

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

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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.

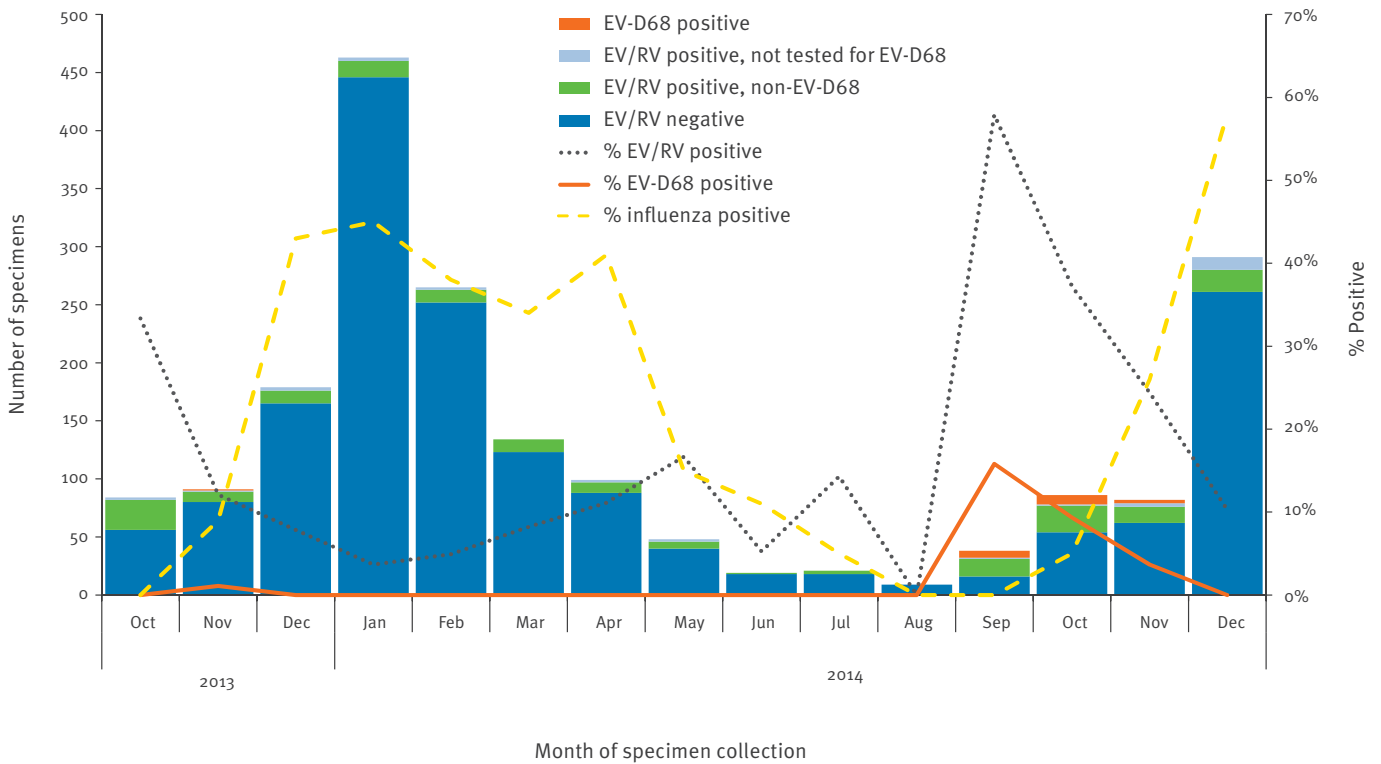
Introduction

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)

