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Successful methodology for large-scale surveillance of severe events following influenza vaccination in Canada, 2011 and 2012

J A Bettinger (jbettinger@cfri.ca)[1], I Rouleau[2], M C Gariépy[2], W R Bowie[3], L Valiquette[4], O G Vanderkooi[5], J D Kellner[5], B L Coleman[6], S A McNeil[7], A McCarthy[8], G De Serres[2,9], On behalf of the Public Health Agency of Canada/Canadian Institutes for Health Research Influenza Research Network

1. Vaccine Evaluation Center, BC Children's Hospital, University of British Columbia, Vancouver, British Columbia, Canada
2. Centre de Recherche du CHU de Québec, Laval University, Canada
3. Vancouver General Hospital, University of British Columbia, Vancouver, British Columbia, Canada
4. Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, Quebec, Canada
5. Alberta Children's Hospital, University of Calgary, Calgary, Alberta, Canada
6. Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada
7. Canadian Center for Vaccinology, IWK Health Centre, Queen Elizabeth II Health Sciences Centre and Dalhousie University, Halifax, Nova Scotia, Canada
8. Ottawa Hospital, Ottawa, Ontario, Canada
9. Institut National de Santé Publique du Quebec, Quebec City, Quebec, Canada

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In 2011 and 2012, a nationwide Canadian vaccine safety surveillance network rapidly collected safety data from healthcare workers (HCW) during the first weeks of the annual influenza vaccination campaign. This network provided the first available post-marketing safety data on seasonal influenza vaccines with information on background rates as a comparator. In 2012, these data were used to investigate a possible safety concern regarding a particular vaccine. An online questionnaire was provided to participating HCW two weeks before the annual influenza vaccination campaign for controls, and eight days after influenza vaccination for vaccinees. Control and vaccinees were requested to report health events occurring in the seven days prior to receiving the questionnaire. Control data were used to calculate background rates. HCW reporting a severe event were followed-up by telephone within 48 hours of the online report to validate the report and check on their health status. More than 22,000 vaccinated HCW were enrolled and surveyed over two seasons and >90% reported no severe event following vaccination. Validated severe event rates were similar in vaccinated HCW and unvaccinated HCW (2.2% vs 2.3%; p<0.70). The questionnaire was accurately completed for most reported symptoms, matched the validated report and was able to detect events of interest. Prior to the safety concern, the implicated vaccine was in use at one centre. Reassuring safety data were provided to public health authorities 48 hours after the vaccine was temporarily suspended. Data from this and similar networks can be used for rapid evaluation of vaccine safety and for safety assessment as required by the European Medicines Agency in 2015.

Introduction

Influenza vaccines are modified yearly to include the influenza viral strains most likely to circulate during the next influenza season. Starting in 2015, European regulatory requirements to evaluate the safety and immunogenicity of seasonal influenza vaccines in small scale clinical trials will be withdrawn [1]. Such trials had insufficient power to adequately evaluate safety concerns arising from annual formulation changes (e.g. adverse events occurring at a rate of 1–2%). These clinical trials are to be replaced by enhanced, preferably active, safety monitoring and vaccine effectiveness assessments [2].

Recognising the need for timely information supporting the seasonal vaccines' safety profiles early in the annual immunisation campaign, a sentinel network was established in Canada in 2009 [3] to conduct online safety monitoring. The goals of the online surveillance are to detect any safety signals and provide an estimate of severe events reported in vaccinated and non-vaccinated individuals.

As part of the sentinel network, methodology for online, active, safety monitoring was further refined and tested and is described here. This study of the network surveillance aimed to assess health events reported following vaccination, by healthcare workers

Table 1

Characteristics of Canadian health care workers enrolled in 2011 and 2012, who responded to an online questionnaire and randomly selected non-responders contacted for validation in 2012, Canada, 2011–2012 (n=43,776 healthcare workers)

	2011/12 influenza season			2012/13 influenza season					
	Controls N (%)	Vaccinees N (%)	P-value[a]	Controls N (%)	Control initial non-responder N (%)	P-value[b]	Vaccinees N (%)	Vaccinee initial non-responder N (%)	P-value
Enrollment and participation									
Enrolled	12,238 (100)	10,070 (100)	–	9,458 (100)	NA	–	12,010 (100)	NA	–
Response (rate)	1,616 (13.2)	7,496 (74.4)	–	2,479 (26.2)	921 (9.7)	–	7,667 (63.8)	994 (8.3)	–
Sex									
Female	1,239 (76.7)	5,462 (72.9)	0.002	1,849 (74.6)	690 (74.9)	0.84	5,634 (73.5)	749 (75.4)	0.28[c]; 0.21[d]
Age group, in years									
<30	270 (16.7)	1,786 (23.8)	<0.001	468 (18.9)	197 (21.4)	0.30	1,882 (24.5)	218 (21.9)	<0.001[c]; <0.001[d]
30–39	351 (21.7)	1,735 (23.2)		609 (24.6)	201 (21.8)		1,830 (23.9)	199 (20)	
40–49	345 (21.4)	1,656 (22.1)		552 (22.3)	200 (21.7)		1,668 (21.8)	210 (21.1)	
50–59	479 (29.6)	1,683 (22.5)		615 (24.8)	228 (24.8)		1,688 (22.0)	260 (26.2)	
≥60	167 (10.3)	632 (8.4)		235 (9.5)	95 (10.3)		599 (7.8)	107 (10.8)	
Occupation									
Physician	210 (13.0)	1,049 (14.0)	<0.001	367 (14.8)	101 (11.0)	<0.001	1,130 (14.7)	88 (8.9)	<0.001[c]; <0.001[d]
Nurse/assistant nurse	292 (18.1)	1,498 (20.0)		439 (17.7)	231 (25.1)		1,537 (20)	251 (25.3)	
Patient care assistant	10 (0.6)	123 (1.6)		19 (0.8)	28 (3.0)		102 (1.3)	47 (4.7)	
Medical technologist	117 (7.3)	543 (7.2)		178 (7.2)	81 (8.8)		537 (7)	81 (8.1)	
Technician/other health professional	293 (18.2)	1,126 (15.0)		405 (16.3)	110 (11.9)		1,192 (15.5)	121 (12.2)	
Housekeeping, logistics/food service	50 (3.1)	357 (4.8)		45 (1.8)	40 (4.3)		211 (2.8)	70 (7)	
Administrative/ office	348 (21.6)	1,281 (17.1)		486 (19.6)	180 (19.5)		1,582 (20.6)	211 (21.2)	
Trainee/student	51 (3.2)	432 (5.8)		139 (5.6)	39 (4.2)		560 (7.3)	56 (5.6)	
Research	148 (9.2)	456 (6.1)		258 (10.4)	49 (5.3)		527 (6.9)	27 (2.7)	
Volunteer	0 (0)	0 (0)		71 (2.9)	24 (2.6)		244 (3.2)	38 (3.8)	
Other	94 (5.8)	353 (4.7)		71 (2.9)	38 (4.1)		45 (0.6)	4 (0.4)	

NA: not applicable.

[a] P-value for difference in characteristics between controls and vaccinees who respectively responded to the online questionnaire in 2011.

[b] P-value for difference in characteristics between controls who responded to the online questionnaire and controls who initially did not respond to the questionnaire (controls initial non-responders) in 2012.

[c] P-value for difference in characteristics between controls and vaccinees who respectively responded to the online questionnaire in 2012.

[d] P-value for difference in characteristics between vaccinees who responded to the online questionnaire and the vaccinees who initially did not respond to the questionnaire (vaccinees initial non-responders) in 2012.

Table 2

Number and rate of health events reported by healthcare workers preceding (controls) or following (vaccinees) influenza vaccination in 2011 and 2012 and health events reported by initial non-responders among controls and vaccinees in 2012, Canada, 2011–2012 (n=1,922 health events)

Type of event reported	2011		2012		Both years			2012			
	Controls (N=1,616) n (%)	Vaccinees (N=7,496) n (%)	Controls (N=2,479) n (%)	Vaccinees (N=7,667) n (%)	Controls (N=4,095) n (%)	Vaccinees (N=15,163) n (%)	P-value[a]	Control initial non-responder (N=921) n (%)	P-value[b]	Vaccinee initial non-responder (N=994) n (%)	P-value[c]
Any event	164 (10.1)	696 (9.3)	232 (9.4)	692 (9.0)	396 (9.7)	1,388 (9.2)	0.31	77 (8.4)	0.38	61 (6.1)	0.002
Severe event[d]	52 (3.2)	155 (2.1)	97 (3.9)	233 (3.0)	149 (3.6)	388 (2.6)	<0.001	36 (3.9)	0.84	27 (2.7)	0.62
Validated event[e]	25 (1.5)	127 (1.7)	69 (2.7)	206 (2.7)	94 (2.3)	333 (2.2)	0.70	NA	–	NA	–
Local reaction	NA	102 (1.4)	NA	99 (1.3)	NA	201 (1.3)	–	NA	–	17 (1.7)	0.30
Severe event[d]	NA	14 (0.2)	NA	17 (0.2)	NA	31 (0.2)	–	NA	–	0 (0.0)	0.61
Validated event[e]	NA	2 (0.03)	NA	5 (0.1)	NA	7 (0.0)	–	NA	–	NA	–
Systemic symptoms	35 (2.2)	239 (3.2)	73 (2.9)	336 (4.4)	108 (2.6)	575 (3.8)	<0.001	28 (3.0)	0.91	28 (2.8)	0.02
Severe event[d]	24 (1.5)	95 (1.3)	52 (2.1)	172 (2.2)	76 (1.9)	267 (1.8)	0.68	14 (1.5)	0.33	17 (1.7)	0.35
Validated event[e]	0 (0.0)	30 (0.4)	11 (0.4)	53 (0.7)	11 (0.3)	83 (0.5)	0.02	NA	–	NA	–
Allergy-like events	1 (0.06)	7 (0.09)	7 (0.3)	4 (0.05)	8 (0.2)	11 (0.1)	0.03	4 (0.4)	0.50	1 (0.1)	0.46
Severe event[d]	0 (0.0)	4 (0.05)	4 (0.2)	2 (0.03)	4 (0.1)	6 (0.0)	0.15	2 (0.2)	0.67	1 (0.1)	0.31
Validated event[e]	0 (0.0)	1 (0.01)	0 (0.0)	1 (0.01)	1 (0.0)	2 (0.0)	0.61	NA	–	NA	–
Respiratory symptoms	31 (1.9)	124 (1.6)	63 (2.5)	131 (1.7)	94 (2.3)	255 (1.7)	0.01	29 (3.2)	0.34	17 (1.7)	>0.99
Severe event[d]	22 (1.4)	74 (1.0)	43 (1.7)	84 (1.1)	65 (1.6)	158 (1.0)	0.004	13 (1.4)	0.65	15 (1.5)	0.26
Validated event[e]	16 (1.0)	58 (0.8)	39 (1.6)	73 (1.0)	52 (1.3)	131 (0.9)	0.02	NA	–	NA	–
GI symptoms	11 (0.7)	67 (0.9)	29 (1.2)	97 (1.3)	40 (1.0)	164 (1.1)	0.56	12 (1.3)	0.73	6 (0.6)	0.09
Severe event[d]	10 (0.6)	39 (0.5)	22 (0.9)	71 (0.9)	32 (0.8)	110 (0.7)	0.71	10 (1.1)	0.56	5 (0.5)	0.21
Validated event[e]	6 (0.4)	20 (0.3)	7 (0.3)	34 (0.4)	13 (0.3)	54 (0.4)	0.71	NA	–	NA	–
ORS[f]	15 (0.9)	191 (2.5)	87 (3.5)	163 (2.1)	102 (2.5)	354 (2.3)	0.56	22 (2.4)	0.10	13 (1.3)	0.09
Severe event[d]	3 (0.2)	21 (0.3)	13 (0.5)	21 (0.3)	16 (0.4)	42 (0.3)	0.24	5 (0.5)	>0.99	3 (0.3)	0.75
Validated event[e]	0 (0.0)	2 (0.03)	0	6 (0.08)	0 (0.0)	8 (0.1)	–	NA	–	NA	–
Paraesthesia[f]	4 (0.3)	165 (2.2)	49 (2.0)	85 (1.1)	53 (1.3)	250 (1.6)	0.11	5 (0.5)	0.002	6 (0.6)	0.18
Severe event[d]	0.7 (0.04)	9 (0.1)	9 (0.4)	7 (0.09)	10 (0.2)	16 (0.1)	0.03	2 (0.2)	0.74	3 (0.3)	0.10
Validated event[e]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	–	NA	–	NA	–
Other	7 (0.4)	54 (0.7)	31 (1.3)	101 (1.3)	38 (0.9)	155 (1.0)	0.59	20 (2.2)	0.05	11 (1.1)	0.66
Severe event[d]	3 (0.2)	16 (0.2)	21 (0.8)	38 (0.5)	24 (0.6)	54 (0.4)	0.04	12 (1.3)	0.24	8 (0.8)	0.24
Validated event[e]	3 (0.2)	14 (0.2)	10 (0.4)	34 (0.4)	13 (0.3)	48 (0.3)	0.99	NA	–	NA	–

GI: gastrointestinal; NA: not applicable; ORS: oculorespiratory syndrome.

