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Start of the 2014/15 influenza season in Europe: drifted influenza A(H3N2) viruses circulate as dominant subtype

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The influenza season 2014/15 started in Europe in week 50 2014 with influenza A(H3N2) viruses predominating. The majority of the A(H3N2) viruses characterised antigenically and/or genetically differ from the northern hemisphere vaccine component which may result in reduced vaccine effectiveness for the season. We therefore anticipate that this season may be more severe than the 2013/14 season. Treating influenza with antivirals in addition to prevention with vaccination will be important.

Influenza activity started increasing in the western part of the World Health Organization (WHO) European Region during week 50 2014, when Malta, the Netherlands and Sweden reported medium intensity of influenza activity which refers to usual activity of influenza season [1]. Rates of influenza-like illness (ILI) and/or acute respiratory infection (ARI) have continued to increase, and in week 2 2015, 13 countries (Albania, Finland, France, Greece, Iceland, Malta, the Netherlands, Portugal, Slovenia, Spain, Sweden, Switzerland, the United Kingdom (UK)) in the WHO European Region reported medium intensity and Albania, the Netherlands, Portugal, Spain and Switzerland had ILI rates above the epidemic threshold for the pre-season [2]. Of the 13 countries reporting medium intensity, six (Finland, the Netherlands, Portugal, Slovenia, Sweden and the UK (England)) reported patterns of widespread activity with laboratory-confirmed influenza cases in 50% or more of their administrative units (or reporting sites).

Influenza surveillance in Europe

Since October 2014, all 53 Member States of the WHO European Region report their epidemiological and virological influenza surveillance data to The European Surveillance System (TESSy), hosted by the European Centre for Disease Prevention and Control (ECDC) [1]. The data are jointly published with the WHO European Regional Office to describe the annual occurrence of

influenza (timing and spread), its impact and severity (groups which are most affected), the predominating influenza type and subtype, as well as analyses of virus strains to support the WHO recommendations for the composition of seasonal influenza vaccines (www.flunewseurope.org). The northern hemisphere influenza vaccine composition recommendation is given by WHO at the end of February each year.

Influenza surveillance in Europe is mainly based on primary care sentinel sites collecting specimens from patients with ILI and/or ARI [1,3]. Data are collected at the national level and reported to the European level according to standardised case definitions [4,5]. The national influenza centres perform antigenic and genetic characterisation of influenza viruses as well as antiviral susceptibility testing of a representative sample of virus isolates.

In addition to the primary care surveillance, particularly since the 2009 influenza A(H1N1) pandemic, hospital surveillance of laboratory-confirmed influenza cases has been conducted, including for this season, in Finland, France, Ireland, Spain, Sweden and the UK. Additionally, sentinel severe acute respiratory infection (SARI) surveillance is in place in 13 countries [1].

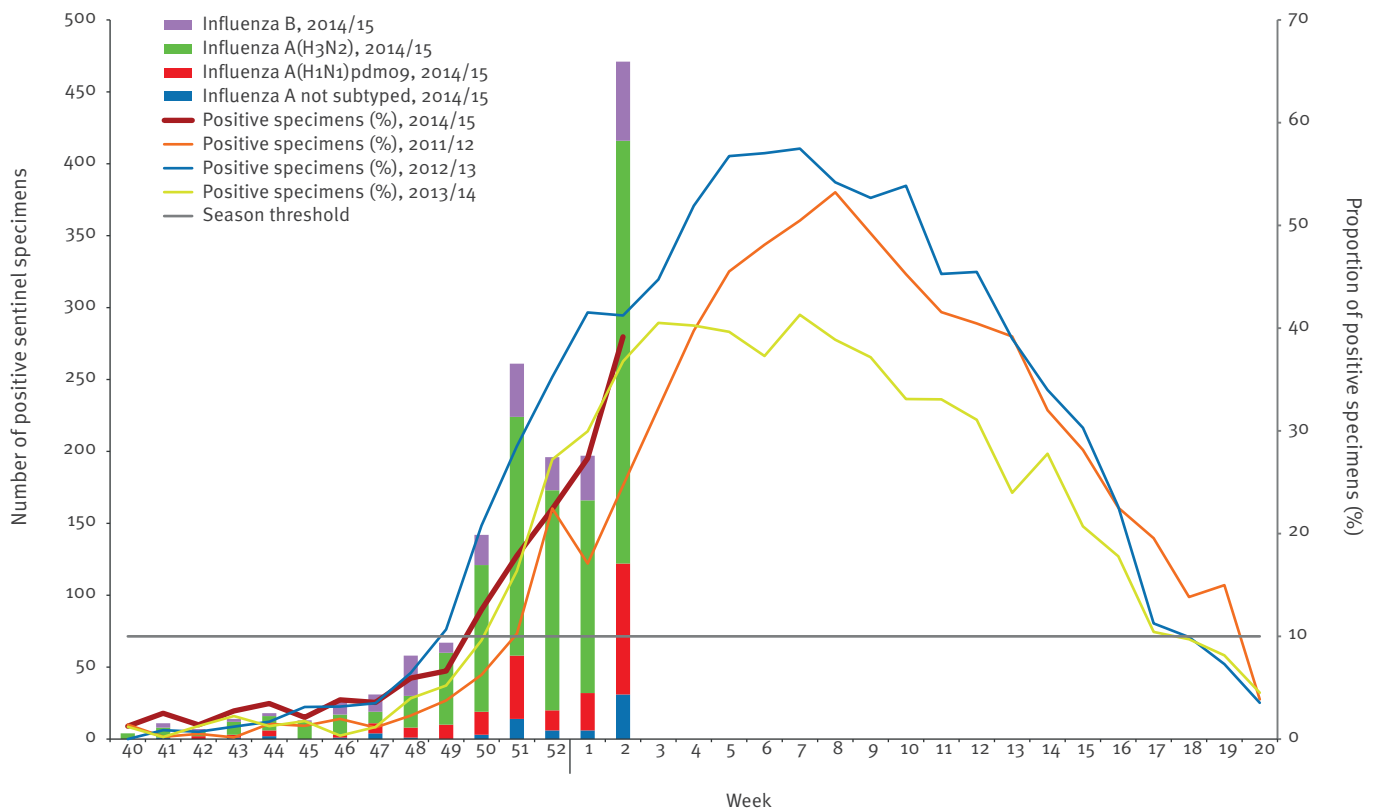
Virological situation in primary healthcare

The overall proportion of influenza-positive sentinel specimens increased from 4% to 39% from week 47 2014 to week 2 2015, indicating the start of the season at a similar time to the previous season (Figure 1). The season threshold of 10% was exceeded in season 2011/12 and 2013/14 in week 51, in 2012/13 in week 49, and in the current season during week 50 (Figure 1).

In most countries, influenza A(H3N2) virus was the dominant subtype in both sentinel and non-sentinel specimens in week 2 2015. In the sentinel systems, since week 40, 1,134 (10%) of the 11,854 specimens

FIGURE 1

Number of influenza virus-positive sentinel specimens by (sub)type and week, and proportion of positive specimens compared to three previous seasons, World Health Organization European Region, weeks 40 2014–2 2015 for season 2014/15



collected in 35 countries tested positive for influenza, 901 (79%) for type A influenza virus and 233 (21%) for type B (Figure 1). Of the 831 type A viruses subtyped, 688 (83%) were A(H3N2) and 143 (17%) were A(H1N1)pdm09 by week 2 2015 (Figure 1). The lineage of 87 type B viruses was determined: six were B/Victoria lineage and 81 B/Yamagata lineage.

The antigenic characteristics of 117 influenza viruses and the genetic characteristics of 202 influenza viruses were reported to TESSy by 16 countries mainly in the western countries of the Region. Of 68 influenza A(H3N2) viruses antigenically characterised, 40 were reported by the national influenza centres as A(H3N2) A/Texas/50/2012-like (vaccine-like) and 26 were A/Switzerland/9715293/2013-like (antigenically different from the vaccine); two viruses could not be ascribed to an antigenic category. All 21 A(H1N1)pdm09 viruses characterised were A/California/7/2009-like (vaccine strain). Of the 30 influenza B viruses characterised, 28 were of the B/Yamagata/16/88-lineage (10 were reported as B/Massachusetts/02/2012-like viruses, one B/Wisconsin/1/2010-like and 17 B/Phuket/3073/2013-like) and two were B/Brisbane/60/2008-like viruses of the Victoria lineage.

Of the 160 genetically characterised A(H3N2) viruses, 110 (69%) fall in two genetic subgroups

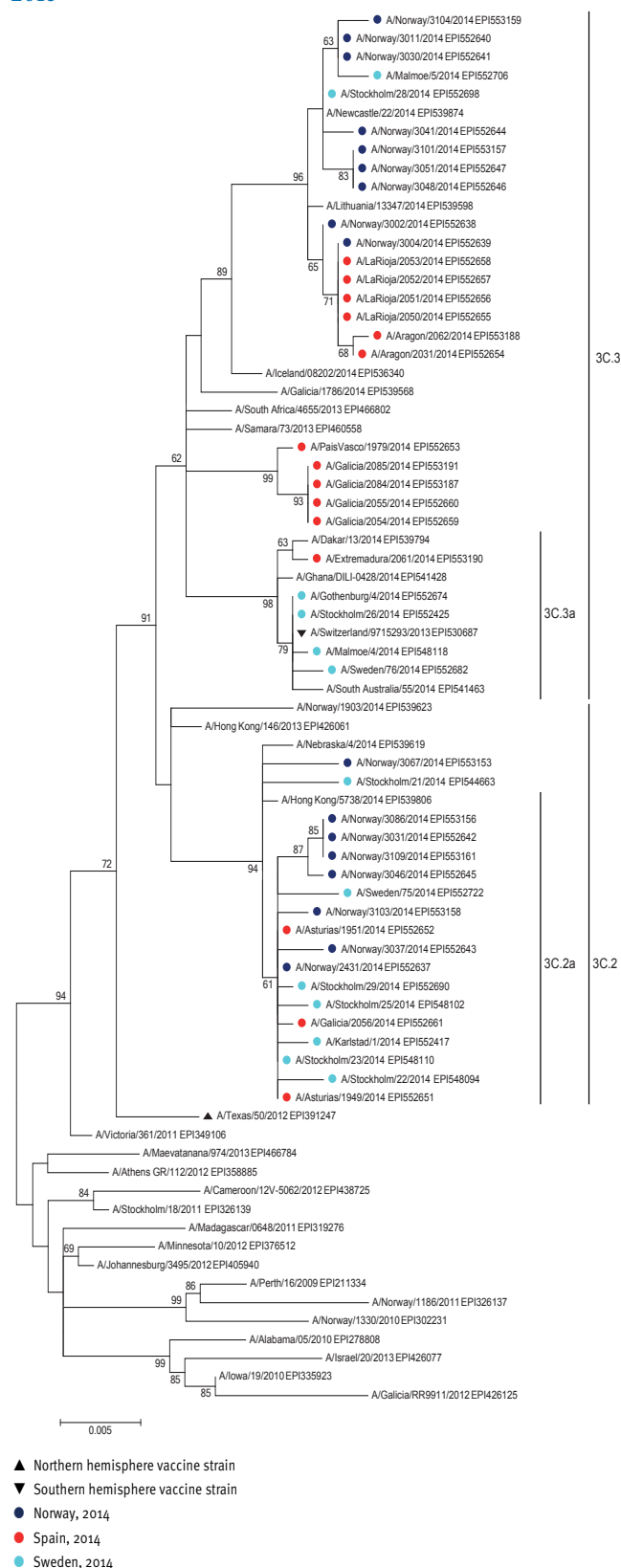
containing antigenic drift variants compared with A/Texas/50/2012, the vaccine component for the northern hemisphere 2014/15 season [6].

For 63 viruses, Norway, Spain and Sweden reported the haemagglutinin gene sequence accession number for the Global Initiative on Sharing All Influenza Data (GISAID) EpiFlu database. The maximum likelihood phylogenetic tree of these viruses together with the A(H3N2) reference viruses shows that the current circulating viruses cluster mainly with the genetic subgroups 3C.3, 3C.3a together with the A/Switzerland/9715293/2013, and 3C.2a with the A/Hong Kong/5738/2014, and show genetic drift from the current vaccine virus (Figure 2). The antigenic drift of viruses clustering with the A/Newcastle/22/2014 has not yet been shown.

Ninety-three influenza A(H3N2) viruses, 20 A(H1N1)pdm09 and four influenza B viruses have been tested phenotypically or genotypically for neuraminidase inhibitor susceptibility. None showed evidence of reduced susceptibility to either oseltamivir or zanamivir.

FIGURE 2

Maximum likelihood phylogenetic tree of haemagglutinin nucleotide sequences (1,063 nucleotides) from influenza A(H3N2) viruses reported to the European Surveillance System and reference A(H3N2) viruses, weeks 40 2014–1 2015



All sequences have been retrieved from GISAID EpiFlu database (accession numbers indicated in the tree)

Laboratory-confirmed hospitalised influenza cases

Current surveillance systems reporting laboratory-confirmed hospitalised influenza cases to TESSy, while not being representative on a population basis in all countries, provide information on groups being hospitalised due to influenza as well as risk factors for severe disease. This season, as of week 40 2014, six countries with a monitoring system for laboratory-confirmed hospitalised influenza cases reported 719 laboratory-confirmed hospitalised cases. In intensive care units (ICU), 671 cases were reported: three in Finland, 101 in France, 20 in Spain, five in Sweden and 542 in the UK. In comparison, for season 2013/14, by week 2 2014, France had reported 77, Ireland two, Spain 227 and Sweden 11 ICU cases. The UK had not reported a single severe case by week 2 2014 and the surveillance system there has not changed.

Of the 719 laboratory-confirmed hospitalised influenza cases, 682 (95%) were positive for influenza A virus (197 subtyped: 149 A(H3N2) and 48 A(H1N1)pdm09) and 37 (5%) for influenza B virus, which reflects the overall predominance of A(H3N2) and co-circulation of the A(H1N1)pdm09 and B viruses.

Of the 671 cases admitted to ICU, 642 (96%) were positive for influenza A virus (170 subtyped: 126 A(H3N2) and 44 A(H1N1)pdm09) and 29 (4%) for influenza B virus. Half of the cases admitted to ICU for which information on age was available (61/128) were aged 65 years or older. The median age at admission to ICU was 64 years (mean 61.6 years, range 1–93 years). In the 2013/14 influenza season (up to week 2 2014 and during the whole season), the majority of ICU cases had been 40–64 years old, with influenza A(H1N1)pdm09 virus as the dominating subtype [7].

Discussion and conclusions

The influenza season in Europe has started and continues to expand according to the clinical, epidemiological and virological indicators. The season is dominated by influenza A(H3N2) viruses, although both A(H1N1)pdm09 and B viruses co-circulate. This is similar to the influenza activity in the other parts of northern hemisphere, e.g. the United States (US), where the influenza activity has continued to increase with influenza A(H3N2) viruses predominating [8].

The last influenza seasons in Europe dominated by A(H3N2) viruses were seasons 2011/12 [9,10] and 2012/13 [3,11], when A(H1N1)pdm09 and A(H3N2) viruses co-dominated. These seasons were estimated as moderately severe based on ILI/ARI consultation rates, although the European Union/European Economic Area (EU/EEA) still lacks agreed criteria for severity of influenza. The current season has started earlier in the US where higher influenza-related hospitalisation rates are being reported as compared with the past A(H3N2)-dominated seasons [12]. As shown for Europe, the 2014/15 season has started at a similar time and

with similar impact in primary care as the previous season. Since A(H3N2)-dominated seasons usually cause more severe outcomes among the elderly and other risk groups than A(H1N1)pdm09 or B seasons [13,14], the current influenza epidemic in Europe is expected to cause an increased number of severe infections, hospitalisations, ICU admissions and deaths in the elderly than the 2013/14 influenza season. This has already been observed in ICU admissions reported from the UK this season in comparison with the previous season.

In September 2014, the WHO consultation and information meeting on the composition of influenza virus vaccines indicated an emergence of two new genetic clades of A(H3N2) viruses (clades 3C.2a and 3C.3a) containing antigenic drift viruses of previously circulating viruses [15]. The US Centers for Disease Control and Prevention subsequently posted a health alert network notification [16], and ECDC issued a risk assessment [17] concerning the continued circulation and transmission of these viruses.

Based on our analysis and the current knowledge of the circulating viruses [18], the northern hemisphere vaccine may not offer desired protection against the circulating A(H3N2) viruses. However, for the A(H1N1)pdm09 and B/Victoria lineage viruses, only limited drift has been observed and protection against the circulating influenza A(H1N1)pdm09 viruses is still conferred by the vaccine.

The vaccine effectiveness for this season for the A(H3N2) and possibly the B component is expected to be reduced as already seen in the US [19] and in previous seasons in Europe [20,21]. However, the vaccine is anticipated to prevent some infections, improve the course or shorten the duration of influenza in infected individuals, and is likely to reduce the number of severe outcomes and mortality. It therefore remains the measure of choice to prevent severe illness and possibly fatal outcomes in risk groups. The circulating viruses are susceptible to the antiviral drugs oseltamivir and zanamivir and these drugs are therefore an important adjunct in the treatment of influenza.

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Conflict of interest

None declared.

Author's contributions

Broberg E: influenza surveillance data maintenance, data analysis and draft of the manuscript; Snacken R: influenza surveillance data maintenance and analysis and seasonal risk assessment, review of the manuscript; Adlhoch C, Beauté J, Galinska M and Pereyaslov D: influenza surveillance data maintenance and analysis, review of the manuscript; Brown C and Penttinen P: surveillance strategy, critical review of the manuscript.

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Interim estimates of 2014/15 vaccine effectiveness against influenza A(H3N2) from Canada's Sentinel Physician Surveillance Network, January 2015

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The 2014/15 influenza season to date in Canada has been characterised by predominant influenza A(H3N2) activity. Canada's Sentinel Physician Surveillance Network (SPSN) assessed interim vaccine effectiveness (VE) against medically attended, laboratory-confirmed influenza A(H3N2) infection in January 2015 using a test-negative case-control design. Of 861 participants, 410 (48%) were test-positive cases (35% vaccinated) and 451 (52%) were test-negative controls (33% vaccinated). Among test-positive cases, the majority (391; 95%) were diagnosed with influenza A, and of those with available subtype information, almost all influenza A viruses (379/381; 99%) were A(H3N2). Among 226 (60%) A(H3N2) viruses that were sequenced, 205 (91%) clustered with phylogenetic clade 3C.2a, considered genetically and antigenically distinct from the 2014/15 A/Texas/50/2012(H3N2)-like clade 3C.1 vaccine reference strain, and typically bearing 10 to 11 amino acid differences from the vaccine at key antigenic sites of the haemagglutinin protein. Consistent with substantial vaccine mismatch, little or no vaccine protection was observed overall, with adjusted VE against medically attended influenza A(H3N2) infection of -8% (95% CI: -50 to 23%). Given these findings, other adjunct protective measures should be considered to minimise morbidity and mortality, particularly among high-risk individuals. Virus and/or host factors influencing this reduced vaccine protection warrant further in-depth investigation.

