK cases. The UK and Canadian cases occurred in people returning from at least 36 hotels on the Riviera Maya coast of Mexico. Drinking water was an unlikely source as several different water networks supply the resorts (some hotels have their own borehole and treatment). Geographical and temporal associations suggest that the outbreak was related to a consumed product(s) distributed throughout the region rather than hygiene deficiencies in individual hotels. A multistate outbreak of cyclosporiasis has also occurred in the US, concurrent with our investigations, in which fresh cilantro from Puebla, Mexico has been implicated as the cause of cluster-associated cases in three US states [19]. Local investigation in Mexico suggests fresh cilantro from Puebla had been distributed to hotels in the Riviera Maya region. Food safety control measures have since been implemented by the Mexican authorities to ensure the safety of cilantro from Puebla state (personal communication to Public Health England: National Focal Point for Emergency INFOSAN in Mexico and National Focal Point for IHR, Mexico, 6 October 2015).

Cyclospora infections are seasonal in England and Wales (Figure 2). Where travel history is known, travel to the Indian subcontinent, Turkey, the Caribbean, Central and South America is commonly reported (Figure 3). Childhood infections are uncommon and case numbers in male and female patients are equivalent (Figure 4). Eleven laboratories detected five or more cases between 2005 and 2014, and many laboratories had no detections. *Cyclospora* oocysts can be detected readily by microscopy, but if screening algorithms are not

followed, cases can go undetected. National External Quality Assessment for *Cyclospora* has improved from 23% in the mid-1990s to 86% by 2011 (personal communication: UK National External Quality Assessment Service, 18 September 2015). Limited information is available on cases in other EU Member States. The FilmArray GI Panel [20] or equivalent PCR array would facilitate faecal screening for *Cyclospora* infections, in part because it does not require the physician or laboratory to specifically request *Cyclospora* testing. Improvements are needed in hygiene control during the production and harvesting of salad and soft fruit products in countries with higher incidence [21].

Acknowledgements

This work was funded in part by the UK Medical Research Council (MRC) and UK Natural Environment Research Council (NERC) for the MEDMI Project; the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Environmental Change and Health at the London School of Hygiene and Tropical Medicine in partnership with Public Health England (PHE), and in collaboration with the University of Exeter, University College London, and the Met Office; the NIHR HPRU in Gastrointestinal Infections; and the European Regional Development Fund Programme and European Social Fund Convergence Programme for Cornwall and the Isles of Scilly (University of Exeter Medical School), Canadian provincial and local public health authorities. PLC is supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. The authors are grateful to staff at the national reference units for expertise in confirming diagnoses and local health protection teams for contributing to collection of risk data.

Conflict of interest

None declared.

Authors' contributions

All authors were involved in the outbreak investigation and contributed to the manuscript. This included expertise in parasitology (PC, GN, RC, KP, GG), epidemiology (GN, MH, RC, JF, KP, CR, GH, JH, CW) and diagnosis (PC, GN, GG, CA).

References

1. ChiodiniPL. A 'new' parasite: human infection with Cyclospora cayetanensis.Trans R Soc Trop Med Hyg. 1994;88(4):369-71. DOI: 10.1016/0035-9203(94)90385-9 PMID: 7570808
2. OrtegaYR, SterlingCR, GilmanRH, CamaVA, DíazF. Cyclospora species--a new protozoan pathogen of humans.N Engl J Med. 1993;328(18):1308-12. DOI: 10.1056/NEJM199305063281804 PMID: 8469253
3. HerwaldtBL. Cyclospora cayetanensis: a review, focusing on the outbreaks of cyclosporiasis in the 1990s.Clin Infect Dis. 2000;31(4):1040-57. DOI: 10.1086/314051 PMID: 11049789
4. LeguaP, SeasC. Cystoisospora and cyclospora.Curr Opin Infect Dis. 2013;26(5):479-83.PMID: 23982239
5. OrtegaYR, SanchezR. Update on Cyclospora cayetanensis, a food-borne and waterborne parasite.Clin Microbiol Rev. 2010;23(1):218-34. DOI: 10.1128/CMR.00026-09 PMID: 20065331
6. Chacín-BonillaL. Epidemiology of Cyclospora cayetanensis: A review focusing in endemic areas.Acta Trop. 2010;115(3):181-93. DOI: 10.1016/j.actatropica.2010.04.001 PMID: 20382099
7. ChalmersRM, NicholsG, RooneyR. Foodborne outbreaks of cyclosporiasis have arisen in North America. Is the United Kingdom at risk?Commun Dis Public Health. 2000;3(1):50-5.
8. Centers for Disease Control and Prevention (CDC),. Outbreak of cyclosporiasis associated with snow peas--Pennsylvania, 2004.MMWR Morb Mortal Wkly Rep. 2004;53(37):876-8.PMID: 15385921
9. HoangLM, FyfeM, OngC, HarbJ, ChampagneS, DixonB, et al. Outbreak of cyclosporiasis in British Columbia associated with imported Thai basil. Epidemiol Infect. 2005;133(1):23-7. DOI: 10.1017/S0950268804003176 PMID: 15724706
10. DöllerPC, DietrichK, FilippN, BrockmannS, DreweckC, VontheinR, et al. Cyclosporiasis outbreak in Germany associated with the consumption of salad. Emerg Infect Dis. 2002;8(9):992-4. DOI: 10.3201/eid0809.010517 PMID: 12194782
11. HoAY, LopezAS, EberhartMG, LevensonR, FinkelBS, da SilvaAJ, et al. Outbreak of cyclosporiasis associated with imported raspberries, Philadelphia, Pennsylvania, 2000. Emerg Infect Dis. 2002;8(8):783-8. DOI: 10.3201/eid0808.020012 PMID: 12141962
12. ManuelD, NeamatullahS, ShahinR, ReymondD, KeystoneJ, CarlsonJ, et al. An outbreak of cyclosporiasis in 1996 associated with consumption of fresh berries- Ontario. Can J Infect Dis. 2000;11(2):86-92.PMID: 18159270
13. AksoyU, AkisuC, SahinS, UslucaS, YalcinG, KuralayF, et al. First reported waterborne outbreak of cryptosporidiosis with Cyclospora co-infection in Turkey. Euro Surveill. 2007;12(2):3142.
14. BaldurssonS, KaranisP. Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2004-2010.Water Res. 2011;45(20):6603-14. DOI: 10.1016/j.watres.2011.10.013 PMID: 22048017
15. DiazE, MondragonJ, RamirezE, BernalR. Epidemiology and control of intestinal parasites with nitazoxanide in children in Mexico.Am J Trop Med Hyg. 2003;68(4):384-5.PMID: 12875284
16. Sánchez-VegaJT, Cabrera-FuentesHA, Romero-OlmedoAJ, Ortiz-FríasJL, SokolinaF, BarretoG. Cyclospora cayetanensis: this emerging protozoan pathogen in Mexico.Am J Trop Med Hyg. 2014;90(2):351-3. DOI: 10.4269/ajtmh.12-0782 PMID: 24379243
17. Orozco-MosquedaGE, Martínez-LoyaOA, OrtegaYR. Cyclospora cayetanensis in a pediatric hospital in Morelia, México.Am J Trop Med Hyg. 2014;91(3):537-40. DOI: 10.4269/ajtmh.13-0535 PMID: 24957545
18. ShahL, MacDougallL, EllisA, OngC, ShyngS, LeBlancL, et al. Challenges of investigating community outbreaks of cyclosporiasis, British Columbia, Canada. Emerg Infect Dis. 2009;15(8):1286-8. DOI: 10.3201/eid1508.081585 PMID: 19751593
19. Centers for Disease Control and Prevention (CDC). Cyclosporiasis Outbreak Investigations — United States, 2015. Atlanta: CDC. [Accessed:22 Oct 2015]. Available from: http://www.cdc.gov/parasites/cyclosporiasis/outbreaks/2015/index.html
20. FilmArray GastrointestinalI Panel Product Sheet. Salt Lake City: BioFire Diagnostics. [Accessed: 22 Oct 2015]. Available from: http://filmarray.com/assets/pdf/Info-Sheet-GI-Panel-MRKT-PRT-0234-07.pdf
21. McClure P, Ronnie N, Waskar M. HACCP, risk assessment, and risk communication. In: Robertson LJ, Smith HV, eds. Foodborne Protozoan Parasites. Hauppauge: Nova Science; 2012. pp. 239-66.

Real-time safety surveillance of seasonal influenza vaccines in children, Australia, 2015

A Pillsbury [1], P Cashman [2 3], A Leeb [4], A Regan [5 6], D Westphal [5 7 8], T Snelling [7 9], C Blyth [7 9 10], N Crawford [11 12 13], N Wood [1 14], K Macartney [1 2 15], on behalf of the AusVaxSafety, surveillance team [16]

1. National Centre for Immunisation Research and Surveillance, The Children's Hospital at Westmead, NSW, Australia
2. Hunter New England Local Health District, NSW, Australia
3. The University of Newcastle, NSW, Australia
4. Illawarra Medical Centre, WA, Australia
5. Communicable Disease Control Directorate, Western Australia Department of Health, WA, Australia
6. School of Pathology and Laboratory Medicine, University of Western Australia, WA, Australia
7. Wesfarmers Centre of Vaccines and Infectious Diseases, Telethon Kids Institute, University of Western Australia, WA, Australia
8. National Centre for Epidemiology and Population Health, Research School of Population Health, The Australian National University, ACT, Australia
9. Princess Margaret Hospital, WA, Australia
10. University of Western Australia School of Paediatrics and Child Health, Princess Margaret Hospital, WA, Australia
11. Department of General Medicine, Royal Children's Hospital, Victoria, Australia
12. SAEFVIC, Murdoch Childrens Research Institute, Victoria, Australia
13. Department of Paediatrics, The University of Melbourne, Victoria, Australia
14. Discipline of Paediatrics and Child Health, University of Sydney, NSW, Australia
15. Department of Microbiology and Infectious Diseases, The Children's Hospital at Westmead, NSW, Australia
16. The members of the group are listed at the end of the article.

Correspondence: Alexis Pillsbury (alexis.pillsbury@health.nsw.gov.au)

Citation style for this article:
Pillsbury A, Cashman P, Leeb A, Regan A, Westphal D, Snelling T, Blyth C, Crawford N, Wood N, Macartney K. Real-time safety surveillance of seasonal influenza vaccines in children, Australia, 2015. Euro Surveill. 2015;20(43):pii=30050. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2015.20.43.30050
2015;20(34):pii=30002. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2015.20.34.30002

Article submitted on 14 October 2015 / accepted on 29 October 2015 / published on 29 October 2015

Increased febrile reactions in Australian children from one influenza vaccine brand in 2010 diminished confidence in influenza immunisation, highlighting the need for improved vaccine safety surveillance. *AusVaxSafety*, a national vaccine safety surveillance system collected adverse events in young children for 2015 influenza vaccine brands in real time through parent/carer reports via SMS/email. Weekly cumulative data on 3,340 children demonstrated low rates of fever (4.4%) and medical attendance (1.1%). Fever was more frequent with concomitant vaccination.

In 2014, a multi-jurisdictional national system, *AusVaxSafety,* was established to undertake enhanced influenza vaccine safety surveillance and report real-time adverse events in children aged six months to four years. This collaborative system was funded by the Australian Government Department of Health. Surveillance (n = 782 children) demonstrated the safety of 2014 seasonal influenza vaccines in a matter of weeks, although most children received one vaccine brand (Vaxigrip, Sanofi Pasteur; 86.2%; n = 674 children) [1,2]. Expansion of the programme in 2015 to incorporate a new data management platform and more participating general practice (GP) sites (GPs provide more than 70% of vaccines given nationally [3]) has enabled reporting of the safety of 2015 southern hemisphere trivalent influenza vaccines for thousands of children receiving multiple manufacturers' vaccines. Here we report the results of our surveillance conducted during the 2015 Australian influenza season.

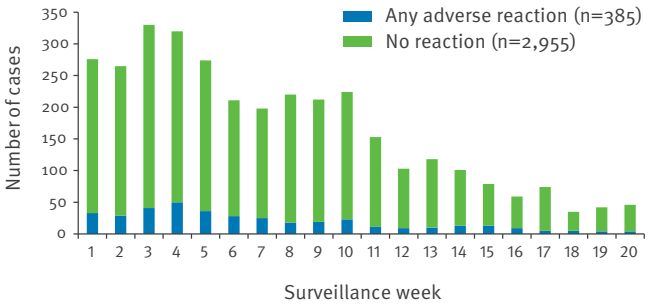
The *AusVaxSafety* vaccine safety surveillance system

In Australia (population 23 million [4]), influenza vaccination is funded under the National Immunisation Program for children aged six months to four years who have medical conditions pre-disposing them to complications and/or for Indigenous children. Only one state, Western Australia (WA), has funded influenza vaccination for this age group since 2008.