[a] P-value for difference in health event rate between controls and vaccinees in both years (2011, 2012). Significance ≤ 0.002 adjusted for multiple comparisons using Bonferroni's correction.

[b] P-value for difference in health event rate between controls who responded to the online questionnaire and controls who initially did not respond (controls initial non-responders) in 2012. Significance ≤ 0.002 adjusted for multiple comparisons using Bonferroni's correction.

[c] P-value for difference in health event rate between vaccinees who responded to the online questionnaire and vaccinees who initially did not respond (vaccinee initial non-responders) in 2012. Significance ≤ 0.002 adjusted for multiple comparisons using Bonferroni's correction.

[d] Severe event is defined as a health event preventing daily activities or causing work absenteeism or requiring a medical consultation, or any combination of these effects.

[e] Validated events are self-reported severe events in the online questionnaire, which remained the primary diagnosis after a nurse follow-up.

[f] Affected per 24 hours. The number of ORS and paraesthesia events for 2011 controls were divided by seven to adjust for the difference in reporting period for these controls. The reporting period for the 2011 controls was seven days, compared with 24 hours for controls in 2012 as well as for vaccinees in 2011 and 2012.

(HCW) participating in an online questionnaire. To establish background rates for health events, non-vaccinated HCW were also recruited to respond to the questionnaire. Telephone follow-up of participants reporting severe health events allowed estimation of the validity of such self-reported events. The representativeness of health events reported by online responders was also assessed by comparing the rates of health events in participants who responded to the online questionnaire to those who did not. The study was conducted during the two immunisation seasons of 2011 and 2012, whereby in 2012, prior to the temporary suspension of a seasonal influenza vaccine [4], some data were collected. The brief, voluntary suspension of the vaccine offered a valuable opportunity to assess the capacity of the Canadian network's ability to detect any signal of severe events post-vaccination and to rapidly provide safety data to public health decision makers.

Methods

Online surveillance system

HCW who received the influenza vaccine in 2011 or 2012 were recruited to participate in an online survey from seven and eight Canadian acute care hospital sites respectively, in Alberta (2012 only), British Columbia, Nova Scotia, Ontario, and Quebec. The HCW were invited to enrol in the study when presenting for vaccination at a participating hospital, and provided their email address, telephone number(s) and informed consent. Enrolled vaccinated HCW were sent an email eight days after vaccination with a link to an online health event questionnaire. Vaccinee non-responders were sent a reminder email three days later.

Two weeks before the start of the 2011 and 2012 vaccination campaigns respectively at seven of the eight sites, HCW immunised in the previous year were invited to serve as a control group to establish the background rates for health events. Conducting the control survey before the start of influenza vaccination allowed for compliance with national recommendations for all HCW to receive the influenza vaccine and provided a comparable control group for vaccinees. Controls were sent an email with an embedded link to the online surveillance questionnaire which remained active until the day before the start of their institution's influenza vaccination programme (SimpleSurvey v2.17.0, OutSideSoft Solutions inc., Saint-Jean-sur-Richelieu, Quebec). Non-responders in the control group were sent a reminder email three days after the initial email link was sent.

Participants were identified by a unique study code and email addresses were not linked with the questionnaire responses. The study was approved by the research ethics boards at each site.

The online questionnaires collected information on demographics (i.e. age, sex, occupation), past influenza vaccination history and occurrence of health events of interest. Health events occurring in the seven days before receiving the questionnaire link were documented by broad categories: local injection site reactions (vaccinated HCW only), systemic symptoms (fever as temperature ≥38.5°C, fatigue, myalgia), respiratory symptoms suggestive of allergy-like events, bronchitis, cold, gastrointestinal symptoms (diarrhoea, nausea, vomiting), influenza, pharyngitis, pneumonia, sinusitis, tonsillitis and any other health event. Symptoms of oculorespiratory syndrome (ORS) and numbness (anaesthesia/paraesthesia) were also solicited [5-7]. ORS was defined according to the National Advisory Committee on Immunization definition [7]. All events were considered severe if they prevented daily activities, resulted in work absenteeism, or required a medical consultation.

Observed health events and capacity for signal investigation

For each study year, rates of health events reported following vaccination were compared with those observed among controls. Symptoms of ORS or paraesthesia beginning within a seven-day observation period for controls in 2011 or during the previous 24 hours for 2012 controls or 24 hours after vaccination for 2011 and 2012 vaccinees were reported (the wording for these questions was changed in 2012 to make the time period in controls and vaccinees comparable). Before comparison, the number of ORS and paraesthesia events in 2011 controls were divided by seven to adjust for the difference in reporting period in 2011 controls. Characteristics and events were compared using chi-squared and Fischer exact tests.

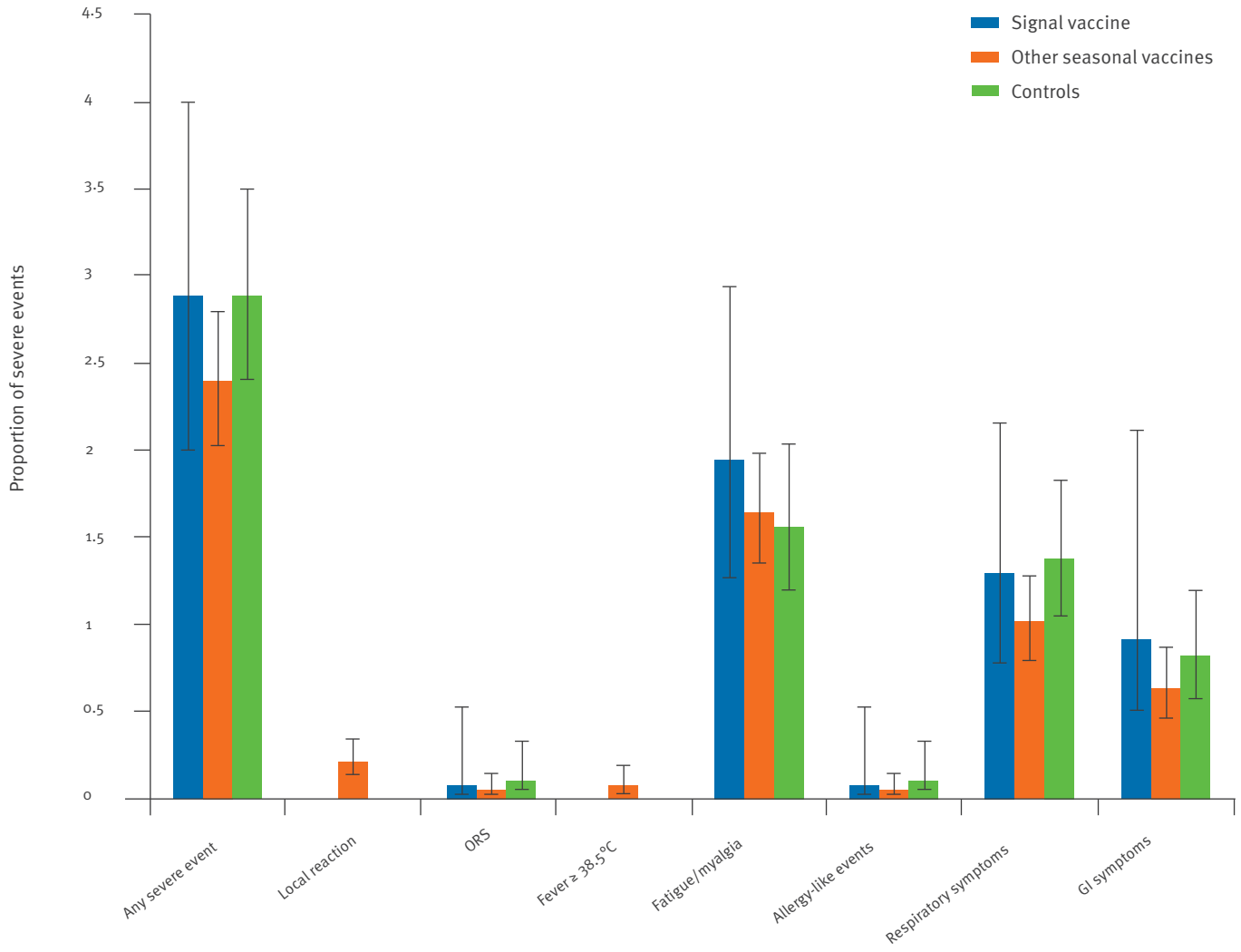
Following a safety signal issued by Italian authorities and a request from the Public Health Agency of Canada, Novartis Vaccines temporarily suspended Canadian distribution of Agriflu on 26 October 2012 [8,9]. Clumping of virus-like particles in the vaccine prompted concerns about the potential for an increase in ORS or allergy-like reactions. The vaccine had already been in use at some of our sites; therefore, we had safety information on the implicated product prior to its temporary suspension. In response to this safety signal, our network compared the event rates observed following vaccination with Agriflu (the signal vaccine) with those observed in controls and after receipt of other seasonal vaccines (Vaxigrip and Fluviral). Individual level data on the vaccines used for HCW vaccination were not available, although 7/8 centres vaccinated HCW with a single product (2 used Fluviral, 4 Vaxigrip and 1 Agriflu exclusively). Only one centre used both Agriflu and Vaxigrip, in unknown proportions and was excluded from this sub-analysis.

Validity of self-reported events by healthcare personnel

Participants who reported any severe health event (i.e. prevented daily activities/work or required a medical consultation) were contacted within 48 hours by a nurse trained in adverse events following vaccination

Figure

Rates of severe health events reported in healthcare workers vaccinated with one seasonal vaccine (n=1,084) compared with respective rates in those vaccinated with other seasonal vaccines (n=6,360) and controls (n=3,400), Canada, 2012



GI: gastrointestinal; ORS: oculorespiratory syndrome.

(AEFI) reporting who verified and documented the health event. Participants were allowed to enter several health events of interest occurring during the week, but nurses were required to choose a single primary event (i.e. the main complaint) based on their clinical judgement after speaking with the participant for each individual case report. Respiratory or gastrointestinal symptoms were considered the primary event when reported in conjunction with systemic symptoms. We excluded acute health events that had an onset before vaccination (for vaccinees) or>1 week before the survey (for controls), as well as events that did not meet the reporting criteria for a severe event, and scheduled medical visits. All events were reviewed and validated centrally by members of the research team (IR, MCG).

Representativeness of events reported by responders

In 2012, 10% of study participants (vaccinees and controls) who did not complete the online questionnaire after the reminder email (non-responders) were randomly selected and contacted by telephone five to 10 days after the reminder email was sent. A minimum of five attempts to contact each non-responder was made on different days and at different times before another non-responder was selected. We compared characteristics and rates of events observed between online responders and non-responders using Bonferonni's correction for the chi-squared and Fischer exact tests.

Results

Study participants

Over the two seasons, 22,080 vaccinated HCW enrolled in the surveillance network (Table 1). Overall, 15,163 (68.7%) responded to the online questionnaire sent eight days following vaccination, although the response rate was statistically higher in 2011 compared with the following year (74.4% vs 63.8%; p<0.001) (Table 1). The characteristics of HCW who responded

to the online surveillance remained stable over the two study years: about three quarters of participants were women, two thirds were between 30 and 59 years of age and the majority (10,397/15,163; 68.5%) were involved in patient care. Most (14,329/15,163; 94.5%) respondents had been vaccinated against influenza in the past and 68.8% (n=10,432/15,163) reported receiving the vaccine annually in the last three years. For the control questionnaire, 12,238 HCW immunised during the previous season were contacted in 2011 and 9,458 in 2012, with response rates of 13.2% and 26.2%, respectively ($p<0.001$) (Table 1).

Demographic characteristics of controls and vaccinees are shown in Table 1. In both years, respondents younger than 30 years of age were slightly more represented in the vaccinated group than in the controls, and there were minor differences with regard to profession/occupation.

Observed health events and capacity for signal investigation

Over the two study years, 1,388 (9.2%) health events were self-reported by vaccinated HCW compared with 396 (9.7%) health events by controls ($p=0.31$) (Table 2). Among vaccinated HCW, 388 reported events (2.6%) were severe enough to result in work absenteeism, prevent daily activities or to require a medical consultation, compared with 149 (3.6%; $p<0.001$) reported among controls. Overall, 43.9% (609/1,388) of events self-reported by vaccinated HCW included respiratory or ORS symptoms, which was similar to the proportion reported by controls (49.5%; 196/396). However, systemic symptoms were more frequently reported by vaccinated HCW (41.4%; 575/1,388) than controls (27.3%; 108/396) respectively ($p<0.001$; Table 2).

The hospitals surveyed in this study conducted their yearly vaccinations campaigns earlier than most jurisdictions in Canada and HCW at one of these hospitals were vaccinated with the vaccine implicated in the safety concern before it was temporarily suspended. This allowed a comparative safety review of the signal vaccine severe event rates with the other seasonal influenza vaccines and the background rates observed in controls within 48 hours of the vaccine suspension.