Background

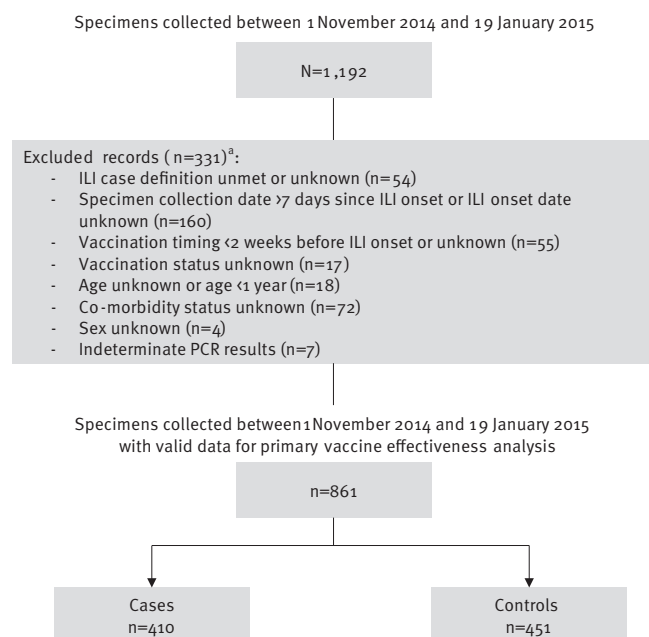
In Canada, the 2014/15 influenza season has been distinguished by an early and intense epidemic due almost exclusively (>90%) to influenza A(H3N2) subtype viruses. Virtually all (>99%) of these A(H3N2) viruses have been characterised as genetically and/or antigenically distinct from the A/Texas/50/2012(H3N2)-like (clade 3C.1) vaccine reference strain used for both the current 2014/15 and prior 2013/14 northern hemisphere influenza vaccines [1].

This profile of dominant influenza A(H3N2) activity is in sharp contrast to the 2013/14 season, when an early epidemic peak also occurred, but was instead due to predominant but antigenically well-conserved A(H1N1) pdm09 viruses [2]. The 2014/15 season more closely resembles that of 2012/13, although the predominant vaccine-mismatched influenza A(H3N2) activity in that season in Canada was related to a different combination of vaccine-virus divergence, notably mutations in that season's egg-adapted vaccine strain used for manufacturing, rather than antigenic drift in circulating viruses [3,4]. In some parts of Canada, an unprecedented number of influenza outbreaks in long-term care facilities (LTCF) were reported in association with vaccine mismatch in 2012/13 [4,5], but the mid-season tally for 2014/15 has already exceeded even that of 2012/13 in some jurisdictions [5].

In response to surveillance signals suggesting suboptimal vaccine performance, Canada's Sentinel Physician

FIGURE 1

Specimen inclusion and exclusion criteria, interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)



ILI: influenza-like illness.

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

Surveillance Network (SPSN) assessed interim influenza vaccine effectiveness (VE) in January 2015. VE findings are presented in the context of in-depth genetic and antigenic characterisation of contributing sentinel influenza A(H3N2) viruses, relevant to the upcoming selection of vaccine strains in February 2015 by the World Health Organization (WHO) for the 2015/16 northern hemisphere influenza vaccine. Findings are also considered in relation to virus-host interactions, notably the effects of influenza vaccination in the previous season on protection by the current season's vaccine.

Methods

Epidemiological estimation of influenza vaccine effectiveness

As previously described [2–4,6,7], a test-negative case-control design was used to estimate VE. Inclusion and exclusion criteria applied to the current dataset are shown in Figure 1. Patients presenting to community-based practitioners at sentinel sites across participating provinces (British Columbia, Alberta, Ontario and Quebec) within seven days of onset of influenza-like illness (ILI) and testing positive for influenza were considered cases; those testing negative were considered controls. ILI was defined as acute onset of respiratory

illness with fever and cough and one or more of the following symptoms: sore throat, arthralgia, myalgia, or prostration. Fever was not an eligibility requirement for elderly adults 65 years and older.

As annual influenza immunisation campaigns typically commence in October across Canada, and increased influenza virus circulation (exceeding 10% test-positivity) typically begins in early November, nasal or nasopharyngeal specimens collected from 1 November 2014 (week 44) were eligible for inclusion in the primary VE analysis. Epidemiological information was obtained from consenting patients or their parent/guardian using a standard questionnaire at specimen collection. Ethics review boards in participating provinces approved this study.

Specimens were tested for influenza A (by subtype) and B viruses at provincial reference laboratories using real-time RT-PCR. Odds ratios (OR) for medically attended, laboratory-confirmed influenza by self-reported vaccination status were estimated by multivariable logistic regression. VE was calculated as $(1 - \text{OR}) \times 100\%$. Vaccine was administered to participants during the seasonal immunisation campaign. Non-adjuvanted, inactivated, split trivalent influenza vaccine (TIV) is primarily used in Canada. Live attenuated influenza vaccine (LAIV) is approved for individuals two to 59 years-old, including the trivalent but for the first time in Canada also the quadrivalent formulation, and was publicly funded in the SPSN provinces of British Columbia, Alberta and Quebec. An adjuvanted subunit TIV is approved for elderly Canadians and publicly funded in British Columbia and Ontario. Participants who received seasonal 2014/15 influenza vaccine at least two weeks before ILI onset were considered vaccinated. Those for whom vaccination timing was unknown or less than two weeks before ILI onset were excluded from primary analysis but explored in sensitivity analyses, as were participants whose comorbidity status was unknown. The effects of prior 2013/14 influenza vaccine receipt on current vaccine protection were explored through indicator variable analysis.

Influenza vaccine manufacturers require an egg-adapted, high-growth reassortant (HGR) version of the reference strain recommended by WHO for further high-yield propagation in embryonated hens' eggs. The HGR version of the WHO-recommended A/Texas/50/2012(H3N2) reference strain [8] used by manufacturers for both the 2014/15 and 2013/14 northern hemisphere influenza vaccines is called X-223A and differs from the A/Texas/50/2012(H3N2) prototype by three amino acids (aa) in antigenic sites of the haemagglutinin (HA) protein.

Laboratory characterisation of contributing sentinel viruses

The HA1 and HA2 regions of the HA gene from a convenience sample of sentinel influenza A(H3N2) viruses

TABLE 1

Reference haemagglutinin sequences obtained from the EpiFlu database of the Global Initiative on Sharing All Influenza Data and used in phylogenetic analysis, 2014/15 Canadian Sentinel Physician Surveillance Network (n = 13)

Segment ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI539806	Hong Kong (SAR)	30 Apr 2014	A/Hong Kong/5738/2014	Government Virus Unit	National Institute for Medical Research	
EPI539576	Hong Kong (SAR)	26 Feb 2014	A/Hong Kong/4801/2014	Government Virus Unit	National Institute for Medical Research	
EPI426061	Hong Kong (SAR)	11 Jan 2013	A/Hong Kong/146/2013	Government Virus Unit	National Institute for Medical Research	
EPI530647	Norway	3 Feb 2014	A/Norway/466/2014	WHO National Influenza Centre	National Institute for Medical Research	
EPI460558	Russian Federation	12 Mar 2013	A/Samara/73/2013	WHO National Influenza Centre Russian Federation	National Institute for Medical Research	
EPI360950	Germany	3 Jul 2011	A/Berlin/93/2011	National Institute for Medical Research	Centers for Disease Control and Prevention	
EPI530687	Switzerland	6 Dec 2013	A/Switzerland/9715293/2013	Hopital Cantonal Universitaire de Geneves	National Institute for Medical Research	
EPI543062	Switzerland	1 Jan 2013	A/Switzerland/9715293/2013 X-247	New York Medical College	Centers for Disease Control and Prevention	
EPI551814	Australia	1 Jan 2014	IVR-176(A/Switzerland/9715293/2013)	CSL Ltd	WHO Collaborating Centre for Reference and Research on Influenza	Deng,Y-M.; Iannello,P.; Spirason,N.; Jelley,L.; Lau,H.; Komadina,N.
EPI377499	United States	15 Apr 2012	A/Texas/50/2012	Texas Department of State Health Services -Laboratory Services	Centers for Disease Control and Prevention	
EPI407126	United States	1 Jan 2012	A/Texas/50/2012 X-223A	New York Medical College	Centers for Disease Control and Prevention	
EPI349103	Australia	24 Oct 2011	A/Victoria/361/2011	Melbourne Pathology	WHO Collaborating Centre for Reference and Research on Influenza	Deng,Y-M; Caldwell,N; Iannello,P; Komadina,N
EPI358038	Australia	1 Jan 2011	IVR-165(A/Victoria/361/2011)	WHO Collaborating Centre for Reference and Research on Influenza	Centers for Disease Control and Prevention	

WHO: World Health Organization.

from original patient specimens contributing to VE analysis were sequenced for phylogenetic and pairwise aa identity analysis based on antigenic maps spanning the 131 aa residues across HA1 antigenic sites A–E [4,6,7,9]. The approximate likelihood method was used to generate the phylogenetic tree of aligned translated sequences in FastTree [10], visualised in FigTree [11], including representative vaccine reference, HGR and clade-specific HA sequences shown in Table 1, kindly made available by the Global Initiative on Sharing All Influenza Data (GISAID), and using clade nomenclature specified by the European Centre for Disease Prevention and Control (ECDC) [12].

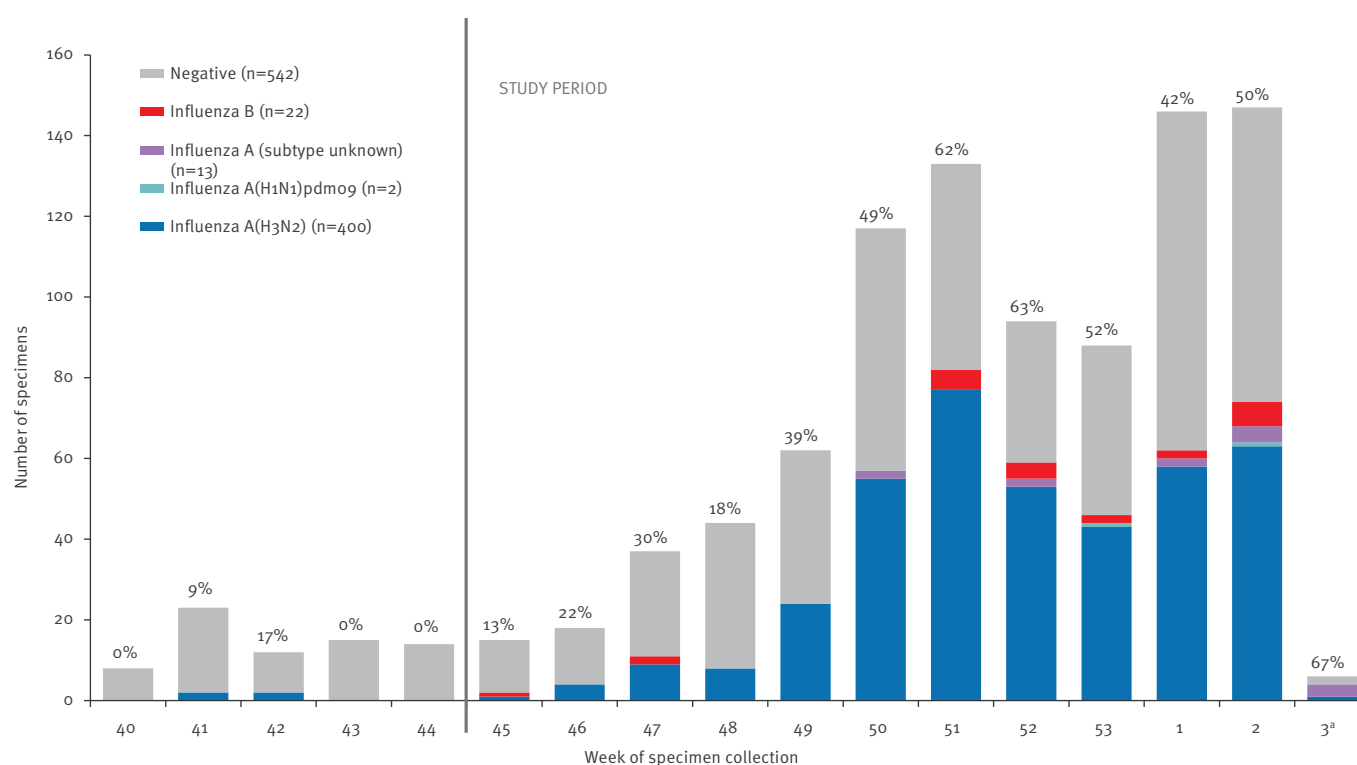
Historically, each new significant antigenic drift variant has, in general, had at least four aa substitutions located in at least two antigenic sites [13]. However, substitutions at antigenic sites A, B and D of the H3

globular head located closest to the receptor-binding site (RBS) are typically considered most influential [14], with site B being emphasised as particularly immunodominant among more recent influenza A(H3N2) strains [15]. Substitutions at just seven antigenic site positions, located in antigenic site A (position 145) and B (positions 155, 156, 158, 159, 189 and 193) have been emphasised in relation to all major A(H3N2) antigenic cluster transitions since 1968 [16]. Substitutions associated with gain or loss of glycosylation may also influence antibody binding [17]. Sequencing findings among sentinel influenza A(H3N2) viruses are thus interpreted within these key antigenic considerations.

A convenience sample of influenza-positive specimens was also inoculated into Madin Darby Canine Kidney (MDCK) (British Columbia, Alberta, Quebec) or Rhesus Monkey Kidney (Ontario) cell culture for virus isolation.

FIGURE 2

Laboratory detections of influenza by week and type/subtype, interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 28 September 2014–19 January 2015 (n = 978)



^a Based on partial week.

Influenza percent positivity by week is shown above bars.

One participant in week 1 had co-infection with influenza A(H3N2) and influenza B; subtotals for influenza A and B will add to more than the total number of influenza positives.

Of the 1,286 nasal or nasopharyngeal specimens collected between week 40 (starting 28 September 2014) and week 3 (starting 18 January 2015), we excluded 308 specimens from the epidemic curve: those failing to meet the influenza-like illness (ILI) case definition or for whom it was unknown (n=58), those whose specimens were collected more than seven days after ILI onset or for whom the interval was unknown (n=173), those whose age was unknown or who were younger than one year (n=20), those with unknown comorbidity status (n=80), those with unknown sex (n=4) and those for whom influenza test results were unavailable or indeterminate (n=9). Specimens were included regardless of the patient's vaccination status or timing of vaccination. Missing collection dates were imputed as the laboratory accession date minus two days, the average time period between collection date and laboratory accession date for records with valid data for both fields.

Note that the epidemic curve displays specimen collection and influenza detections from week 40 and regardless of the patient's vaccination status or timing; as such, tallies do not match those in the text.

Aliquots of virus isolates were submitted to the National Microbiology Laboratory (NML), Canada's influenza reference laboratory, for antigenic characterisation by haemagglutination inhibition (HI) assay using guinea pig erythrocytes [4,18] in relation to the cell-passaged A/Texas/50/2012(H3N2)-like clade 3C.1 vaccine reference strain and the A/Switzerland/9715293/2013(H3N2)-like clade 3C.3a reference strain recommended for the 2015 southern hemisphere vaccine [8]. To address potential neuraminidase-mediated binding of influenza A(H3N2) viruses to erythrocytes, the HI assay was conducted in the presence of 20 nM oseltamivir carboxylate following re-growth of viruses in MDCK-SIAT1 cells [19]. HI titres were recorded as the reciprocal of the highest ferret serum dilution at which inhibition of haemagglutination was detected. Previously, a ≥ 4 -fold reduction in post-infection ferret HI-antibody titre was considered a signal of antigenic distinction between the field

isolate and vaccine reference strain, but this has more recently been revised to a ≥ 8 -fold titre reduction [18]. Due to difficulties this season in growing influenza A(H3N2) viruses to sufficient titres for antigenic characterisation by HI assay in the presence of oseltamivir carboxylate, genetic characterisation by sequencing at the NML and provincial public health laboratories was performed to infer antigenic properties of sentinel viruses, as also reported in national laboratory-based surveillance summaries in the United States [20] and Canada [1] for the current 2014/15 season.

Results

Epidemiological findings

A total of 1,192 specimens were submitted within the VE study period, of which 861 (72%) were included in primary VE analyses with collection dates between 3

TABLE 2

Influenza virus characterisation by type and subtype, interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

Specimen	Alberta n (%)	British Columbia n (%)	Ontario n (%)	Quebec n (%)	Overall n (%)
Total	262	156	228	215	861
Influenza-negative	128 (49)	89 (57)	130 (57)	104 (48)	451 (52)
Influenza-positive	134 (51)	67 (43)	98 (43)	111 (52)	410 (48)
Influenza A ^a	131 (98)	63 (94)	96 (98)	101 (91)	391 (95)
A(H3N2)	130 (99)	57 (90)	95 (99)	97 (96)	379 (97)
A(H1N1)pdm09	0 (0)	0 (0)	1 (1)	1 (1)	2 (1)
Subtype unknown	1 (1)	6 (10)	0 (0)	3 (3)	10 (3)
Influenza B ^a	3 (2)	4 (6)	2 (2)	11 (10)	20 (5)
Antigenic characterisation of A(H3N2) sentinel viruses by HI assay^b					
Total	6	1	0	0	7
A/Texas/50/2012-like ^c	0	0	0	0	0
<4-fold reduced titre	0	0	0	0	0
≥4-fold reduced titre	5	0	0	0	5
≥8-fold reduced titre	5	0	0	0	5
Insufficient volume for HI assay	1	1	0	0	2
A/Switzerland/9715293/2013-like ^c	6	1	0	0	7
<4-fold reduced titre	3	1	0	0	4
≥4-fold reduced titre	3	0	0	0	3
≥8-fold reduced titre	0	0	0	0	0
Genetic characterisation of A(H3N2) sentinel viruses by sequencing					
Total	104	30	28	64	226
Clade 3C.2a	98 (94)	17 (57)	27 (96)	63 (98)	205 (91)
Clade 3C.3x	5 (5)	13 (43)	0 (0)	1 (2)	19 (8)
Clade 3C.3	1 (1)	0 (0)	1 (4)	0 (0)	2 (1)

HI: haemagglutination inhibition.

^a One participant in Quebec had co-infection with influenza A(H3N2) and influenza B; subtotals for influenza A and B will add to more than the total number of influenza positives.

^b 37 additional specimens (34 Alberta, 3 Quebec) submitted to the National Microbiology Laboratory for antigenic characterisation had insufficient titre to characterise by HI assay.

^c In two-way HI assay, anti-sera raised to the cell-passaged A/Switzerland/9715293/2013(H3N2) referent virus inhibited the homologous antigen at a titre of 320, equivalent to the titre in inhibiting the heterologous cell-passaged A/Texas/50/2012(H3N2) antigen. Conversely, anti-sera raised to the A/Texas/50/2012(H3N2) referent strain inhibited homologous antigen at an HI titre of 1280 and the heterologous A/Switzerland/9715293/2013(H3N2) antigen at a titre of 80, a 16-fold titre reduction. These referent strains are antigenically distinct.

November 2014 (week 45: 2–8 November 2014) and 19 January 2015 (week 3: 18–24 January 2015) (Figure 1, Figure 2). Of these, 410 (48%) were test-positive cases and 451 (52%) were test-negative controls. Among test-positive cases, the majority (n=391; 95%) were influenza A, and of those with subtype information available, almost all (379/381; 99%) were A(H3N2) (Figure 2, Table 2).