For the purposes of *AusVaxSafety* surveillance, children aged six months to four years receiving seasonal influenza vaccine from participating GP sites (n = 54), hospitals (n = 6), public clinics (n = 2) and primary healthcare providers such as Aboriginal Medical Services (n = 7) in four states (New South Wales (NSW), Victoria, South Australia and WA) were eligible for inclusion. Parent/carer-reported adverse events in children were solicited within three days of vaccination using two computer-based data management platforms, Vaxtracker [5] and SmartVax [6]. Both systems sent automated SMS messages (and/or emails for Vaxtracker) and received parent/carer-completed questionnaire responses via reply SMS with a URL link to smartphone survey (SmartVax)

Figure

AusVaxSafety participants with and without post-vaccine reaction, by week of vaccination, and cumulative percentage of participants, Australia, 1 April–31 August 2015 (n = 3,340)



Surveillance Week 1 included all participants vaccinated prior to the official rollout of the influenza vaccine for the 2015 season (20 April 2015) and captured children vaccinated from 1 to 19 April 2015. After that, each surveillance week consisted of seven days, with Week 2 including 20–26 April, etc. Week 20 included eight days (24–31 August 2015).

or web-based survey (Vaxtracker). Demographic details were obtained, as well as information regarding vaccine brand, medical conditions, concomitant vaccines, reactions and healthcare consultations required after vaccination (including follow-up visit to a GP, emergency department (ED) or hospitalisation).

Serious adverse events (SAE) were categorised according to predefined criteria, which included any untoward medical event that resulted in death, was life-threatening or required hospitalisation [7]. We also included seizures requiring medical attendance (ED and/or hospitalisation) as medically important events. SAEs were reported to state/territory health departments and the Therapeutic Goods Administration as required by legislation. For this report, data were compiled from 1 April through 31 August 2015 and cumulative data reported to health authorities weekly. After week 4 of surveillance, progressive results were periodically made publically available online and shared via immunisation provider networks.

For rapid signal detection, fast initial response cumulative summation (FIR CUSUM) and Bayesian methods [8,9] were employed weekly to estimate the probability that any potential safety signal was true or false based on predetermined expected and threshold rates of two objective outcome measures (fever and medical advice/attendance sought) in relation to the number of reports received. Expected and threshold rates were set according to previous surveillance results and published studies. For fever, the expected rate was 6% and the threshold rate for triggering a signal was 13% [5,10-12].

Results

Approximately 75% of the 4,441 parents/carers invited agreed to participate, resulting in 3,340 post-vaccination reports (Figure). The majority of parent/carers responded within two hours of being queried. Descriptive details of participants are presented in Table 1.

Weekly analysis using FIR CUSUM and Bayesian methods (conducted 1 April through 5 July 2015) did not demonstrate a safety signal at any time. After the third week of surveillance (n = 877 cumulative reports), fever rates remained less frequent than 5% each week and medical advice/attendance rates remained lower than 2%.

Parent/carer-reported fever was recorded by 4.4% (n = 148); medical advice/attendance was sought by 1.1% (n = 35). Details on reactions and medical advice/attendance sought are included in Table 2.

Of the 35 children who received medical advice/attendance, 23 reported fever. Five children experienced seizures, four of whom had a history of seizures (three: underlying neurological conditions; one: previous febrile seizures). The fifth seizure case occurred in a child diagnosed with a febrile viral illness. Only three of the children with seizures sought medical attendance and were thus classified as having SAEs; all attended an ED only. One additional SAE was recorded in a child hospitalised with an influenza-like illness and fever. Two of the four children experiencing an SAE had received Vaxigrip, one had received Fluarix and the other received Influvac. All reported improvement within days.

No significant difference was identified between children who had received one of the two most commonly used vaccine brands, Vaxigrip or Fluarix, and who experienced fever or sought medical advice/attendance. All other brands had been administered in insufficient numbers to reliably report on differences (Table 3). Children receiving other vaccines concomitantly were significantly more likely to experience fever (60/687; 8.7%) than those who did not (87/2,618; 3.3%) (p = 0.000). There was no difference between children with and without an underlying condition regarding fever (29/400 (7.3%) vs 56/721 (7.8%)) or medical advice/attendance sought (9/400 (2.3%) vs 17/721 (2.4%)).

Discussion

Our novel system of active, prospective vaccine safety surveillance, *AusVaxSafety,* has demonstrated in real time that 2015 southern hemisphere influenza vaccines registered for use in young Australian children were safe and well-tolerated. Adverse event rates reported by parents/carers remained low and within expected ranges throughout the surveillance period. The fever rate was lower than the pooled estimate (6.7%) in a recent systematic review of randomised control trials of children aged six to 35 months receiving the first dose of a trivalent influenza [12].

Table 1

Demographic details of *AusVaxSafety* participants, Australia, 1 April–31 August 2015 (n = 3,340)

Variable	Response	Number	Percentage
Median age (range)	23.0 months (6.0–59.9)		
Sex [a]	Male	1,781/3,314	53.7%
Ethnicity [b]	Indigenous	119/2,519	4.7%
Underlying medical condition [c]	Yes	400/1,121	35.7%
Concomitant vaccine(s) received [d]	Yes	687/3,305	20.8%

[a] Sex unknown for 26 of 3,340 participants.

[b] Ethnicity unknown for 821 of 3,340 participants.

[c] Underlying medical condition not available for 2,219 of 3,340 participants (SmartVax data management system does not currently collect this variable).

[d] Data on whether concomitant vaccine was received unknown for 35 of 3,340 participants.

Table 2

Adverse events reported by 2015 *AusVaxSafety* participants within three days of vaccination, Australia, 1 April–31 August 2015 (n = 3,340)

Adverse event		Number	Percentage
Any adverse event		385/3,340	11.5%
Fever		148/3,340	4.4%
Seizure [a]		5/3,340	0.2%
Injection site reaction		67/3,340	2.0%
Vomiting/abdominal pain		41/3,340	1.2%
Rash		36/3,340	1.1%
Participants who sought any medical advice and/or required any medical attendance		35/3,340	1.1%
Highest medical advice and/or attendance reported	Participants attending a medical facility for consultation with a general practitioner or other medical practitioner	23/3,340	0.7%
	Participants telephoning a medical facility or a medically staffed helpline for advice	4/3,340	0.1%
	Participants presenting to an emergency department (not admitted) [a]	6/3,340	0.2%
	Participants hospitalised [b]	2/3,340	0.1%

[a] Of the five children with seizures reported, three presented to an emergency department and were thus classified as having a serious adverse event.

[b] One child was hospitalised with an unrelated condition not deemed a serious adverse event. The other hospitalised child had an influenza-like illness.

Active, prospective vaccine safety surveillance is superior to traditional post-marketing vaccine safety surveillance which typically relies on passive reporting. In Australia, SMS technology has also been used to study vaccine reactions among healthcare workers and pregnant women [13,14]. One study in the United States also used SMS follow-up of parents, detecting increased fever rates in children who had concomitantly received trivalent influenza vaccine and 13-valent pneumococcal vaccine compared with those who received each vaccine alone [15]. Similarly, we reported an increased (although low) rate of fever when influenza vaccine was administered together with other vaccines. This was also associated with a significantly higher likelihood of seeking medical advice and warrants further investigation.

Because large volumes of influenza vaccine are distributed annually within short, defined periods, active surveillance provides the opportunity to gain early, reliable assessments of the safety profiles of new vaccines. As the number of available influenza vaccines increases, obtaining timely safety data becomes more important, particularly as strain composition may vary from season to season. In 2010 in Australia, an unexpected increase in febrile reactions following receipt of influenza vaccination in young children led to a three month suspension of all national paediatric influenza immunisation programmes [16]. Epidemiological and laboratory studies linked these reactions to one manufacturer's vaccine (Fluvax or Afluria, bioCSL) which is no longer registered for use in young children [16,17]; however, confidence in all influenza vaccines was negatively impacted [18,19]. In response to these safety concerns which have resulted in low uptake of

Table 3

Details of influenza vaccines administered to *AusVaxSafety* participants, Australia, 1 April–31 August 2015 (n = 3,340)

Brand[a] (manufacturer)	Vaccine type	Number of vaccines administered (n = 3,336)		Number of participants with fever by brand		Number of participants who sought medical advice/attendance by brand	
		n	%	n/N	%	n/N	%
Vaxigrip (Sanofi-Pasteur)	Trivalent	3,075	92.2	133/3,075[c]	4.3%	28/3,075[d]	0.9%
Fluarix (GlaxoSmithKline)	Trivalent	189	5.7	9/189	4.8	4/189	2.1
Influvac (BGP Products)	Trivalent	47	1.4	5/47	NR	2/47	NR
Agrippal (Novartis Vaccines and Diagnostics)	Trivalent	11	0.3	0/11	NR	0/11	NR
FluQuadri[b] (Sanofi Pasteur)	Quadrivalent	14	0.4	1/14	NR	1/14	NR

NR: not relevant.

[a] Brand unknown for four participants.

[b] All administered vaccines except for FluQuadri were trivalent. Quadrivalent vaccines (FluQuadri/ FluQuadri Junior and Fluarix Tetra (GlaxoSmithKline)) were available for use for the first time in Australia in 2015 but were not funded under the National Immunisation Program.

[c] p = 0.775 for rates of fever among those who received Vaxigrip (4.3%) compared with those who received Fluarix (4.8%) calculated using Pearson's chi-square test.

[d] p = 0.102 for rates of medical advice/attendance sought among those who received Vaxigrip (0.9%) compared with those who received Fluarix (2.1%) calculated using Fisher's exact test.

seasonal influenza vaccines in children, *AusVaxSafety* surveillance data have been able to provide reassuring results.

Data obtained from parental reporting should be interpreted with care. Consequently, *AusVaxSafety* reports on outcomes which are the most objective: fever and medical advice/attendance sought within three days of vaccination. Although these provide less precision than results obtained in more formal follow-up such as clinical trials, this is unlikely to reduce our system's sensitivity for detecting SAEs, of which medical advice/attendance sought can be considered a good proxy. This was demonstrated in the epidemiological investigation of the 2010 increase in febrile reactions [16].

An advantage of our system is its potential adaptability for monitoring new vaccines, such as live attenuated influenza vaccine, although this is not yet available in the southern hemisphere. Another advantage is its ability to provide rapid real-time feedback to inform programme rollout and vaccine promotion. In addition, *AusVaxSafety*'s flexibility may be valuable in situations where vaccine safety data are limited, such as for pandemic vaccines. The timeliness of our results also makes them valuable beyond Australia; our data may be of interest to counterparts in the northern hemisphere preparing for 2015/16 vaccination using vaccines comprised of the strains administered in the 2015 southern hemisphere season.

Our system, which is able to report adverse events within days of vaccination, is as near to real time as possible. Such timeliness is feasible thanks to the strong collaboration with parents/carers and providers and the use of SMS technology for reporting reactions. We anticipate being able improve our system by including more participants in future years. To our knowledge, *AusVaxSafety* is the only active influenza vaccine safety surveillance system for young children analysing and reporting data on a weekly basis, allowing safety deliberations on vaccines within mere weeks of influenza vaccination commencing. Our ability to provide early and reliable safety profiles of seasonal influenza vaccines for children is likely to improve public confidence and vaccine uptake, which we will continue to assess.

AusVaxSafety 2015 surveillance team

Karen Orr, Gulam Khandaker, Kevin Yin, David Durrheim, Craig Dalton, Sally Munnoch, Michelle Butler, Jody Stephenson, Stephen Clarke, Keira Glasgow, Lauren Dalton, Brendan McMullan, Geraldine Dunne, Jim Buttery, Gowri Selvaraj, Annette Alafaci, Peter Eizenberg, Paul Effler, Peter Richmond, Peter Jacoby, Parveen Fathima, Lauren Tracey, Gabriela Willis, Jennifer Kent, Ian Peters, Rachel West, Kari Jarvinen, Susan Vlack, Deborah Judd, Melinda Hassall, Julia Clark, Stephen Lambert, Michael Gold, Gabriella Lincoln, Rosalind Webby, Kaylene Prince.

Acknowledgements

AusVaxSafety surveillance was funded under a contract with the Australian Government Department of Health. We would like to thank the *AusVaxSafety* Steering Committee members for their contribution to oversight of the 2015 surveillance effort. We would also like to express our gratitude to the staff

at our participating hospitals, clinics and general practices, as well as the parents/carers of children who participated in and supported 2015 *AusVaxSafety* surveillance.

Conflict of interest

None declared.

Authors' contributions

AP served as *AusVaxSafety* surveillance coordinator for 2015, drafted the manuscript, and conducted data analysis and interpretation of results. PC contributed to the design and implementation of the *AusVaxSafety* surveillance system, served as coordinator/recruiter of the Hunter New England area surveillance sites, reviewed and contributed to the manuscript draft. AL contributed to the design and implementation of the *AusVaxSafety* surveillance system, recruited general practice site participants, reviewed and contributed to the manuscript draft. AR contributed to the design and implementation of the *AusVaxSafety* surveillance system, served as a coordinator of the Western Australia surveillance sites, conducted data collection and analysis, and reviewed and contributed to the manuscript draft. DW served as a coordinator of the Western Australia surveillance sites, conducted data collection and analysis, and reviewed and contributed to the manuscript draft. TS contributed to the design and implementation of the *AusVaxSafety* surveillance system, conducted data analysis, and reviewed the manuscript draft. CB contributed to the design and implementation of the *AusVaxSafety* surveillance system and reviewed and contributed to the draft manuscript. NC contributed to the design and implementation of the *AusVaxSafety* surveillance system and reviewed and contributed to the draft manuscript. KM contributed to the design, implementation and coordination of the *AusVaxSafety* surveillance system, and drafted the manuscript.