A sub-analysis of the 2012 dataset was conducted including the vaccinees who responded to the questionnaire (n=7,667), the vaccinated initial online non-responders who subsequently provided information (n=994), but excluding the one site with mixed vaccine use (n=1,217). This confirmed the interim findings. A total of 1,084 of the 7,444 (15%) vaccinees received the signal vaccine. The rate of self-reported severe events among HCW vaccinated with the signal vaccine was 2.9% (31/1,084), which was similar to 2.4% (151/6,360; $p=0.40$) in HCW vaccinated at institutions using other seasonal vaccines and to all controls at 3.9% (133/3,400; $p=0.11$). The clinical nature of severe health events reported by HCW vaccinated with the signal vaccine was similar to those reported after other seasonal vaccines (Figure).

Validity of self-reported events

Over the two study seasons, nurses were able to complete follow-up calls with 93% (500/537) of participants reporting severe events online (Table 3). This resulted in 90% (134/149) of controls and 94% (366/388) of vaccinated HCW being followed-up ($p=0.09$). Following the nurse interviews, 30% (40/134) of controls and 9% (33/366) of vaccinees reporting severe events were excluded, leaving a total of 427 participants with eligible severe health events (94 controls and 333 HCW). Reasons for exclusions were that these events (i) started >1 week before the survey (for controls) or prior to vaccination (for vaccinees), (ii) did not prevent daily activities/work or require a medical consultation or (iii) were previously scheduled medical visits. In both years, the proportion of events that were excluded was significantly higher among controls than that observed among vaccinated individuals (30% vs 9%; $p<0.001$). Participants who were excluded did not vary according to the type of event or clinical presentation, with the notable exception of paraesthesia, which was a pre-existing condition in all controls not considered.

The accuracy and validity of the online reported severe health events are shown in Table 3. Among 427 participants reporting eligible severe health events, 45% (n=193) had respiratory symptoms. For 79% (n=153) of these, respiratory symptoms remained the primary diagnosis after talking to the nurse (i.e. validated event). Gastrointestinal symptoms were reported by 28% (121/427) of participants with eligible severe health events, and of those reports, 54% (65/121) remained as the primary diagnosis. Eligible severe systemic symptoms were frequently reported (59% in controls and 71% in vaccinated HCW) (Table 3). However, systemic symptoms reported by controls were more often secondary to another health problem (most often respiratory or gastrointestinal symptoms), and only 15% of controls reporting such symptoms were validated. In contrast, for vaccinated HCW one third (76/281) of systemic events remained the primary diagnosis. While 27 vaccinated HCW (8%) who reported an eligible severe health event had a local reaction (Table 3), 21 of them missed work or consulted a physician for other health issues.

Observed health events

The overall reporting rate for validated severe events was similar ($p=0.7$) between vaccinees (2.2%) and controls (2.3%) (Table 2). Among validated severe events 84.5% (361/427) prevented daily activities or resulted in work absenteeism alone and 15.4% (66/427) required a medical consultation with or without absenteeism. Most medical consultations were clinic visits (83.3%; 55/66), while 1.5% (1/66) were emergency department visits. At the time of follow-up, the reported problem had either resolved (64.4%; 275/427) or was improving (28.5%; 122/427) in participants. 3.7% (16/427) of

Table 3

Accuracy and validity of severe health events reported online by vaccinated and control healthcare workers, Canada, 2011–2012 (n=537 participants)

Type of severe health event	Controls					Vaccinated healthcare workers				
	Severe events reported or persons reporting N	Severe events or persons followed-up by nurse N	Reporting errors[a] or persons concerned N	Eligible events[b,c] or eligible persons N (%)	Validated events[c,d] or person concerned N (%)	Severe events reported or persons reporting N	Severe events or persons followed-up by nurse N	Reporting errors[a] or persons concerned N	Eligible events[b,c] or eligible persons N (%)	Validated events[c,d] or persons concerned N (%)
Local reaction	NA	NA	NA	NA	NA	31	31	4	27 (8)	6 (2)
Systemic symptoms	76	74	19	55 (59)	11 (15)	267	255	20	235 (71)	76 (27)
Allergy-like events	4	4	2	2 (2)	0 (0)	6	6	1	5 (2)	1 (<1)
Respiratory symptoms	65	62	13	49 (52)	39 (55)[e]	158	153	9	144 (43)	114 (41)[e]
GI symptoms	32	32	8	24 (26)	12 (17)	110	107	10	97 (29)	53 (19)
ORS	34	27	9	18 (20)	0 (0)	42	42	3	39 (12)	6 (2)
Paraesthesia	14	11	11	0 (0)	0 (0)	16	15	3	12 (4)	0 (0)
Other	24	22	7	15 (16)	9 (13)	54	54	6	48 (14)	25 (9)
Total persons[f]	**149**	**134**	**40**	**94 (100)**	**71 (100)**	**388**	**366**	**33**	**333 (100)**	**281 (100)**

GI: gastrointestinal; NA: not applicable; ORS: oculorespiratory syndrome.

[a] The event reported was not considered, because it either did not prevent daily activities/work or require a medical consultation, or a medical consultation was pre-existing, or symptoms started prior to vaccination for vaccinees or prior to the reporting period for controls.

[b] Eligible events are events remaining after taking into account reporting errors.

[c] The denominators for the percentages are the total persons for the column in question.

[d] Validated events are events self-reported in online questionnaire that remained the primary diagnosis after a follow-up with a nurse.

[e] Significant difference between validated events in controls and vaccinees at $p<0.05$.

[f] Each person could report more than one health event, so the total number of persons is not equal to the total of reported events.

participants reported no change or worsening of their health problem, a proportion that was the same for both vaccinated HCW and controls, and 3.3% (14/427) did not answer this question. In the two seasons under study, one participant was hospitalised in the week following vaccination for gastrointestinal symptoms that started six hours after vaccination. This individual was diagnosed with appendicitis resulting in an emergency appendectomy. No deaths were reported.

Representativeness of events reported

Study participants who did not respond to the 2012 online questionnaire (i.e. initial non-responders) were contacted by telephone. No difference in age ($p=0.3$) and sex ($p=0.84$) could be observed for controls between initial non-responders and responders. Vaccinee initial non-responders were slightly older than vaccinee online responders ($p<0.001$) but similar in sex ($p=0.21$) (Table 1). Overall, initial non-responders differed by hospital occupation group ($p<0.001$).

Vaccinated participants who responded online reported more health events (9.0%) across all types than vaccinated non-responders (6.1%), although this difference was not observed among controls (Table 2). The rate of severe events, however, was generally similar in both responders and initial non-responders. Vaccinee non-responders reported not answering the online questionnaire due to circumstantial factors, mainly because they reported being too busy (38.2%; 380/994), did not recall receiving the email (30.6%; 304/994), reported that the embedded link to the questionnaire did not work (11.9%; 118/994) or were away or did not check email regularly (8.1%; 80/994).

Discussion

Online monitoring offers an economical and sustainable platform to conduct large-scale electronic surveillance of vaccinated individuals, allows rapid identification of AEFI and minimises human resource needs. However, rapid large-scale surveillance of vaccine safety poses challenges which require a careful balance between information needs and feasibility. The quantity and validity of the information collected must be sufficient to allow stakeholders to detect and interpret safety signals in a timely manner, while requesting a minimal amount of information to obtain sufficient response rates from participants. Self-reported severe events offer the advantage of improved efficiency, but unless validated, may under- or overestimate AEFI reporting rates

Our results demonstrate that online safety surveillance can be used to effectively monitor influenza vaccine safety in a large number of vaccinees, despite the methodological limitations of relying on self-reported health events. As shown during the influenza A(H1N1)pdm09 pandemic, internet-based safety questionnaires are uniquely suited to rapid collection and analyses and can be adapted to provide monitoring for seasonal influenza vaccines [3,10-12]. The rapid collections of data, early in the mass vaccination campaigns that occur simultaneously across Canada allow for ongoing monitoring and analysis throughout the first weeks of activities and provides an opportunity to detect signals before widespread vaccine use. The ability of online surveillance to detect rare events will depend on the total number of respondents. Our study was able to detect events with a frequency of 1 per 1,000.

Although public health officials were concerned about the possibility of an increase in oculorespiratory syndrome among Agriflu recipients in 2012, event rates observed among HCW vaccinated in centres using this vaccine were similar to the rates observed in centres using other seasonal vaccines and to rates observed in the control group. This was later confirmed by passive surveillance results from the United States Vaccine Adverse Event Reporting System and the Canadian Adverse Event Following Immunization Surveillance System [4,13]. The 2012 interim analysis of data, in response to the temporary suspension of the Agriflu vaccine, confirmed that the network can provide timely evaluation of safety signals and adequately support decision makers. At this time, our network remains the largest able to provide active monitoring of influenza vaccine safety both nationally and internationally. Our findings confirm that influenza vaccines used in Canada for both the 2011 and 2012 seasons were safe and that their safety profiles were consistent with those expected following influenza vaccination.

We also showed that most of the eligible severe events self-reported by vaccinated HCW were consistent with the nurse interviews and had indeed prevented daily activities, resulted in missed work or required a medical consultation. The higher error rate in the control questionnaire reflects the difficulty controls may have in identifying the time period under surveillance and indicates a reference point, through a reminder email, may be needed for this group. Vaccinees have the advantage of a well-defined observation period starting at the vaccination event from which to start tracking any new or exacerbated symptoms. This discrepancy was particularly evident for the paraesthesia questions where control symptoms starting more than one week before the questionnaire period were frequently reported, indicating background rates for chronic conditions or illness may be more difficult to separate from new events using an online questionnaire. This shortcoming was addressed in the severe event follow-up where the difference between controls and vaccinees disappeared when more accurate questioning elicited precise event windows. Reassuringly, most primary diagnoses had indeed been reported by participants, but the main difficulty we encountered in validating health events reported by both controls and vaccinated HCW was in distinguishing the primary complaint from all other health events that occurred during the observation period. This problem was particularly evident for local reactions and systemic symptoms, which often accompanied respiratory and gastrointestinal symptoms, but which alone did not prevent daily activities or lead to absenteeism or medical consultations. The more specific events or symptom questions on the online questionnaire (respiratory symptoms, gastroenteritis, etc.) were more likely to accurately capture a true event than nonspecific event or symptom questions (fever, myalgia, etc.).

The inclusion of a control group in our study is an added strength of the network. It provides background rates for health events just before the start of the influenza vaccination campaign in a similar population and enables precise calculation of risk estimates. Moreover, age and sex specific background rates can be estimated. Importantly background event rates can be compared over multiple years to address fluctuations in events or temporal variations, a potential weakness of the staggered data collection periods of controls and vaccinees.

The similarity in severe event rates between initial non-responders and online responders indicates our online survey participants were representative of their respective vaccine and control groups. This suggests the rates of severe events elicited with our online survey is representative of the group overall.

Limitations

We did not track the total number of individuals who presented at each institution for vaccination or the characteristics of those who were vaccinated but did not enrol in our study. Therefore, we cannot determine whether selection bias occurred at recruitment. Even if our sample is not representative of all HCW, we would not expect the rate of severe events to occur differentially among those who participated and those who did not. In our control group, we had fewer controls that were under the age of 30 years, but the proportion in the remaining age categories was similar, therefore we would not expect this to affect our estimates for severe events. Moreover our severe event rates mirror those seen in other studies collected by different methods [11,14].

The importance of individual-level vaccine information became immediately apparent with the temporary suspension of one vaccine product. Fortunately for our study, only one among the healthcare centres considered used multiple influenza vaccines, so we were able to infer which product individuals received based upon where they were immunised. However, our experience from institutional vaccination of HCW using a single

product or vaccine lot may not hold true for children and adults vaccinated in the community. In subsequent years, as a wider range of vaccine products become available, individual-level vaccine product data will be necessary.

HCW who participated in our surveillance constitute a unique group of vaccine recipients, which may not be representative of community vaccinees. Almost 70% of our participants have medical training or are involved in patient care. This likely enables them to better evaluate health problems and communicate chief complaints which may have improved the validity of the online survey. The validity of self-reported events by non-HCW populations may not be similar. Evaluation of this methodology in cohorts of children and non-HCW adults are needed.

Conclusions

Online surveillance can provide rapid assessment of influenza vaccine safety and is highly acceptable to the HCW participating in this activity. The addition of a control group enhances internal validity and establishes background rates for common events of interest. This methodology works particularly well in a mass vaccination setting where large numbers of individuals can be rapidly enrolled and followed-up and meets the new enhanced surveillance requirements as outlined by the EMA.

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Conflict of interest

JAB none. IR none. MCG none. WRB none. LV received research grants from Pfizer and Cubist, consultation fees from Cubist and is involved in sponsored clinical trials with Merck and Sanofi. OGV received research grants from Pfizer, Merck, and Sanofi and has participated in advisory boards for Novartis and Pfizer. JDK none. BLC none. SAM has received research grants from GlaxoSmithKline, Sanofi Pasteur and Pfizer. AM none. GDS received research grants from GlaxoSmithKline (GSK) and travel fee reimbursement to attend an ad hoc GSK Advisory Board without honorarium.