As in previous SPSN publications, adults 20–49 years-old contributed the largest proportion of specimens (40%) (Table 3) [2–4,6,7]. However, compared with the 2013/14 mid-season analysis [2], a significantly lower proportion of participants in 2014/15 were 20–49 years-old (40% vs 50%; $p < 0.01$), more notable among cases (36% vs 53%; $p < 0.01$) than controls (44% vs

48%; $p > 0.05$). Conversely, a greater proportion of participants were elderly adults 65 years and older (16% vs 8%; $p < 0.01$), again more notable among cases (16% vs 4%; $p < 0.01$) than controls (15% vs 12%; $p > 0.05$) [2]. The proportion of female participants (62%) and those with chronic comorbidity (24%) were comparable to observations in the 2013/14 mid-season analysis (63% and 22%, respectively) [2].

When vaccination status was assessed without regard to timing of ILI onset, 166 of 470 (35%) controls self-reported receipt of the 2014/15 influenza vaccine, comparable to the 2013/14 mid-season analysis (32%) [2] and the most recent influenza immunisation coverage survey for the general adult population in Canada (37%) [21]. Overall, 291 (34%) participants self-reported

TABLE 3A

Profile of participants included in interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

	Distribution by case status n (%)				Vaccination coverage within strata n (%) vaccinated ^a			
	Overall	Cases	Controls	p value ^b	Overall	p value ^b	Cases	Controls
N (%)	861	410 (48)	451 (52)		291 (34)		144 (35)	147 (33)
Age group (years)				0.08		< 0.01		
1–8	102 (12)	48 (12)	54 (12)		18 (18)		12 (25)	6 (11)
9–19	109 (13)	62 (15)	47 (10)		19 (17)		13 (21)	6 (13)
20–49	344 (40)	146 (36)	198 (44)		93 (27)		36 (25)	57 (29)
50–64	172 (20)	87 (21)	85 (19)		64 (37)		36 (41)	28 (33)
≥ 65	134 (16)	67 (16)	67 (15)		97 (72)		47 (70)	50 (75)
Median (range)	39 (1–103)	39 (1–103)	39 (1–94)	0.98	NA		NA	NA
Sex				< 0.01		< 0.01		
Female	533 (62)	228 (56)	305 (68)		201 (38)		90 (39)	111 (36)
Male	328 (38)	182 (44)	146 (32)		90 (27)		54 (30)	36 (25)
Co-morbidity ^c				0.43		< 0.01		
No	655 (76)	307 (75)	348 (77)		180 (27)		86 (28)	94 (27)
Yes	206 (24)	103 (25)	103 (23)		111 (54)		58 (56)	53 (51)
Province				0.11		< 0.01		
Alberta	262 (30)	134 (33)	128 (28)		107 (41)		58 (43)	49 (38)
British Columbia	156 (18)	67 (16)	89 (20)		39 (25)		14 (21)	25 (28)
Ontario	228 (26)	98 (24)	130 (29)		87 (38)		42 (43)	45 (35)
Quebec	215 (25)	111 (27)	104 (23)		58 (27)		30 (27)	28 (27)

ILI: influenza-like illness; LAIV: live attenuated influenza vaccine; NA: not applicable.

^a Participants who received seasonal 2014/15 influenza vaccine at least two weeks before ILI onset were considered vaccinated; participants who received seasonal 2014/15 influenza vaccine less than two weeks before ILI onset were excluded from primary analysis but explored in sensitivity analysis. Vaccination status was based on self/parent/guardian report. Details related to special paediatric dosing requirements was not sought.

^b Differences between cases and controls or vaccinated and unvaccinated participants (based on overall sample to explore potential confounding) were compared using the chi-squared test or Wilcoxon rank-sum test.

^c Chronic co-morbidities that place individuals at higher risk of serious complications from influenza as defined by Canada's National Advisory Committee on Immunization, including heart, pulmonary, renal, metabolic, blood, cancer and immunocompromising conditions or those that compromise management of respiratory secretions, or morbid obesity. Questionnaire answers were 'yes,' 'no,' or 'unknown' without specifying the co-morbidity.

receipt of the 2014/15 vaccine at least two weeks before ILI onset and were considered vaccinated for the purpose of VE analysis. Among vaccinated participants reporting vaccine type, the proportion that received LAIV was 10% (16/165) in those two to 59 years-old and 47% (16/34) in those two to 19 years-old (i.e. all LAIV recipients were two to 19 years-old) (Table 3). The proportion of vaccinated participants overall did not differ significantly between cases and controls (35% vs 33%; $p=0.43$). As observed in previous publications of the SPSN [2-4,6,7], the vast majority of vaccinated participants in 2014/15 were repeat recipients, including 251 of 283 (89%) who had also been vaccinated in 2013/14 and 237 of 269 (88%) also vaccinated in 2012/13.

Crude VE against influenza A was –17% (95% CI: –55 to 12%), and –21% (95% CI: –61 to 9%) against the dominant circulating A(H3N2) viruses (Table 4). With full adjustment for covariates, VE estimates increased to –4% (95% CI: –45 to 25%) and –8% (95% CI: –50 to 23%) for influenza A and A(H3N2), respectively.

Calendar time was the covariate most influential on adjusted VE. In sensitivity analyses, adjusted VE estimates remained within 10% of the primary analysis with confidence intervals slightly wider but consistently overlapping zero (Table 4). Among participants immunised in 2014/15 only, crude and adjusted VE estimates were higher at ca 40–50% (vs unvaccinated participants) compared with those immunised in 2013/14 only or in 2013/14 and 2014/15 (<10%); however, confidence intervals were wide and overlapping with the further reduced sample size (Table 4).

Laboratory findings

In total, 44 of 379 (12%) influenza A(H3N2)-positive specimens were submitted to Canada's NML, of which just seven of 44 (16%), collected between 17 November and 18 December 2014, had sufficient titre for antigenic characterisation by HI assay when tested in the presence of oseltamivir carboxylate. All viruses were considered antigenically distinct from the cell-passaged A/Texas/50/2012-like vaccine reference strain and

TABLE 3B

Profile of participants included in interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

	Distribution by case status n (%)				Vaccination coverage within strata n (%) vaccinated ^a			
	Overall	Cases	Controls	p value ^b	Overall	p value ^b	Cases	Controls
N (%)	861	410 (48)	451 (52)		291 (34)		144 (35)	147 (33)
Collection interval				< 0.01		0.51		
≤ 4 days	642 (76)	337 (82)	305 (68)		213 (33)		118 (35)	95 (31)
5–7 days	219 (25)	73 (18)	146 (32)		78 (36)		26 (36)	52 (36)
Median (range)	3 (0–7)	3 (0–7)	3 (0–7)	< 0.01	NA		NA	NA
Calendar time ^d				< 0.01		0.06		
Week 45–46	31 (4)	5 (1)	26 (6)		5 (16)		1 (20)	4 (15)
Week 47–48	72 (8)	16 (4)	56 (12)		17 (24)		3 (19)	14 (25)
Week 49–50	173 (20)	78 (19)	95 (21)		57 (33)		31 (40)	26 (27)
Week 51–52	217 (25)	135 (33)	82 (18)		84 (39)		51 (38)	33 (40)
Week 53–1	221 (26)	102 (25)	119 (26)		74 (33)		32 (31)	42 (35)
Week 2–3	147 (17)	74 (18)	73 (16)		54 (37)		26 (35)	28 (38)
Received 2014/15 influenza vaccine ^a								
Any vaccination ^e	326/896 (36)	160/426 (38)	166/470 (35)	0.49	NA		NA	NA
≥ 2 weeks before ILI onset	291 (34)	144 (35)	147 (33)	0.43	NA		NA	NA
Received LAIV ^f	16/165 (10)	11/85 (13)	5/80 (6)	0.15	NA		NA	NA
Received adjuvanted vaccine ^g	27/51 (53)	11/21 (52)	16/30 (53)	0.95	NA		NA	NA
Prior vaccination history								
Received 2013/14 vaccine ^h	358/804 (45)	177/388 (46)	181/416 (44)	0.55	251/358 (70)	< 0.01	131/177 (74)	120/181 (66)
Received 2012/13 vaccine ⁱ	343/761 (45)	178/377 (47)	165/384 (43)	0.24	237/343 (69)	< 0.01	127/178 (71)	110/165 (67)

^a Participants who received seasonal 2014/15 influenza vaccine at least two weeks before ILI onset were considered vaccinated; participants who received seasonal 2014/15 influenza vaccine less than two weeks before ILI onset were excluded from primary analysis but explored in sensitivity analysis. Vaccination status was based on self/parent/guardian report. Details related to special paediatric dosing requirements was not sought.

^b Differences between cases and controls or vaccinated and unvaccinated participants (based on overall sample to explore potential confounding) were compared using the chi-squared test or Wilcoxon rank-sum test.

^c Chronic co-morbidities that place individuals at higher risk of serious complications from influenza as defined by Canada's National Advisory Committee on Immunization, including heart, pulmonary, renal, metabolic, blood, cancer and immunocompromising conditions or those that compromise management of respiratory secretions, or morbid obesity. Questionnaire answers were 'yes,' 'no,' or 'unknown' without specifying the co-morbidity.

^d Based on week of specimen collection. Missing collection dates were imputed as the laboratory accession date minus two days, the average time period between collection date and laboratory accession date for records with valid data for both fields. Week 3 of 2015 based on partial week.

^e Participants who received seasonal 2014/15 influenza vaccine less than two weeks before ILI onset or for whom vaccination timing was unknown were excluded from the primary analysis. They were included for assessing 'any' immunisation, regardless of timing, for comparison with other sources of vaccination coverage. The denominator is shown for 'any' immunisation.

^f Among participants 2–59 years-old who received 2014/15 influenza vaccine at least two weeks before ILI onset and had valid data for type of vaccine. All 16 participants who received LAIV were 2–19 years of age. Among vaccinated participants 2–19 years-old, 16 of 34 (47%) overall received LAIV including 11 of 24 cases (46%) and five of 10 controls (50%).

^g Among participants 65 years and older who received 2014/15 influenza vaccine at least two weeks before ILI onset and had valid data for receipt of adjuvanted vaccine.

^h Children younger than two years in 2014/15 were excluded from 2013/14 vaccine uptake analysis as they may not have been eligible for vaccination during the immunisation campaign in autumn 2013 on the basis of age under six months.

ⁱ Children younger than three years in 2014/15 were excluded from 2012/13 vaccine uptake analysis as they may not have been eligible for vaccination during the immunisation campaign in autumn 2012 on the basis of age under six months.

were instead antigenically similar to the cell-passaged A/Switzerland/9715293/2013-like reference strain (Table 2). Based on phylogenetic analysis, five of these viruses clustered with clade 3C.2a and two with an emerging clade of viruses awaiting official ECDC clade-level designation and thus temporarily labelled in the current analysis as 3C.3x. Both clade 3C.3x viruses had

an L157S substitution in antigenic site B and an N122D substitution in antigenic site A, as discussed below.

Of the 379 sentinel A(H3N2) viruses collected between 11 November 2014 and 10 January 2015, 226 (60%) were sequenced; 205 (91%) belonged to clade 3C.2a, 19 (8%) to our provisionally named clade 3C.3x, and two (1%) to clade 3C.3 (Table 2, Figure 3, Figure 4).

TABLE 4A

Interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

Model	Influenza (any)	Influenza A	Influenza A(H3N2)
	VE (95% CI)	VE (95% CI)	VE (95% CI)
Primary analysis			
N [n case (% vac); n control (% vac)]	861 [410 (35); 451 (33)]	842 [391 (36); 451 (33)]	830 [379 (37); 451 (33)]
Unadjusted	–12 (–49 to 16)	–17 (–55 to 12)	–21 (–61 to 9)
Age group (1–8, 9–19, 20–49, 50–64, ≥ 65 years)	–11 (–51 to 18)	–17 (–60 to 14)	–22 (–67 to 10)
Sex (female/male)	–19 (–58 to 11)	–24 (–65 to 7)	–29 (–73 to 4)
Comorbidity (no/yes)	–10 (–47 to 18)	–15 (–54 to 14)	–19 (–60 to 12)
Province (Alberta, British Columbia, Ontario, Quebec)	–12 (–49 to 16)	–15 (–54 to 14)	–19 (–59 to 11)
Collection interval (≤ 4/5–7 days)	–14 (–52 to 14)	–19 (–59 to 11)	–23 (–65 to 8)
Calendar time (2-week interval)	0 (–34 to 25)	–4 (–39 to 23)	–8 (–45 to 20)
Age, sex, comorbidity, province, interval, time	–1 (–40 to 28)	–4 (–45 to 25)	–8 (–50 to 23)
Sensitivity analysis – vaccination timing			
Vaccination defined without regard to vaccination timing (i.e. any vaccination)			
N [n case (% vac); n control (% vac)]	896 [426 (38); 470 (35)]	876 [406 (38); 470 (35)]	861 [391 (39); 470 (35)]
Unadjusted	–10 (–45 to 16)	–14 (–51 to 13)	–16 (–54 to 12)
Fully adjusted ^a	0 (–37 to 27)	–2 (–41 to 26)	–5 (–44 to 24)
Participants vaccinated < 2 weeks before ILI onset recoded as ‘unvaccinated’			
N [n case (% vac); n control (% vac)]	887 [422 (34); 465 (32)]	867 [402 (35); 465 (32)]	853 [388 (36); 465 (32)]
Unadjusted	–12 (–48 to 15)	–17 (–55 to 12)	–22 (–62 to 8)
Fully adjusted ^a	1 (–38 to 28)	–3 (–43 to 26)	–8 (–51 to 22)
Participants vaccinated < 2 weeks before ILI onset recoded as ‘vaccinated’			
N [n case (% vac); n control (% vac)]	887 [422 (37); 465 (35)]	867 [402 (38); 465 (35)]	853 [388 (38); 465 (35)]
Unadjusted	–11 (–46 to 16)	–15 (–52 to 13)	–18 (–56 to 11)
Fully adjusted ^a	–2 (–41 to 26)	–4 (–44 to 24)	–7 (–48 to 23)
Sensitivity analysis – comorbidity			
N [n case (% vac); n control (% vac)]	910 [433 (35); 477 (31)]	890 [413 (36); 477 (31)]	878 [401 (37); 477 (31)]
Includes participants with unknown comorbidity			
Unadjusted	–17 (–54 to 11)	–22 (–61 to 8)	–26 (–67 to 5)
Fully adjusted ^b	–7 (–47 to 23)	–10 (–52 to 20)	–14 (–58 to 18)
Participants with unknown comorbidity recoded as ‘no’			
Unadjusted	–17 (–54 to 11)	–22 (–61 to 8)	–26 (–67 to 5)
Fully adjusted ^a	–5 (–46 to 24)	–9 (–51 to 21)	–13 (–56 to 19)
Participants with unknown comorbidity recoded as ‘yes’			
Unadjusted	–17 (–54 to 11)	–22 (–61 to 8)	–26 (–67 to 5)
Fully adjusted ^a	–6 (–46 to 23)	–9 (–51 to 21)	–13 (–57 to 18)

CI: confidence interval; ILI: influenza-like illness; VE: vaccine effectiveness; % vac: percentage vaccinated.

^a Adjusted for age group, sex, comorbidity, province, collection interval, and calendar time.

Clade 3C.2a viruses comprised the majority (>90%) of viruses in all contributing SPSN provinces, with the exception of British Columbia, where there was more equal contribution of clade 3C.2a (17/30; 57%) and clade 3C.3x (13/30; 43%). None of the 226 sentinel A(H3N2) viruses contributing to the VE analysis that were sequenced belonged to the northern hemisphere 2014/15 A/Texas/50/2012(H3N2) vaccine clade 3C.1, nor to the 2015 southern hemisphere A/Switzerland/9715293/2013(H3N2) vaccine

clade 3C.3a. However, as described above, all seven viruses that could be characterised by HI assay were considered antigenically similar to the A/Switzerland/9715293/2013(H3N2) strain, even though none of those seven viruses clustered within clade 3C.3a.

Relative to the X-223A HGR, sentinel clade 3C.2a viruses typically differed by 10 or 11 antigenic site aa substitutions as itemised in Figure 3. In addition to the

TABLE 4B

Interim 2014/15 influenza vaccine effectiveness evaluation, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 861)

Model	Influenza (any)	Influenza A	Influenza A(H3N2)
	VE (95% CI)	VE (95% CI)	VE (95% CI)
Stratified analysis – restricted to non-elderly adult participants 20–64 years old			
N [n case (% vac); n control (% vac)]	516 [233 (31); 283 (30)]	506 [223 (32); 283 (30)]	496 [213 (33); 283 (30)]
Unadjusted	–4 (–52 to 29)	–11 (–62 to 24)	–16 (–71 to 20)
Fully adjusted ^a	11 (–35 to 41)	6 (–43 to 38)	2 (–49 to 36)
Stratified analysis – restricted to specimens collected from week 50 onward			
N [n case (% vac); n control (% vac)]	699 [365 (36); 334 (36)]	682 [348 (37); 334 (36)]	670 [336 (38); 334 (36)]
Unadjusted	1 (–34 to 28)	–4 (–42 to 24)	–8 (–48 to 21)
Fully adjusted ^c	–3 (–47 to 28)	–9 (–55 to 24)	–13 (–61 to 21)
Indicator variable analysis – effect of prior 2013/14 influenza vaccine receipt on 2014/15 VE ^d			
Unvaccinated both seasons			
N [n case (%); n control (%)]	414 [201 (52); 213 (51)]	400 [187 (51); 213 (51)]	392 [179 (50); 213 (51)]
Unadjusted/fully adjusted	Reference	Reference	Reference
Current 2014/15 influenza vaccine only			
N [n case (%); n control (%)]	32 [10 (3); 22 (5)]	32 [10 (3); 22 (5)]	32 [10 (3); 22 (5)]
Unadjusted	52 (–4 to 78)	48 (–12 to 76)	46 (–17 to 75)
Fully adjusted ^a	49 (–15 to 78)	46 (–24 to 76)	43 (–29 to 75)
Prior 2013/14 influenza vaccine only			
N [n case (%); n control (%)]	107 [46 (12); 61 (15)]	105 [44 (12); 61 (15)]	105 [44 (12); 61 (15)]
Unadjusted	20 (–23 to 48)	18 (–27 to 47)	14 (–33 to 44)
Fully adjusted ^a	8 (–47 to 42)	8 (–47 to 43)	4 (–54 to 40)
Both 2013/14 and 2014/15 influenza vaccine			
N [n case (%); n control (%)]	251 [131 (34); 120 (29)]	248 [128 (35); 120 (29)]	247 [127 (35); 120 (29)]
Unadjusted	–16 (–58 to 15)	–21 (–67 to 12)	–26 (–73 to 8)
Fully adjusted ^a	–8 (–56 to 26)	–11 (–62 to 23)	–15 (–67 to 21)

CI: confidence interval; ILI: influenza-like illness; VE: vaccine effectiveness; % vac: percentage vaccinated.

^a Adjusted for age group, sex, comorbidity, province, collection interval, and calendar time.

^b Adjusted for age group, sex, province, collection interval, and calendar time; not adjusted for comorbidity.

^c Adjusted for age group, sex, comorbidity, province, and collection interval; not adjusted for calendar time.

^d Based on same exclusion criteria as primary analysis, with further restriction to participants aged ≥ 2 years in 2014/15 and those with data for 2013/14 and 2014/15 influenza vaccine receipt.