References

1. AusVaxSafety. AusVaxSafety Final report to the Australian Government Department of Health for Contract HEALTH/082/2014. Sydney: National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases; Dec 2014.
2. Khandaker G. Going national: AusVaxSafety 2014 and beyond. Active surveillance for adverse events following immunisation - new methods in vaccine pharmacovigilance. Conference on Vaccine safety: active surveillance for adverse events following immunisation – new methods in vaccine pharmacovigilance; 29 Oct 2014; North Sydney, Australia.
3. Hull B, Dey A, Beard F, Menzies R, Brotherton J, McIntyre P. Annual immunisation coverage report 2013. Westmead: National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases; 2013. Available from: http://ncirs.edu.au/assets/surveillance/coverage/2013-coverage-report-final.pdf
4. Australian demographic statistics, Mar 2015. Sydney: Australian Bureau of Statistics. [Accessed: 30 September 2015]. Available from: http://www.abs.gov.au/ausstats/abs@.nsf/mf/3101.0
5. CashmanP, MoberleyS, DaltonC, StephensonJ, ElvidgeE, ButlerM, et al. Vaxtracker: Active on-line surveillance for adverse events following inactivated influenza vaccine in children. Vaccine. 2014;32(42):5503-8. DOI: 10.1016/j.vaccine.2014.07.061 PMID: 25077424
6. LeebA, ReganAK, PetersIJ, LeebC, LeebG, EfflerPV. Using automated text messages to monitor adverse events following immunisation in general practice.Med J Aust. 2014;200(7):416-8. DOI: 10.5694/mja13.11166 PMID: 24794676
7. MahajanD, DeyA, CookJ, HarveyB, MenziesRI, MacartneyKM. Surveillance of adverse events following immunisation in Australia, 2012.Commun Dis Intell Q Rep. 2014;38(3):E232-46. PMID: 25391415
8. LucasJ, CrosierR. Fast initial response for cusum quality-control schemes: give your cusum a head start.Technometrics. 1982;24(3):199-205. DOI: 10.1080/00401706.1982.10487759
9. GriggOA, FarewellVT, SpiegelhalterDJ. Use of risk-adjusted CUSUM and RSPRT charts for monitoring in medical contexts. Stat Methods Med Res. 2003;12(2):147-70.PMID: 12665208
10. BlythCC, MarkusTY, EfflerPV, RichmondPC. Ensuring safety of the 2011 trivalent influenza vaccine in young children.Med J Aust. 2011;195(1):52.PMID: 21728948
11. WoodNJ, BlythCC, WillisGA, RichmondP, GoldMS, ButteryJP, et al. The safety of seasonal influenza vaccines in Australian children in 2013. Med J Aust. 2014;201(10):596-600. DOI: 10.5694/mja13.00097 PMID: 25390267
12. Li-Kim-MoyJ, YinJK, RashidH, KhandakerG, KingC, WoodN, et al. Systematic review of fever, febrile convulsions and serious adverse events following administration of inactivated trivalent influenza vaccines in children. Euro Surveill. 2015;20(24):21159. DOI: 10.2807/1560-7917.ES2015.20.24.21159 PMID: 26111238
13. TraceyLE, ReganAK, MakDB, EfflerPV. Adverse events following influenza immunization reported by healthcare personnel using active surveillance based on text messages.Infect Control Hosp Epidemiol. 2015;36(5):608-10. DOI: 10.1017/ice.2015.16 PMID: 25652211
14. ReganAK, BlythCC, MakDB, RichmondPC, EfflerPV. Using SMS to monitor adverse events following trivalent influenza vaccination in pregnant women.Aust N Z J Obstet Gynaecol. 2014;54(6):522-8. DOI: 10.1111/ajo.12266 PMID: 25306915
15. StockwellMS, BroderK, LaRussaP, LewisP, FernandezN, SharmaD, et al. Risk of fever after pediatric trivalent inactivated influenza vaccine and 13-valent pneumococcal conjugate vaccine. JAMA Pediatr. 2014;168(3):211-9. DOI: 10.1001/jamapediatrics.2013.4469 PMID: 24395025
16. ArmstrongPK, DowseGK, EfflerPV, CarcioneD, BlythCC, RichmondPC, et al. Epidemiological study of severe febrile reactions in young children in Western Australia caused by a 2010 trivalent inactivated influenza vaccine. BMJ Open. 2011;1(1):e000016. DOI: 10.1136/bmjopen-2010-000016 PMID: 22021725
17. RockmanS, DysonA, KoernigS, BecherD, NgM, MorelliAB, et al. Evaluation of the bioactivity of influenza vaccine strains in vitro suggests that the introduction of new strains in the 2010 Southern Hemisphere trivalent influenza vaccine is associated with adverse events. Vaccine. 2014;32(30):3861-8. DOI: 10.1016/j.vaccine.2014.03.032 PMID: 24928062
18. BlythCC, RichmondPC, JacobyP, ThorntonP, ReganA, RobinsC, et al. The impact of pandemic A(H1N1)pdm09 influenza and vaccine-associated adverse events on parental attitudes and influenza vaccine uptake in young children. Vaccine. 2014;32(32):4075-81. DOI: 10.1016/j.vaccine.2014.05.055 PMID: 24877764
19. Government of Western Australia Department of Health. Child influenza vaccination rate low despite rise in uptake. Disease WAtch. 2013;17(4). Available from: http://www.health.wa.gov.au/diseasewatch/vol17_issue4/child_vaccination.cfm

Research article

Systematic community- and hospital-based surveillance for enterovirus-D68 in three Canadian provinces, August to December 2014

DM Skowronski [1], C Chambers [1], S Sabaiduc [1], M Murti [2], R Gustafson [3], S Pollock [4], D Hoyano [5], S Rempel [2,6], S Allison [7], G De Serres [8], JA Dickinson [9], R Tellier [10], K Fonseca [10], SJ Drews [11], C Martineau [8], F Reyes-Domingo [6], T Wong [12,13], P Tang [1], M Krajden [1]

1. 1. British Columbia Centre for Disease Control, Vancouver, British Columbia, Canada
2. Fraser Health Authority, Surrey, British Columbia, Canada
3. Vancouver Coastal Health Authority, Vancouver, British Columbia, Canada
4. Interior Health Authority, Kelowna, British Columbia, Canada
5. Vancouver Island Health Authority, Victoria, British Columbia, Canada
6. Public Health Agency of Canada, Ottawa, Ontario, Canada
7. Northern Health Authority, Prince George, British Columbia, Canada
8. Institut national de santé publique du Québec, Québec, Canada
9. University of Calgary, Alberta, Canada
10. Alberta Provincial Laboratory, Calgary, Alberta, Canada
11. Alberta Provincial Laboratory, Edmonton, Alberta, Canada
12. Health Canada, Ottawa, Ontario, Canada
13. Formerly affiliated with: Public Health Agency of Canada, Ottawa, Ontario, Canada

Correspondence: Danuta M Skowronski (danuta.skowronski@bccdc.ca)

Citation style for this article:
Skowronski DM, Chambers C, Sabaiduc S, Murti M, Gustafson R, Pollock S, Hoyano D, Rempel S, Allison S, De Serres G, Dickinson JA, Tellier R, Fonseca K, Drews SJ, Martineau C, Reyes-Domingo F, Wong T, Tang P, Krajden M. Systematic community- and hospital-based surveillance for enterovirus-D68 in three Canadian provinces, August to December 2014. Euro Surveill. 2015;20(43):pii=30047. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2015.20.43.300472015;20(34):pii=30002. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2015.20.34.30002

Article submitted on 04 August 2015 / accepted on 04 October 2015 / published on 29 October 2015

Respiratory specimens collected from outpatients with influenza-like illness in three Canadian provinces (British Columbia (BC), Alberta and Quebec) participating in a community-based sentinel surveillance network were prospectively screened for enterovirus-D68 (EV-D68) from 1 August to 31 December 2014 and compared to specimens collected from 1 October 2013 to 31 July 2014. Eighteen (1%) of 1,894 specimens were EV-D68-positive: 1/348 (0.3%) collected from October to December 2013 and 11/460 (2.4%) from October to December 2014, an eight-fold increase in detection rates (p=0.01), consistent with epidemic circulation in autumn 2014. The remaining EV-D68 detections were in September 2014 (6/37). Enhanced passive surveillance was also conducted on all inpatient and outpatient EV-D68 cases (n=211) detected at the BC provincial reference laboratory from 28 August to 31 December 2014. Incidence of hospitalisations was 3/100,000 overall and 21, 17, 4 and 1/100,000 among those <5, 5–9, 10–19 and ≥20-years-old with male-to-female ratios >1 among paediatric but not adult cases. Three cases in BC with comorbidity or co-infection died and five exhibited neurological features persisting >9 months. Active surveillance in outpatient and inpatient settings is needed from more areas and additional seasons to better understand EV-D68 epidemiology and potential at-risk groups for severe or unusual manifestations.

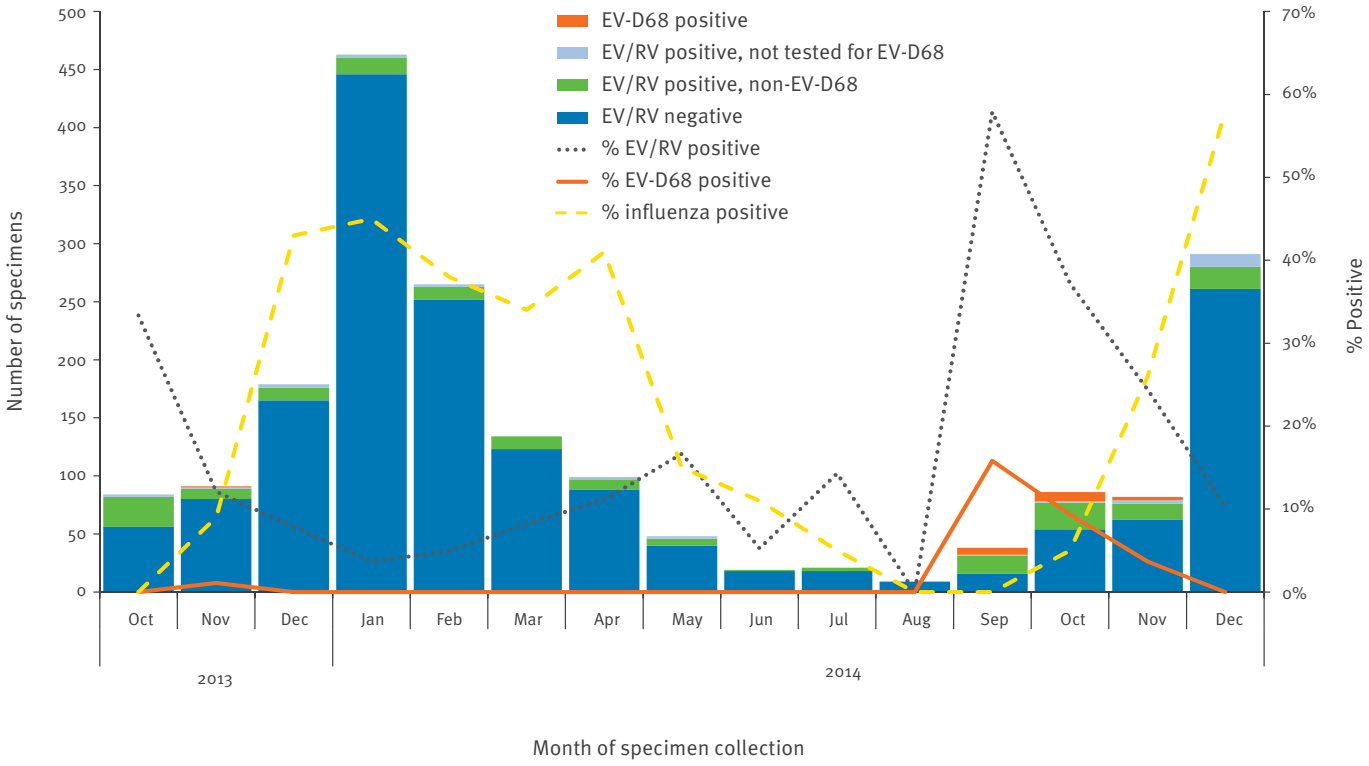
Introduction

Enterovirus-D68 (EV-D68) is a non-polio enterovirus that shares biological characteristics with enteroviruses (EVs) and rhinoviruses (RVs) [1]. First identified in California in 1962, EV-D68 was only sporadically detected in subsequent decades [2-4]. Between 2008 and mid-2014, however, EV-D68 was associated with several clusters of severe respiratory illness globally, disproportionately affecting children less than 5-years-old [5-12].

In mid-August 2014, two paediatric hospitals in the United States (US) reported increases in severe EV-D68-associated respiratory illness [13] that were followed by a nationwide outbreak notable for affecting children with asthma in particular [14]. Several countries in Europe also reported EV-D68 activity during the summer and autumn of 2014, including one country, Norway, where EV-D68 was also associated with an increase in hospitalisations for severe respiratory illness [9,15-18]. In September 2014, the US Centers for Disease Control and Prevention (US CDC) additionally began investigating reports of acute flaccid myelitis of unknown aetiology in children, detecting EV-D68 in some, but not all, of these patients [19-22]; sporadic cases of EV-D68-associated neurological illness were also reported from France (n=1) and Norway (n=2) [15,23,24].