Authors' contribution

JAB was involved in the conception and design of the study, acquisition of the data, the analysis and interpretation of the data and wrote the article. IR was involved in the conception and design of the study, the analysis and interpretation of the data, and writing and revision of the manuscript. MCG was involved in the acquisition of the data, the analysis and interpretation of the data, and writing and revision of the manuscript. WRB was involved in the design of the study, acquisition of the data and revision of the manuscript. LV was involved in the design of the study, acquisition of the data and revision of the manuscript. OGV was involved in the design of the study, acquisition of the data and revision of the manuscript. JDK was involved in the design of the study, acquisition of the data and revision of the manuscript. BLC was involved in the design of the study, acquisition of the data and revision of the manuscript. SAM was involved in the design of the study, acquisition of the data and revision of the manuscript. AM was involved in the design of the study, acquisition of the data and revision of the manuscript. GDS was involved in the conception and design of the study, acquisition of the data, the analysis and interpretation of the data, and revision of the manuscript.

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Large and prolonged food-borne multistate hepatitis A outbreak in Europe associated with consumption of frozen berries, 2013 to 2014

E Severi (Ettore.severi@ecdc.europa.eu)[1,2], L Verhoef[3], L Thornton[4], B R Guzman-Herrador[5], M Faber[6], L Sundqvist[7], R Rimhanen-Finne[8], A M Roque-Afonso[9], S L Ngui[10], F Allerberger[11], A Baumann-Popczyk[12], L Muller[13], K Parmakova[14], V Alfonsi[15], L Tavoschi[1], H Vennema[3], M Fitzgerald[4], M Myrmel[16], M Gertler[6], J Ederth[7], M Kontio[8], C Vanbockstael[17], S Mandal[10], M Sadkowska-Todys[12], M E Tosti[15], B Schimmer[3], J O'Gorman[18], Kathrine Stene-Johansen[5], J J Wenzel[19], G Jones[17], K Balogun[10], A R Ciccaglione[15], L O'Connor[20], L Vold[5], J Takkinen[1], C Rizzo[15]

1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
2. Karolinska Institutet, Stockholm, Sweden
3. National Institute of Public Health and the Environment, Bilthoven, the Netherlands
4. Health Protection Surveillance Centre, Dublin, Ireland
5. Norwegian Institute of Public Health, Oslo, Norway
6. Robert Koch Institute, Berlin, Germany
7. Public Health Agency of Sweden, Solna, Sweden
8. Institute for Health and Welfare, Helsinki, Finland
9. National Reference Centre for Hepatitis A, Paul Brousse Hospital, Villejuif, France
10. Public Health England, London, United Kingdom
11. Austrian Agency for Health and Food Safety (AGES), Vienna, Austria
12. National Institute of Public Health - National Institute of Hygiene, Warsaw, Poland
13. Statens Serum Institut, Copenhagen, Denmark
14. National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria
15. Istituto Superiore di Sanità (ISS), Rome, Italy
16. Norwegian University of Life Sciences, Oslo, Norway
17. Institut de Veille Sanitaire (InVS) region Picardy, Amiens, France
18. National Virus Reference Laboratory, Dublin, Ireland
19. Regensburg University Medical Center, Regensburg, Germany
20. Food Safety Authority of Ireland, Dublin, Ireland

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In May 2013, Italy declared a national outbreak of hepatitis A, which also affected several foreign tourists who had recently visited the country. Molecular investigations identified some cases as infected with an identical strain of hepatitis A virus subgenotype IA. After additional European Union/European Economic Area (EU/EEA) countries reported locally acquired and travel-related cases associated with the same outbreak, an international outbreak investigation team was convened, a European outbreak case definition was issued and harmonisation of the national epidemiological and microbiological investigations was encouraged. From January 2013 to August 2014, 1,589 hepatitis A cases were reported associated with the multistate outbreak; 1,102 (70%) of the cases were hospitalised for a median time of six days; two related deaths were reported. Epidemiological and microbiological investigations implicated mixed frozen berries as the vehicle of infection of the outbreak. In order to control the spread of the outbreak, suspected or contaminated food batches were recalled, the public was recommended to heat-treat berries, and post-exposure prophylaxis of contacts was performed. The outbreak highlighted how large food-borne hepatitis A outbreaks may affect the increasingly susceptible EU/EEA general population and how, with the growing international food trade, frozen berries are a potential high-risk food.

Introduction

Hepatitis A virus (HAV) is a hepatovirus of the *Picornaviridae* family with a linear single-stranded genome of 7,500 nucleotides (nt) [1]. HAV mutation rate is low and therefore its genome is relatively conserved over time [2]. Six HAV genotypes have been defined: genotypes I to III infect humans and are divided in subgenotypes A and B. Subgenotype IA is the predominant subgenotype circulating in Europe [1,3].

HAV is generally transmitted to humans through the faecal–oral route. The hepatitis A (HA) incubation period is approximately 28–30 days (range: 15–50). The disease, acute and generally self-limiting, affects the liver and is characterised by fever, diarrhoea and

Box

Hepatitis A virus infection: European outbreak case definition (2013–14)

According to the European outbreak hepatitis A virus (HAV) infection case definition, a **confirmed case** is defined as:

An EU/EEA resident with laboratory-confirmed HAV genotype IA
and
date of symptom onset (or date of testing if onset date not available) on or after 1 January 2013
and
at least one of the following conditions:

(i) identical sequence (i.e. 100.0%) to the 2013 HAV genotype IA outbreak strain (GenBank accession number KF182323) based on a fragment of 460 nucleotides (nt) at the region of VP1-2a[a]

(ii) 99.8% similarity to this sequence (i.e. one nt difference in 460 nt) from 2,915 to 3,374 on NC_001489.

(iii) identical sequence (i.e. 100.0%) on a shorter fragment of at least 174 nt at the region of VP1-2a from 2,967 to 3,191 on NC_001489.

According to the European epidemic HAV infection case definition, a **probable (suspect/possible) case** is defined as:

An EU/EEA resident with laboratory-confirmed HAV infection
and
date of symptom onset (or date of testing if onset date unavailable) on or after 1 January 2013[b]
and
fulfilling, within 15–50 days before symptom onset, at least one of the following epidemiological criteria:

(i) having been in a country experiencing the outbreak during the indigenous outbreak period[c];

(ii) person-to-person contact with a confirmed case (secondary case).

The following **exclusion criteria** for probable cases are applied:

(i) HAV confirmed case who has a different sequence type to the 2013 HAV genotype IA outbreak strain;

(ii) existence of an epidemiological link to a person excluded for the reason given in criterion number i;

(iii) history of travel outside EU/EEA/EFTA countries within 15–50 days before symptom onset.

EU/EEA: European Union/European Economic Area; EFTA: European Free Trade Association.

[a] For Norwegian isolates, identical sequence to GenBank number KF773842 based on a fragment of 466 nucleotides at the region VP3-VP1.

[b] As for confirmed cases: at the time of writing this report (December 2014), outbreak cases were still being reported by at least one EU/EEA country, hence no end date for the outbreak case definition could be defined.

[c] As at 30 June 2014, these are: Finland from January to June 2014; Ireland from January to October 2013; Italy from January 2013 onward; Netherlands from August to December 2013; Norway from November 2013 to April 2014.

jaundice. The proportion of symptomatic infections is very low in young children (under six years of age) but clinical expression and severity increases with age; the overall case fatality rate is about 0.3% but can be as high as 1.8% in adults over 50 years and in immunocompromised patients [4].

HAV is rather stable in the environment and is resistant to acidification, drying, freezing and other food preservation methods, and it has therefore a good potential to cause food-borne outbreaks [5-7].

In May 2013, Germany reported through the European Commission's Early Warning Response System (EWRS) seven HA cases in travellers to northern Italy. Following the German alert, other European Union/European Economic Area (EU/EEA) countries reported cases associated with travel to Italy and, simultaneously, Italy declared a national outbreak. Some cases were identified as infected with an identical strain through molecular characterisation (sequencing); identical sequences had previously been detected only in 2008, in travellers returning from the Czech Republic (data not shown). A multistate outbreak investigation team was established under the European Centre for Disease Prevention and Control (ECDC) coordination and including members of public health institutes of all 13 EU/EEA countries reporting associated cases during 2013 and 2014 [8-13].

The aim of this paper is to describe results, challenges and lessons learnt from the public health side of the epidemiological and microbiological multinational outbreak investigation. The paper presents new insights into this large and prolonged outbreak and gathers together information from different investigations and from an extensive food trace-back carried out at national levels and by the European Food Safety Authority (EFSA) [8-12,14]. It also offers a number of recommendations to improve harmonisation of procedures in HA outbreak investigations across Europe.

Methods

The European outbreak case definition defined confirmed and probable cases on the basis of symptom onset (on or after 1 January 2013), sequencing, travel history and epidemiological link with other cases (Box).

HA is a mandatorily notifiable disease in all EU/EEA countries reporting cases associated with this outbreak. HA cases are investigated by local public health departments and information, including clinical status and potential exposure, is reported to the national surveillance systems. In Italy, there is also exist an enhanced surveillance system for acute viral hepatitis (SEIEVA), complementing the national surveillance system, which monitors potential risk factors associated with HA [15].

HAV infection is laboratory confirmed through serological testing for anti-HAV IgM or by PCR at local laboratory level in all countries affected by this outbreak. Molecular characterisation is performed through sequencing of a genomic fragment. Sequencing was always performed in national reference laboratories, apart from Italy, where it was also carried out in regional laboratories. Sequencing is routinely performed on all available samples in England, the Netherlands and Finland, in all samples from clusters or outbreaks in Denmark, France and Norway and, in Sweden, in all samples from patients infected in Sweden or other EU/EEA countries. Sequencing is not routinely performed in Bulgaria, Italy and Poland. In all the other countries involved, sequencing is performed for a subset of samples. Following the identification of a nationwide HA increase in May 2013, sequencing of a subset of isolates was introduced in Italy, particularly isolates collected from May 2013 to January 2014; however, for the rest of 2014, sequencing operations were notably reduced, with only a few samples characterised on a monthly basis [9]. Similarly, following identification of the European outbreak strain in Ireland in July 2013 and in Norway and Sweden in February 2014, these three countries opted for sequencing available samples from all HA serologically confirmed cases in the previous months and for part of 2014 [10]. Also, in May 2013, Poland sequenced five isolates from cases with a history of travel to Italy.

HAV sequencing was performed according to national (and also subnational for Italy) protocols. Comparison of the sequencing results focused on a genomic fragment in the region VP1–2A. In order to be categorised as the outbreak strain, a sample needed to have either (i) ≥ 99.8% identity with the outbreak reference sequence (GenBank access number KF182323) in a 460 nt fragment at the region VP1–2A, or (ii) 100% identity with a shorter fragment of at least 174 nt in the same region.

In England, Finland, France, the Netherlands and Norway, cases infected with the European outbreak strain, or their family members, were interviewed, when possible, using an adapted version of a questionnaire initially developed in Ireland in September 2013 [9]. In Sweden, cases were re-interviewed using a questionnaire developed for the HA outbreak in Nordic countries that had occurred earlier in 2013 [16]. Soon after the Italian national outbreak was declared in May 2013 [8], microbiological evidence that frozen berries were the vehicle of HAV was soon obtained in Italy. Consequently in Austria, Bulgaria, Germany, Italy and Poland additional information on consumption of berries was gathered for cases infected with the outbreak strain or reporting a travel history to Italy in or after spring 2013.

On the basis of the hypothesis generated from patients' interviews and trace-back investigations, three separate matched case–control studies were conducted in Italy, Ireland and Norway, in July 2013, September 2013 and April 2014 respectively [9,11].

We describe cases by case classification, time of symptom onset, sex, age, travel history, reported exposure to berries, clinical symptoms and outcome of hospitalisation (defined as being in hospital care at least overnight) or death. We also provide additional insights into epidemiological investigations conducted during the outbreak.