N145S site A cluster-transition substitution distinguishing all clade 3C.2 (and 3C.3) viruses generally, differences between clade 3C.2 viruses and X-223A include N128T (gain of glycosylation) and P198S site B substitutions. The latter two substitutions are the result of having switched the vaccine prototype strain from A/Victoria/361/2011(H3N2) (a clade 3C virus) in 2012/13 to A/Texas/50/2012(H3N2) (a clade 3C.1 virus) since the 2013/14 season. Clade 3C.2 viruses also differ from X-223A at positions 186 (site B), 219 (site D) and 226 (site D) due to mutations in the egg-adapted HGR. Sentinel viruses within the dominant 3C.2a subgroup were further distinguished through an N144S (site A) substitution associated with loss of glycosylation, an additional F159Y (site B) cluster-transition mutation and an adjacent K160T (site B) substitution associated with the gain of a potential glycosylation site, as well as Q311H (site C) and N225D substitutions, the latter

within the RBS (but not within defined antigenic sites A–E [4,6,9]). Other substitutions relative to X-223A were scattered through antigenic sites A, C and E.

The provisionally named clade 3C.3x sentinel viruses typically differed from X-223A by 12 antigenic site aa substitutions, as also shown in Figure 3. Of note, in addition to the L157S substitution at antigenic site B that distinguishes this emerging subgroup, 18 of 19 clade 3C.3x viruses also bore an N122D antigenic site A substitution associated with loss of glycosylation.

Discussion

Interim VE estimates from the Canadian SPSN show little or no protection from the 2014/15 influenza vaccine against the A(H3N2) epidemic strain. The disappointing 2014/15 mid-season VE of –8%, with 95% confidence intervals (CI) overlapping zero and extending to just

FIGURE 3A
Influenza A(H3N2) haemagglutinin (HA) antigenic site pairwise sequence and per cent amino acid identity comparisons, relative to the 2014/15 high growth reassortant vaccine strain X-223A, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 217)

Antigenic site		A										B										C										D										E										F										G										H										I										J										K										L										M										N										O										P										Q										R										S										T										U										V										W										X										Y										Z										aa										ab										ac										ad										ae										af										ag										ah										ai										aj										ak										al										am										an										ao										ap										aq										ar										as										at										au										av										aw										ax										ay										az										ba										bb										bc										bd										be										bf										bg										bh										bi										bj										bk										bl										bm										bn										bo										bp										bq										br										bs										bt										bu										bv										bw										bx										by										bz										ca										cb										cc										cd										ce										cf										cg										ch										ci										cj										ck										cl										cm										cn										co										cp										cq										cr										cs										ct										cu										cv										cw										cx										cy										cz										da										db										dc										dd										de										df										dg										dh										di										dj										dk										dl										dm										dn										do										dp										dq										dr										ds										dt										du										dv										dw										dx										dy										dz										ea										eb										ec										ed										ee										ef										eg										eh										ei										ej										ek										el										em										en										eo										ep										eq										er										es										et										eu										ev										ew										ex										ey										ez										fa										fb										fc										fd										fe										ff										fg										fh										fi										fj										fk										fl										fm										fn										fo										fp										fq										fr										fs										ft										fu										fv										fw										fx										fy										fz										ga										gb										gc										gd										ge										gf										gg										gh										gi										gj										gk										gl										gm										gn										go										gp										gq										gr										gs										gt										gu										gv										gw										gx										gy										gz										ha										hb										hc										hd										he										hf										hg										hh										hi										hj										hk										hl										hm										hn										ho										hp										hq										hr										hs										ht										hu										hv										hw										hx										hy										hz										ia										ib										ic										id										ie										if										ig										ih										ii										ij										ik										il										im										in										io										ip										iq										ir										is										it										iu										iv										iw										ix										iy										iz										ja										jb										jc										jd										je										jf										jg										jh										ji										jj										jk										jl										jm										jn										jo										jp										jq										jr										js										jt										ju										jv										jw										jx										jy										jz										ka										kb										kc										kd										ke										kf										kg										kh										ki										kj										kk										kl										km										kn										ko										kp										kq										kr										ks										kt										ku										kv										kw										kx										ky										kz										la										lb										lc										ld										le										lf										lg										lh										li										lj										lk										ll										lm										ln										lo										lp										lp										lr										ls										lt										lu										lv										lw										lx										ly										lz										ma										mb										mc										md										me										mf										mg										mh										mi										mj										mk										ml										mn										mo										mp										mq										mr										ms										mt										mu										mv										mw										mx										my										mz										na										nb										nc										nd										ne										nf										ng										nh										ni										nj										nk										nl										nm										nn										no										np										np										nr										ns										nt										nu										nv										nw										nx										ny										nz										oa										ob										oc										od										oe										of										og										oh										oi										oj										ok										ol										om										on										oo										op										op										or										os										ot										ou										ov										ow										ox										oy										oz										pa										pb										pc										pd		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MHGR: high-growth reassortant; MDCK: Madin Darby Canine Kidney cell-passaged virus; WHO: World Health Organization.

Analysed viruses were a convenience sample of those collected by the Canadian Sentinel Physician Surveillance Network, contributing to vaccine effectiveness analyses and fully sequenced across all antigenic sites.

The comparator virus specified in bold is the 2014/15 influenza A(H3N2) HGR X-223A vaccine strain used by manufacturers. Sentinel influenza A(H3N2) viruses (n = 217, total of all four provinces) are compared against this strain with respect to antigenic site aa substitutions. Only antigenic site residues with substitutions in sentinel or vaccine viruses relative to the anchoring X-223A HGR are displayed. The aa residues 145, 156 and 159 shaded in black are recognised H3 antigenic cluster transition sites.

Viruses labelled clade 3C.3x bear the L157S substitution +/- N122D substitution but have not yet received official clade level-specific designation. They are temporarily labelled clade 3C.3x for this manuscript.

^a #aa signifies the number of aa substitutions between the sentinel virus sequence and the X-223A HGR at H3 antigenic sites, A-E.

^p % identity calculated as $[1 - (\text{number of aa substitutions in antigenic sites} / (\text{total number of antigenic site aa residues}))] \times 100\%$, relative to the X-223A HGR. The total number of A-E antigenic site aa residues is 131 for H3 viruses.

A/Victoria/361/2011 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2012/13 influenza vaccine.

^d IVR-165 is the egg-adapted HGR version of A/Victoria/361/2011 used by vaccine manufacturers.

A/Texas/50/2012 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2014/15 influenza vaccine

X-223A is the egg-adapted HGR version of A/Texas/50/2012 used by manufacturers, shown in bold as the strain against which sentinel influenza A(H3N2) virus antigenic site aa are compared.

^g A/Switzerland/9715293/2013 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the southern hemisphere's 2015 influenza vaccine.

IVR-176 and X-247 are egg-adapted HGR versions of A/Switzerland/9715293/2013 for vaccine manufacturers.

FIGURE 3B

Influenza A(H3N2) haemagglutinin (HA1) antigenic site pairwise sequence and per cent amino acid identity comparisons, relative to the 2014/15 high growth reassortant vaccine strain X-223A, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 217)

Antigenic site	C		E					A	B	A					B					A	D	B					D					E	C					Clade	# aa (% identity) ^{a,b}		
	48	53	62	63	78	83	88	91	94	122	128	137	138	140	142	144	145	156	157	159	160	168	171	186	192	198	207	208	213	214	219	226	261	278	279	309	311	312			
A/Victoria/361/2011 (MDCK) ^c	I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	H	L	F	K	M	N	G	I	S	K	R	V	I	S	I	R	N	S	V	Q	S	3C	6 (95.4%)	
2012-13 HGR: A/Victoria/361/2011 (IVR-165) ^d	I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	Q	L	F	K	M	N	V	I	S	K	R	V	I	Y	I	R	N	S	V	Q	S	3C	6 (95.4%)	
A/Texas/50/2012 (MDCK) ^e	I	D	E	N	G	K	V	S	Y	N	N	S	A	I	R	N	N	H	L	F	K	M	N	G	I	P	K	R	V	I	S	I	R	K	S	V	Q	S	3C.1	3 (97.7%)	
HGR: A/Texas/50/2012 (X-223A) ^f	I	D	E	N	G	K	V	S	Y	N	N	S	A	I	R	N	N	H	L	F	K	M	N	V	I	P	K	R	V	I	F	N	R	K	S	V	Q	S	3C.1	-	
A/Switzerland/9715293/2013 (MDCK) ^g	I	D	E	N	G	K	V	S	Y	N	A	S	S	I	G	N	S	H	L	S	K	M	N	G	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	9 (93.1%)	
2015 HGR: A/Switzerland/9715293/2013 (IVR-176) ^h	I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	R	L	S	K	M	N	V	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	10 (92.4%)	
2015 HGR: A/Switzerland/9715293/2013 (X-247) ^h	I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	H	L	S	K	M	N	V	I	S	K	R	V	I	F	I	R	K	S	V	Q	S	3C.3a	8 (93.9%)	
Alberta		n																																							
A/Alberta/57/2014	48										T					S	S		Y	T			G	S	S						S	I					H		3C.2a	10 (92.4%)	
A/Alberta/56/2014	11									T						S	S		Y	T			G	S	S						S	I					H		3C.2a	11 (91.6%)	
A/Alberta/77/2014	16								I		T					S	S		Y	T			G	S	S						S	I					H		3C.2a	11 (91.6%)	
A/Alberta/113/2014	1								I		T					S	S		Y	T			G	S	S						S	I					H		3C.2a	10 (92.4%)	
A/Alberta/55/2014	1									T						S	S		Y	T	I		G	S	S						S	I					H		3C.2a	11 (91.6%)	
A/Alberta/60/2014	1									T						S	S		Y	T			G	S	S						S	I					H		3C.2a	11 (91.6%)	
A/Alberta/67/2014	1									T						S	S		Y	T			G	S	S						S	I					H		3C.2a	11 (91.6%)	
A/Alberta/100/2014	1								H		T					S	S		Y	T			G	S	S						S	I					H		3C.2a	11 (91.6%)	
A/Alberta/119/2014	1	N								T						S	S		Y	T			G	S	S						S	I	L				H		3C.2a	12 (90.8%)	
A/Alberta/120/2014	1									T						S	S		Y	T			G	S	S						S	I					H		3C.2a	11 (91.6%)	
A/Alberta/127/2014	3									T	A					S	S		Y	T			G	S	S						S	I					H		3C.2a	11 (91.6%)	
A/Alberta/133/2014	1															S	S		Y	T			G	S	S						S	I	P				H		3C.2a	11 (91.6%)	
A/Alberta/138/2014	3									S	T					S	S		Y	T			G	S	S						S	I					H		3C.2a	11 (91.6%)	
A/Alberta/159/2014	1									T						S	S		Y	T			G	S	S						S	I					F		3C.2a	11 (91.6%)	
A/Alberta/166/2014	1								I		T					S	S		Y	T			G	S	S						S	I					H		3C.2a	12 (90.8%)	
A/Alberta/124/2014	2	T								T						S	S		Y	T			G	S	S						S	I					H		3C.2a	11 (91.6%)	
A/Alberta/88/2014	1									A						G	S						G	S	S						S	I	Q						3C.3	8 (93.9%)	
A/Alberta/124/2014	4			K						D	A					G	S		S				G	S	S						S	I	Q							3C.3x	12 (90.8%)

HGR: high-growth reassortant; MDCK: Madin Darby Canine Kidney cell-passaged virus; WHO: World Health Organization.

Analyzed viruses were a convenience sample of those collected by the Canadian Sentinel Physician Surveillance Network, contributing to vaccine effectiveness analyses and fully sequenced across all antigenic sites.

The comparator virus specified in bold is the 2014/15 influenza A(H3N2) HGR-X-223A vaccine strain used by manufacturers. Sentinel influenza A(H3N2) viruses (n = 217; total of all four provinces) are compared against this strain with respect to antigenic site aa substitutions. Only antigenic site residues with substitutions in sentinel or vaccine viruses relative to the anchoring X-223A HGR are displayed. The aa residues 145, 156 and 159 shaded in black are recognised H3 antigenic cluster transition sites. Viruses labelled clade 3C.3x bear the L157S substitution +/- N122D substitution but have not yet received official clade level-specific designation. They are temporarily labelled clade 3C.3x for this manuscript.

^a #aa signifies the number of aa substitutions between the sentinel virus sequence and the X-223A HGR at H3 antigenic sites, A–E.

^b % identity calculated as [(number of aa substitutions in antigenic sites) / (total number of antigenic site aa residues)] × 100%, relative to the X-223A HGR. The total number of A–E antigenic site aa residues is 131 for H3 viruses.

^c A/Victoria/361/2011 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2012/13 influenza vaccine.

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^h IVR-176 and X-247 are egg-adapted HGR versions of A/Switzerland/9715293/2013 for vaccine manufacturers.

FIGURE 3C

Influenza A(H3N2) haemagglutinin (HA1) antigenic site pairwise sequence and per cent amino acid identity comparisons, relative to the 2014/15 high growth reassortant vaccine strain X-223A, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 217)

Antigenic site	Antigenic site										C	E				A	B				A	D	B		D				E	C				Clade	# aa (% Identity) ^{a,b}																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
	Amino acid number HA1											48	53	62	63		78	83	88	91			94	122	128	137	138	140		142	144	145	156			157	159	160	168	171	186	192	198	207	208	213	214	219	226	261	278	279	309	311	312																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
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Viruses labelled clade 3C.3x bear the L157S substitution +/- N122D substitution but have not yet received official clade level-specific designation. They are temporarily labelled clade 3C.3x for this manuscript.

^a #aa signifies the number of aa substitutions between the sentinel virus sequence and the X-223A HGR at H3 antigenic sites, A–E.

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^h IVR-176 and X-247 are egg-adapted HGR versions of A/Switzerland/9715293/2013 for vaccine manufacturers.

FIGURE 3D

Influenza A(H3N2) haemagglutinin (HA1) antigenic site pairwise sequence and per cent amino acid identity comparisons, relative to the 2014/15 high growth reassortant vaccine strain X-223A, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n = 217)

Antigenic site		C			E				A	B	A				B				A	D	B				D				E	C				Clade	# aa (% identity) ^{a,b}																
		48	53	62	63	78	83	88	91	94	122	128	137	138	140	142	144	145	156	157	159	160	168	171	186	192	198	207	208	213	214	219	226	261	278	279	309	311	312												
A/Victoria/361/2011 (MDCK) ^c	Amino acid number HA1																																																		
		I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	H	L	F	K	M	N	G	I	S	K	R	V	I	S	I	R	N	S	V	Q	S	3C	6 (95.4%)										
	2012-13 HGR: A/Victoria/361/2011 (IVR-165) ^d	I	D	E	N	G	K	V	S	Y	N	T	S	A	I	R	N	N	Q	L	F	K	M	N	V	I	S	K	R	V	I	Y	I	R	N	S	V	Q	S	3C	6 (95.4%)										
A/Texas/50/2012 (MDCK) ^e		I	D	E	N	G	K	V	S	Y	N	N	S	A	I	R	N	N	H	L	F	K	M	N	G	I	P	K	R	V	I	S	I	R	K	S	V	Q	S	3C.1	3 (97.7%)										
HGR: A/Texas/50/2012 (X-223A) ^f		I	D	E	N	G	K	V	S	Y	N	N	S	A	I	R	N	N	H	L	F	K	M	N	V	I	P	K	R	V	I	F	N	R	K	S	V	Q	S	3C.1	-										
A/Switzerland/9715293/2013 (MDCK) ^g		I	D	E	N	G	K	V	S	Y	N	A	S	S	I	G	N	S	H	L	S	K	M	N	G	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	9 (93.1%)										
2015 HGR: A/Switzerland/9715293/2013 (IVR-176) ^h		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	R	L	S	K	M	N	V	I	S	K	R	V	I	S	I	R	K	S	V	Q	S	3C.3a	10 (92.4%)										
2015 HGR: A/Switzerland/9715293/2013 (X-247) ^h		I	D	E	N	G	K	V	S	Y	N	A	S	S	R	G	N	S	H	L	S	K	M	N	V	I	S	K	R	V	I	F	I	R	K	S	V	Q	S	3C.3a	8 (93.9%)										
Quebec	n																																																		
A/Quebec/55/2014	15											T					S	S			Y	T			G	S	S															3C.2a	10 (92.4%)								
A/Quebec/36/2014	26										T					S	S			Y	T			G	S	S																3C.2a	11 (91.6%)								
A/Quebec/110/2014	1										T					S	S			Y				G	S	S																3C.2a	10 (92.4%)								
A/Quebec/35/2014	13	T									T					S	S			Y	T			G	S	S																3C.2a	11 (91.6%)								
A/Quebec/112/2014	1	T									T					S	S			Y	T			G	S	S																3C.2a	11 (91.6%)								
A/Quebec/59/2014	1										T					S	S			Y	T			G	S	S																3C.2a	11 (91.6%)								
A/Quebec/76/2014	2										T					S	S			Y	T			G	S	S		R														3C.2a	11 (91.6%)								
A/Quebec/109/2014	1										T					S	S			Y	T			G	A	S																3C.2a	11 (91.6%)								
A/Quebec/117/2014	1															S	S			Y	T			G	S	S																3C.2a	12 (90.8%)								
A/Quebec/44/2014	1			K							D	A				G	S		S		S			G	S	S																3C.3x	10 (92.4%)								

HGR: high-growth reassortant; MDCK: Madin Darby Canine Kidney cell-passaged virus; WHO: World Health Organization.

Analysed viruses were a convenience sample of those collected by the Canadian Sentinel Physician Surveillance Network, contributing to vaccine effectiveness analyses and fully sequenced across all antigenic sites.

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^d IVR-165 is the egg-adapted HGR version of A/Victoria/361/2011 used by vaccine manufacturers.

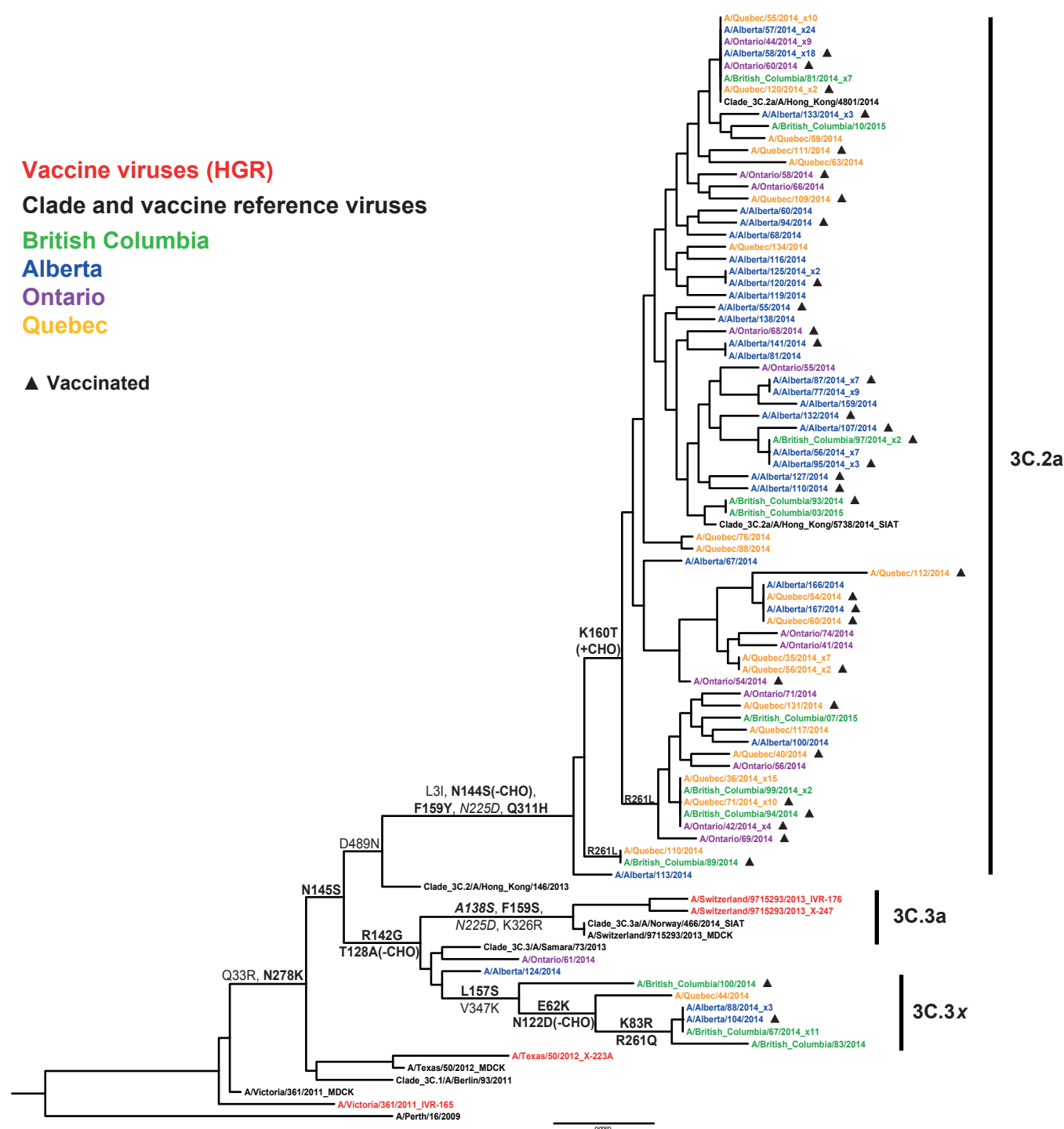
^e A/Texas/50/2012 (MDCK) is the influenza A(H3N2) vaccine prototype recommended by the WHO for the northern hemisphere's 2014/15 influenza vaccine.