Figure 1

Epidemic curve of EV/RV, EV-D68 and influenza detections by month of specimen collection, Canadian Sentinel Practitioner Surveillance Network, British Columbia, Alberta and Quebec, 1 October 2013–31 December 2014 (n=1,909)[a]



EV: enterovirus; RV: rhinovirus; RVP: Respiratory Virus Panel.

[a] Excludes 169 specimens not tested for EV/RV: 133 influenza-positive specimens from Alberta not tested by Luminex xTAG RVP during 2014/15 season, 20 specimens with insufficient viral load for testing, and 16 specimens from British Columbia that were tested only for EV-D68 (not other EV/RV) using an EV-D68-specific PCR assay (all EV-D68 negative), as per protocol indicated in Table 1.

Figure 2

Epidemic curve by week of specimen collection and hospitalisation status, laboratory-based enhanced passive surveillance, British Columbia, 28 August–31 December 2014 (n=211)

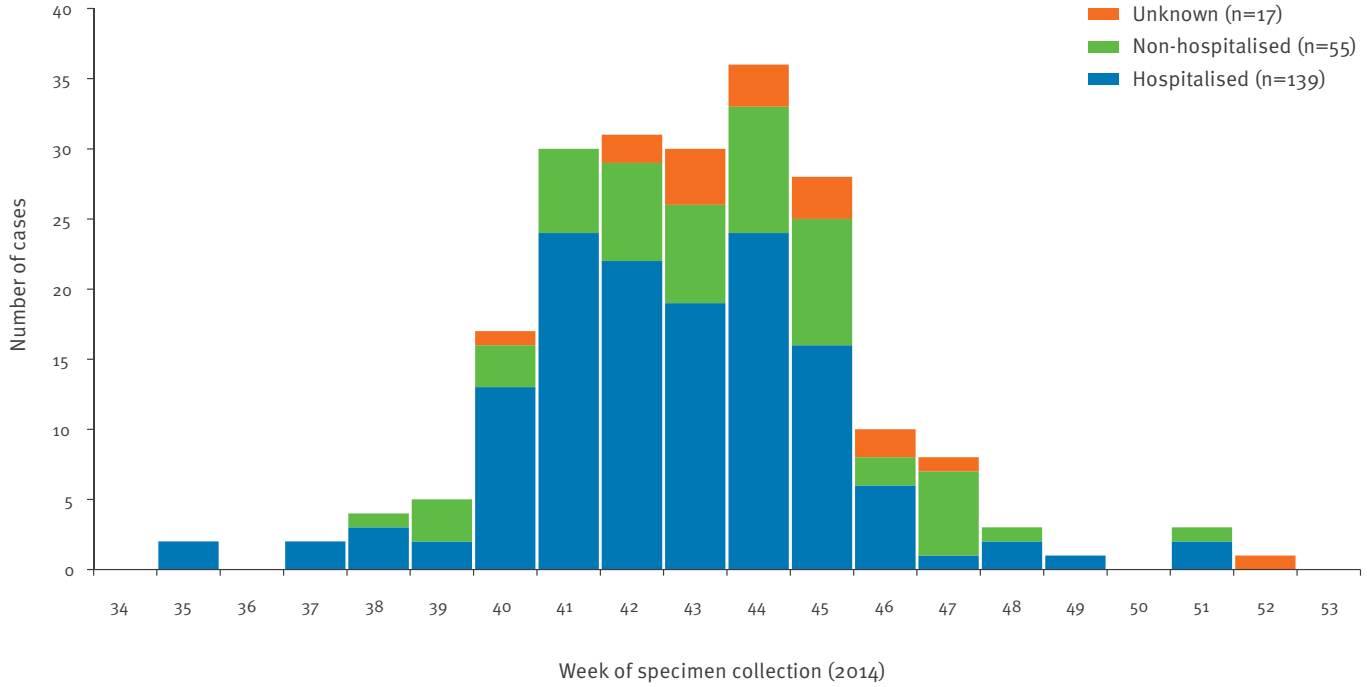


Table 1

Overview of active community-based sentinel surveillance and laboratory-based enhanced passive surveillance used for EV-D68 detection and characterisation, Canada, 2014

Attribute	Canadian Sentinel Practitioner Surveillance Network	British Columbia Public Health Microbiology and Reference Laboratory
Type of surveillance	Active, community-based sentinel	Enhanced passive, laboratory-based
Setting	Outpatient sentinel sites	Inpatient and outpatient specimens submitted to BC PHMRL
Province(s)	BC, Alberta, Quebec	BC
Time period [a]	1 August–31 December 2014	28 August–31 December 2014[b]
Period of comparison [a]	1 October 2013–31 July 2014	None
Patient population	Patients presenting within seven days of ILI[c] onset to sentinel practitioners	All laboratory-confirmed cases of EV-D68 in BC residents[d] Age restrictions for testing: 9 Sep 2014–18 Sep 2014: Inpatients: <5 years old Outpatients: <5 years old 19 Sep 2014–20 Oct 2014: Inpatients: no age restriction Outpatients: <20 years old 21 Oct 2014–31 Dec 2014: Inpatients: no age restriction Outpatients: no age restriction
Source of data	Patient/provider self-report; standard questionnaire completed at time of specimen collection	Patient/provider self-report and/or electronic medical record; enhanced surveillance form completed at case detection
Specimen type	Respiratory (nasal/nasopharyngeal)	Respiratory at clinician discretion
Laboratory testing	All provinces: EV/RV screening by Luminex xTAG RVP BC PHMRL: EV-specific [31] and/or EV-D68-specific RT-PCR assay and partial VP1 sequencing [10], as per BC PHMRL protocol Alberta[e]: EV-specific PCR assay [27] and VP4/partial VP2 sequencing [28-30] Quebec: Partial VP1 sequencing [32]	9 Sep 2014–20 Oct 2014: Luminex xTAG RVP and partial 5' UTR/VP1 sequencing [10] 21 Oct 2014– 13 Nov 2014: EV-specific RT-PCR assay [31], EV-D68-specific RT-PCR assay and partial VP1 sequencing [10] 14 Nov 2014–31 Dec 2014: EV-D68-specific RT-PCR assay and partial VP1 sequencing [10]

BC: British Columbia; EV: enterovirus; ILI: influenza-like illness; PHMRL: Public Health Microbiology and Reference Laboratory; RVP: Respiratory Virus Panel; RV: rhinovirus; SPSN: Sentinel Practitioner Surveillance Network.

[a] Defined using specimen collection date.

[b] Full enhanced surveillance case report form completed until 31 October 2014; partial enhanced surveillance data (basic demographic data and hospitalisation status) collected until 31 December 2014.

[c] Defined as acute respiratory illness with fever and cough and at least one of sore throat, arthralgia, myalgia or prostration.

[d] EV-D68 cases in out-of-province residents, sentinel patients detected through the Canadian SPSN, and those with unknown/missing geographic information were excluded from enhanced passive surveillance analysis.

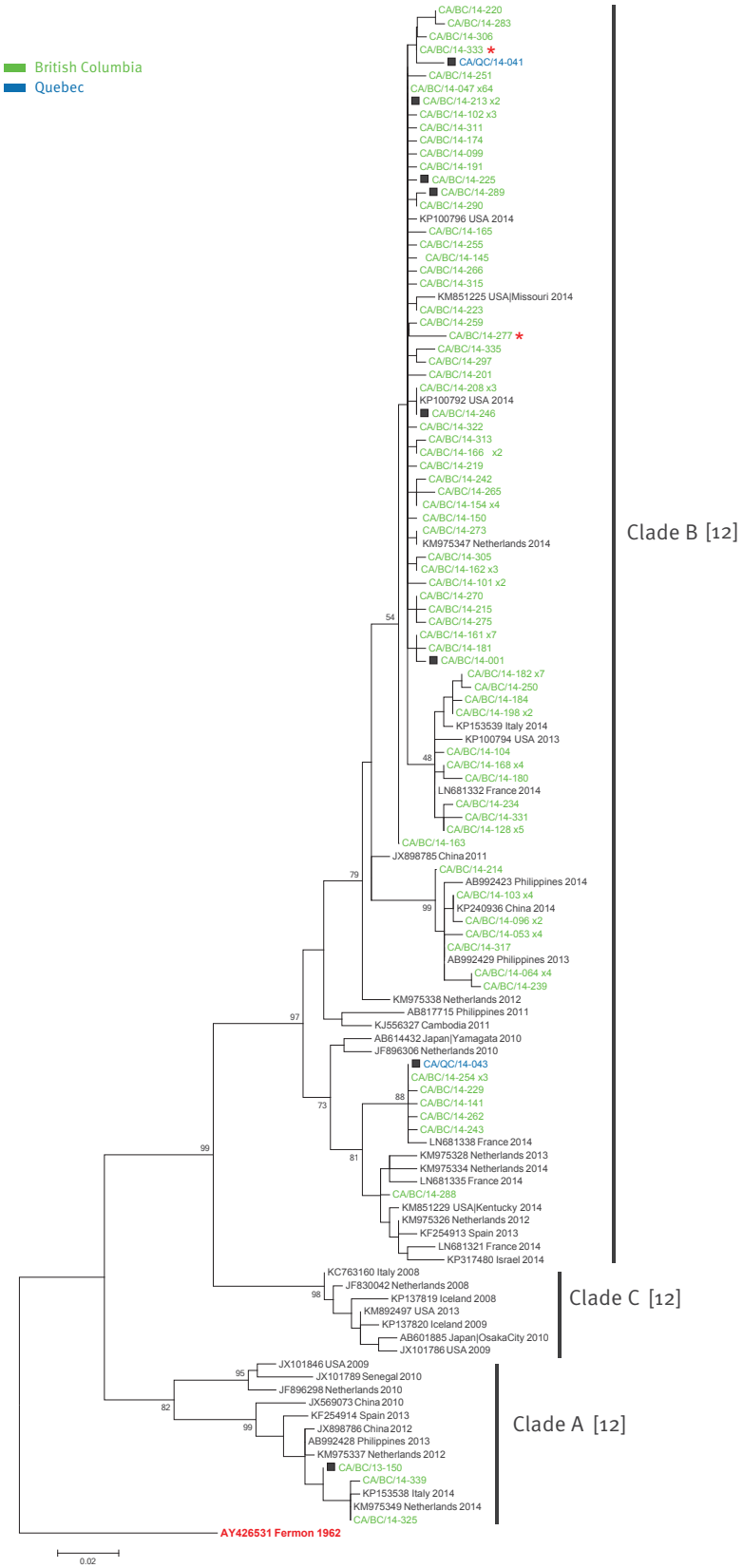
[e] EV/RV screening by Luminex xTAG RVP not performed on influenza-positive specimens collected during 2014/15 influenza season (1 October 2014–31 December 2014) in Alberta.

EV-D68 infection is not generally notifiable and laboratory testing for EV-D68 was not widely performed prior to the 2014 outbreak. Consequently, localised clusters of paediatric hospitalisations have largely shaped current understanding of EV-D68 illness, skewing impressions of typical disease severity. Few countries have utilised existing general practice sentinel surveillance schemes for influenza-like illness (ILI) or acute respiratory illness (ARI) to systematically assess outpatient illness due to EV-D68 infection, as conducted in the Netherlands and Germany [8,9,18].

In Canada, pre-existing infrastructure for standardised screening of respiratory specimens was mobilised in response to the alerts from the US. Respiratory specimens collected from ILI patients of all ages attending sentinel outpatient practices in three participating provinces (British Columbia (BC) (population: 4.4 million [25]), Alberta (population: 3.6 million [25]) and Quebec (population: 7.9 million [25])) of the community-based Canadian Sentinel Practitioner Surveillance Network (SPSN) were retrospectively and prospectively screened for EV-D68 before and during the 2014 August-to-December epidemic period. In addition, laboratory-based enhanced passive surveillance was conducted in BC on all detections of EV-D68 from inpatient and outpatient specimens tested at the BC Public Health Microbiology and Reference Laboratory (BC PHMRL) during the 2014 epidemic period. Here we report findings from these two surveillance approaches to inform the epidemiology of EV-D68 including the spectrum of illness, population-based incidence, and potential at-risk groups for severe or unusual sequelae, including neurological or fatal outcomes.