Results

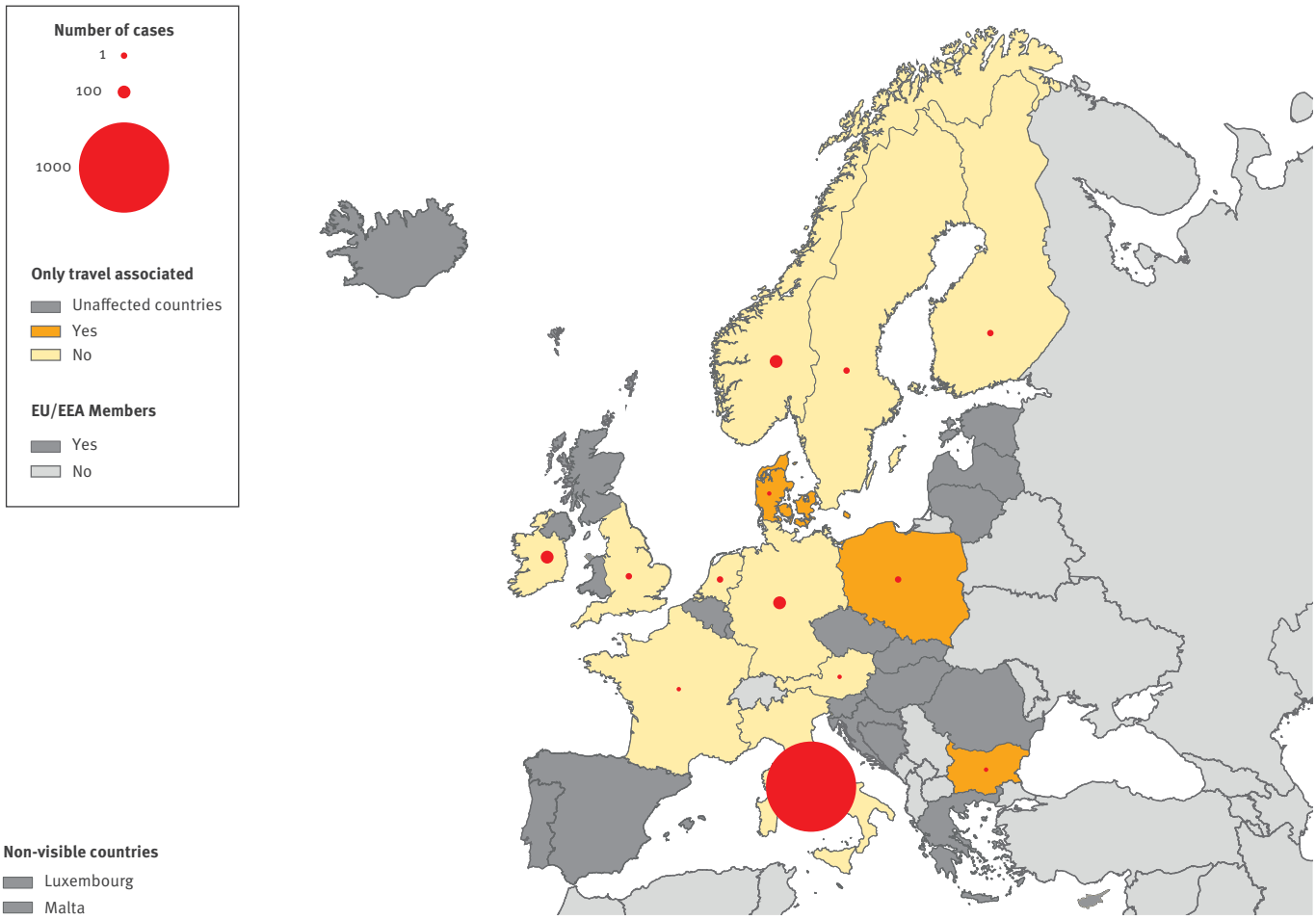
From 1 January 2013 to 31 August 2014, a total of 1,589 HA cases were reported as associated with this outbreak from 13 EU/EEA countries (Figure 1, Figure 2); most of the cases (n = 1,438; 90%) were reported in Italy. Germany, Ireland and Norway each reported around 30 cases and all other countries reported fewer cases, with Austria, Bulgaria and Denmark each reporting a single case associated with this outbreak. In most of the affected countries, cases were geographically distributed nationally.

The outbreak strain (from Italy in May 2013) sequence GenBank number was KF182323 and the subgenotype was IA. Sequences from 361 viral isolates were found to be identical to the outbreak strain (Table): individuals with this strain were classified as 'confirmed', whereas the remaining 1,228 cases were classified as 'probable' (Box). Apart from Bulgaria, Germany and Italy, where the proportion of confirmed cases was lower than 30% of the total number of cases, all other countries sequenced at least 75% of strains from all reported cases (Table, Figure 3). National HAV sequencing protocols were not harmonised during the outbreak, thus in different countries, genomic fragments of different length were characterised: all fragments were at least 300 nt, except for Italy where 93 isolates (38% of all sequenced isolates in Italy (n = 247)) were characterised in regional laboratories for a length of at least 174 nt (Table).

The monthly number of reported cases peaked from March to October 2013, when the highest number of

Figure 1

Hepatitis A cases by reporting country and cases' travel history, European Union/European Economic Area countries, 1 January 2013–31 August 2014 (n = 1,589)



EU/EEA: European Union/European Economic Area.

Source: data from European Centre for Disease Prevention and Control (ECDC). Administrative boundaries from EuroGraphics and GAUL (global administrative unit layers).

cases per month was reported in Italy. Most cases were reported in this period also in Ireland, particularly from June to August 2013, and in the Netherlands, particularly from August to October 2013. Although in Italy the monthly number of reported cases in 2014 halved compared with that in 2013, most cases in Norway, Finland and Sweden were reported in the second year of the outbreak: most Norwegian cases were reported from February to April 2014 and most of the Finnish and Swedish cases from April to June 2014.

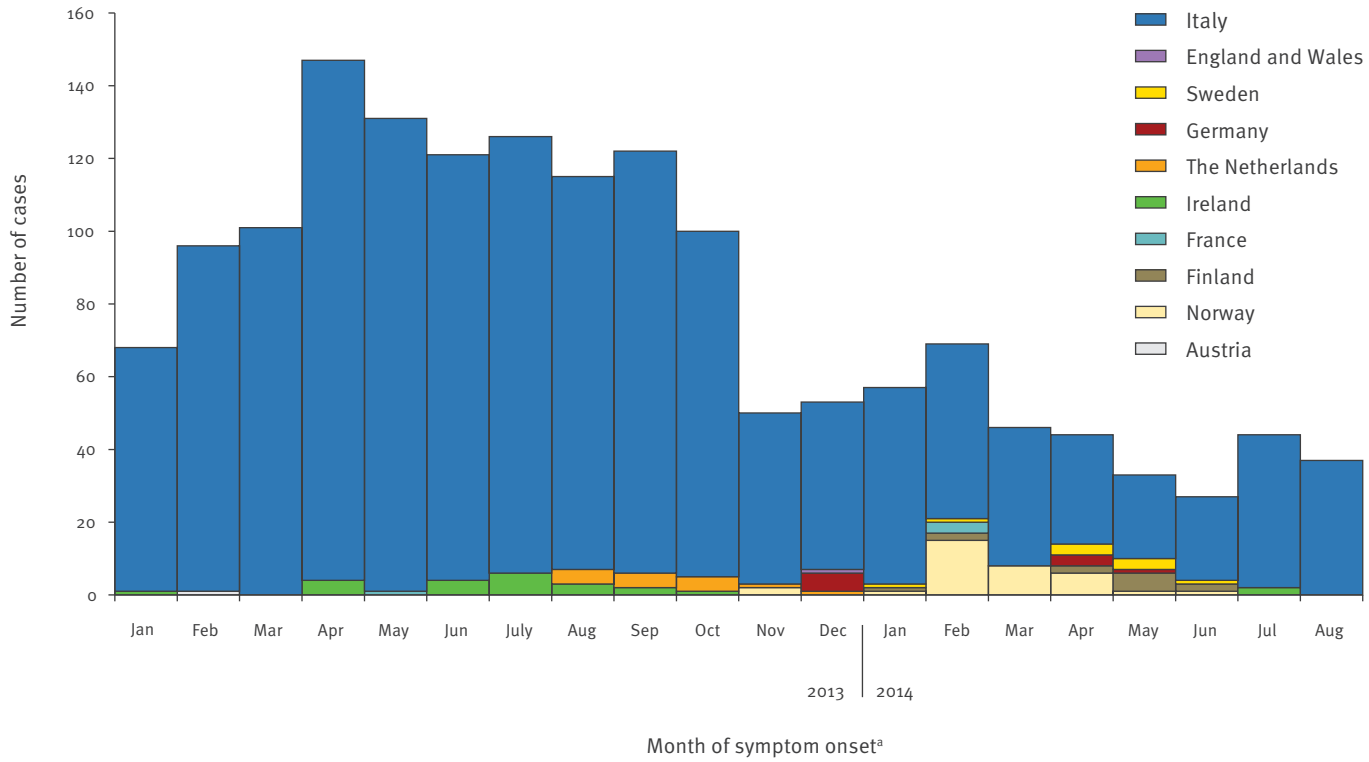
Information on sex was available for 1,576 cases and on age for 1,579 cases: of these, 54% were male (n = 852) and 77% were aged between 20 and 65 years (n = 1,213). The median age of both types of cases was 36 years (range: 1–92), 39 years for confirmed cases (range: 8–68) and 34 years (range: 1–92) for outbreak-probable cases. Of the 908 cases with available information, 96% were primary cases (n = 869) and 4% secondary cases (n = 39). A total of 43 cases reported having travelled during the exposure period (within 15–50 days before symptom onset) to another EU/EEA country experiencing the HAV outbreak (Box), 42 to Italy and one to Norway. Apart from Bulgaria, Denmark and Poland, where all cases had a travel history to Italy, in all other countries there was evidence of local transmission, with all or part of the cases not reporting any history of travel.

Overall, 70% of the cases (n = 1,102) were hospitalised following infection. The median duration of hospitalisation was six days (range: 1–49) among the 568 cases with documented information on hospitalisation. Two cases were reported to have died with or due to HAV infection.

Of the 788 patients reporting information on possible exposure, 495 (63%) from all 13 countries reported exposure to berries. The majority of the primary cases indicated consumption of frozen berries, often in smoothies or cakes. In several countries, clusters of cases were found to be associated with a common exposure to frozen berries: four of the nine autochthonous cases reported in Germany implicated the

Figure 2

Hepatitis A cases by probable country of infection and month of symptom onset[a], European Union/European Economic Area countries, 1 January 2013–31 August 2014 (n = 1,587[b])



[a] Or month of testing when symptom onset date was unavailable.

[b] Information on month of symptom onset was unavailable for two cases.

same food item identified as the vehicle of infection in the Norwegian outbreak (described below). Three French cases identified in the Aisne district in February 2014 were found to be linked to a catering service producing a fruit tart containing mixed berries. Three Swedish cases reported consumption of smoothies at the same resort. In contrast to the other countries, in the Netherlands, all 10 locally infected primary cases reported consumption of fresh soft fruits: this was higher than expected, particularly in autumn months (about 30% expected) [16]. Nonetheless, seven of the 10 cases in this cluster also reported consuming frozen berries or products possibly containing frozen berries.

Following declaration of outbreaks in each country, Italy, Ireland and Norway performed matched case–control studies to identify the vehicle of infection. In Italy, a matched case–control study was carried out to test the hypothesis that cases were associated with consumption of frozen berries. The study found that confirmed cases were more likely to have been exposed to frozen berries (adjusted matched (Adjm) odds ratio (OR): 4.2; 95% confidence interval (CI): 2.5–7.0), raw seafood (AdjmOR: 3.8; 95% CI: 2.2–6.8) and travel (AdjmOR: 2.0; 95% CI: 1.2–3.4). A restricted statistical analysis conducted on 24 early confirmed cases (with symptom onset from 1 January to May 2013) and 82 matched controls, confirmed berries as the highest independent risk factor for HA (matched (m) OR: 4.99; 95% CI: 1.32–18.92) [11]. The case–control study carried out in Ireland tested the hypothesis that cases were associated with consumption of either fresh or frozen berries. The results indicated that products containing frozen berries were implicated in the outbreak. Among 11 cases, 10 had consumed at least one of four products containing frozen berries, compared with 16/42 of controls (AdjmOR: 12; 95% CI: 1.5–94) [9]. The Norwegian study tested the hypothesis that cases were associated with consumption of a particular cake identified through trace-back investigations that was topped with non-heat-treated mixed frozen berries. The matched case–control study confirmed an association between the cake and HA disease (mOR: 13; 95% CI: 1.7–110) [10].

Discussion

We have described the epidemiological and microbiological investigations of a prolonged HA outbreak affecting more than 1,500 patients in 13 EU/EEA countries during 2013 and part of 2014. To the best of our knowledge, this is the largest food-borne HA outbreak involving such a wide geographical area in Europe reported in the scientific literature.

Confirmed cases were identified through molecular characterisation, allowing for detection of otherwise

Table

Characteristics of hepatitis A outbreak cases and viral genetic region sequenced, European Union/European Economic Area countries, 1 January 2013–31 August 2014 (n = 1,589)

Country	Number of reported cases	Number of confirmed cases	Number of cases who travelled to outbreak country[a]	Median age in years (range)	Number of male cases	Occurrence of symptom onset	Number reported hospitalised (number of deaths)	Length of genomic fragment sequenced (region)
Austria	1	1	0	48 (NA)	0	Feb 2013	1 (0)	397 nt (VP1–2a)
Bulgaria	1	0	1	40 (NA)	1	Apr 2013	1 (0)	NA
Denmark	1	1	1	60 (NA)	1	Nov 2013	1 (0)	400–1231 nt (VP1 region)
England	5	5	3	36 (26–66)	2	Nov 2013–Aug 2014	4 (0)	505 nt (VP1–2A)
Finland	12	9	0	56 (25–82)	9	Jan–Jun 2014	10 (1)	328 nt (VP1–2A)
France	5	5	0	39 (19–68)	2	May 2013–Feb 2014	3 (0)	508 nt (VP1–2A)
Germany	34	10	25	46 (8–69)	20	Mar 2013–May 2014	21 (0)	397 nt (VP1–2A)
Ireland	27	23	4	35 (21–64)	13	Jan 2013–Jul 2014	14 (0)	400 nt (VP1–2A)
Italy	1,438	246	0	35 (1–99)	769	Jan 2013–Aug 2014	1,015 (1)	460 nt (VP1–2A)
The Netherlands	15	15	1	30 (10–80)	8	Apr–Dec 2013	4 (0)	460 nt (VP1–2A)
Norway	33	33	0	45 (24–71)	18	Nov 2013–Jun 2014	18 (0)	490 nt (VP1–2A) and/or 476 nt (VP1 N-terminal)
Poland	6	3	6	47 (30–51)	4	Apr–Jul 2013	6 (0)	460 nt (VP1–2A)
Sweden	11	10	2	42 (9–62)	5	Jul 2013–Jun 2014	4 (0)	1,252 nt (VP1 gene + parts of VP3 and 2A)
Total	**1,589**	**361**	**43**	**36 (1–99)**	**852**	**Jan 2013–Aug 2014**	**1,102 (2)**	**NA**

NA: not applicable; nt: nucleotides.