^f X-223A is the egg-adapted HGR version of A/Texas/50/2012 used by manufacturers, shown in bold as the strain against which sentinel influenza A(H3N2) virus antigenic site aa are compared.

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^h IVR-176 and X-247 are egg-adapted HGR versions of A/Switzerland/9715293/2013 for vaccine manufacturers.

Phylogenetic tree of influenza A(H3N2) viruses 2014/15, Canadian Sentinel Physician Surveillance Network, 1 November 2014–19 January 2015 (n=215)



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23%, is in striking contrast to the 2013/14 mid-season VE analysis. During that season's interim analysis with comparable sample size, we measured substantial and statistically significant VE of 74% (95% CI: 58–83%) against the dominant but antigenically well-conserved A(H1N1)pdm09 epidemic strain [2]. The VE point estimate reported here for the 2014/15 seasonal vaccine is the lowest component-specific estimate reported by the Canadian SPSN against any seasonal strain of the past 10 years, including other recent influenza A(H3N2) vaccine-mismatched seasons in 2012/13 (VE=45% mid-season [3], 41% end-of-season [4]) or 2010/11 (VE=39%) [7].

Consistent with the low VE we report for 2014/15, virtually all (99%) of the sentinel influenza A(H3N2) viruses contributing to VE analysis showed genetic and/or antigenic evidence of vaccine mismatch. Although only seven SPSN viruses contributing to VE analysis grew to sufficient titre for antigenic characterisation by HI assay, the high proportion of vaccine-mismatched viruses reported here is similar to reports from national laboratory-based surveillance summaries for Canada [1]. Of the 62 A(H3N2) viruses HI-characterised in the presence of oseltamivir carboxylate and reported to date nationally by Canada's NML (including non-SPSN viruses), 61 (98%) have shown reduced titres to the A/Texas/50/2012(H3N2) vaccine strain [1]. The majority of these viruses have clustered with clade 3C.2a, and the remainder with what we have provisionally labelled here as clade 3C.3x. Nationally, based on genetic characterisation of viruses unable to grow to sufficient titre for HI assay, 393 of 395 (99%) viruses to date have been found to belong to one of these two genetic groups (foremost clade 3C.2a) and are considered antigenically distinct from the vaccine strain [1]. The approach used this season to impute vaccine mismatch based on phylogenetic findings follows that established by the United States Centers for Disease Control and Prevention (US CDC) where only 64% of circulating A(H3N2) viruses so far this season have been considered antigenically distinct from the vaccine strain [20]. This substantial difference between Canada and the US in the proportion of A(H3N2) viruses that are considered vaccine-mismatched may explain the higher (albeit still suboptimal) VE estimate reported in mid-season analysis by the US CDC (22%) [22]; however, other methodological, demographic or immunological differences should also be considered.

As in previous seasons, non-elderly adults contributed most (60%) to our VE analyses, although elderly participants were slightly more represented (16%) this season compared to previous years (10% or less) [2-4,6,7]. The adult predominance in our sample may be relevant to consider when comparing our 2014/15 mid-season VE estimates to those from the US CDC, where there was a greater paediatric contribution (43% of the overall sample) [22]. Children are less likely to have had prior influenza vaccine or virus exposure history and are more likely to have received LAIV. LAIV has been

associated with better efficacy than inactivated vaccine in the very young [23-27], although the opposite was observed against influenza A(H1N1)pdm09 in the US during the 2013/14 season [28] and relative effectiveness in the context of substantial vaccine mismatch or with history of prior repeat immunisation is uncertain. Our VE estimate against influenza A(H3N2) in non-elderly adults of 2% is comparable to (within 10% of) the US mid-season VE estimate for adults 18–49 years-old (12%), although neither country's estimate in adults is statistically significant and confidence intervals overlap. More nuanced evaluation of age and other influences on VE will be important to explore with larger sample size in end-of-season analyses.

At the genetic level, vaccine-virus divergence in 2014/15 was defined among Canadian SPSN viruses by a substantial number of aa differences (10–11) in the dominant (>90%) clade 3C.2a viruses relative to the vaccine component, including substitutions at pivotal antigenic, cluster-transition and receptor-binding sites and/or in association with potential gain or loss of glycosylation, each of which may influence antibody recognition. Substitutions evident in the vaccine strain, notably associated with egg-adaptation and HGR generation, may also have compounded the effects of antigenic drift in circulating viruses [4]. The emerging but as yet minor subgroup of viruses bearing the L157S +/- N122D mutation (here labelled clade 3C.3x) also warrants close monitoring. Although position 157 has not been identified historically as a cluster-transition residue, it is within the same pocket as other key residues (i.e. 155, 156, 158, 159) and may be of emerging significance [16]. The loss of glycosylation associated with the N122D substitution may also be influential [17]. Clade 3C.3 viruses with this particular combination of aa substitutions have not previously been identified by the Canadian SPSN, but were detected in Spain during the 2013/14 season, cited in association with the low VE (13%) against A(H3N2) viruses in mid-season analysis from that country [29]. Compared with Spanish sequences from 2013/14, clade 3C.3x viruses characterised by the Canadian SPSN in 2014/15 have acquired an additional three aa mutations in antigenic site E, an antigenic site distant from the RBS and not typically considered immuno-dominant but possibly relevant to overall virus fitness.

As published previously by the Canadian SPSN [4,6] and US CDC and other investigators [30-33], we observed variability in VE by prior vaccination history. In particular, VE against influenza A(H3N2) among those who received the 2014/15 influenza vaccine without prior vaccination in 2013/14 was higher (43%) than among participants who were vaccinated with the same A(H3N2) vaccine component in both 2013/14 and 2014/15 (–15%). Although none are statistically significant, these substantial differences in VE based on prior immunisation are consistent with the antigenic distance hypothesis articulated by Smith et al. [34]. That hypothesis suggests that negative interference from

prior immunisation may be more pronounced when the antigenic distance is small between successive vaccine components but large between vaccine and circulating strains. Such is the scenario for the current 2014/15 season for which the identical A(H3N2) vaccine component was used as during the 2013/14 season, poorly matched to the 2014/15 epidemic strain. However, limited sample size precludes definitive conclusions, particularly since a large proportion (nearly 90%) of vaccinated SPSN participants are repeat vaccine recipients [2-4,6,7]. There may also be other unrecognised differences across subgroups of participants with differing immunisation histories. Further evaluation is required across additional study settings and seasons and with greater sample size to confirm these findings, assess possible underlying immunological interactions, and inform implications for vaccine reformulation and policy recommendation.

There are limitations to this study, notably related to sample size, in particular in subgroup analyses. Mid-season analysis was undertaken with the recognition that sample size was sufficient to provide 80% statistical power to detect a VE of at least 40%, given vaccine coverage typically spanning 30 to 40% in our setting. The absence of statistical significance with much lower VE is not unexpected given that in order to measure a VE of 10% in either direction from zero with the same statistical power would require more than 10,000 participants and more than 1 million participants would be required to show a significant VE of 1%. Our findings are thus consistent with a VE close to zero, where a precise estimate may never be resolved statistically. Higher VE may be observed in final end-of-season analyses, particularly if other influenza types or subtypes for which the trivalent vaccine is a better match circulate through the remainder of the 2014/15 season. Vaccine status in this study was based on self-reporting which may introduce some misclassification bias. However, this information was collected at the time of specimen collection, before the test result was known, minimising differential misclassification. As in prior seasons' analyses by the SPSN, the predominance of adults and repeat influenza vaccine recipients among our study participants is relevant to consider in the generalisation of our findings to other settings where the population profile may differ. Although we uniquely characterised more than half of our sentinel A(H3N2) viruses to the level of clade specification, and our virological profile reflected that of national surveillance summaries for Canada [1], we cannot rule out systematic differences in viruses available for genetic or antigenic characterisation, a problem for all laboratory-based surveillance. The validity of VE estimates derived by the test-negative approach has been previously demonstrated [35,36] but the design remains observational and bias and confounding cannot be ruled out.

In summary, interim VE findings from the Canadian SPSN indicate that the 2014/15 influenza vaccine

has provided little or no protection against medically attended illness due to predominant and substantially mismatched A(H3N2) viruses this season. Given limited vaccine protection, other adjunct protective measures should be considered to minimise associated morbidity and mortality, particularly among high-risk individuals. The virological and/or host factors influencing reduced vaccine protection against influenza A(H3N2) during the 2014/15 season warrant further in-depth investigation.

GenBank Accession Numbers

Viruses from original specimens with complete or partial sequences of the haemagglutinin (HA) gene (HA1 and HA2) provided by provincial laboratories and contributing to the 2014/15 interim influenza vaccine effectiveness analysis by the Canadian Sentinel Physician Surveillance Network were deposited in GenBank with accession numbers KP701523–KP701743.

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Conflict of interest

Within 36 months of manuscript submission, GDS received research grants from GlaxoSmithKline (GSK) for unrelated vaccine studies. JG has received a research grant from Pfizer. MK has received research grants from Roche, Merck, GenProbe and Siemens. SS and TLK are funded by the Canadian Institutes of Health Research Grant (TPA-90193). The other authors declare that they have no competing interests to report.

Authors' contributions

Principal investigator (epidemiology): DMS (National and British Columbia); GDS (Québec); JAD (Alberta); ALW (Ontario). Investigators (laboratory): JBG (Ontario); HC and CM (Québec); MP and MK (British Columbia); SD and KF (Alberta); YL and NB (national). National database coordination: TLK. Data analysis: CC and DMS (epidemiology); SS and AE (phylogenetic). Preparation of first draft: DMS. Draft revision and approval: all.

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Multistate foodborne hepatitis A outbreak among European tourists returning from Egypt– need for reinforced vaccination recommendations, November 2012 to April 2013

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A multistate outbreak of hepatitis A virus (HAV) among European travellers returning from Egypt occurred between November 2012 and April 2013. A total of 14 European Union (EU)-European Free Trade Association (EFTA) countries reported 107 cases. Twenty-one cases from six countries were affected by strains of sub-genotype 1B harbouring identical RNA sequences, suggesting a common source outbreak. An international outbreak investigation team interviewed a number of cases with a trawling questionnaire to generate hypotheses on potential exposures. Some of these exposures were further tested in a case–control study based on a more specific questionnaire. Both trawling and case–control questionnaires aimed to collect cases' vaccination details as well as epidemiological information. Most cases participating in either questionnaire (35/43) had been staying in all-inclusive hotels located along the Red Sea. The case–control study found cases associated with exposure to strawberries or mango (multivariable analysis *p* value: 0.04). None of the 43 cases interviewed in any of the two questionnaires had been vaccinated. The most common reasons for non-vaccination was unawareness that HAV vaccination was recommended (23/43, 53%) and perceiving low infection risk in all-inclusive luxury resorts (19/43, 44%). Vaccination had not been recommended to five of the six cases who sought travel medical advice before travelling. Public health authorities should strongly reinforce measures to remind travellers, travel agencies and healthcare providers of the importance of vaccination before visiting HAV-endemic areas, including Egypt.

Introduction

Hepatitis A is an acute illness caused by hepatitis A virus (HAV), which is characterised by dark urine, discoloured faeces, fatigue, fever and jaundice. Transmission mainly occurs through ingestion of contaminated food and water, and via the faecal–oral route among close contacts to infected persons. The incubation period for hepatitis A is approximately 30 days and can range from 15 to 50 days [1]. The risk of developing symptomatic illness following HAV infection is related to age: in young children (≤ 5 years of age), HAV infection is usually asymptomatic but among older children and adults, infection usually causes clinical disease with jaundice occurring in more than 70% of cases [2].

The incidence of HAV infection has been declining in most countries of the European Union (EU) during recent decades, reflecting improved hygiene and living conditions, and was estimated at 2.5 per 100,000 in European Economic Area-EU countries in 2011 [3]. However, hepatitis A remains one of the most common travel-related diseases among European travellers [4,5]. An effective and safe vaccine against HAV infection is available on the market since the early 1990s [2,6].

On 15 April 2013, the Norwegian Institute of Public Health communicated through the Epidemic Intelligence Information System for Food- and Waterborne Diseases and Zoonoses platform (EPIS-FWD) of the European Centre for Disease Prevention and Control (ECDC) an increase in HAV infections in travellers returning from Egypt compared with the normal annual rate [7].

Following Norway's notification, several EU-European Free Trade Association (EFTA) countries reported cases with disease onset after 1 November 2012 and recent travel history to Egypt (mainly to resorts in the Red Sea). Some of these cases shared identical viral RNA sequences (genotype IB) to the outbreak strain isolated in four Norwegian patients. Egypt, and particularly the Red Sea area, is a popular tourist destination for European travellers [7]; HAV infection in Egypt remains highly endemic and the virus is frequently detected in the environment [8].

The significant increase of travel-related cases compared with the historical baseline at country level, together with the identification of the same HAV sequence in cases from different countries, suggested a multistate outbreak [9]. An outbreak investigation coordinated by the ECDC and involving several public health institutes in EU-EFTA, the World Health Organization (WHO) Regional Offices (RO) for Europe and the Eastern Mediterranean as well as Egyptian public health authorities, was initiated to identify common exposures among cases. Moreover, to determine if vaccination recommendations to travellers should be reinforced, the vaccination details of cases were sought. While the first cases and outbreak strain sequence were described in a preliminary report, which was published when the epidemic was ongoing [7] more detailed findings of the epidemiological investigation are presented here, including the results of a case-control study.

Methods

Case definition and case finding

After the initial alert in EPIS-FWD, the following EU-EFTA epidemic case definition was established: A probable case was defined as a symptomatic person with a laboratory-confirmed HAV infection (presence of IgM and/or polymerase chain reaction (PCR)-positive), with onset of symptoms (or date of testing if onset date not available) after 1 November 2012, with travel history to Egypt two to six weeks before onset of symptoms (or date of testing if onset date not available) and no other known hepatitis A exposure. A confirmed case was defined as a probable case with HAV RNA sequence matching the outbreak sequence first isolated from Norwegian cases [7]. Cases, for which typing was performed but resulted in a sequence different to the outbreak strain, were excluded. Countries were asked to report to ECDC via the EPIS-FWD platform the number of HAV cases meeting the outbreak case definition together with available information on demographic characteristics, clinical features and travel details.

Trawling questionnaire

The International Outbreak Investigation Team (IOT) developed a trawling questionnaire in order to generate hypotheses on potential risk exposures. The primary aim was to interview confirmed cases clustered in the same hotels, but countries were encouraged to

interview also probable cases from these hotels when this was not possible. Trawling questionnaire collected information on basic demographics, symptoms, vaccination status, travel details (travel agencies, airports, airlines), holiday activities (swimming, scuba diving, snorkelling, day/night trips) and food and drink consumption at the hotel food services during the stay in Egypt. Questionnaires were translated to respective languages, and staff from the cases' respective national public health authorities conducted the trawling interviews.

Case-control study

We designed a case-control study in order to identify risk factors associated with disease transmission by testing hypotheses on exposures found through the trawling questionnaire. Both confirmed and probable cases staying at the same hotels during the same time period (between January and March 2013) as at least one confirmed case were eligible for inclusion in the case-control study.

Cases eligible for the case-control study were asked to nominate as controls travel companions staying at the same hotel during the same period, a form of convenience sampling. Controls were excluded if they reported history of HAV vaccination or had knowingly been infected with HAV (to exclude as many controls who would have been protected against infection as possible) or were under 16 years of age (to minimise misclassification due to asymptomatic infection).

The IOT developed the case-control questionnaire (available on request), which included the same questions on basic demographics, symptoms and vaccination details as the trawling questionnaire; however, it comprised more detailed questions on consumption of food and drink items most frequently mentioned in the answers to the trawling questionnaire. Cases recruited for the case-control study, which had not been previously investigated with the trawling questionnaire, were interviewed with the case-control questionnaire. For cases considered in the case-control study which had already participated in the trawling questionnaire, we developed a supplementary questionnaire including only the detailed questions on exposures of interest. Cases either completing the case-control questionnaire, or the trawling questionnaire followed by the supplementary questionnaire, were included in the case-control study.

Assessment of vaccination details among cases

As all countries affected by the outbreak had explicit HAV vaccination recommendations for travellers to Egypt, we aimed to study the vaccination status and reasons for non-vaccination among cases in order to find out if awareness of these recommendations should be improved. Both the trawling and case-control questionnaire collected information on vaccination (vaccination status, year of vaccination and dose, allowing calculation whether vaccination was protective at time

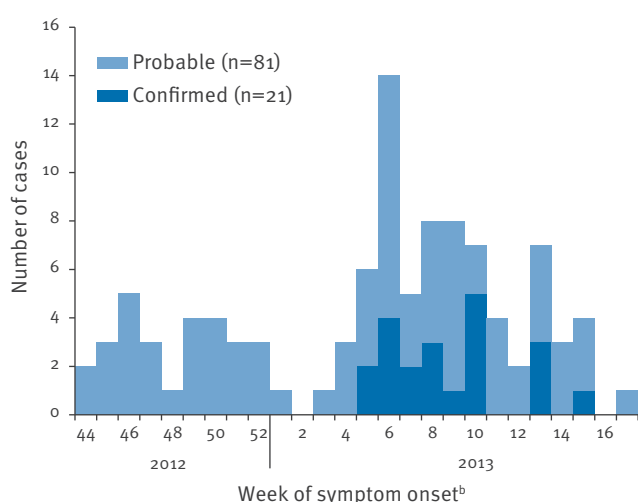
of travel). Participants were also asked whether advice was sought from medical professionals before their trip to Egypt and if yes, whether vaccination was recommended, not recommended or not discussed during the consultation. Furthermore, the cases were asked to specify reasons for non-vaccination. All cases interviewed in the trawling questionnaire or in the case-control study were included in the descriptive analyses on vaccination.