Figure 3

Phylogenetic analysis of EV-D68 partial VP1 sequences, enhanced passive surveillance (British Columbia), 28 August–31 December 2014, and Canadian Sentinel Practitioner Surveillance Network (British Columbia and Quebec), 1 October 2013–31 December 2014

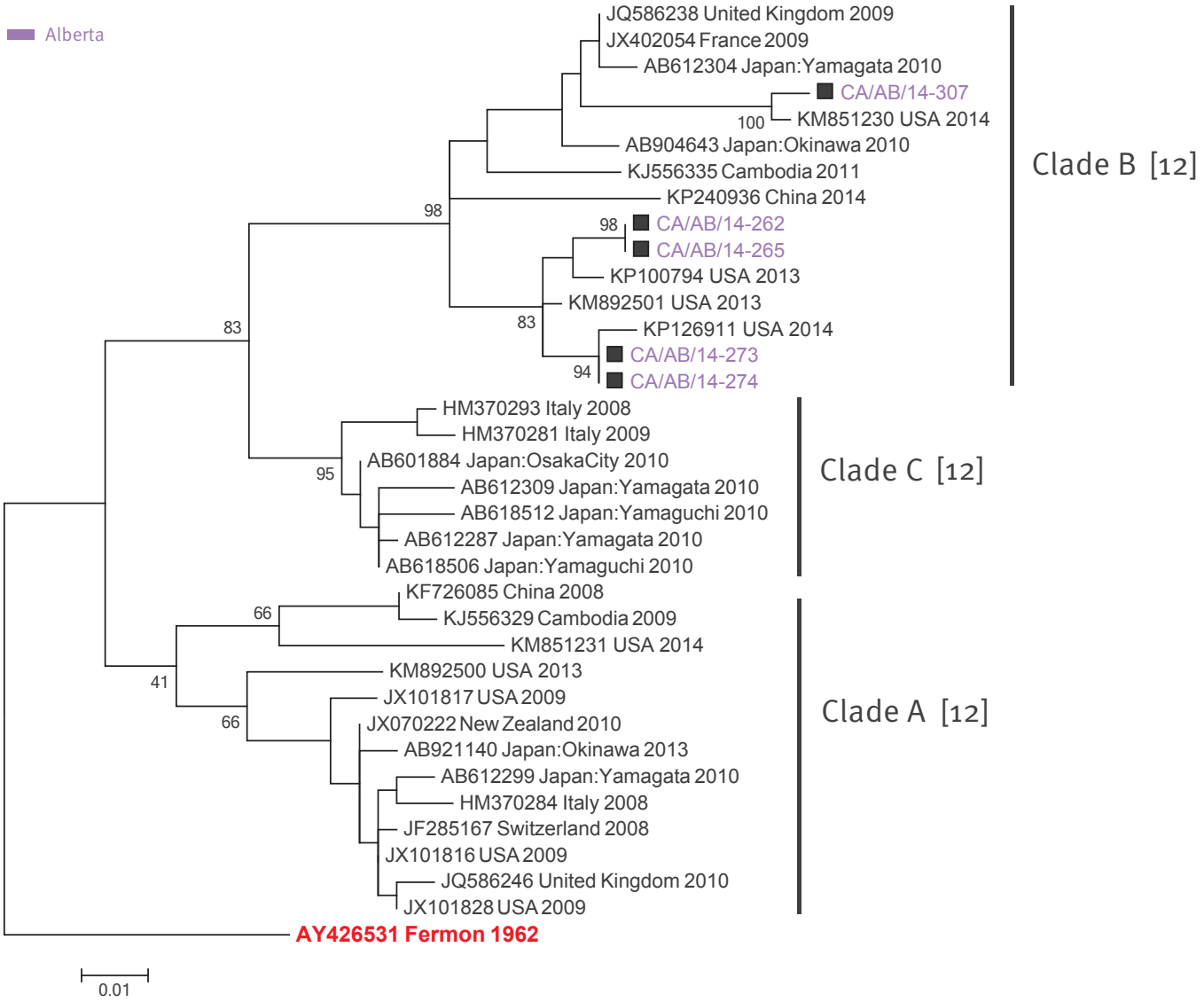


Canadian EV-D68 sequences are colour coded by province of specimen collection, global sequences available in GenBank are shown in black and the Fermon reference strain (1962) is shown in red. Sentinel viruses collected through the Canadian Sentinel Practitioner Surveillance Network are indicated with black squares. Red asterisks denote sequences with adjacent asparagine deletions at positions 144-145 in the D-E immunogenic loop.

Phylogenetic clusters are labelled clade A, B, and C as described in Tokarz et al. [12], corresponding to major group 3, 1, and 2 as described in Meijer et al. [9] and lineage 2, sub-lineage 1.2, and sub-lineage 1.1 as described in Lauinger et al. [7], respectively.

Figure 4

Phylogenetic analysis of EV-D68 VP4/partial VP2 sequences, Canadian Sentinel Practitioner Surveillance Network (Alberta), 1 October 2013–31 December 2014



Canadian EV-D68 sequences are colour coded by province of specimen collection, global sequences available in GenBank are shown in black and the Fermon reference strain (1962) is shown in red. Sentinel viruses collected through the Canadian Sentinel Practitioner Surveillance Network are indicated with black squares.

Phylogenetic clusters are labelled clade A, B, and C as described in Tokarz et al. [12], corresponding to major group 3, 1, and 2 as described in Meijer et al. [9] and lineage 2, sub-lineage 1.2, and sub-lineage 1.1 as described in Lauinger et al. [7], respectively.

Methods

Epidemiological sampling and laboratory testing protocols are outlined in Table 1 and are summarised below.

Active community-based sentinel surveillance

Community-based sentinel practitioners of the Canadian SPSN collect nasal or nasopharyngeal specimens year-round from patients presenting within seven days of ILI onset defined as acute respiratory illness with fever and cough and at least one of the following symptoms: sore throat, arthralgia, myalgia or prostration [26]. Epidemiological information is collected from consenting patients/guardians using a standard questionnaire at the time of specimen collection. Research ethics board (REB) approval is obtained in each participating province.

The public health laboratories in the provinces of BC, Alberta and Quebec conducted prospective EV-D68 testing of EV/RV-positive SPSN specimens collected between 1 August and 31 December 2014. Additionally, they retrospectively tested specimens collected between 1 October 2013 and 31 July 2014 that were EV/RV-positive. The latter period was chosen as comparison because routine SPSN influenza surveillance

Table 2

Characteristics of patients with specimens collected by the Canadian Sentinel Practitioner Surveillance Network and tested for EV-D68, British Columbia, Alberta and Quebec, 1 August–31 December 2014 (n=506)

Characteristic, n (% by column)	EV-D68-positive[a]	EV-D68-negative
N[b] (% by row)	17 (3)	489 (97)
Age group (years)		
<5	1	42 (9)
5-9	2	35 (7)
10-19	3	61 (12)
20-59	10	260 (53)
≥60	1	90 (18)
Unknown	0	1 (0)
Median (range)	23 (1–64)	36 (0–94)
Sex		
Male	10	192 (39)
Paediatric (<20 years)	2	53 (28)
Adult (≥20 years)	8	138 (72)
Female	7	295 (60)
Paediatric (<20 years)	4	84 (28)
Adult (≥20 years)	3	211 (72)
Unknown	0	2 (0)
Specimen collection interval (days)[c]		
≤4	12	345 (71)
5-7	5	144 (29)
Median (range)	4 (0-7)	3 (0–7)
Chronic comorbidity		
No	15	335 (69)
Yes	1	117 (24)
Unknown	1	37 (8)
Province		
BC	10	126 (26)
Alberta	5	177 (36)
Quebec	2	186 (38)

All values are number (n) and percentage (%) by column where displayed (except in initial header row), unless otherwise specified.

BC: British Columbia; EV: enterovirus; RV: rhinovirus.

[a] One EV-D68 detection in a specimen collected from an adult in British Columbia in November 2013 is not included in this table which is restricted to specimens collected during the 2014 epidemic period.

[b] EV-D68 typing was not performed on 16 specimens with insufficient viral load; 16 BC specimens were tested only for EV-D68 (not other EV/RV) using an EV-D68-specific PCR assay as per protocol in Table 1.

[c] Interval between onset of influenza-like illness and collection of nasal/nasopharyngeal specimen.

typically commences on 1 October each year. Although the SPSN is primarily designed for influenza surveillance, public health laboratories in these provinces also routinely test SPSN specimens for a panel of respiratory viruses, including EV/RV, influenza A (H1, H3 and H1N1pdm09 subtypes), influenza B, respiratory syncytial virus, parainfluenza types 1–4, human metapneumovirus, adenovirus, coronaviruses NL63, HKU1, 229E and OC43, and bocavirus (BC and Quebec only) using versions of the Luminex xTAG Respiratory Virus Panel (RVP) (Luminex Corp., US). During the 2014/15 influenza season, Alberta made the a priori decision to restrict routine EV/RV testing to influenza-negative patients for resource reasons.

Provincial protocols for EV-D68 detection among EV/RV-positive specimens are specified in Table 1 [10,27-32]. These assays have comparable sensitivities and limits of detection are considered within one $\log_{10}$ copy/mL, as demonstrated in a national validation study [33]. Specific EV-D68 typing was established by partial sequencing of structural capsid viral protein (VP) namely VP1 in BC [10] and Quebec [32] and VP2 in Alberta [28-30].

Laboratory-based enhanced passive surveillance in British Columbia

The BC PHMRL is the only site to provide confirmatory diagnosis of EV-D68 in BC. Such testing is usually conducted upon physician request; until 19 September 2014, this was predominantly to confirm diagnosis among inpatients at the province's tertiary paediatric hospital. From 9 September 2014 onwards, this also included routine screening of all respiratory specimens collected from inpatients or outpatients less than 5-years-old. On 19 September 2014, routine EV-D68 screening was extended to all respiratory specimens submitted to the BC PHMRL from all inpatients of any age across the province. Outpatient specimens were also included in routine EV-D68 screening with staggered implementation as shown in Table 1. EV-D68 typing was performed as specified in Table 1 [10,31].

Detailed case report forms were completed on all EV-D68 detections by the BC PHMRL for specimens collected between 28 August and 31 October 2014, with only basic demographic and hospitalisation status recorded thereafter to 31 December 2014. Forms were completed by local public health practitioners based primarily upon the electronic medical record and reported to the BC Centre for Disease Control (BCCDC) as part of outbreak investigation, exempt from REB approval. Patients presenting with neurological or fatal outcome notified to BCCDC as part of enhanced passive surveillance are further described as a case series based upon information in the electronic medical record and supplemented by direct patient, guardian and/or clinician interview.

Phylogenetic analyses

Partial VP1 sequences (nucleotides 133-471), including B-C and D-E immunogenic loops, from BC and Quebec were aligned to a subset of representative VP1 sequences in GenBank to establish clade designation [2,12,34]. Since no recombination was observed within VP4, the complete VP4 and partial VP2 (first 215 nucleotides of 5' end) sequences from Alberta were used to align with VP4/VP2 sequences in GenBank and divided

Table 3

Characteristics of EV-D68 cases with full case report forms completed, laboratory-based enhanced passive surveillance, British Columbia, 28 August–31 October 2014 (n=146)

Characteristic, n (% by column)	Overall[a]	Hospitalised cases			Non-hospitalised cases		
		All cases	Paediatric (<20 years)	Adult (≥20 years)	All cases	Paediatric (<20 years)	Adult (≥20 years)
N (% by row)	146	111	91 (82)	20 (18)	35	26 (74)	9 (26)
Age group (years)							
<5	53[b] (36)	39 (35)	39[c] (43)	NA	14	14[d]	NA
5-9	39 (27)	32 (29)	32 (35)	NA	7	7	NA
10-19	25 (17)	20 (18)	20 (22)	NA	5	5	NA
≥20	29 (20)	20 (18)	NA	20	9	NA	9
Median age (range)	8 (0–90)	8 (0–82)	5 (0–19)	35.5 (21–82)	7 (0–90)	4 (0–19)	42 (20–90)
Sex							
Male	85 (58)	65 (59)	59 (65)	6	20	18	2
Female	61 (42)	46 (41)	32 (35)	14	15	8	7
Month of specimen collection							
August	2 (1)	2 (2)	2 (2)	0	0	0	0
September	19 (13)	14 (13)	14 (15)	0	5	5	0
October	125 (86)	95 (86)	75 (82)	20	30	21	9
Median (range) hospital stay (days)[e]	NA	3 (1–18)	3 (1–18)	4 (1–12)	NA	NA	NA
Admitted to ICU							
Yes	NA	9 (8)	7 (8)	2	NA	NA	NA
No	NA	87 (78)	71 (78)	16	NA	NA	NA
Unknown	NA	15 (14)	13 (14)	2	NA	NA	NA
Clinical presentation[f]							
Respiratory illness	135 (92)	103 (93)	83 (91)	20	32	24	8
Pneumonia diagnosis	27 (18)	25 (23)	19 (21)	6	2	2	0
Neurological illness[g]	4 (3)	4 (4)	4 (4)	0	0	0	0
Immunocompromised[f]	4 (3)	4 (4)	3 (3)	1	0	0	0
Travel outside Canada[f,h]	5 (3)	4 (4)	4 (4)	0	1	1	0
Co-infection[f,i]	10 (7)	10 (9)	9 (10)	1	0	0	0
Asthma prevalence[f]	55 (38)	47 (42)	39 (43)	8	8	5	3
Among males	33 (39)	31 (48)	28 (47)	3	2	2	0
Among females	22 (36)	16 (35)	11 (34)	5	6	3	3

All values are number (n) and percentage (%) by column where displayed (except in initial header row), unless otherwise indicated.

ICU: intensive care unit; NA: not applicable.

[a] Restricted to non-sentinel cases in British Columbia residents with enhanced surveillance case report forms with valid hospitalisation information collected as at 31 October 2014. Six cases missing information on hospitalisation status were excluded.