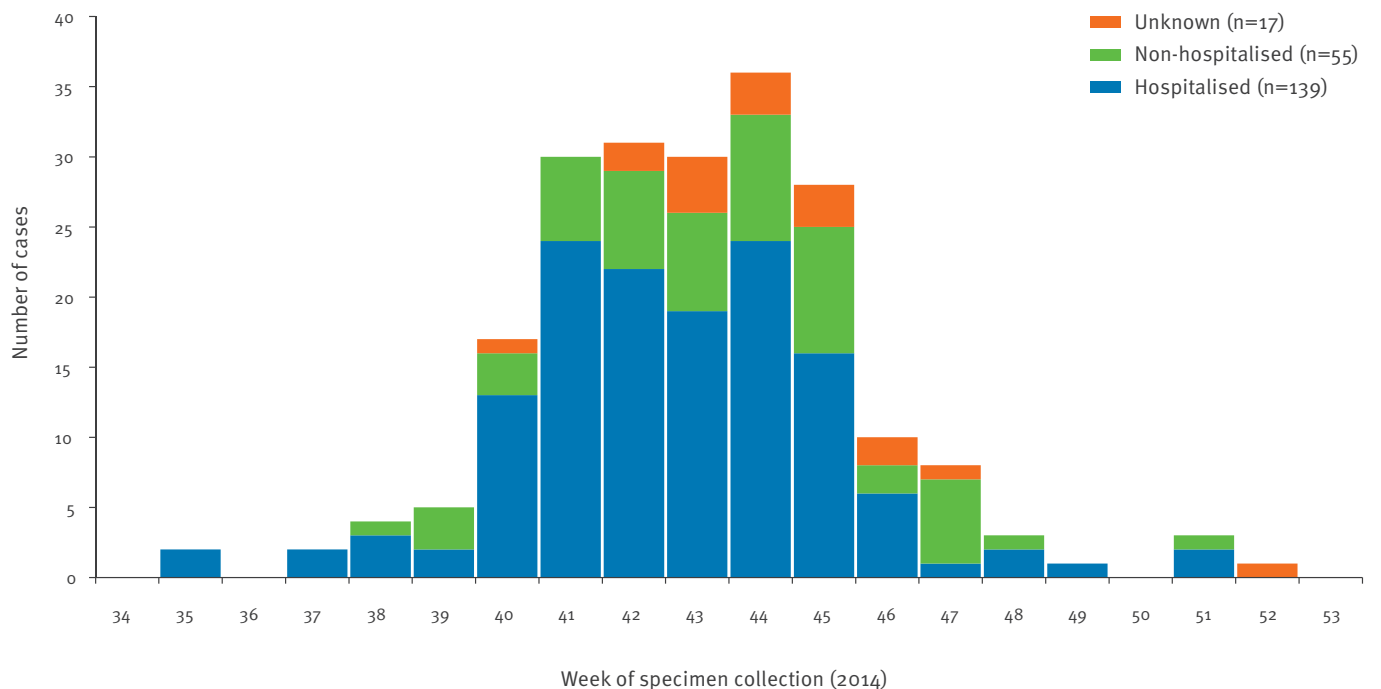


TABLE 1

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.

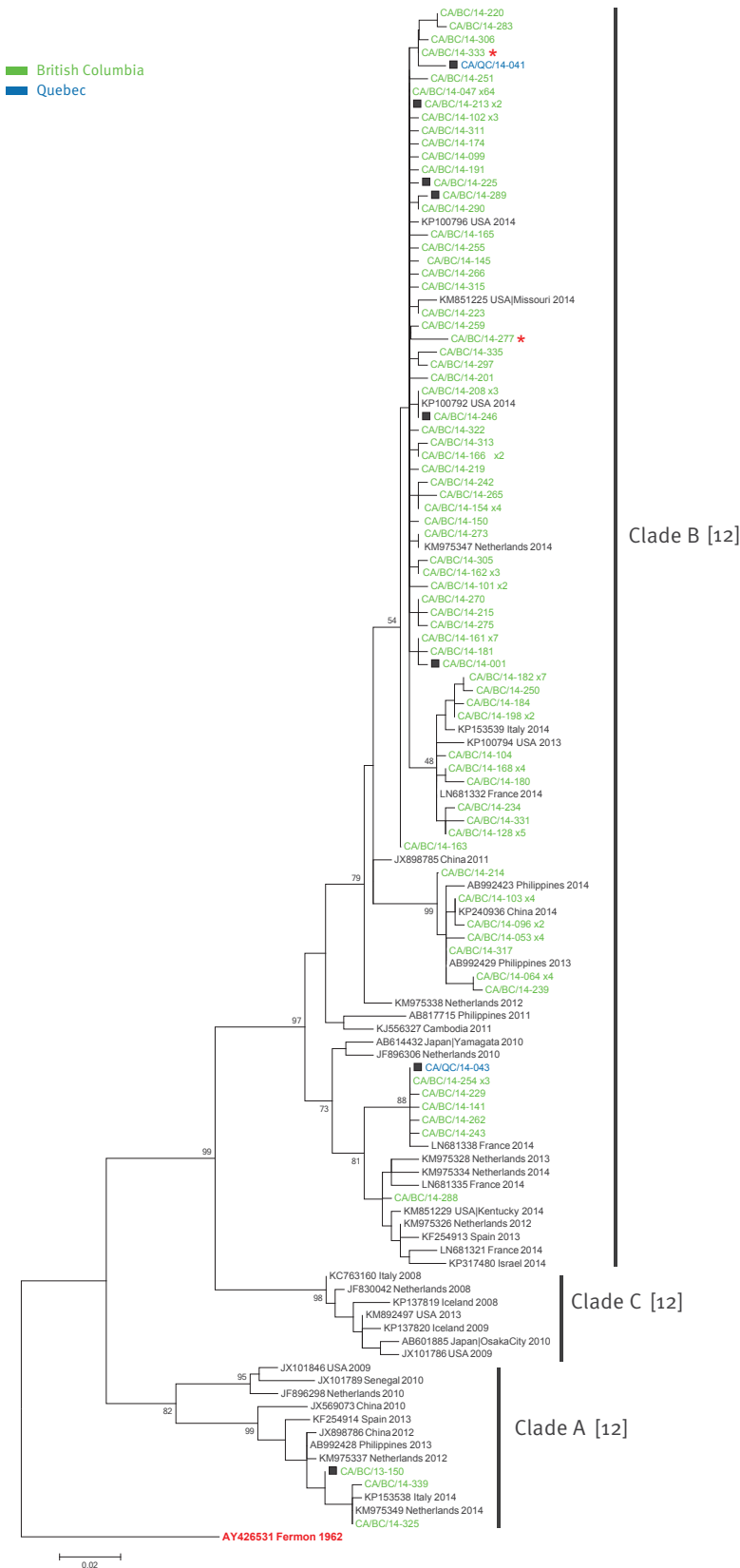
EV-D68 infection is not generally notifiable and laboratory testing for EV-D68 was not widely performed prior to the 2014 outbreak. Consequently, localised clusters of paediatric hospitalisations have largely shaped current understanding of EV-D68 illness, skewing impressions of typical disease severity. 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 VPI 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