Data analysis

We describe cases and controls in terms of demographics (age, sex and country of origin), clinical symptoms (cases) and exposures of interest through crude numbers and proportions. Food items served in different forms (e.g. strawberries as fresh fruit, in smoothies, pastries or in fruit sauce) were recoded into a single exposure variable. In the univariate analysis, we assessed the associations between outcome and exposures of interest by calculating odds ratios (OR) and 95% confidence intervals (CI), and determined the p-value with the Fisher exact test. To adjust for potential confounders, we fitted into a multivariable logistic regression model any exposure positively associated with the outcome with a p-value < 0.25, excluding those with fewer than 10 cases [10]. We used STATA version 12 (Statacorp, College Station, Texas) to perform the analysis.

FIGURE 1

Weekly distribution of probable and confirmed hepatitis A infection cases with travel history to Egypt, European Union-European Free Trade Association, November 2012–April 2013 (n = 102)^a

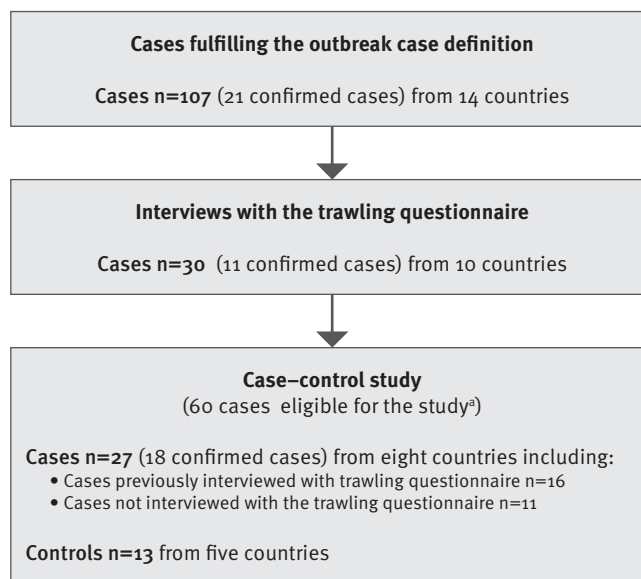


^a Five probable cases are not included in the figure due to missing information on onset date and testing date. Cases were reported from following countries: Denmark (n = 8), Estonia (n = 1), Finland (n = 2), France (n = 9), Germany (n = 44), Ireland (n = 2), Latvia (n = 1), Lithuania (n = 3), the Netherlands (n = 10), Norway (n = 7), Slovakia (n = 2), Sweden (n = 6), Switzerland (n = 3), United Kingdom (n = 9).

^b Date of testing for hepatitis A virus was used if symptom onset date was not available (n = 13 cases).

FIGURE 2

Numbers of cases recruited to participate in the trawling questionnaire and number of cases and controls for the case-control study, European travellers to Egypt, 2012–2013



^a Both confirmed and probable cases staying at the same hotels during the same time period (January–March 2013) as at least one confirmed case were eligible for inclusion in the case-control study.

Laboratory methods

Laboratory confirmation of HAV infection included a positive IgM and/or PCR result, which was determined by standard serological or virological methods in the respective countries. If feasible, the RNA sequence of HAV (442 nucleotides in VP1/2A region) was determined in laboratories in different countries following respective protocols. The outbreak strain sequence has been published previously [7].

Results

Description of the outbreak

A total of 107 cases (21 confirmed and 86 probable) were reported from the following EU-EFTA countries: Denmark (n = 8), Estonia (n = 1), Finland (n = 2), France (n = 9), Germany (n = 44), Ireland (n = 2), Latvia (n = 1), Lithuania (n = 3), the Netherlands (n = 10), Norway (n = 7), Slovakia (n = 2), Sweden (n = 6), Switzerland (n = 3) and the United Kingdom (UK) (n = 9). For the 102 cases with available information, the date of symptom onset, or date of testing (if onset date was not available), ranged from 2 November 2012 (week 44) to 26 April 2013 (week 17) (Figure 1). Most cases (n = 72, 71%) occurred between January and April (weeks 3 to 15) 2013 with a peak in case numbers in week six (February). Six countries (Denmark, France, Ireland, the Netherlands, Norway, and the UK) identified confirmed cases (n = 21); all were reported in 2013 (Figure 1). Confirmed cases

TABLE 1

Demographic characteristics and clinical symptoms (if applicable) of hepatitis A cases and controls, European travellers to Egypt, November 2012–April 2013

Characteristics	All reported cases (n=107)	Cases in case–control study (n=27) ^a	Controls in case–control study (n=13)
Female n/N ^b (%)	50/101 (50)	16/27 (59)	6/13 (46)
Median age in years (range)	36 (4–76)	39 (5–72)	48 (27–70)
Confirmed case n/N ^b (%)	21/107 (20)	14/27 (52)	NA
Median length of stay in Egypt in days (range)	7 (1–80)	7 (6–14)	7 (6–14)
Symptoms			
Dark urine or coloured stools n/N ^b (%)	–	27/27 (100)	NA
Jaundice or yellow eyes n/N ^b (%)	–	27/27 (100)	NA
Abdominal pain n/N ^b (%)	–	19/27 (70)	NA
Vomiting n/N ^b (%)	–	13/27 (48)	NA
Fever >38°C n/N ^b (%)	–	17/24 (71)	NA
Diarrhoea n/N ^b (%)	–	13/26 (50)	NA
Median duration of illness in days (range)	–	21 (4–60)	NA
Hospitalised n/N ^b (%)	–	18/27 (67)	NA

NA: not applicable; –: not available.

^a Includes 16 cases which had also been interviewed with trawling questionnaire.

^b Total cases with respective available information.

reported staying in seven different hotels in three different geographically dispersed locations in Egypt; Taba, Sharm El Sheikh and Hurghada.

Trawling questionnaire

A flowchart summarising the number of cases recruited for the trawling questionnaire and the case–control study is shown in Figure 2.

Thirty cases were interviewed with the trawling questionnaire in May 2013, including 11 confirmed and 19 probable (Figure 2). Travel details and activities during the holiday did not suggest common activities and exposures among cases which stayed at different hotels in Egypt and came from different countries. Consumption of several food items at the hotel services was frequently mentioned by cases such as fresh fruits and berries, raw vegetables, different salads and orange juice. These exposures were included in the case–control study questionnaire.

Case–control study

Twenty-seven of the 60 cases eligible for the study were interviewed with the questionnaire between June and August 2013. Thirteen controls were included in the study (Figure 2). Cases and controls stayed at six and four different hotels, respectively. Participants' characteristics are presented in Table 1.

At univariate level, cases were more likely than controls to have consumed strawberries, raspberries and mango in any form (p value ≤ 0.05 , Table 2) with strawberry exposure mentioned by 17 of 21 cases. Exposure to fresh strawberries, mango, and to orange juice, were more common among cases but these associations

were not statistically significant. The frequency of other exposures repeatedly mentioned in the trawling questionnaire (>70% exposure) including to different salads, jam and marmalade, ice (water-based), cooked fish, sandwiches, eggs and raw vegetables, were similar between cases and controls (data not shown). The multivariable model included exposures to strawberries, mango and orange juice: exposure to strawberries and mango remained independently significant (Table 2). Cases and controls did not significantly differ in age or sex distribution and these variables were not adjusted for in the model (p values >0.16).

Vaccination status among cases and reasons for non-vaccination

All cases interviewed with the trawling questionnaire ($n=30$) or with only the case–control questionnaire ($n=13$ Figure 2), were included in this study. Among the 43 cases, none were vaccinated. The most common reason for not being vaccinated was not knowing that HAV vaccination was recommended, (23/43), followed by not perceiving a high risk of infection in an all-inclusive luxury resort (19/43) (Figure 3). Thirty-five of the 43 interviewed cases stayed at resorts or hotels, which were all-inclusive. Six cases sought professional medical advice before travel and for five cases vaccination was not recommended. These cases represented four different countries. When the vaccine was not recommended, a general practitioner (GP) was indicated as the specific source of advice for three of the cases, while for the two remaining, the details on source of information were missing. For the sixth case, vaccination was recommended by a GP, but this advice was eventually ignored.

TABLE 2

Univariate and multivariable associations between food/drink exposures and hepatitis A infection, European travellers to Egypt, November 2012–2013

Exposure item	Exposure		Univariate analysis		Multivariate analysis	
	Cases n=27 n/N (%)	Controls n=13 n/N (%)	Crude OR (95%CI)	P value (Fisher exact test)	Adjusted OR (95% CI)	P value (Wald test)
Strawberries ^a	17/21 (81)	4/11 (36)	7.4 (1.4–38.4)	0.02	10.1 (1.1–93)	0.04
Fresh strawberries ^b	14/24 (58)	3/13 (23)	4.6 (0.9–31.9)	0.08	–	–
Mango ^a	10/17 (59)	2/11 (18)	6.4 (1.1–39.3)	0.05	21 (1.1–409)	0.04
Fresh mango ^b	10/22 (45)	2/13 (15)	4.5 (0.7–50.1)	0.14	–	–
Raspberries ^{a,c}	6/14 (43)	0/9 (0)	NA	0.05	–	–
Orange juice	21/25 (84)	7/13 (54)	4.5 (0.97–20.7)	0.06	7.0 (0.4–107.3)	0.17

CI: confidence interval; OR: odds ratio; NA: not applicable.

Denominators represent persons for whom data were available for the given variable.

^a As fresh fruits, in smoothies, pastries or fruit sauce.

^b Fresh mango and fresh strawberries were not included in the multivariable model due to collinearity with the variables combining the different forms of fruits consumed.

^c Exposure to raspberries was not included in the multivariable analysis as the number of cases exposed was under 10.

Discussion

We describe a multistate outbreak of HAV sub-genotype 1B infection among European travellers returning from Egypt. The outbreak highlighted the risk of hepatitis A for non-immune Europeans visiting a highly endemic country. A persistent common source of infection was suspected as identical HAV strains were isolated from several cases over a period of several weeks. Unfortunately, HAV genotyping is not routinely done in many EU countries and some laboratory-confirmed cases in the context of the outbreak may have been overlooked by the retrospective investigation. Transmission likely occurred through contaminated food, with contaminated strawberries or possibly mangos being likely vehicles of the outbreak. Cases were not aware of the vaccine recommendations or did not perceive a high risk of infection in all-inclusive holiday resorts, despite all the affected countries having explicit HAV vaccination recommendations for Egypt. Moreover, for the six cases identified among 43 interviewed in our study, which sought medical advice before travel, vaccination was not recommended for five.

The vaccine against HAV is highly immunogenic and effective [2] and the infection can be prevented when travelling to high-risk destinations by following national vaccination recommendations in nearly every European country. Our study pointed out that awareness of vaccine recommendations should clearly be improved, including among GPs giving advice on vaccinations to travellers. It is also important to reinforce that vaccination is needed in countries with high endemicity of HAV even in settings that the public consider safe, namely all-inclusive holiday resorts. A study on travel-associated HAV infections in Switzerland indicated that risk of contracting hepatitis A is often underestimated

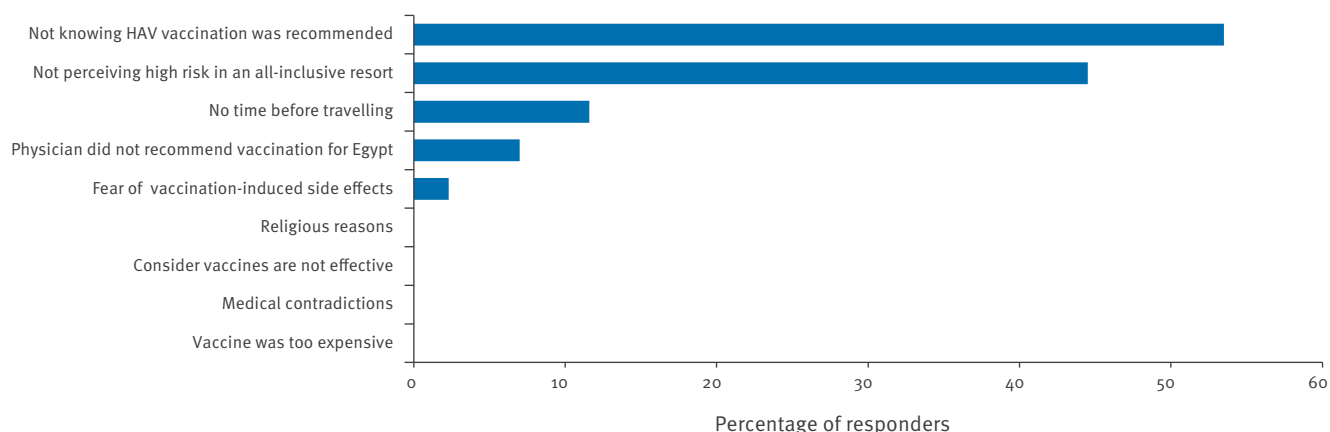
in nearby regions for Europeans despite endemicity, such as northern Africa [4]. Another study showed that Swedish tourists travelling to Egypt were less frequently vaccinated than Swedish travellers to other high-risk areas, probably due to low risk perception [11], corroborating our findings.

HAV infection can lead to severe illness, particularly in older adults [1], and the direct medical costs and indirect costs of lost productivity associated with hepatitis A can be substantial [3]. Two-thirds of the cases in our study were hospitalised, showing a hospitalisation rate that is higher than usual for hepatitis A [12,13]. This probably reflects that only cases with a severe infection visit healthcare and are notified to the national surveillance systems. Infection in travellers may further lead to secondary transmission back in home countries, thereby further increasing the burden of travel-associated infections. Particularly, if infected travellers work in settings such as food-handling, transmission may spread through the larger community [14]. Some secondary cases related to this outbreak were reported but we were not able to assess the full extent of secondary transmission due to our case definition restrictions in case finding.

The outbreak was most likely foodborne with transmission occurring on hotel premises, as other exposures did not concur between cases. Most cases only ate at the hotels and never left hotel grounds. Since the genetic substitution rate in HAV is considered unusually low [15] and several cases with identical HAV-strains were identified, a common source was suspected. However, multiple sources cannot be excluded and we were not able to compare full-length sequences of the HAV strains, which could have confirmed the genetic relatedness between the outbreak

FIGURE 3

Reasons for not getting vaccinated against hepatitis A virus, European travellers to Egypt, November 2012–2013 (n=43)



isolates. Food-borne transmission of HAV has been implicated in several outbreaks [14,16–18], including a very large hotel outbreak in Egypt in 2004 affecting travellers [19]. In that outbreak, the food vehicle also was an Egyptian produced fruit preparation (citrus juice). The recent outbreaks in Nordic countries and Italy in 2013 were associated with the consumption of frozen strawberries and mixed berries, respectively [20,21]. Phylogenetic analysis indicated that the outbreak sequences from the Nordic outbreak clustered with the strain identified in this study and with other strains previously isolated from travellers returning from Egypt, suggesting these strains may have a common ancestor in Egypt or surrounding countries [20].

Our case–control study indicated that exposure to strawberries and possibly mango was associated with being infected with HAV. However, raspberries cannot be ruled out as more controls could have allowed further analyses with this exposure. Both strawberries and mango were often consumed as fresh products. Information on dose of exposure was collected but often missing and thus not analysed. The association to strawberries appears to be the most likely source of infection, given that the strawberry production season in Egypt spans from December to April, which coincides with the timing of this outbreak. In contrast, mango season is in the summer and local fresh mango thus unavailable in the winter. Strawberries are also biologically plausible, since they are ready to eat and hard to effectively wash. Contamination could have occurred upon water irrigation or rinsing near the place of production – local contamination in the various hotels seems unlikely, given the single outbreak strain. There is no evidence of a link between this outbreak and the other concurrent strawberry-associated outbreaks in Europe [20,21] as the HAV strains differ. We were not able to obtain information from the hotels affected regarding menus, possibly sick food handlers, or possible distribution chains of the implicated food items, which could have explained clustering of cases

in certain hotels, and enabled to further assess the findings from our analytical study.

The results from the analytical study must be interpreted with caution. The time between interviews and exposure to infection was several months and cases were possibly more likely to remember exposures than controls. Recall bias may have resulted in overestimated measures of associations. Several cases were included in both the trawling interviews and in the analytical study, which should normally be avoided but this was not feasible in our study due to restricted sample size. We were not able to retrieve lists of healthy guests from the affected hotels for control sampling. The number of controls in the case–control study was small and we could not take into account the fact that controls did not represent all the same hotels as cases. Selecting controls from travel companions may have resulted in over-matching and possibly underestimation of the strength of associations; however, this choice ensured that controls had the same chance as the cases to be exposed to the infection. Moreover, attributable to small sample size and missing data, the number of observations used in the multivariable model was rather small leading to limited power. Media attention around strawberries and HAV infection in Nordic countries (Sweden, Norway, Denmark and Finland) due to the concurrent strawberry-associated outbreak may have had an effect on recalling food exposures in our study. However, the frequency of exposure to strawberries among cases from Nordic countries was not higher than other cases in our study; in fact, it was lower (data not shown) and thus, media bias probably did not have major influence on our findings. Our study design was not specifically designed to study vaccination status and reasons for non-vaccination among travellers to Egypt in general. Therefore we were only able to study vaccination details among cases in our study and cannot generalise findings to travellers to Egypt in general. It would have been of interest to analyse reasons for non-vaccination by country of cases but this was not done due to small sample size.

This outbreak investigation was conducted in collaboration with several public health institutes in Europe, ECDC, WHO and local Egyptian authorities. The cooperation, involving both epidemiological and microbiological investigations, was essential in an outbreak affecting several countries and occurring in a popular holiday destination and should be promoted and reinforced if similar multistate outbreaks occur in the future. However, outbreaks affecting Europeans outside EU are often challenging to investigate. For instance, the collection of important information can be cumbersome and, as also applies to outbreaks within the EU, performing environmental investigations is not always possible. Initial difficulties should not, however, discourage the investigations of events putting European travellers at risk, especially when recurrently happening in very popular destinations.

The results of our investigation suggest that public health authorities should reinforce the importance of vaccination before visiting HAV-endemic areas with information campaigns targeting travellers, travel agencies and healthcare providers, particularly the GPs often mentioned as the source of advice in this study. Hotels and travel agencies should monitor the hygiene practices in food handling and preparation more carefully and yet unvaccinated tourists should avoid food items considered likely vehicles of HAV. After previous outbreaks affecting travellers returning from Egypt, it was suggested that travel agencies should consider adding reminders of vaccination upon booking holidays to Egypt [22-24]. Recently revised proposal of an EU directive on package travel also highlights that the retailer should provide proper health information to travellers [25]. This outbreak evidently showed that more efficient actions are needed in order to improve vaccine uptake and prevent future outbreaks of HAV among travellers.

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Conflict of interest

None declared.

Authors' contributions

JS designed the study, coordinated the data collection, analysed the data and wrote the manuscript, EM contributed to the study design, collected data and wrote the manuscript, LV contributed to the study design, supervised the study in Norway and wrote the manuscript, CG contributed to study design, coordinated data collection and wrote the manuscript, ES designed the study, analysed data, coordinated

data collection and wrote the manuscript. Members of the International Outbreak Team contributed with data from respective countries and reviewed the study design and manuscript.