[a] Having been in a country experiencing the outbreak during the indigenous outbreak period.

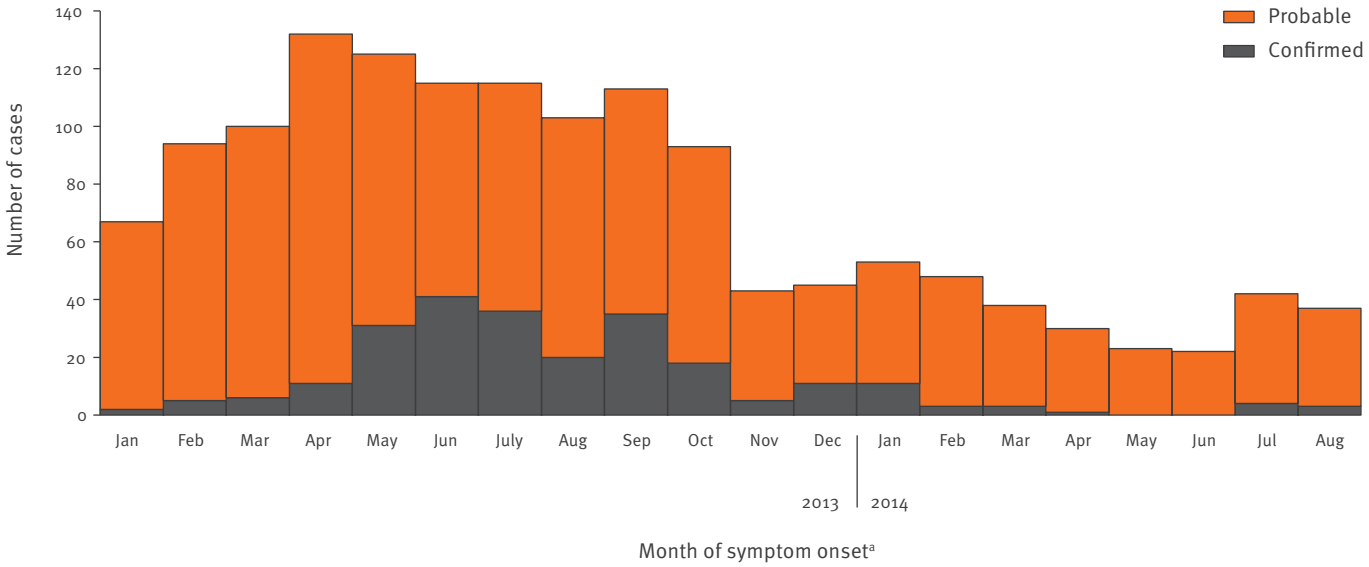
unnoticeable links between cases occurring at different times and in distant countries. Such a case definition, grounded on molecular characterisation, is highly specific. In order to include as confirmed cases only those food-borne cases infected through exposure to contaminated berries, the outbreak response team decided to require complete sequence homology or one single nucleotide difference as compared with the outbreak strain reference sequence. All isolates sequenced during the multinational investigation were longer than 300 nt, apart for 93 isolates sequenced in Italian regional laboratories.

As only some EU/EEA countries perform molecular typing, and often only on a subset of cases, it is likely that additional cases associated with this outbreak were missed in those countries not performing routine sequencing of isolates from HA patients. In support of this, a confirmed case reported by Ireland in August 2014 was most likely infected in Romania, a country not performing molecular characterisation of HAV isolates and not reporting cases associated with this outbreak. On the other hand, the number of cases reported in Italy as associated with this outbreak may have been over-estimated due to the enhancement of national surveillance during the outbreak and the absence of routine sequencing. Of the 1,438 cases reported associated with this outbreak, 17% were diagnosed with the outbreak sequence. Therefore, some of the probable cases were possibly infected by other HAV strains not associated with this outbreak and independently circulating in Italy. It is also plausible that, after such a long circulation of the outbreak strain, some of the confirmed cases may have been infected through a different transmission mechanism than food-borne [17].

On the basis of the epidemiological and microbiological evidence implicating mixed frozen berries as the vehicle of infection in this outbreak, the European Commission gave EFSA the mandate to lead an extensive European-wide trace-back exercise to identify the

Figure 3

Hepatitis A cases by confirmed/probable status and month of symptom onset[a], Italy, 1 January 2013–31 August 2014 (n = 1,438)



[a] Or month of testing when symptom onset date was unavailable.

contaminated berry type and its place of production [14]. During French, Italian and Norwegian environmental investigations, HAV contamination was detected in 14 lots of frozen mixed berries and in two lots of mixed berry cakes. The EFSA trace-back could not indicate a single point source of contamination but identified Bulgarian blackberries and Polish redcurrants as the most common ingredients in the lots of berries associated with cases. Due to the inconclusive findings of the trace-back, it was not possible to trace back and trace forward the contaminated berry product. Apart from Italy, where cases were reported over the whole outbreak period, locally infected HA cases occurred in well-defined waves over a period of one year in at least five different countries. This was most likely due to the distribution of contaminated frozen berries at different times in different countries and shows the complexity of the frozen berry market in Europe.

Sporadic outbreak cases detected in summer 2014 in Ireland and Italy support the possibility of contaminated lots remaining on the market or in consumers' freezers, posing a challenge to declaring the outbreak over, although the monthly number of HA cases reported in the affected countries had returned to the pre-2013 baseline. It is particularly challenging to declare the outbreak over in Italy, where, due to the size and duration of the outbreak, the strain may have become endemic [17].

In the last decade, multistate HA outbreaks have been reported in the EU, mostly in subpopulations at increased risk of HAV infection such as travellers abroad, people who inject drugs, men who have sex with men or ethnic minorities [18-23]. In the past five years, large multistate food-borne HA outbreaks were associated with consumption of food items distributed in different EU/EEA countries and Australia [24-26]. In addition, three HAV subgenotype IB outbreaks, associated with strains different from the outbreak strain described here, occurred in 2012 and 2013 in different EU/EEA countries, Canada and the US, and implicated frozen and fresh strawberries, and pomegranate arils [26-28]. Frozen berries were also implicated with different HA and norovirus multistate outbreaks in the past three decades in the EU [29].

This outbreak had substantial implications in direct and indirect costs for the healthcare systems and the patients affected. All reported cases were symptomatic. Hospitalisation was reported in an estimated 70% of the cases with a median hospitalisation period of about a week (range: 1–20). Most of the hospitalised patients were adults of working age, thus resulting in considerable societal and individual costs due to the disease. Two of the reported hospitalised patients were known to have died.

The investigations led to a number of measures to halt the outbreak: recall of food batches found or suspected to be contaminated, risk communication to the general public and catering sector recommending heat-treating berries, and post-exposure prophylaxis to contacts of cases to reduce secondary transmission, according to national guidelines.

The investigation benefited from excellent collaboration among public health institutes and food safety authorities of the affected countries, who shared proactively and in a timely fashion the available information. Both Ireland and Italy provided their questionnaires for adaptation and use in other countries.

The HA laboratory network (HAVNET) [30], in May 2013, shared a common sequencing protocol for human samples, to enhance comparability of sequencing results between countries from then on. ECDC coordinated the European investigation and prepared three rapid risk assessments to inform and alert countries about this outbreak [31-33].

Regarding lessons learnt from this outbreak investigation, a number of follow-up actions have been identified on how to handle similar situations better in the future. Most countries initially used different protocols for HAV sequencing from human samples, and for food samples a protocol specifically developed for all food products possibly involved was not always available. This practice hampered strain comparison within and between human and food isolates. Following the outbreak, a multidisciplinary expert group agreed to promote the use of the well-developed standard sequencing protocol by HAV-NET for human HAV samples, and, when possible, for food samples. The protocol has been distributed to all national public health laboratories in the EU/EEA countries to help compare and exchange information on sequencing results, and to speed up molecular investigations in future outbreaks.

This and other recently occurring HA outbreaks highlight that frozen berries and frozen soft-fruit in general should be considered potentially high-risk food items in Europe [24,26,34]. Both consumption of frozen berries and the frequency of reporting of outbreaks associated with this food item rapidly increased in the past 15 years in Europe [29]. In order to avoid opportunities for contamination or commercialisation of contaminated products, it is important that countries producing berries establish and monitor appropriate hygiene standards and HA awareness for berry pickers. It is similarly important that commercial berry producers consider the risk of contamination with HAV and other viruses in their Hazard Analysis of Critical Control Points (HACCP) programmes. In addition, more work is needed to enhance the sensitivity of the detection of HAV in food samples and in particular in fresh and frozen berries, as it proves challenging due to low-level and unevenly spread contamination. A coordinated approach in risk-communication could be considered: any signals of contamination in frozen berries known to be distributed in the retail market could trigger recommendations by food safety authorities for heat-treatment of berries before consumption in consumers' homes, as well as in commercial food outlets and mass catering kitchens, as occurred in several affected countries during this and previous HA outbreaks related to frozen berries.

Finally, in consideration of the changing HAV epidemiology, emerging sources of exposure and the increasing size of the susceptible adult population, vaccination recommendations may need to be reconsidered in the EU/EEA. In the interests of public health, options such as universal childhood vaccination and/or targeted vaccination of workers in the berry production chain may be considered at national level.

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Conflict of interest

None declared.

Authors' contributions

LVe, LTo, BRGH, MF, LS, RRF, AMRA,SLN, FA, ABP, LM, KP, VA, HV, MF, MM, MG, JE, MK, CV, SM, MST, MET, BS, JO'G, KSJ, JJW, GJ, KB, ARC, LO'C, LVo, CR contributed with data and analysis from respective countries, wrote country sections and reviewed the overall manuscript. ES coordinated the data collection, analysed the data and wrote the manuscript. LTa and JT contributed to the data analysis and the manuscript writing.

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Food-borne diseases associated with frozen berries consumption: a historical perspective, European Union, 1983 to 2013

L Tavoschi (Lara.Tavoschi@ecdc.europa.eu)[1], E Severi[1], T Niskanen[1], F Boelaert[2], V Rizzi[2], E Liebana[2], J Gomes Dias[1], G Nichols[1,3], J Takkinen[1], D Coulombier[1]

1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
2. European Food Safety Authority (EFSA), Parma, Italy
3. Current affiliation: Public Health England (PHE), Colindale, London, United Kingdom

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Epidemiological investigations of outbreaks of hepatitis A virus (HAV) and norovirus (NoV) infections in the European Union/European Economic Area (EU/EEA) in the last five years have highlighted frozen berries as a vehicle of infection. Given the increasing berry consumption in the EU over the last decades, we undertook a review of the existing evidence to assess the potential scale of threat associated with this product. We searched the literature and four restricted-access online platforms for outbreak/contamination events associated with consumption of frozen berries. We performed an evaluation of the sources to identify areas for improvement. The review revealed 32 independent events (i.e. outbreak, food contamination) in the period 1983–2013, of which 26 were reported after 2004. The identified pathogens were NoV, HAV and *Shigella sonnei*. NoV was the most common and implicated in 27 events with over 15,000 cases reported. A capture–recapture analysis was performed including three overlapping sources for the period 2005–2013. The study estimated that the event-ascertainment was 62%. Consumption of frozen berries is associated with increasing reports of NoV and HAV outbreaks and contamination events, particularly after 2003. A review of the risks associated with this product is required to inform future prevention strategies. Better integration of the available communication platforms and databases should be sought at EU/EEA level to improve monitoring, prevention and control of food-borne-related events.

Introduction

In the past few years, several European Union/European Economic Area (EU/EEA) countries reported food-borne outbreaks and clusters of hepatitis A virus (HAV) and norovirus (NoV) infections. Analytical epidemiological studies conducted as part of the outbreak investigations identified frozen berries as the main vehicle of infection in several of them [1,2]. In these outbreaks, molecular typing of the isolated viral strains was pivotal in identifying a multinational dimension. Preliminary food trace back investigations revealed large scale distribution of these products in the EU/EEA area, and pointed to producers in countries both inside and outside of the EU/EEA. In 2013, outbreaks affecting an unprecedented large number of people in a number of countries have occurred in the EU/EEA and beyond, highlighting the role of frozen berries as a vehicle of infection [3-5].