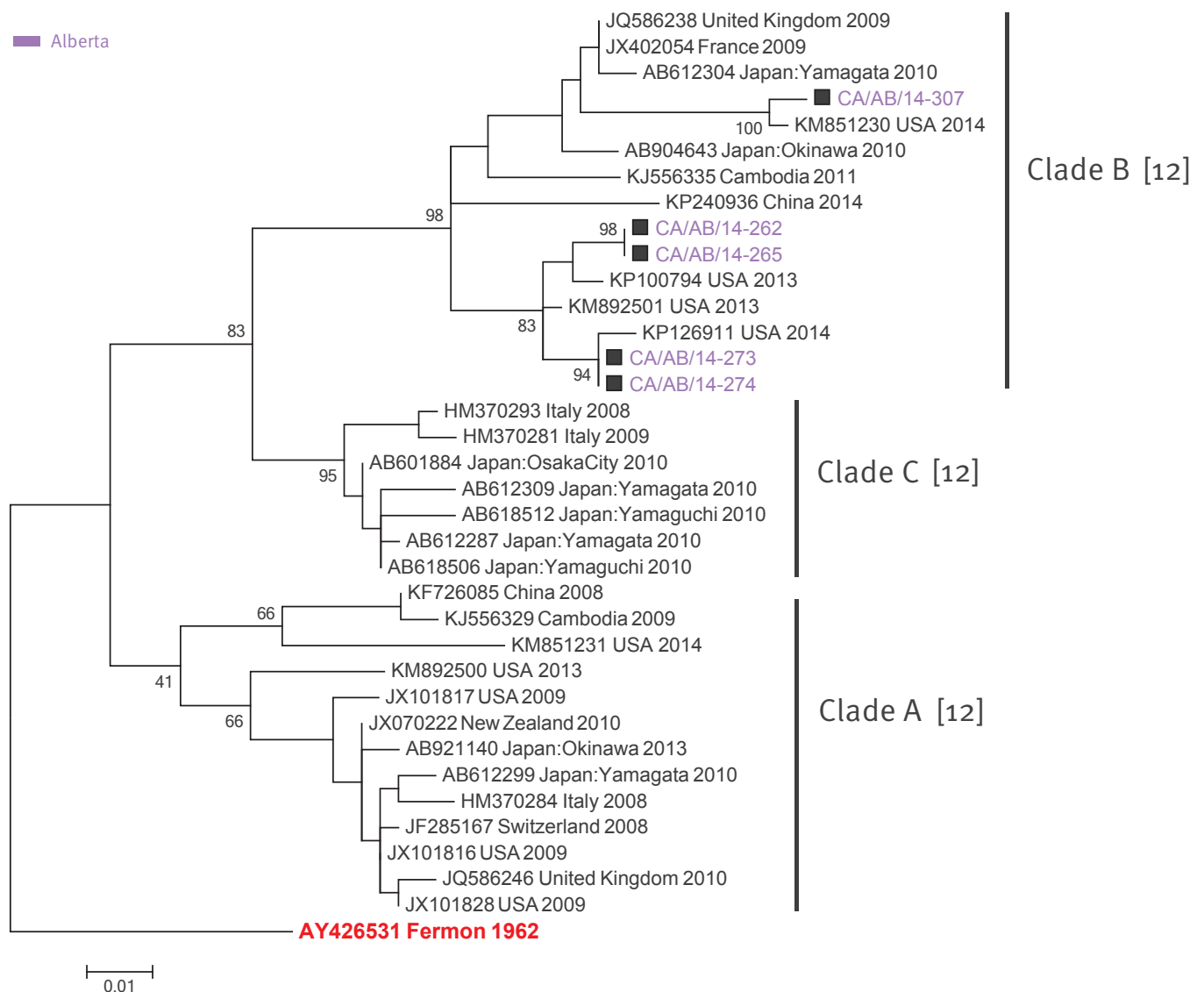


Canadian EV-D68 sequences are colour coded by province of specimen collection, global sequences available in GenBank are shown in black and the Fermon reference strain (1962) is shown in red. Sentinel viruses collected through the Canadian Sentinel Practitioner Surveillance Network are indicated with 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 log₁₀ 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

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
IVIIG 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.

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Conflicts of interest

MK has received research grants from Roche, Merck, Hologic, Siemens and Boehringer 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.

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