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Trends in Human Leptospirosis in Denmark, 1980 to 2012

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Leptospirosis in humans is a mandatory notifiable disease in Denmark. To identify changing trends in human leptospirosis in Denmark, we analysed data from the passive laboratory surveillance and clinical notifications from a 32-year period (1980–2012). In that period, 584 cases of leptospirosis were laboratory-diagnosed, an average annual incidence rate of 0.34 cases/100,000 population (range: 0.07–1.1/100,000 population). Seventy per cent of patients were male. Overall, Patoc was the predominant serogroup diagnosed (32%) but over time, the *Leptospira* serogroup distribution has changed. In recent years Icterohaemorrhagiae and Sejroe have been diagnosed most frequently, in contrast to Patoc and Sejroe in earlier years. Notification data for 170 cases showed that work-related exposures were reported in 48% of infections, with fish farming (44%) and farming (22%) as the most frequently mentioned professions. Other common exposures were related to travel (13%), recreation (8%) and sewage (7%). Geomapping of cases showed a geographical clustering for some exposures. Future preventive measures could include raising awareness among clinicians about the risks and prevention of exposure in specific groups (fish farmers, farmers and travellers) to reduce leptospirosis in Denmark.

Introduction

Leptospirosis is a serious, acute febrile disease caused by spirochaetes from different species of pathogenic *Leptospira* bacteria. Leptospirosis is recognised as an emerging public health problem worldwide [1]. It is considered a zoonotic disease, as pathogenic *Leptospira* live in the kidneys of many host animals, including livestock and rodents. In Denmark, rats and mice are the most common carriers of leptospires, but many other animals, including cows and dogs can carry the bacteria. It has also been shown that in 2006 and 2007 in certain suburban sewage areas of Copenhagen, the

prevalence of *Leptospira* spp. in rats ranged between 48% and 89% [2]. The bacteria are shed into the environment via urine and can survive in fresh water like rivers and lakes, but not in sea water. The optimal environment for *Leptospira* are warm and humid conditions, but they survive in temperate climates as well. Infection in humans can occur through direct contact with an infected animal or its excrements (primarily urine) or through contaminated fresh or sewage water [3].

A *Leptospira* strain with unique antigens is termed a serovar, and several serovars with related antigens are placed in a serogroup. Approximately 30 serogroups are recognised containing ca 300 different serovars [4]. *Leptospira* spp. serovars are often specific to particular hosts and can therefore indicate a probable source of infection in humans [4,5], e.g. Icterohaemorrhagiae from rats, Sejroe and Saxkoebing from mice, Canicola from dogs, Hardjo from cows, and Pomona and Bratislava from pigs.

The disease has a mild and a severe form. Most commonly, the symptoms are non-specific and include fever, abdominal and chest pain and nausea, but can in severe cases lead to renal failure and haemorrhage, known as Weil's disease. As the disease resembles several other acute infections, the differential diagnosis includes influenza, viral meningitis, acute abdominal infection, glomerulonephritis [4], but also other zoonotic diseases occurring in the same epidemiological and ecological context, such as hanta virus infection, brucellosis and Q-fever, should be considered. For correct diagnosis it is essential to focus on the patient's travel history, activities, and exposure to animals. Culturing can be difficult, time consuming and requires specialised growth media, and is therefore not recommended for a quick routine diagnosis, but

it remains essential for a direct diagnosis of leptospirosis as well as for surveillance of resistance profiles. Leptospirosis diagnosis without culturing is conventionally performed serologically [6] and, more recently, by PCR [7,8].

Leptospirosis has been studied and diagnosed in Europe for a long time and historical reviews from Germany, France, Portugal and the Netherlands [9-12] have provided insight into the epidemiology of leptospirosis. The aim of this study was to describe the incidence of human leptospirosis in Denmark over time and analyse possible sources of exposure.

Methods

Laboratory diagnosis

Culturing for *Leptospira* is normally performed from blood, urine and spinal fluid in either Korthof medium or EMJH medium (DIFCO). In this study, patient sera were tested for specific antibodies by microagglutination test (MAT) with a variety of *Leptospira* strains which represented the strains Danish patients are typically infected with [4,5]. Over time, the serovars included in the MAT have changed. The MAT included *Leptospira* spp. of serovars: Ballum, Bataviae, Bratislava, Canicola, Grippotyphosa, Hardjo, Hurstbridge, Icterohaemorrhagiae, Poi, Pomona, Saxkoebing and Sejroe from 1980 to 2011 and included locally selected strains. The use of serovar Poi was discontinued in 2008 because of poor growth of the strain. In order to apply the World Health Organization standards and to be able to compare the Danish data with other European reference laboratories, serovars included in the MAT were switched in 2012 to the strains ordered from the Royal Tropical Institute in Amsterdam and now include Autumnalis, Bataviae, Cynopteri, Canicola, Castellonis, Copenhageni, Grippotyphosa type Moskva, Hardjo, Hurstbridge, Icterohaemorrhagiae, Javanica, Pomona, Sejroe and Tarassovi. In addition to the mentioned serovars, the sera were also tested against the non-pathogenic strain Patoc, which has antigens common to many serovars in the *Leptospira* family. Patoc does not cause leptospirosis in humans, but agglutination with Patoc acts as a marker for an infection by a pathogenic *Leptospira* strain. The following dilutions of sera (in titre) 1:30, 1:100, 1:300, 1:1,000, 1:3,000, 1:10,000, and 1:30,000 were tested by MAT. The test was considered positive if the highest observed titre was $\leq 1:100$ for at least one of the serovars [4,13]. The infecting serogroup was deduced from the highest titre against at least one serovar in the MAT. Cross reactions between serovars are known [14], therefore we report the infecting serogroups. Since 2009, leptospirosis has also been diagnosed by an in house PCR test using an improved method of DNA extraction [8,15].

Data collection

Data from all cases of leptospirosis diagnosed from 1 January 1980 to 31 December 2012 in Denmark were retrieved from Statens Serum Institut (SSI), the sole

diagnostic laboratory in the country. As cases we included only patients with a laboratory confirmation of leptospirosis and living in Denmark. Clinical diagnosis and detection of *Leptospira* and/or specific antibodies against *Leptospira* is notifiable under Danish law to the local medical officer of health (embedslæge) and to the Department of Infectious Diseases Epidemiology, SSI. The notification includes information on the infected person, location and timeframe of disease onset, documentation of admission to the hospital, as well as data pertaining to the patient's profession and/or workplaces, travel abroad, and any specific information referring to the source of infection.

Population data for the five Danish administrative regions including sex and age group distribution were provided by Denmark's Statistics and the population as of 1 January of each year (www.dst.dk) was used for analysis. For the years before 2007, population data per county (in 2007, 16 counties became five regions) was acquired and calculated into population per region.

Geomapping and geocoding

QGIS 1.8.0_Lisboa (www.qgis.org) was used for the spatial analysis of leptospirosis cases and plotting of incidence per region and per county. A geographical database with county and region borders in vector format (SHP file) was obtained from the Danish Geodata Agency (GST). Leptospirosis cases were geocoded using the Central Population Registry (CPR registry) and the geocoding of addresses from GST. The address data used for the study originated from the Danish Geodata Agency and were built on Official Standard Addresses and Coordinates (OSAK). The standard addresses from the public information server (den offentlige informationsserver, OIS, the basis for the OSAK addresses) are constructed from address data that the Danish municipalities provide. The addresses from all municipalities are gathered in OIS. In OSAK, these data are supplied with extra information such as

FIGURE 1
Annual incidence rate of leptospirosis, Denmark, 1980–2012

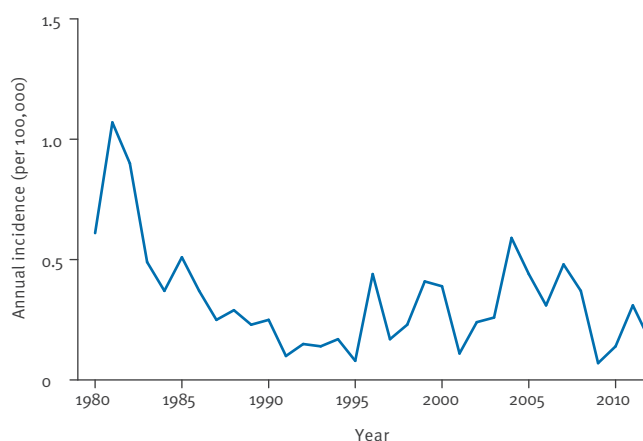
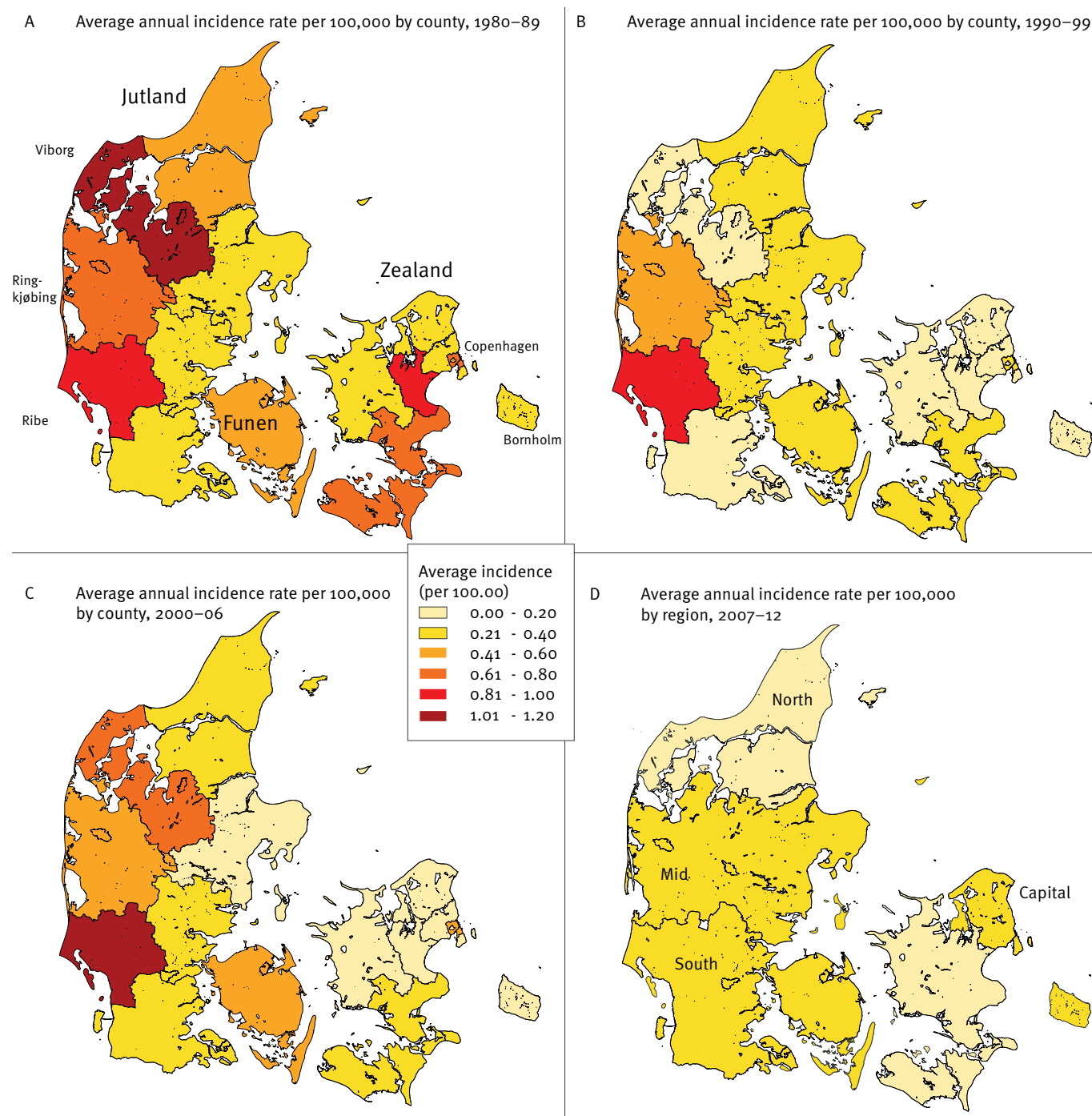


FIGURE 2

Geographical distribution of leptospirosis over time, Denmark, 1980–2012



postal codes and are therefore well suited for national use. An OSAK address consists of an address with an address point attached, which is defined with a set of Universal Transverse Mercator (UTM) coordinates. Furthermore, the register contains information about road code, road number and municipality code [16]. Addresses were joined to cases based on the date of disease notification or the date of diagnosis.

Results

Occurrence and incidence of leptospirosis

From 1980 to 2012, 584 Danish cases of leptospirosis were diagnosed in Denmark. The annual number of leptospirosis cases in Denmark peaked in 1981 with 55 cases, and then decreased until the early 1990s. Since then, the annual number of cases has varied from four to 32 cases per year. Over the whole period from 1980 to 2012, the average annual number of cases was 17.

This corresponds to an average annual incidence rate of 0.34/100,000 inhabitants, with the highest annual incidence in 1981 (1.1/100,000) and the lowest annual incidence in 2009 (0.07/100,000) (Figure 1).

Analysis of the incidence rate per county was possible for the period from 1980 to 2006, before Denmark has been organised in five regions. This showed that Ribe county had the highest incidence of 0.91/100,000 population, followed by Viborg county (0.56/100,000) and Ringkjøbing county (0.50/100,000), which are all located on the western coast of Jutland (Figure 2A). The incidence over time showed that Ribe county consistently had the highest incidence (Figure 2 B–C), while no cases were diagnosed in Viborg county between 1990 and 1999 (Figure 2B). Analysis of the incidence rate per region showed that the incidence rate was highest in the region South Denmark (0.44/100,000) and lowest in the region North Denmark (0.26/100,000) and the Capital Region (0.27/100,000) (Figure 2D). The highest annual incidence rate was observed in 1981 in Central Denmark (1.62/100,000).

Male patients accounted for 70% of all infections. Data on the age of the patient was available for 582 cases (99.7%) and the median age was 49.0 years (range: 0–87 years). The incidence rate per age group showed a clear peak for men in the age group 50–59 years, with 0.84/100,000 as a maximum, while the incidence rate for women increased with age, with a maximum incidence rate of 0.47/100,000 in the age group ≥70 years (Figure 3).

Leptospirosis diagnostic tests

The number of tests performed and persons tested could be obtained for the years 2005 to 2012. In those years, 4,438 tests had been performed at SSI on samples from 3,364 individuals. Some testing is performed on persons not residing in Denmark; between 2005 and 2012, 169 persons (5%) tested were not

Danish residents and were therefore excluded from the study. The annual number of tests performed per year increased from almost 400 per year in the period 2005 to 2007, to just below 600 in the period 2008 to 2010. In 2011, the number of tests increased abruptly to 1,018 after media reporting of a death due to leptospirosis. In 2012, the number of tests was 642. This shows that the number of samples submitted for testing has been stable over recent years.

Serogroups over time

Among all 584 diagnosed cases, the most commonly diagnosed serogroups were Patoc (n=187; 32.0%), Icterohaemorrhagiae (n=168; 28.8%) and Sejroe (n=146; 25.0%). However, the distribution of serovar over time has changed. Patoc has become less dominant among the serogroups; while it contributed 50.2% between 1980 and 1989, only 14.7% of the cases were identified as serogroup Patoc in the past 13 years (Table 1). At the same time, Icterohaemorrhagiae has become predominant: The contribution of this serogroup increased from 14.9% between 1980 and 1989 to 47.1% of the cases between 2010 and 2012. In recent years, more cases have had an unknown serogroup, following the introduction of PCR diagnostics in 2008. From all patients diagnosed by PCR, additional samples are requested for serogroup identification by MAT, but samples are not always available.

The monthly distribution of the cases with the most dominant serogroups Icterohaemorrhagiae, Sejroe and Patoc showed an incidence peak in the months August to November for Icterohaemorrhagiae, while the Sejroe and Patoc cases were scattered throughout the year (Figure 4).

Notifications, hospitalisations and deaths

Leptospirosis is a notifiable disease in Denmark and between 1980 and 2012, 170 of 584 diagnosed (30%) leptospirosis cases were notified to the Department of Infectious Diseases Epidemiology at SSI. In recent years, the proportion of notified cases among all diagnosed cases has increased, as between 2004 and 2012, on average 53% of the annual laboratory-diagnosed cases were notified.

For analysis of exposure, hospitalisations and deaths, the information provided in the notification was used. Among the 170 notified cases, 139 (82%) were hospitalised and four deaths (2.3%) were reported between 1980 and 2012.

Source of infection and serogroup

The notification data included information on the most likely source of infection. Among the 170 notified cases, 19 (11%) were female and 151 (89%) male. The sources of exposure are grouped into work (82 cases), travel abroad (22 cases), recreation (14 cases), sewage water (11 cases) and other (10 cases). The likely source of exposure was unknown for 31 notified cases (Table

FIGURE 3
Leptospirosis incidence rate by age and sex, Denmark, 1980–2012 (n = 582)

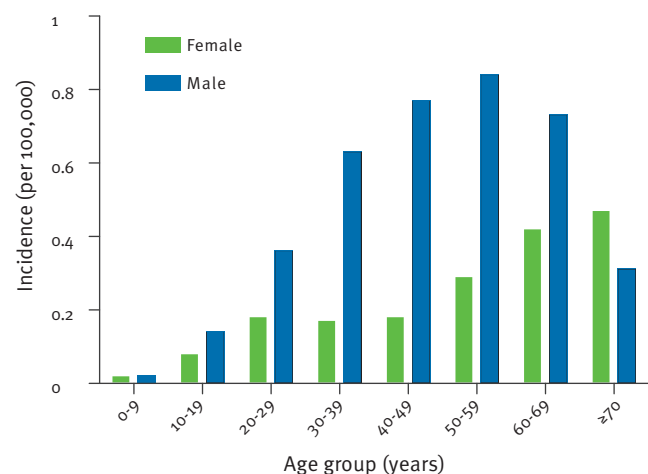


TABLE 1

Serogroup distribution of leptospirosis cases, Denmark, 1980–2012 (n = 584)

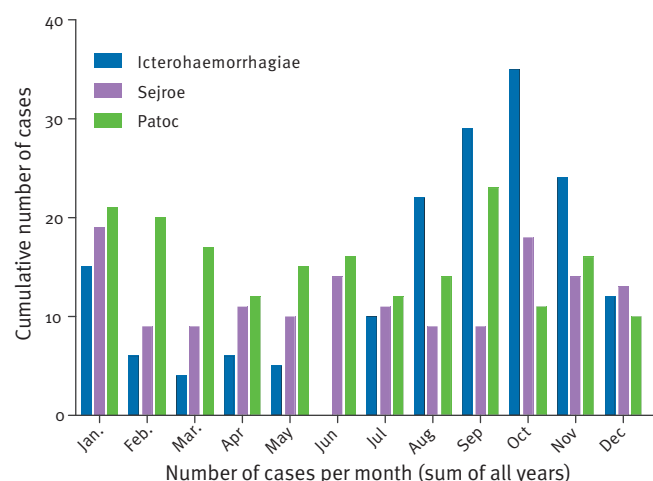
Serogroup	1980–89		1990–99		2000–09		2010–12		Total	
	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%
Ballum	1	0.4	0	0.0	2	1.1	1	2.9	4	0.7
Bataviae	0	0.0	1	0.9	0	0.0	1	2.9	2	0.3
Bratislava	11	4.2	8	7.1	6	3.4	0	0.0	25	4.3
Canicola	0	0.0	0	0.0	2	1.1	0	0.0	2	0.3
Grippotyphosa	0	0.0	0	0.0	3	1.7	0	0.0	3	0.5
Hardjo	0	0.0	1	0.9	2	1.1	0	0.0	3	0.5
Hurstbridge	0	0.0	2	1.8	7	4.0	0	0.0	9	1.5
Icterohaemorrhagiae	39	14.9	52	46.4	61	34.5	16	47.1	168	28.8
Patoc	131	50.2	25	22.3	26	14.7	5	14.7	187	32.0
Poi	5	1.9	5	4.5	11	6.2	0	0.0	21	3.6
Pomona	1	0.4	0	0.0	4	2.3	0	0.0	5	0.9
Sejroe	73	28.0	18	16.1	52	29.4	3	8.8	146	25.0
Unknown ^a	0	0.0	0	0.0	1	0.6	8	23.5	9	1.5
Total	261	100	112	100	177	100	34	100	584	100

^a Unknown serogroups are cases diagnosed by PCR only, since 2008.