[b] Includes 14 infant cases <1 year-old and 20 cases 1–2-years-old (i.e. 34 cases <3-years-old).

[c] Includes nine hospitalised infant cases <1 year-old and 16 hospitalised cases 1–2 years-old (i.e. 25 cases <3-years-old).

[d] Includes five non-hospitalised infant cases <1 year-old and four non-hospitalised cases 1–2 years-old (i.e. nine cases <3-years-old).

[e] Five patients remained in hospital at time of reporting and were excluded from length of stay calculations.

[f] Proportions displayed are among those with known information only.

[g] One adult case with neurological illness with specimen collection in November 2014 not included in this table which is restricted to specimens collected from 28 August to 31 October 2014 with full enhanced surveillance case report forms.

[h] In 30 days prior to symptom onset; all five patients reported travel to the United States.

[i] Influenza A(H3N2) (n=1), respiratory syncytial virus (n=1), and *Streptococcus pneumoniae* (n=8, including one detection in blood and seven in upper respiratory specimen).

into clades corresponding to VP1 designations [12]. Phylogenetic trees were constructed in MEGA6, rooted to the 1962 prototype Fermon strain using the maximum-likelihood method with a Jukes-Cantor substitution model and 1,000 bootstrap replicates [35].

Results

Active community-based sentinel surveillance

Among 2,078 specimens submitted to the Canadian SPSN, meeting ILI criteria and collected between 1 October 2013 and 31 December 2014, 1,909 (92%) were screened for EV/RV and 221 (12%) tested EV/RV-positive. There was a mirror-image pattern of EV/RV versus influenza test-positivity by month, reflecting their differences in seasonality (Figure 1).

During this period, 1,894 of 2,078 (91%) specimens were assessed for EV-D68, of which 18 (1%) tested positive. This includes 1 of 348 (0.3%) collected from October to December 2013 and 11 of 460 (2.4%) from October to December 2014, a significant eight-fold increase in detection rates across two successive years (Chi-square test p=0.01). The remaining EV-D68 detections were from specimens collected in September 2014 (6/37) (Figure 1). There were no EV-D68 co-infections with other respiratory viruses included on the Luminex xTAG RVP panel.

Six of the 17 EV-D68-positive cases detected during the 2014 epidemic period were <20-years-old. Detection rates among adults 20 to 59-years-old (10/270; 4%) did not differ from rates among children <20-years-old (6/144; 4%). A single EV-D68 case was detected among adult patients ≥60-years old (1/91; 1%). Two of six paediatric EV-D68 cases were male compared to 8 of 11 adult cases. Other patient characteristics among EV-D68 test-positive and test-negative specimens for the August-to-December 2014 epidemic period are shown in Table 2.

Laboratory-based enhanced passive surveillance in British Columbia

Of 3,716 respiratory specimens collected between 28 August and 31 December 2014 in BC and tested at the BC PHMRL, 239 (6%) were positive for EV-D68, representing 218 unique patients (n=211 excluding seven detected also by the SPSN). The majority (172/211; 82%) had specimens collected between weeks 40 to 45 (early October–early November) (Figure 2) and 72% (139/194) of those with known information were hospitalised.

Based on 2014 BC population estimates [36], incidence of EV-D68 hospitalisations across the 2014 surveillance period was 3 per 100,000 overall and 21, 17, 4 and 1 per 100,000 among those <5, 5–9, 10–19 and ≥20-years-old, with male-to-female ratios of 1.4 overall and 2.1, 1.4, 1.8 and 0.7 by age group, respectively. Hospitalisation rates were essentially unchanged when the period of specimen collection was restricted to 19 September to 31 December 2014, during which screening of respiratory specimens submitted from inpatients of all ages was undertaken: 3 per 100,000 overall and 20, 16, 4 and 1 per 100,000 by age category, with male-to-female ratios of 1.4 overall and 2.2, 1.5, 1.4 and 0.7 by age category, respectively.

Between 28 August and 31 October 2014, there were 152 EV-D68 detections by the BC PHMRL. Among these, 146 (96%) case report forms with valid hospitalisation information were submitted to the BCCDC. As shown in Table 3, most of these patients (117/146; 80%) were in paediatric age groups with a median age among the 146 cases of eight years (range: 0-90 years). Males were over-represented among paediatric (77/117; 66%) but not adult (8/29) cases. Most cases were hospitalised (111/146; 76%) with a median length-of-stay of three days (range: 1–18 days) among those patients (106/111; 95%) discharged at time of reporting. Among the 111 hospitalised patients, nine (8%) required admission to an intensive care unit (ICU); information on ICU admission was unknown for 15 patients. Asthma history was reported among 47 of 111 (42%) hospitalised patients and less frequently among non-hospitalised patients (8/35).

Case series of EV-D68-associated neurological or fatal outcomes

Five EV-D68 cases (paediatric (n=4); male (n=4)) identified through enhanced passive surveillance in BC were associated with neurological illness including acute flaccid limb weakness (n=3), generalised paralysis (n=1) or head/neck paralysis (n=1) (Table 4) [37]. All had preceding respiratory illness and one had concurrent gastrointestinal illness. All were admitted to hospital; three required ventilation support. None were fatal. All cases were up-to-date for age with polio vaccination. Magnetic resonance imaging in four cases identified hyperintensity predominantly affecting central grey matter of the cervical but also thoracic cord; this was not assessed in Case 4 (adult). Examination of the cerebrospinal fluid (CSF) revealed pleocytosis but normal glucose and, in Cases 1, 2 and 4, elevated protein.

Upper respiratory specimens were EV-D68-positive in all five neurological cases and EV-D68 was also detected by PCR in whole blood from Case 5. All CSF specimens were negative for EV by RT-PCR. One stool specimen was assessed (Case 2) and was EV-D68 negative. Other investigations undertaken at clinician discretion included a range of viral, bacterial and/or fungal pathogens (information available from authors upon request). No alternative aetiologies were identified. Of note, Cases 1, 2 and 5 were *Mycoplasma pneumoniae* IgM-reactive but, among these, Cases 1 and 2 were *M. pneumoniae* PCR-negative in respiratory specimens and Cases 2 and 5 were *M. pneumoniae* PCR-negative in CSF specimens. *Streptococcus pneumoniae* was detected by PCR in nasopharyngeal specimens from Cases 3 and 5; however, CSF was PCR-negative in

Table 4

Summary of clinical and epidemiological findings for EV-D68 cases associated with neurological illness, laboratory-based enhanced passive surveillance, British Columbia, 28 August–31 December 2014 (n=5)

Findings	Case 1 [37]	Case 2	Case 3	Case 4	Case 5
Age group (years)	5–9	15–19	<5	50–59	10–14
Month of onset (2014)	August	August	October	October	October
Hospitalisation	Yes	Yes (twice, same episode)	Yes	Yes	Yes
Length of hospital stay	8 days	5 days / 45 days	>6 months	47 days	6 days
ICU admission	No	Yes (ventilation)	Yes (ventilation)	Yes (ventilation)	No
Clinical presentation	Acute flaccid paralysis LUL: hypotonic, areflexic	Generalised paralysis all limbs; cranial neuropathy	Acute flaccid paralysis RUL; unable to lift head	Paralysis head/neck; weakness face/throat	Weakness RUL
Initial diagnosis	Acute flaccid paralysis	Acute transverse myelitis	Acute flaccid paralysis	Bulbar paresis	Acute transverse myelitis
Sensory involvement	Numbness, tingling; normal sensory nerve function	Tingling (mild)	None	Sensory loss in face	None
Respiratory illness	Yes	Yes	Yes	Yes	Yes
ILI symptoms	Fever, cough, rhinorrhoea, congestion, headache	Mild fever, aches, fatigue	Fever, cough, congestion, fatigue	Fever, rigors, neck stiffness, headache	Mild cough and rhinorrhoea
Interval from respiratory to neurological illness onset	5 days	Intermittent cough 2-3 months prior	10 days	3 weeks	2 days
Comorbidity	None	None	None	Gout	Asthma
IVIG treatment	Yes	Unknown; plasmapheresis undertaken	Yes	Yes	No
Polio vaccination	Up-to-date for age	Up-to-date for age	Up-to-date for age	Up-to-date for age	Up-to-date for age
Magnetic resonance imaging / EMG	Hyperintensity C2 to T1; nerve conduction and EMG consistent with anterior horn involvement	Hyperintensity C1-C2 to T2; predominantly central grey but also white matter; examination consistent with anterior horn involvement	Hyperintensity of central grey matter from medulla to T11; multiple enhancing nerve roots C2-C4, cauda equina	Not assessed	C2 to C7 spinal lesion; central grey matter involvement
CSF	Mild pleocytosis; elevated protein; normal glucose	Pleocytosis; elevated protein; normal glucose	Pleocytosis; normal protein; normal glucose	Pleocytosis; elevated protein; normal glucose	Pleocytosis; normal protein; normal glucose
EV-D68 test result in respiratory specimens	NP swab – positive	NP swab and tracheal aspirate – positive	NP swab – positive	NP swab – positive	NP swab – positive
EV test result in CSF	Negative	Negative	Negative	Negative	Negative
Neurological status (as at July 2015)	Partial recovery, residual deficit	Partial recovery, residual deficit	Partial recovery, residual deficit	Partial recovery, residual deficit	Partial recovery, residual deficit
Other notes	Preceding head/neck pain	Shingles late spring	Preceding neck pain	None	Preceding neck pain; asthma exacerbation; concurrent GI illness

CSF: cerebrospinal fluid; EMG: electromyography; EV: enterovirus; GI: gastrointestinal illness; ICU: intensive care unit; ILI: influenza-like illness; IVIG: intravenous immunoglobulin; LUL: left upper limb; NP: nasopharyngeal; RUL: right upper limb.

Case 3 and *S. pneumoniae* was not isolated from CSF of Cases 3 or 5. As at July 2015 (›9-11 months post onset), all five cases had ongoing neurological deficit.

Three EV-D68 cases in BC with illness onset between August and October 2014 had fatal outcome including: an adult (20-29-years-old) with respiratory failure following acute asthma exacerbation; an adult (≥65-years-old) with multiple comorbidities and respiratory failure following acute chronic obstructive pulmonary disease exacerbation; and a child (‹5-years-old) in whom death was ultimately attributed to Group A streptococcal sepsis.

Phylogenetic analysis

Most (184/187; 98%) of the viruses that were sequenced clustered phylogenetically within clade B (GenBank identifiers: KT873535-KT873716; KT587195-KT587199) [12]. This includes 179 of 182 (98%) VP1 sequences (including all eight patients with neurological or fatal outcomes) with percent nucleotide identity of 90.6–100% compared to recent isolates from the US, France, Italy, the Netherlands, and the Philippines (Figure 3). All five VP4/partial VP2 sequences from Alberta also clustered with strains associated with clade B (Figure 4) [12]. Because VP2 sequences from Alberta were partial, clade markers at positions 142-143 described in Lauinger et al. could not be confirmed [7].

Of the clade B VP1 sequences, 8 of 179 (4%) had a T146S substitution in the immunogenic D-E loop, and two had adjacent asparagine deletions at positions 144-145 in the D-E loop not found in other published sequences; neither the substitution nor the deletion was found in sequences obtained from neurological or fatal cases. Three VP1 sequences from BC, one SPSN specimen from 2013 and two enhanced passive surveillance specimens from 2014, clustered instead in clade A with recent isolates from France, Italy, and the Netherlands, characterised by an asparagine deletion at position 140 in the D-E loop [7,8]. Phylogenetic analysis did not suggest clustering by month, severity, inpatient/outpatient status or asthma history.

Discussion

Canadian investigators used two surveillance approaches to inform risk assessment related to EV-D68-associated illness. These dual surveillance approaches revealed epidemic features of EV-D68 in Canada during the period spanning from August to December 2014. Active sentinel surveillance showed increased EV-D68 detection among outpatient ILI cases affecting all age groups while enhanced passive surveillance showed severe respiratory and neurological disease requiring hospitalisation that occurred at higher incidence, but not exclusively, among children.

The ILI case definition used by the SPSN to standardise outpatient respiratory specimen collection is relatively specific for influenza. It requires fever and cough and at least one other defining symptom such as sore throat [38]. By applying this case definition, we will have missed milder illness caused by other respiratory viruses, for which fever may not be a cardinal feature. Accordingly, the actual number of EV-D68 detections by the SPSN was small (n=18). Among outpatient cases of EV-D68 detected through sentinel surveillance in the Netherlands where an ARI case definition was used, fever or cough were each experienced by 13 of 16 cases and sore throat by 8 of 16 [9]; in Germany, which also used an ARI case definition, 16 of 24 EV-D68 cases experienced fever and cough and 11 of 24 experienced fever, cough and sore throat combined [18]. With comparable community prevalence, community-based sentinel systems that apply a broader ARI case definition will certainly detect more EV-D68 cases. However, sentinel surveillance systems are not intended for the detection of rare events or for quantifying absolute disease burden [39]. They perform best in the detection of highly prevalent conditions and are most valuable for trend analysis. For that purpose, a consistently applied case definition, whether ILI or ARI, is most important. While a single occurrence of a pathogen may reflect chance sporadic detection, multiple case detections across a geographically dispersed network are an indication of widespread community circulation. As such, the eight-fold increase in EV-D68 detection rates by the SPSN in 2014 compared to 2013, even with an ILI case definition, is indicative of epidemic circulation. Nevertheless, it is acknowledged to be an under-estimate of true incidence.