The 2006 European Commission (EC) report on the soft fruit processing sector notes that the EU berry consumption has experienced a 4.5 fold increase in volume from 1988 to 2005. The import into the EU of frozen berries has seen a particularly steep increase in the last decade; this was also due to the growth in popularity of fruit-based products like smoothies, ice creams and yogurts [6]. The most traded soft fruits are strawberries, blackberries, blueberries, currants and raspberries. The main producers of berries imported into the EU are China, Morocco and Serbia while, within the EU, two thirds of berries are produced in Poland [6-8]. A recent scientific opinion published by the European Food Safety Authority (EFSA) on the risk of contamination of berries [7] highlights that this food commodity often receives no or only minimal processing. Berry production is labour-intensive and berries are often cultivated in small farms [6]. Contamination and cross-contamination via equipment, water (irrigation and washing) and particularly via food handlers have been identified as the main risk factors.

This paper provides a historical overview on contamination of frozen berries and the related outbreaks in the EU/EEA, through an analysis of the scientific literature and of relevant EU-operated databases. In addition we

Box

Methodology for the selection of records in the literature to review food-borne events associated with frozen berries consumption, EU/EEA, 1983–2013

PubMed

#1 "Disease Outbreaks"[Mesh] OR outbreak*[tiab]

#2 Berries[tiab] OR berry[tiab] OR "Fragaria"[Mesh] OR fragaria*[tiab] OR strawberr*[tiab] OR raspberr*[tiab] OR blackberr*[tiab] OR "Blueberry Plant"[Mesh] OR blueberr*[tiab] OR "Punicaceae"[Mesh] OR pomegranate*[tiab] OR cranberr*[tiab] OR "Vaccinium macrocarpon"[Mesh] OR "Ribes"[Mesh] OR gooseberr*[tiab] OR ribes[tiab] OR "black currant"[tiab] OR "black currants"[tiab] OR "Sambucus"[Mesh] OR sambucus[tiab] OR elderberr*[tiab] OR punicaceae[tiab] OR "Vaccinium vitis-idaea"[Mesh] OR "Vaccinium vitis idaea"[tiab] OR "lingon berry"[tiab] OR "lingon berries"[tiab] OR lingonberr*[tiab] OR ((juice[tiab] OR juices[tiab]) AND (fruit[tiab] OR fruits[tiab] OR "Fruit"[Mesh]))

#3 "Hepatitis A"[Mesh] OR "Hepatitis A virus"[Mesh] OR "hav"[tiab] OR "hepatitis a"[tiab] OR "hepatitis type a"[tiab] OR "Salmonella"[Mesh] OR "Salmonella Infections"[Mesh] OR salmonella[tiab] OR salmonellosis[tiab] OR salmonelloses[tiab] OR "Typhoid Fever"[Mesh]OR typhoid[tiab] OR typhoids[tiab] OR "enteric fever"[tiab] OR "enteric fevers"[tiab] OR "Norovirus"[Mesh] OR norovirus[tiab] OR noroviruses[tiab] OR "Norwalk virus"[tiab] OR "norwalk viruses"[tiab] OR NoV[tiab] OR hNoV[tiab] OR "Caliciviridae"[Mesh] OR Caliciviridae[tiab] OR calicivirus[tiab] OR "Escherichia coli"[Mesh] OR "Escherichia coli"[tiab] OR "E coli"[tiab] OR "e.coli"[tiab] OR "e. coli"[tiab] OR "Chagas Disease"[Mesh] OR "Trypanosoma cruzi"[Mesh] OR chagas[tiab] OR "trypanosoma cruzi"[tiab] OR "Cyclosporiasis"[Mesh] OR "Cyclospora"[Mesh] OR cyclosporiasis[tiab] OR cyclosporiases[tiab] OR cyclospora[tiab] OR cyclosporas[tiab] OR "Foodborne Diseases"[Mesh] OR "Foodborne Disease"[tiab] OR "Foodborne Diseases"[tiab] OR "Food borne Diseases"[tiab] OR "Food borne Disease"[tiab] OR "Foodborne illness"[tiab] OR "Foodborne illnesses"[tiab] OR "Food borne illness"[tiab] OR "Food borne illnesses"[tiab])

#4 #1 AND #2 AND #3

Embase

#1 outbreak*:ab,ti

#2 'berry'/exp OR berry:ti,ab OR berries:ti,ab OR 'strawberry'/exp OR fragaria*:ti,ab OR strawberr*:ti,ab OR 'raspberry'/exp OR raspberr*:ti,ab OR 'blueberry'/exp OR blueberry*:ti,ab OR 'blackberry'/exp OR blackberr*:ti,ab OR 'pomegranate'/exp OR pomegranate*:ti,ab OR punicaceae:ti,ab OR 'cranberry'/exp OR cranberr*:ti,ab OR 'Vaccinium macrocarpon':ti,ab OR 'gooseberry'/exp OR ribes:ti,ab OR 'black currant':ti,ab OR 'black currants':ti,ab OR 'Sambucus'/exp OR sambucus:ti,ab OR elderberr*:ti,ab OR 'lingonberry'/exp OR 'lingon berry':ti,ab OR 'lingon berries':ti,ab OR lingonberr*:ti,ab OR 'Vaccinium vitis idaea':ti,ab OR 'fruit juice'/exp OR ((fruit:ti,ab OR fruits:ti,ab) AND (juice:ti,ab OR juices:ti,ab))

#3 'hepatitis a'/exp OR 'hepatitis a virus'/exp OR hav:ab,ti OR 'hepatitis a':ab,ti OR 'hepatitis type a':ab,ti OR 'salmonella'/exp OR 'salmonellosis'/exp OR salmonella:ab,ti OR salmonellosis:ab,ti OR salmonelloses:ab,ti OR typhoid:ab,ti OR typhoids:ab,ti OR 'enteric fever':ab,ti OR 'enteric fevers':ab,ti OR 'norovirus'/exp OR 'norovirus infection'/exp OR norovirus:ab,ti OR noroviruses:ab,ti OR 'norwalk virus':ab,ti OR 'norwalk viruses':ab,ti OR nov:ab,ti OR hnov:ab,ti OR 'calicivirus'/exp OR 'calicivirus infection'/exp OR caliciviridae:ab,ti OR calicivirus:ab,ti OR 'escherichia coli'/exp OR 'escherichia coli infection'/exp OR 'escherichia coli':ab,ti OR 'e coli':ab,ti OR 'e.coli':ab,ti OR 'e. coli':ab,ti OR 'chagas disease'/exp OR chagas:ab,ti OR 'trypanosoma cruzi'/exp OR 'trypanosoma cruzi':ab,ti OR 'cyclospora'/exp OR 'cyclosporiasis'/exp OR cyclosporiasis:ab,ti OR cyclosporiases:ab,ti OR cyclospora:ab,ti OR cyclosporas:ab,ti OR 'food poisoning'/exp OR 'foodborne disease':ab,ti OR 'foodborne diseases':ab,ti OR 'food borne diseases':ab,ti OR 'food borne disease':ab,ti OR 'foodborne illness':ab,ti OR 'foodborne illnesses':ab,ti OR 'food borne illness':ab,ti OR 'food borne illnesses':ab,ti

#4 #1 AND #2 AND #3

evaluated the different data sources to identify potential areas for improving outbreak monitoring at EU/EEA level.

Methods

In order to review the available evidence of outbreaks of HAV and NoV and/or other relevant food-borne diseases associated with consumption of frozen berries, the scientific literature and relevant EU-based databases were searched.

Literature review

A comprehensive literature search was conducted in PubMed and Embase on 25 October 2013, using keywords and Medical Subject Heading (MESH) terms as described in the Box. No time, language or geographical limits were applied. Additional studies were identified through manual search of references and personal communications from experts in the EU/EEA Member States. Articles retrieved were screened by title/abstract and full text and included if (i) an outbreak/contamination event was reported; (ii) the vehicle of infection was identified to be frozen berries; (iii) at least one EU/EEA country was involved. All reports of outbreaks confined to non-EU/EEA countries were excluded. The same information obtained from notifications, were also extracted from included articles.

EU-based databases

Four relevant restricted-access online platforms exist at EU-level that collect information on contamination events and/or on human cases of diseases and outbreaks, namely: Epidemic Intelligence System for Food and Waterborne Diseases and Zoonoses (EPIS FWD), ECDC Threat Tracking Tool (TTT), European Food Safety Authority (EFSA) database on human food-borne

Table 1

European Union-operated databases and their purposes, historical perspective on food-borne events associated with frozen berries consumption, 1983–2013

EU-level database	Purpose
Epidemic Intelligence System for Food- and Waterborne Diseases and Zoonoses (EPIS FWD)	Communication platform of the ECDC for preliminary human health risk assessment of food and waterborne diseases, including notification of outbreaks and unusual increases of cases of disease at the national level. It was set up in 2010, and reporting is done on a voluntary basis [41].
ECDC Threat Tracking Tool (TTT)	ECDC database for epidemic intelligence purpose to keep track of events with potential public health impact at EU/EEA level. It was set up in 2005. An ECDC epidemic intelligence team is responsible for capturing relevant events into the database.
European Food Safety Authority (EFSA) database on human food-borne outbreaks	Database of Member States' annual reports on food-borne outbreaks in the EU/EEA. It was established in 2005, when reporting of food-borne outbreaks became mandatory in the EU/EEA.
Rapid Alert System for Food and Feed (RASFF)	European Commission communication platform to share information about existing threats/alerts posed by a food (or feed) item which is still on the market (e.g. pathogen-contaminated food item). It was set up in 1979. Reporting of any information about a serious health risk from food or feed is mandatory for EU/EEA countries (http://ec.europa.eu/rasff and http://ec.europa.eu/food/safety/rasff/index_en.htm).

ECDC: European Centre for Disease Prevention and Control; EEA: European Economic Area; EU: European Union.

outbreaks, Rapid Alert System for Food and Feed (RASFF). EFSA's scientific reports and opinions published on EFSA website were also consulted (Table 1).

The online platforms were searched by extracting all the notifications related to frozen berries involving at least one EU/EEA country (latest access date 31 October 2013) using the built-in search options. From each notification identified, the following information was extracted if available: time (year/month), country/ies involved, type of berry, number of associated human cases, result of molecular investigation and country of origin of the foodstuff. RASFF notifications of border control or other routine food safety checks were included in the analysis even in the absence of evidence of associated human cases, and listed in the output table as pathogen contamination incidents.

The Early Warning and Response System (EWRS) restricted platform of the EC was also searched, but not included in the analysis due to complete overlap with TTT and EPIS platforms.

Identification of independent events

We defined an event as: (i) notification of a food vehicle contamination associated with one or more outbreaks involving human cases; (ii) notification of a contamination of a food vehicle with no associated human cases (reported in Table 2 as a contamination incident). Once eligible entries were identified from the different sources, a manual record-linkage was performed based on the following variables: country reporting the outbreak; period (year, month); pathogen (species, genotype if available); vehicle (type of berry); reported cases. Each event identified can include one or more outbreaks and a cumulative number of reported cases.

A capture–recapture analysis [9] on three sources, namely the literature, RASFF, and EFSA database, was carried out for the period 2005 to 2013. The analysis was performed using three-source log-linear models, incorporating pairwise independencies in order to reduce possible bias. Multiple reports of the same event by any of the sources were treated as a single event in the analysis.