2). Work-related cases accounted for almost half of the notified leptospirosis cases. The professions that were notified most frequently were fish farmers (45% of work-related cases), farmers (22%) and sewage workers (11%). Not work-related exposure to sewage water, e.g. cleaning of flooded areas after heavy rainfall, was also reported as exposure (6.5%). Among travel-related cases, Asia was the most common destination, but travel in Europe was also reported. In the group with recreational exposure, most exposures were related to fresh water activities.

FIGURE 4

Monthly distribution of leptospirosis cases of the three most dominant serogroups, Denmark, 1980–2012 (n=501)



Although the numbers per group are small, we analysed whether certain serogroups were more common in connection with a certain type of exposure. This showed that fish farmers in 27 of 37 cases were infected with serogroup Icterohaemorrhagiae, while farmers were infected in 10 of 18 cases with serogroup Sejroe. Sewage workers showed a variety of serogroups, while persons exposed to sewage at home were in nine of 11 cases infected with serogroup Icterohaemorrhagiae. Cases related to travel abroad showed more variety of serogroups, although Icterohaemorrhagiae and Sejroe were most commonly reported. Persons exposed during recreation in Denmark were infected with Icterohaemorrhagiae in eight of 14 cases.

Mapping of place of residence at the time of disease onset or of diagnosis was possible for 335 (57.4%) cases. Figure 5 shows the cases in Denmark and indicates the source of exposure. Fish farmers mostly resided in Ribe county and the rest of Jutland where most fish farms are located, while the cases with non-work related exposure to sewage were mainly located in the Copenhagen area (eight of 11 cases). Farmers as well as cases related to travel and recreation were more spread out over the country.

Discussion

Overall, leptospirosis incidence in Denmark has not changed remarkably in the past 32 years. About a third of all leptospirosis cases from 1980 to 2012 were female, similar to what is reported in Portugal (67% male cases) [12], while in the Netherlands, less than 10% of the cases are female [11]. The reported incidence ratio of male vs female cases was 5:1 in Germany

TABLE 2

Sex, possible exposures, and *Leptospira* serogroups of notified leptospirosis cases, Denmark, 1980–2012 (n = 170)

Exposures	Total (%)	Sex		Serogroup				
		M	F	IH	Sejroe	Patoc	Other	Unknown (PCR only)
Work	82 (48.2)	81	1	45	17	1	17	2
Fish farmer	37	37	0	27	2	1	6	1
Farmer	18	18	0	6	10	0	2	0
Sewage worker	9	9	0	2	2	0	4	1
Hunter	3	3	0	3	0	0	0	0
Mink farmer	2	2	0	1	0	0	1	0
Other	13	12	1	6	3	0	4	0
Travel abroad	22 (12.9)	16	6	8	6	1	3	4
Asia (Malaysia, Nepal, Thailand, Bali, Cambodia)	15	13	2	4	6	1	1	3
Europe (Italy, Poland, Spain)	4	1	3	2	0	0	1	1
Central America (Tobago, Venezuela)	2	2	0	1	0	0	1	0
World	1	0	1	1	0	0	0	0
Recreation in Denmark	14 (8.2)	14	0	8	1	2	3	0
Canoe/kayak	4	4	0	2	1	0	1	0
Fishing	3	3	0	2	0	0	1	0
Other water	4	4	0	2	0	1	1	0
Hunting and Fishing	1	1	0	1	0	0	0	0
Other (snake owner, orchard)	2	2	0	1	0	1	0	0
Sewage-related	11 (6.5)	9	2	9	0	0	0	2
Cleaning (after flooding)	9	7	2	7	0	0	0	2
Cleaning (not specified)	2	2	0	2	0	0	0	0
Other exposures	10 (5.9)	6	4	3	1	1	5	0
Farm (living/visiting)	9	5	4	3	0	1	5	0
Combination travel/sewage worker	1	1	0	0	1	0	0	0
Unknown exposure	31 (18.2)	25	6	12	9	4	5	1
Total	170 (100)	151	19	85	34	9	33	9

F: female; IH: Icterohaemorrhagiae; M: male.

and 10:1 in France and Italy [17]. Whether these numbers reflect the true number of cases in these countries is unclear, as it has been reported that clinical leptospirosis is typically more severe in men, which may lead to systematic underinvestigation and undertreatment of female cases [18]. As leptospirosis is known as a neglected disease we analysed whether the number of tests performed had dropped over recent years, but no decline has been observed in Denmark since 2005. In contrast, a strong increase in the number of tests occurred after media reporting on a death due to leptospirosis in 2011.

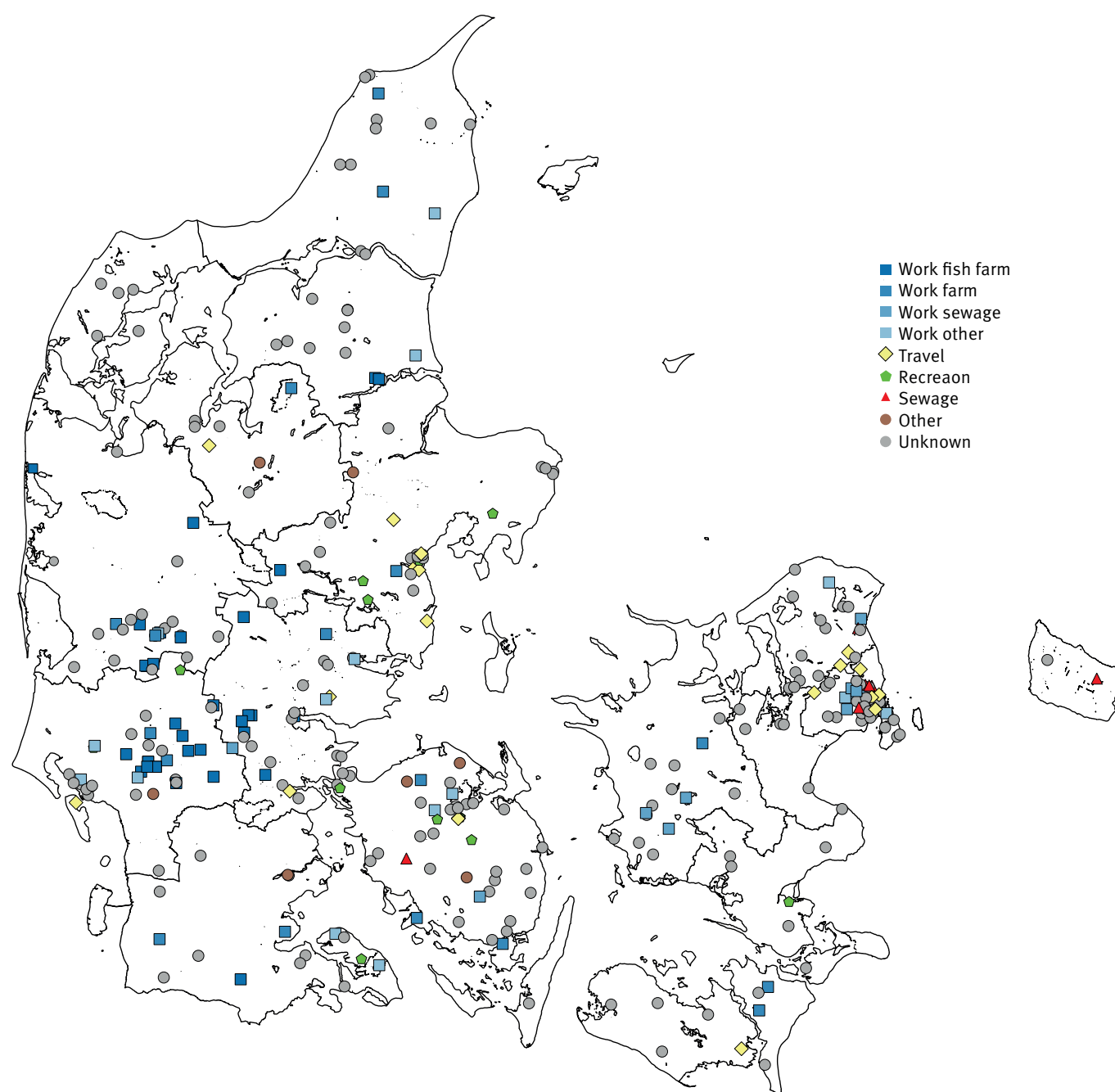
The trend in *Leptospira* serogroup distribution has changed over the past 32 years. In recent years, Icterohaemorrhagiae has replaced serogroup Patoc, which cross-reacts with most pathogenic *Leptospira* not included in the MAT panel. In Denmark, Icterohaemorrhagiae and Sejroe have become the predominant serogroups during the past 12 years,

while in France and the Netherlands, the two most predominant serogroups are Icterohaemorrhagiae and Grippotyphosa [10,11] and Ireland reports Icterohaemorrhagiae and Hardjo as the dominant serogroups [19]. Unfortunately, since the introduction of PCR diagnostics, we have seen an increase in cases without a known serogroup, as MAT is not performed for all PCR-positive cases.

Overall in Denmark, work-related exposure comprises a larger part of the leptospirosis cases than in other countries, where travel and recreational activities are far more important exposures [9,11,19]. The work-related exposure has decreased compared with an earlier report from Denmark [20], partially due to an increase in travel-related exposure, as could be expected due to an increase in international travel in recent years. Travel-related exposure was seen in 13% of our cases and is observed in other European countries, as illustrated by a recent report of two confirmed

FIGURE 5

Geographical mapping of leptospirosis cases by exposure, Denmark, 1980–2012 (n = 335)



cases after travel in Spain [21]. A recent review also shows a clear increase in the proportion of travel-associated leptospirosis over time [22].

The highest incidence of leptospirosis over the years was observed in Ribe county, as reported previously [5,20]. Geomapping of our cases by exposure showed that most cases in Ribe county are fish farmers. Fish farms only exist in the Danish peninsula of Jutland and constitute an attractive environment for rats. Despite the decline in fresh-water fish farms from more than 700 in the early 1980s, to 196 in 2011 [23], it is still

an important industry in Denmark. Fish farmers constituted 36% of work-related exposures between 2000 and 2009, while no cases among fish farmers have been observed since 2009. Farmers and sewage workers represent the other important work-related exposures, as has been reported from Germany and Ireland [9,19]. Analysis of *Leptospira* serogroup distribution for the predominant types of exposure showed that fish farmers were most commonly infected with serogroup Icterohaemorrhagiae, the serogroup linked to rats. In contrast, farmers were most commonly infected with serogroup Sejroe, suggesting that mice

may be involved in transmission of leptospirosis. Borg-Petersen first isolated serovar Saxkoebing of the Sejroe serogroup in Denmark from the yellow-necked mouse *Apodemus flavicollis* [24], and infection has been reported on rare occasions in man [25]. As rats are well-known carriers of leptospirosis in Denmark, we analysed whether a correlation existed between the annual number of reported rat sightings, as proxy for the rat population in Denmark, and the annual number of leptospirosis cases. Data on the rat population was provided by the Danish Ministry of the Environment in the form of the number of reports of rat sightings per year between 1996 and 2012 (personal communication: Kirsten Søndergaard, July 2014). No correlation between the annual number of rat sightings and leptospirosis cases was found (data not shown). However, as leptospirosis serogroups associated with rats and mice were most frequent in Denmark, rodent control and attention to the risk of infection from rodents' habitats could help prevent the infection.

Exposure to sewage, either work-related or at home (12% of notified cases) can also be a factor in acquiring leptospirosis in Denmark. A recent study addressing illness after cleaning of flooded areas in Copenhagen in July 2011 showed that 56 of 257 (22%) of the involved professionals developed symptoms of illness [26]. A cluster of five leptospirosis cases was detected in Copenhagen after the flooding and one person died. Although only 6.5% of the notified cases in our study were exposed to sewage or flooded areas at home, this could increase in the future, as data from the Danish Meteorological Institute indicate that rising temperatures worldwide could result in more frequent extreme rainfall and storms in Denmark, resulting in more frequent flooding and thus possible exposure to leptospirosis [27]. Reports describing a link between leptospirosis and extreme weather such as heavy rainfall and flooding have been published recently [28,29].

One limitation of our study was that only a proportion of diagnosed cases were notified clinically. Among the notified cases, only 11% were female, while overall, women comprised 30% of the cases. Furthermore, the serogroup distribution was different when comparing all cases with the group of notified cases, as serogroup Patoc was observed in only nine of 170 (5.3%) notified cases, while overall, serogroup Patoc was identified in 32% of cases, and in 14.7% of the cases in the past 13 years. This indicates that the notified cases may not give a true representation of all cases and it is possible that we lack important information on possible exposure among female patients and patients with certain serogroups of *Leptospira*. The discrepancy between the diagnosed cases and the notified cases may reflect differences in disease severity, where a clinically more severe case may be more readily notified. This hypothesis is supported by the fact that fewer women were observed in this group and serogroup Icterohaemorrhagiae was overrepresented among our notified cases. More detailed clinical information on

the severity of disease would have been very useful, but unfortunately clinical information is very limited on the notification forms used in Denmark and was not available for this study.

Leptospirosis is a serious disease, as reflected by the hospitalisation rate of 81% and four reported deaths among our notified cases. The severity of acute infection is obvious, but the long-term effects of Leptospirosis are unknown and chronic infections with *Leptospira* have been previously reported [30]. Leptospirosis has also been implicated as a cause of uveitis in humans [31]. Therefore, it is possible that *Leptospira* may have so far unknown similar chronicity and sequelae as seen in other infections with spirochaetes such as *Borrelia* and *Treponema*.

The non-specific symptoms make the disease likely to be underdiagnosed. In addition, the incidence could increase in the future due to predicted extreme weather conditions and the increase in adventure travels which can include water sports in exotic destinations. However, there is also potential for prevention. To prevent leptospirosis in Denmark, it is recommended to raise awareness among specific groups, such as fish farmers and travellers to Asia, about the risks and prevention of exposure. In addition, awareness should be raised among clinicians about the risk of leptospirosis exposure among these groups.

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Conflict of interest

None declared.

Authors' contributions

LBvA, AK and CK have collected the leptospirosis data. LBvA, TC, JK, SE and KAK have analysed the data. LBvA wrote the first draft of the manuscript. All other authors have contributed to further versions of the manuscript and approved the final version before submission.

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New developments of influenza surveillance in Europe

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The influenza season 2014/15 has started in Europe [1] and developments can be followed closely via the joint European Centre for Disease Prevention and Control (ECDC) and World Health Organization (WHO) influenza bulletin. The bulletin was launched in October 2014 and is available from www.flunewseurope.org.

All 53 European countries have been invited to report through the single joint entry point to the European Surveillance System (TESSy), the ECDC system for reporting data. Data from TESSy are then used to publish the joint weekly bulletin in English and Russian. Data transfer to the WHO global platforms FluNet and FluID will continue to be managed by the WHO Regional Office for Europe.

Since week 40 in 2014, 53 European countries have the opportunity to report influenza surveillance data to a single platform with the analysis posted in a new European joint weekly bulletin as well as in the WHO global influenza update. Extended influenza surveillance in Europe will provide more data to better estimate the burden of the disease.

The new bulletin includes features such as a format for interactive consultation by country, and a better description of data sets from different surveillance systems.

Collaboration among European countries for creating a harmonised network of influenza surveillance started in the 1990s and has evolved incrementally with additional countries and extended objectives [2]. Since 2008, the European Influenza Surveillance Network (EISN), covering 30 EU/EEA countries, has been managed by the ECDC. In parallel, the WHO Regional Office for Europe has been covering the 53 countries of the European Region, including all EU/EEA countries. In this structure, countries initially reported data derived from sentinel and other clinical and laboratory surveillance systems via two different platforms. Each organisation has also published separate bulletins (the Weekly Influenza Surveillance Overview and Euroflu, respectively). This dual reporting resulted in unavoidable discrepancies [3].

In 2010, the European Surveillance System (TESSy), was established at ECDC as a single data entry point in order to synchronise reporting for both platforms [4] and in 2014, based on feedback from key stakeholders, ECDC and the WHO Regional Office for Europe decided to publish a single joint influenza bulletin for the WHO European Region and EU/EEA Member States.

Activities of the influenza surveillance will continue to be streamlined by means of influenza surveillance meetings, ad hoc working groups to tackle specific topics and a range of laboratory strengthening activities including external quality assessment and training.

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The 2013 joint ECDC/EFSA report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks published

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On 28 January 2015, the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) joint summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks, was published. The report presents the results of zoonoses monitoring activities carried out in 2013 in 32 European countries, 28 European Union (EU) Member States and four non-Member States [1].

The report shows that campylobacteriosis remains the most commonly reported zoonosis in the EU. After several years of an increasing trend, the campylobacteriosis notification rate has stabilised around the 2012 level. *Campylobacter*, the causative agent of campylobacteriosis, is mostly found in chicken meat.

The number of reported listeriosis cases, 1,763, represents an 8.6 percent increase between 2012 and 2013 and reflects an increasing EU trend in 2009-2013. Although the number of confirmed cases is relatively low, it is still a cause for concern as the reported *Listeria* infections are mostly severe, invasive forms of the disease with higher death rates than for other foodborne diseases.

The number of confirmed verocytotoxigenic *Escherichia coli* (VTEC) infections in humans also increased. In 2013 reported cases of VTEC infection rose by 5.9 percent compared to 2012. This may reflect the effect of increased awareness in Member States following the 2011 outbreak, which translated into better testing and reporting.

The decreasing EU trend in confirmed human salmonellosis cases observed in recent years continued. The reported number of salmonellosis fell for the eighth year in a row with a 7.9 percent decrease between 2012 and 2013. Most Member States met their *Salmonella* reduction targets for poultry. The report also shows a continued 2009-2013 decreasing EU trend in confirmed yersiniosis cases. Positive findings for *Yersinia* were mainly reported in pig meat and pig meat products.

In total, 5,196 food-borne and water-borne outbreaks were reported in the EU in 2013. *Salmonella* was the most common causative agent in foodborne outbreaks with known origin, followed by viruses, bacterial toxins and *Campylobacter*. In 28.9 % of all outbreaks the causative agent was unknown. Eggs and egg products, followed by mixed food, and fish and fish products were the most important food vehicles in food-borne outbreaks.

The report further summarises trends and sources along the food chain caused by zoonoses such as *Brucella*, *Trichinella*, *Echinococcus*, *Toxoplasma*, rabies, *Coxiella burnetii* (Q fever), West Nile virus and tularemia, as well as on cases of tuberculosis caused by *Mycobacterium bovis*.

Read more about food and waterborne diseases and zoonoses on the [ECDC website](#).

References

1. EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2015. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2013. EFSA Journal 2015;13(1):3991, 162 pp. doi:10.2903/j.efsa.2015.3991 Available from: <http://www.efsa.europa.eu/en/efsajournal/doc/3991.pdf>