In Canada, adults ≥20-years-old are predominant SPSN participants (›70-75% [26]) and correspondingly comprised two-thirds of outpatient EV-D68 detections. Among specimens obtained from non-elderly adults 20 to 59-years-old and paediatric cases ‹20-years-old, however, the proportion that tested positive for EV-D68 was equivalent (4%). This suggests that children and adults are at comparable risk for outpatient EV-D68 illness that presents as ILI, although there may be differences based on other presentations. The EV-D68 age distribution we describe as extending to adults is similar to outpatient sentinel surveillance observations reported from the Netherlands and Germany [8,9,18]. Although few countries outside of Canada or within Europe have used existing sentinel schemes to explore historic and current EV-D68 patterns [8,9,18], such infrastructure could prove highly informative if invoked elsewhere and may also be efficient for characterising other pathogens displaying sudden, unexpected but widespread activity.

Passive surveillance systems are most sensitive to severe disease, particularly if involving children or clusters. Enhanced passive surveillance conducted in BC also reflected this paediatric hospital-based skew, driven by specimen collection largely initiated at clinician discretion and laboratory-confirmation protocols that initially prioritised specimens collected in hospital and from children. All confirmatory testing for EV-D68 in BC was conducted at the BC PHMRL and this served

as the trigger for enhanced data collection in 2014. However, from 19 September 2014, all inpatient respiratory specimens submitted to the BC PHMRL were screened for EV-D68, allowing unique population-based comparison of hospitalisation incidence by age and sex. Ultimately, about three quarters of EV-D68 detections were hospitalised patients among whom more than 80% were in paediatric age groups, consistent with the age distribution of prior documented outbreaks [5-7,11,13,15,17]. While this pattern likely reflects a tip-of-the-iceberg hospital-based surveillance phenomenon, children may indeed be at higher risk owing to greater exposure opportunities and lower likelihood of pre-existing cross-reactive immunity, an immuno-epidemiological hypothesis that still requires evaluation.

The majority of EV-D68 cases were detected in early-October to early-November 2014, also consistent with prior documented outbreaks in North America and Europe [3,8-11,17]. However, labour action among teachers in September 2014 in BC resulted in school closures extending to October 2014 that may have delayed EV-D68 circulation in school-aged children. In order to detect possible seasonal recurrence in 2015, all inpatient and outpatient respiratory specimens collected in BC from 1 August to 29 September 2015 were routinely screened with an EV-D68-specific RT-PCR assay at the BC PHMRL; however, none of 709 specimens screened in 2015 were EV-D68-positive. Conversely, between 28 August and 29 September 2014, 18 EV-D68 cases were already detected, including 13 hospitalisations and two neurological events, reinforcing the exceptional activity in 2014.

Males were over-represented among paediatric but not adult cases in the 2014 BC enhanced surveillance data, a pattern that has been documented before [3,6-9,11,17], but was neither discernible in the small outpatient sentinel series reported here nor in data from Germany [18]. Male predominance may reflect increased pre-pubertal prevalence of asthma in boys [40,41]. Overall 38% of EV-D68 cases described here reported asthma compared to 7% of the general BC population [40]. Asthma is a recognised risk factor for severe EV-D68 illness [1,6,13,15,28], although underlying mechanisms are unclear. Other respiratory viruses, notably RVs with which EV-D68 shares biological features, are associated with acute exacerbation and more severe lower respiratory illness in asthmatic individuals. This effect is thought to be mediated through Th2-skewed pro-inflammatory cytokine production and impaired antiviral responses [42,43]. *S. pneumoniae* was co-detected in upper respiratory specimens from at least 7 of our 111 (6%) hospitalised EV-D68 cases for whom we had complete enhanced surveillance data. While *S. pneumoniae* is believed to enhance RV-induced disease severity, including asthma exacerbation [44], our finding likely reflects background carriage rates of *S. pneumoniae* in children.

EV-D68 was associated with fatal outcome in three BC patients, including two adults with underlying comorbidity and one child in whom bacterial co-infection played a role. Enhanced passive surveillance in BC also detected five cases (four paediatric) of EV-D68-associated neurological illness, all with preceding respiratory symptoms and prolonged neurological deficit. The number of EV-D68 cases with neurological manifestations or fatal outcome detected in BC is higher per capita than reported elsewhere during the 2014 epidemic [20,23,24]. This likely reflects the centralised, province-wide laboratory screening and enhanced public health follow-up that was undertaken compared to other cluster-driven analyses, although we cannot rule out true regional differences. Other EVs are known causes of neurological disease, particularly poliovirus and EV-A71, but also EV-D94, which is closely related to EV-D68 [45,46]. However, only two cases of EV-D68-associated neurological illness had been documented globally prior to the 2014 epidemic, both from the US. One involved acute flaccid paralysis in a young adult in 2005 and the second involved a fatal meningomyeloencephalitis in a child in 2008 [3,47]. EV-D68 was detected in the CSF of both these cases [3,47].

Clinical features in our neurological case series are consistent with reports elsewhere in 2014. They include acute flaccid limb weakness, bulbar weakness, and/or cranial nerve dysfunction in association with the detection of EV-D68 in respiratory specimens [19-24]. Also similar to other reports, magnetic resonance imaging findings showed grey matter involvement in multiple spinal levels but mostly affecting the cervical spine [19-24]. EV-D68 was detected in whole blood from one BC patient, but in the absence of EV-D68 detection in CSF specimens, a causal role for EV-D68 remains unproven in this case series, as elsewhere in 2014 [19-24]. It should be noted, however, that recovery of poliovirus from the CSF has also only rarely been reported in cases of paralytic poliomyelitis [48]. The finding of detectable *M. pneumoniae* IgM in three of four neurological cases described here is intriguing given similar serologic findings in a small proportion of other recent EV-D68 neurological case reports [20,21] and the independent association between *M. pneumoniae* and neurological illness [49,50]. Like other recent reports [20,21], however, *M. pneumoniae* was not detected in respiratory or CSF specimens suggesting that antibody findings we report likely reflect prior infection or potentially a false-positive or cross-reactive antibody response.

Phylogenetic analysis of EV-D68 viruses from both outpatients and inpatients showed clade B viruses predominated in Canada during the 2014 epidemic period, clustering with recent (2013–2014) sequences from the US and elsewhere but distinct from more historical sequences [9,12]. Increased variability in the VP1 region, inclusive of the immunogenic B-C and D-E loops, may have enabled EV-D68 to escape antibody recognition [2,8,9,12,34]. We did not identify mutations

or clustering by severe outcome, but full genome analysis would be required to assess other neurotropic or virulence markers, such as in the 5'-untranslated region [7,12,51].

There are limitations to our analyses, foremost related to surveillance methods. Heightened awareness through media and other clinician communications likely influenced patient and provider behaviours related to care-seeking, index of suspicion and testing during the 2014 epidemic period. Nevertheless, neither will the surveillance systems have captured all cases nor will the findings reflect true incidence or disease burden. Small numbers limit our power to test statistical associations. A causal versus contributory or coincidental role for EV-D68 in severe illness cannot be concluded; investigations and their timing were mostly at clinician discretion and other aetiologies, including co-infection, may have been under-recognised. We did not assess other types of EV among EV/RV-positive specimens to compare with the EV-D68 experience. Molecular diagnostic testing for EV-D68 was not routinely performed historically and EV-D68 typing assays were developed and validated real-time in response to the evolving 2014 epidemic, also influencing comparisons across space and time. Laboratory protocols showed comparable performance in a national validation study, and all specimens included in the SPSN analysis were collected within seven days of ILI onset (71% within four days); nevertheless, other variation in specimen collection (e.g. type, viral load), handling, transport and processing may have influenced detection rates between participating provinces. These considerations are, however, relevant to all laboratory-based surveillance. Epidemiological data collection and/or reporting were incomplete particularly when drawn from electronic medical records (as per enhanced passive surveillance) rather than from direct patient/clinician interview (as per active sentinel surveillance). Analyses restricted to patients with known information will have underestimated the proportion with some risk factors/conditions.

Despite these limitations, the dual surveillance approaches we report suggest generalised increase in EV-D68-associated outpatient illness across a broad age distribution during the 2014 epidemic period. Severe respiratory and neurological illness requiring hospitalisation predominantly, but not exclusively, affected children, with possible fatal outcome among those with comorbidity or co-infection. Active surveillance, including both outpatient and inpatient settings, is needed from more areas and additional seasons to further inform EV-D68 incidence, spectrum of illness, and potential at-risk groups for severe or unusual outcomes.

Acknowledgements

Funding for the Canadian SPSN was provided by the Canadian Institutes of Health Research (grant # TPA-90193), British Columbia Centre for Disease Control, Alberta Health and Wellness, Ministère de la santé et des services sociaux du Québec, Institut national de santé publique du Québec, and the Public Health Agency of Canada.

The authors would like to thank the patients and their families who contributed to the SPSN and enhanced passive surveillance data; the public health nurses and epidemiologists in the regional health authorities in BC who completed the enhanced surveillance case report forms; the sentinel physicians participating in the Canadian SPSN in BC, Alberta, and Quebec; the provincial coordinators for the Canadian SPSN at the BC Centre for Disease Control, TARRANT Viral Watch in Alberta, and the Institut national de santé publique du Quebec; the laboratory technicians who conducted the EV-D68 sequencing and serotyping at the BC Public Health Microbiology and Reference Laboratory, the Alberta Provincial Laboratory (namely Kanti Pabbaraju and Sallene Wong), and the Quebec Provincial Laboratory and the input into EV-D68 surveillance from national partners in other provinces and the Public Health Agency of Canada. We gratefully acknowledge the authors, originating and submitting laboratories of the sequences obtained from GenBank used in phylogenetic analyses.

Conflicts of interest

MK has received research grants from Roche, Merck, Hologic, Siemens and Boerhinger Ingelheim for unrelated studies. All other authors declare that they have no conflicts of interest.

Authors' contributions

Conception or design of the work: DMS, CC, GDS, JAD, FRD, TW; data acquisition: DMS, MM, RG, SP, DH, SR, SA, GDS, JAD; data/specimen analysis: DMS, CC, SS, GDS, RT, KF, SJD, CM, PT, MK; interpretation of the data for the work: DMS, CC, SS, MM, RG, SP, DH, SR, SA, GDS, JAD, RT, KF, SJD, CM, PT, MK; drafting the work or revising it critically for important intellectual content: All; approval of manuscript submission: All.

References

1. ObersteMS, MaherK, SchnurrD, FlemisterMR, LovchikJC, PetersH, et al. Enterovirus 68 is associated with respiratory illness and shares biological features with both the enteroviruses and the rhinoviruses. J Gen Virol. 2004;85(Pt 9):2577-84.DOI: 10.1099/vir.0.79925-0 PMID: 15302951
2. ImamuraT, OshitaniH. Global reemergence of enterovirus D68 as an important pathogen for acute respiratory infections.Rev Med Virol. 2015;25(2):102-14.DOI: 10.1002/rmv.1820 PMID: 25471236
3. Centers for Disease Control and Prevention,KhetsurianiN, Lamonte-FowlkesA, OberstS, PallanschMA. Enterovirus surveillance--United States, 1970-2005.MMWR Surveill Summ. 2006;55(8):1-20.PMID: 16971890
4. SchiebleJH, FoxVL, LennetteEH. A probable new human picornavirus associated with respiratory diseases.Am J Epidemiol. 1967;85(2):297-310.PMID: 4960233
5. Centers for Disease Control and Prevention (CDC),. Clusters of acute respiratory illness associated with human enterovirus 68--Asia, Europe, and United States, 2008-2010.MMWR Morb Mortal Wkly Rep. 2011;60(38):1301-4.PMID: 21956405
6. HasegawaS, HiranoR, Okamoto-NakagawaR, IchiyamaT, ShirabeK. Enterovirus 68 infection in children with asthma attacks: virus-induced asthma in Japanese children.Allergy. 2011;66(12):1618-20.DOI: 10.1111/j.1398-9995.2011.02725.x PMID: 21958204
7. LauingerIL, BibleJM, HalliganEP, AaronsEJ, MacMahonE, TongCY. Lineages, sub-lineages and variants of enterovirus 68 in recent outbreaks.PLoS One. 2012;7(4):e36005.DOI: 10.1371/journal.pone.0036005 PMID: 22536453

8. MeijerA, van der SandenS, SnijdersBE, Jaramillo-GutierrezG, BontL, van der EntCK, et al. Emergence and epidemic occurrence of enterovirus 68 respiratory infections in The Netherlands in 2010. Virology. 2012;423(1):49-57.DOI: 10.1016/j.virol.2011.11.021 PMID: 22177700
9. MeijerA, BenschopKS, DonkerGA, van der AvoortHG. Continued seasonal circulation of enterovirus D68 in the Netherlands, 2011-2014.Euro Surveill. 2014;19(42):20935.DOI: 10.2807/1560-7917.ES2014.19.42.20935 PMID: 25358039
10. Rahamat-LangendoenJ, Riezebos-BrilmanA, BorgerR, van der HeideR, BrandenburgA, SchölvinckE, et al. Upsurge of human enterovirus 68 infections in patients with severe respiratory tract infections. J Clin Virol. 2011;52(2):103-6.DOI: 10.1016/j.jcv.2011.06.019 PMID: 21802981
11. RenoisF, BouinA, AndreolettiL. Enterovirus 68 in pediatric patients hospitalized for acute airway diseases.J Clin Microbiol. 2013;51(2):640-3.DOI: 10.1128/JCM.02640-12 PMID: 23224095
12. TokarzR, FirthC, MadhiSA, HowieSR, WuW, SallAA, et al. Worldwide emergence of multiple clades of enterovirus 68. J Gen Virol. 2012;93(Pt 9):1952-8.DOI: 10.1099/vir.0.043935-0 PMID: 22694903
13. MidgleyCM, JacksonMA, SelvaranganR, TurabelidzeG, ObringerE, JohnsonD, et al. Severe respiratory illness associated with enterovirus D68 - Missouri and Illinois, 2014. MMWR Morb Mortal Wkly Rep. 2014;63(36):798-9.PMID: 25211545
14. Centers for Disease Control and Prevention (CDC). Enterovirus D68 in the United States, 2014. Atlanta: CDC. [Accessed 3 August 2015]. Available from: http://www.cdc.gov/non-polio-enterovirus/about/ev-d68.html
15. BragstadK, JakobsenK, RojahnAE, SkramMK, VainioK, Holberg-PetersenM, et al. High frequency of enterovirus D68 in children hospitalised with respiratory illness in Norway, autumn 2014. Influenza Other Respir Viruses. 2015;9(2):59-63. DOI: 10.1111/irv.12300 PMID: 25534826
16. ESCV-ECDC EV-D68 study group,PoelmanR, SchuffeneckerI, Van Leer-ButerC, JossetL, NiestersHG, LinaB. European surveillance for enterovirus D68 during the emerging North-American outbreak in 2014.J Clin Virol. 2015;71:1-9.DOI: 10.1016/j.jcv.2015.07.296 PMID: 26364237
17. MidgleySE, ChristiansenCB, PoulsenMW, HansenCH, FischerTK. Emergence of enterovirus D68 in Denmark, June 2014 to February 2015.Euro Surveill. 2015;20(17):21105. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21105DOI: 10.2807/1560-7917.ES2015.20.17.21105 PMID: 25955773
18. ReicheJ, BöttcherS, DiedrichS, BuchholzU, BudaS, HaasW, et al. Low-level circulation of enterovirus D68-associated acute respiratory infections, Germany, 2014. Emerg Infect Dis. 2015;21(5):837-41.DOI: 10.3201/eid2105.141900 PMID: 25898320
19. Division of Viral Diseases, National Centers for Immunization and Respiratory Diseases, CDC; Division of Vector-Borne Diseases, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, CDC; Children's Hospital Colorado; Council of State and Territorial Epidemiologists,LeshemE. Notes from the field: acute flaccid myelitis among persons aged ≤21 years - United States, August 1-November 13, 2014. MMWR Morb Mortal Wkly Rep. 2015;63(53):1243-4.PMID: 25577990
20. GreningerAL, NaccacheSN, MessacarK, ClaytonA, YuG, SomasekarS, et al. A novel outbreak enterovirus D68 strain associated with acute flaccid myelitis cases in the USA (2012-14): a retrospective cohort study. Lancet Infect Dis. 2015;15(6):671-82.DOI: 10.1016/S1473-3099(15)70093-9 PMID: 25837569
21. Centers for Disease Control and Prevention (CDC),AyscueP, Van HarenK, SheriffH, WaubantE, WaldronP, YagiS, et al. . Acute flaccid paralysis with anterior myelitis - California, June 2012-June 2014.MMWR Morb Mortal Wkly Rep. 2014;63(40):903-6. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6340a6.htm?s_cid=mm6340a6_wPMID: 25299608
22. Centers for Disease Control and Prevention (CDC),PastulaDM, AliabadiN, HaynesAK, MessacarK, SchreinerT, MaloneyJ, et al. . Acute neurologic illness of unknown etiology in children - Colorado, August-September 2014.MMWR Morb Mortal Wkly Rep. 2014;63(40):901-2.PMID: 25299607
23. LangM, MirandA, SavyN, HenquellC, MaridetS, PerignonR, et al. Acute flaccid paralysis following enterovirus D68 associated pneumonia, France, 2014. Euro Surveill. 2014;19(44):20952. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20952DOI: 10.2807/1560-7917.ES2014.19.44.20952 PMID: 25394254
24. PfeifferHC, BragstadK, SkramMK, DahlH, KnudsenPK, ChawlaMS, et al. Two cases of acute severe flaccid myelitis associated with enterovirus D68 infection in children, Norway, autumn 2014. Euro Surveill. 2015;20(10):21062. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21062DOI: 10.2807/1560-7917.ES2015.20.10.21062 PMID: 25788251
25. Statistics Canada. Population and dwelling counts, for Canada, provinces and territories, 2011 and 2006 censuses. Ottawa: Statistics Canada; 2015. Available from: http://www12.statcan.gc.ca/census-recensement/2011/dp-pd/hlt-fst/pd-pl/Table-Tableau.cfm?LANG=Eng&T=101&S=50&O=A
26. SkowronskiDM, ChambersC, SabaiducS, De SerresG, WinterAL, DickinsonJA, et al. Integrated sentinel surveillance linking genetic, antigenic, and epidemiologic monitoring of influenza vaccine-virus relatedness and effectiveness during the 2013-2014 influenza season. J Infect Dis. 2015;212(5):726-39.DOI: 10.1093/infdis/jiv177 PMID: 25784728
27. PabbarajuK, WongS, WongAA, TellierR. Detection of enteroviruses and parechoviruses by a multiplex real-time RT-PCR assay.Mol Cell Probes. 2015;29(2):81-5.DOI: 10.1016/j.mcp.2015.02.001 PMID: 25681753
28. DrewsSJ, SimmondsK, UsmanHR, YeeK, FathimaS, TipplesG, et al. Characterization of enterovirus activity, including that of enterovirus D68, in pediatric patients in Alberta, Canada, in 2014. J Clin Microbiol. 2015;53(3):1042-5.DOI: 10.1128/JCM.02982-14 PMID: 25588657
29. CoirasMT, AguilarJC, GarcíaML, CasasI, Pérez-BreñaP. Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays.J Med Virol. 2004;72(3):484-95.DOI: 10.1002/jmv.20008 PMID: 14748074
30. PabbarajuK, WongS, ChanENY, TellierR. Genetic characterization of a Coxsackie A9 virus associated with aseptic meningitis in Alberta, Canada in 2010.Virol J. 2013;10(1):93. Available from: http://www.virologyj.com/content/10/1/93DOI: 10.1186/1743-422X-10-93 PMID: 23521862
31. GregoryJB, LitakerRW, NobleRT. Rapid one-step quantitative reverse transcriptase PCR assay with competitive internal positive control for detection of enteroviruses in environmental samples.Appl Environ Microbiol. 2006;72(6):3960-7.DOI: 10.1128/AEM.02291-05 PMID: 16751503
32. NixWA, ObersteMS, PallanschMA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens.J Clin Microbiol. 2006;44(8):2698-704.DOI: 10.1128/JCM.00542-06 PMID: 16891480
33. HatchetteTF, DrewsSJ, GrudeskiE, BoothT, MartineauC, DustK, et al. Detection of enterovirus D68 in Canadian laboratories. J Clin Microbiol. 2015;53(5):1748-51.DOI: 10.1128/JCM.03686-14 PMID: 25740765
34. ImamuraT, OkamotoM, NakakitaS, SuzukiA, SaitoM, TamakiR, et al. Antigenic and receptor binding properties of enterovirus 68. J Virol. 2014;88(5):2374-84.DOI: 10.1128/JVI.03070-13 PMID: 24371050
35. TamuraK, StecherG, PetersonD, FilipskiA, KumarS. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0.Mol Biol Evol. 2013;30(12):2725-9.DOI: 10.1093/molbev/mst197 PMID: 24132122
36. BC Stats. Population estimates for 2014. Victoria: BC Stats. [Accessed 3 August 2015]. Available from: http://www.bcstats.gov.bc.ca/StatisticsBySubject/Demography/PopulationEstimates.aspx
37. SherwoodMD, GanttS, ConnollyM, DobsonS. Acute flaccid paralysis in a child infected with enterovirus D68: a case report.B C Med J. 2014;56:495-8. Accessed 3 August 2015. Available from: http://www.bcmj.org/sites/default/files/BCMJ_56_Vol10_flaccid_paralysis.pdf
38. JiangL, LeeVJ, LimWY, ChenMI, ChenY, TanL, et al. Performance of case definitions for influenza surveillance. Euro Surveill. 2015;20(22):21145.DOI: 10.2807/1560-7917.ES2015.20.22.21145 PMID: 26062645
39. Centers for Disease Control and Prevention (CDC). Manual for the surveillance of vaccine-preventable diseases. Enhancing Surveillance, Chapter 19. 5th ed., 2011. Atlanta: CDC. [Accessed 24 September 2015]. Available from: http://www.cdc.gov/vaccines/pubs/surv-manual/index.html
40. ChenY, JohansenH, ThillaiampalamS, SambellC. Asthma. Statistics Canada Catalogue no. 82-003.Health Rep. 2005;16:43-6.
41. KynykJA, MastronardeJG, McCallisterJW. Asthma, the sex difference.Curr Opin Pulm Med. 2011;17(1):6-11.DOI: 10.1097/MCP.0b013e3283410038 PMID: 21045697
42. BusseWW, LemanskeRF, GernJE. Role of viral respiratory infections in asthma and asthma exacerbations.Lancet.

2010;376(9743):826-34.DOI: 10.1016/S0140-6736(10)61380-3 PMID: 20816549

43. JacksonDJ, JohnstonSL. The role of viruses in acute exacerbations of asthma.J Allergy Clin Immunol. 2010;125(6):1178-87, quiz 1188-9.DOI: 10.1016/j.jaci.2010.04.021 PMID: 20513517
44. KloepferKM, LeeWM, PappasTE, KangTJ, VrtisRF, EvansMD, et al. Detection of pathogenic bacteria during rhinovirus infection is associated with increased respiratory symptoms and asthma exacerbations. J Allergy Clin Immunol. 2014;133(5):1301-7, 1307.e1-3.PMID: 24698319
45. Richman DD, Whitley RJ, Hayden FG. Clinical Virology. New York: Churchill Livingston, 1997.
46. SmuraTP, JunttilaN, BlomqvistS, NorderH, KaijalainenS, PaananenA, et al. Enterovirus 94, a proposed new serotype in human enterovirus species D. J Gen Virol. 2007;88(Pt 3):849-58.DOI: 10.1099/vir.0.82510-0 PMID: 17325357
47. KreuterJD, BarnesA, McCarthyJE, SchwartzmanJD, ObersteMS, RhodesCH, et al. A fatal central nervous system enterovirus 68 infection. Arch Pathol Lab Med. 2011;135(6):793-6.PMID: 21631275
48. Centers for Disease Control and Prevention (CDC). Epidemiology and Prevention of Vaccine-Preventable Diseases. Hamborsky J, Kroger A, Wolfe S, eds. 13th ed. Washington D.C. Public Health Foundation, 2015. p. 299. Atlanta: CDC. [Accessed 3 August 2015]. Available from: http://www.cdc.gov/vaccines/pubs/pinkbook/downloads/polio.pdf
49. KoskiniemiM. CNS manifestations associated with Mycoplasma pneumoniae infections: summary of cases at the University of Helsinki and review.Clin Infect Dis. 1993;17(Suppl 1):S52-7.DOI: 10.1093/clinids/17.Supplement_1.S52 PMID: 8399938
50. HelyMA, WilliamsonPM, TerentyTR. Neurological complications of Mycoplasma pneumoniae infection.Clin Exp Neurol. 1984;20:153-60.PMID: 6439446
51. NorderH, MagniusL. Can sequence data predict enterovirus D68 infection outcome?Lancet Infect Dis. 2015;15(6):620-1.DOI: 10.1016/S1473-3099(15)70107-6 PMID: 25837570

ESCAIDE registration open until 1 November

Eurosurveillance editorial team [1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Correspondence: Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)

Citation style for this article:
ESCAIDE registration open until 1 November . Euro Surveill. 2015;20(43):pii=30049. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2015.20.43.30049

Article published on 29 Ocotber 2015

The registration for the 11–13 November 2015 European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), organised in Stockholm, Sweden, will close on 1 November. The topics of the plenary sessions this year are:

- Antibiotic resistance: a tragedy of the commons
- Social Media: a toy or a useful tool?
- Ensuring that evidence leads to public health protection - special session on occasion of the 20th EPIET anniversary
- Emerging challenges to vaccine programmes: antigen escape and non-specific immune effects, and
- Public Health Event 2015: Ebola and MERS-CoV – recent advances and remaining challenges.

In addition to the above topics, there will be pre-ESCAIDE symposia on communications during outbreak management and bridging epidemiology to public health policy, on 10 November.

Register here: http://registration.escaide.eu/

Read more here: http://ecdc.europa.eu/en/ESCAIDE/programme/Pages/overview.aspx