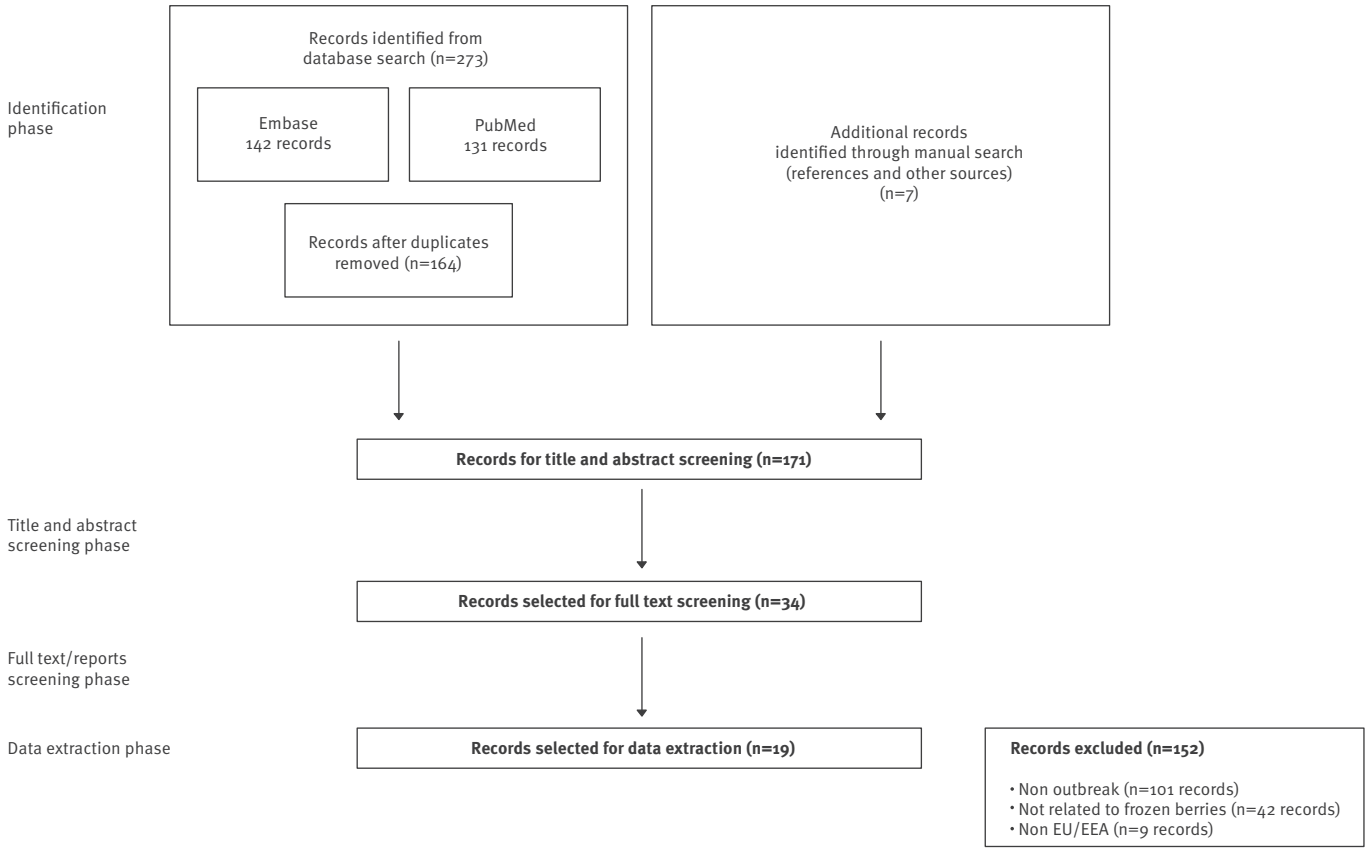
Results

Literature review

The literature search retrieved 273 articles: 131 from PubMed and 142 from Embase. After manual removal of duplicates and search for additional records, 171 were screened for the title and abstract, and 34 were

Figure 1

Flowchart showing the selection of records in the literature to review food-borne events associated with frozen berries consumption, EU/EEA, 1983–2013



EEA: European Economic Area; EU: European Union.

screened for the full text. Nineteen articles were included in the analysis (Figure 1).

Analysis of the reported events

The triangulation of the evidence collected from the various sources revealed 32 independent events, including 27 events with human cases reported, associated with contaminated frozen berries and five contamination incidents with no reported human cases. The identified pathogens were NoV, HAV and *Shigella sonnei*. The overall study period covered 30 years, from 1983 to 2013, however, 26 of 32 reported events, were between 2005 and 2013. The findings are summarised in Table 2.

Frozen berry contamination with NoV was implicated in 27 events during the period from 1998 to 2013. Of these 27, four were detected during routine food safety control and not associated, according to the available evidence, with human cases. Three of these four events occurred in 2013 and resulted in border rejection of the food consignment or in product recall. The remaining 23 events were distributed over a 15-year period from 1998 to 2013, and caused almost 14,000 reported human cases in 70 outbreaks in six EU countries, namely Denmark, Finland, France, Germany, the Netherlands and Sweden [2,10-19]. The frozen berries implicated as food vehicles in 23 of 27 of the reported outbreak events were frozen raspberries.

HAV contamination of frozen berries was first reported in an outbreak in 1983 in the United Kingdom [20], although there had been previous suggestions of associations [21]. However, there have been no reports between then and 2012–2013 when two multinational outbreaks occurred. The first outbreak affected four Nordic countries and was associated with the consumption of frozen strawberries; the second outbreak affected Italy and 12 additional EU/EEA countries and was associated with the consumption of frozen mixed berries [1,20,22-25]. The number of cases associated with these two events is estimated to be well above 1,500.

Finally, a *Shigella sonnei* outbreak linked to frozen mixed berries was reported to have affected 21 people in Sweden in 1996.

Analysis of the information sources

When the sources of information were taken into consideration, 12 out of 32 events were reported through RASFF only, five events were reported in the literature

Table 2

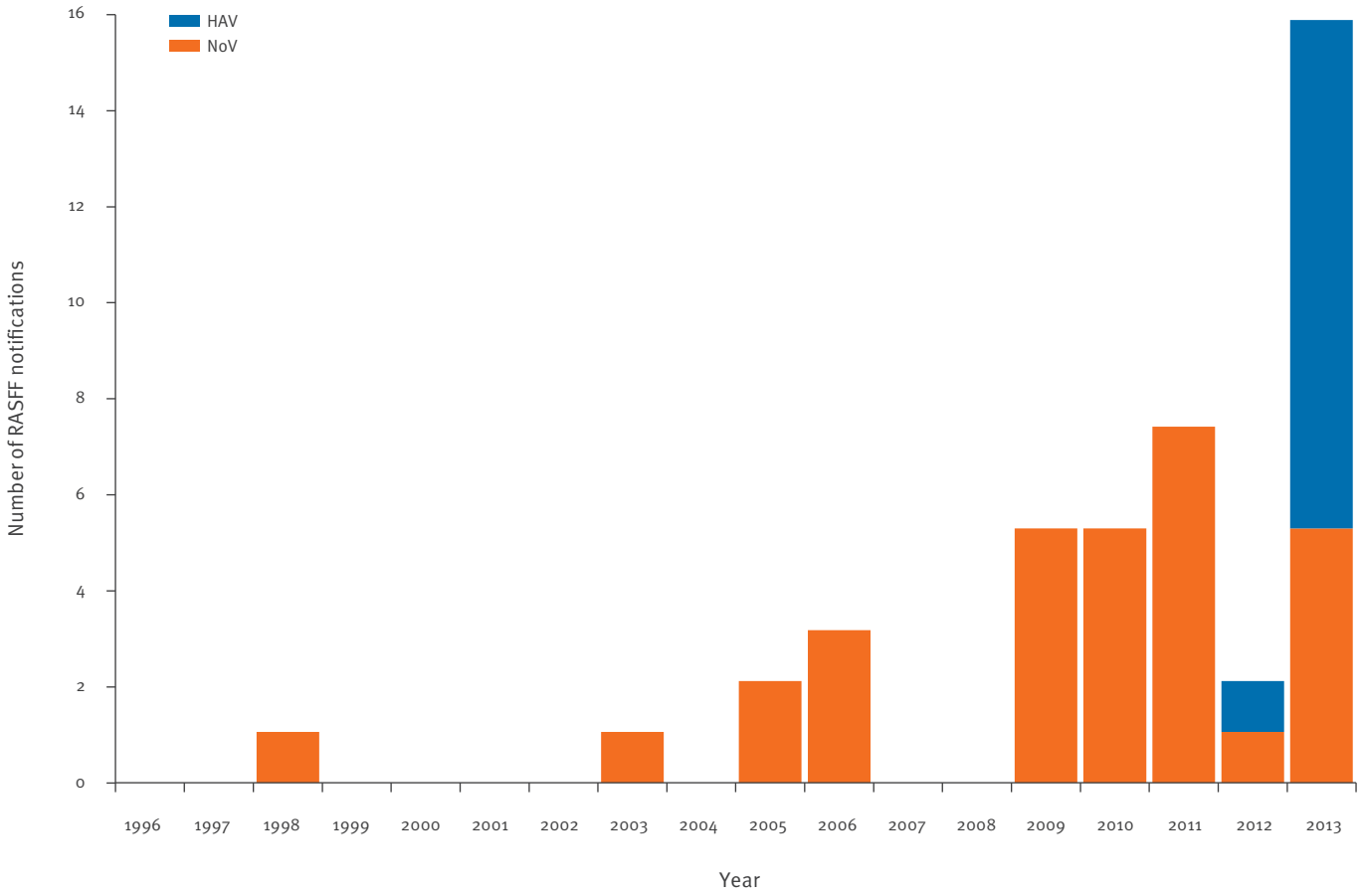
Reported outbreaks and contamination incidents with food-borne pathogens associated with frozen berries in the EU/EEA, 1983–2013

Listing of independent events	Source	Year	Pathogen	Vehicle	Number of cases reported	Number of reported outbreaks	Affected country/ies	Country/ies of origin of food vehicle
1	Literature [20]	1983	HAV	Frozen raspberries	24	1	United Kingdom	na
2	RASFF	1996	*Shigella sonnei*	Frozen mixed berries	21	1	Sweden	na
3	Literature [10,42]	1998	NoV	Frozen raspberries	509	1	Finland	na
4	RASFF	1998	NoV	Frozen raspberries	265	2	Sweden, Finland	Serbia, Montenegro
5	Literature [11]	2001	NoV	Frozen raspberries	30	1	Sweden	na
6	RASFF	2003	NoV	Frozen raspberries	>50	1	Sweden	Serbia, Montenegro
7	Literature [12]	2005	NoV	Frozen raspberries	75	1	France	na
8	TTT, RASFF, Literature [13,14]	2005	NoV	Frozen raspberries	>1,000	6	Denmark	Poland
9	Literature [15]	2005	NoV	Frozen blueberries	241	1	Germany	na
10	TTT, RASFF	2006	NoV	Frozen raspberries	25	1	Denmark	Serbia, Montenegro
11	TTT, RASFF, Literature [16,43]	2006	NoV	Frozen raspberries	43	4	Sweden	China
12	RASFF	2006	NoV	Frozen raspberries	45	1	The Netherlands	Chile
13	EFSA	2007	NoV	Frozen raspberries	9	1	Denmark	na
14	RASFF, EFSA, Literature [17-19]	2009	NoV	Frozen raspberries	1,093	22	Finland	Poland
15	RASFF, EFSA	2009–2010	NoV	Frozen raspberries	96	9	Denmark, Sweden	Serbia, Bosnia and Herzegovina
16	EFSA	2010	NoV	Frozen raspberries	60	1	Denmark	na
17	EFSA	2010	NoV	Frozen raspberries	133	2	Finland	na
18	RASFF	2010	NoV	Frozen raspberries	>1	1	Sweden	Poland
19	RASFF, EFSA	2011	NoV	Frozen raspberries	8	1	Denmark	China
20	RASFF	2011	NoV	Frozen raspberries	NA contamination incident	NA	Finland	Serbia
21	RASFF, EFSA	2011	NoV	Frozen raspberries	201	8	Denmark	Serbia
22	EFSA	2011	NoV	Frozen raspberries	52	2	Denmark	na
23	EFSA	2011	NoV	Frozen raspberries	18	1	Germany	Germany
24	TTT, RASFF, EFSA, Literature [2]	2012	NoV	Frozen strawberries	10,950	1	Germany	China
25	RASFF	2012	HAV	Frozen strawberries	NA contamination incident	NA	Belgium	China
26	EPIS FWD Literature [1,22]	2012–2013	HAV	Frozen strawberries	103	1	Finland, Denmark, Sweden,Norway	Egypt, Morocco
27	EPIS FWD, RASFF, Literature [23-25]	2013	HAV	Frozen mixed berries	>1,000	1	France, Italy,Ireland, the Netherlands, United Kingdom	Poland, Bulgaria
28	RASFF	2013	NoV	Frozen strawberries	NA contamination incident	NA	Lithuania	China
29	RASFF	2013	NoV	Frozen strawberries	NA contamination incident	NA	Denmark	China
30	RASFF	2013	NoV	Frozen raspberries	NA contamination incident	NA	The Netherlands	Poland
31	RASFF	2013	NoV	Frozen raspberries	29	1	Finland	Poland
32	RASFF	2013	NoV	Frozen raspberries	13	1	Denmark	Poland, Serbia

EEA: European Economic Area; EPIS FWD: Epidemic Intelligence System for Food and Waterborne Diseases and Zoonoses; EU: European Union; HAV: hepatitis A virus; na: not available; NA: not applicable; NoV: norovirus; RASFF: Rapid Alert System for Food and Feed; TTT: Threat Tracking Tool.

Figure 2

Distribution of number of RASFF notifications for norovirus and hepatitis A virus contamination in frozen berries, by year and implicated pathogen, EU/EEA, 1996–2013 (n=42)



HAV: hepatitis A virus; NoV: norovirus; RASFF: Rapid Alert System for Food and Feed.

only, and five events were reported only through EFSA. Finally, 10 events were reported by more than one source, including three events notified in EPIS FWD in the period from 2010 to 2013. Twenty-one events were linked to at least one RASFF notification, encompassing the three identified pathogens in the period from 1996 to 2013.

The number of RASFF notifications linked to contaminated frozen berries has increased over time, as shown in Figure 2 below. Among the events reported, five were linked to more than one RASFF notification, and up to 10 for one single multinational event (event number 27 in Table 2). The cumulative number of RASFF notifications was 42.

The geographic distribution of events shows a specific pattern, with countries affected by outbreaks of NoV and/HAV associated with the consumption of contaminated frozen berries being reported predominantly in Nordic countries, and in particular Denmark, Sweden and Finland (Figure 3). The food trace-back activities have pointed to Serbia, Poland or China as the country of origin of the implicated berries in 19 of the events (Figure 4).

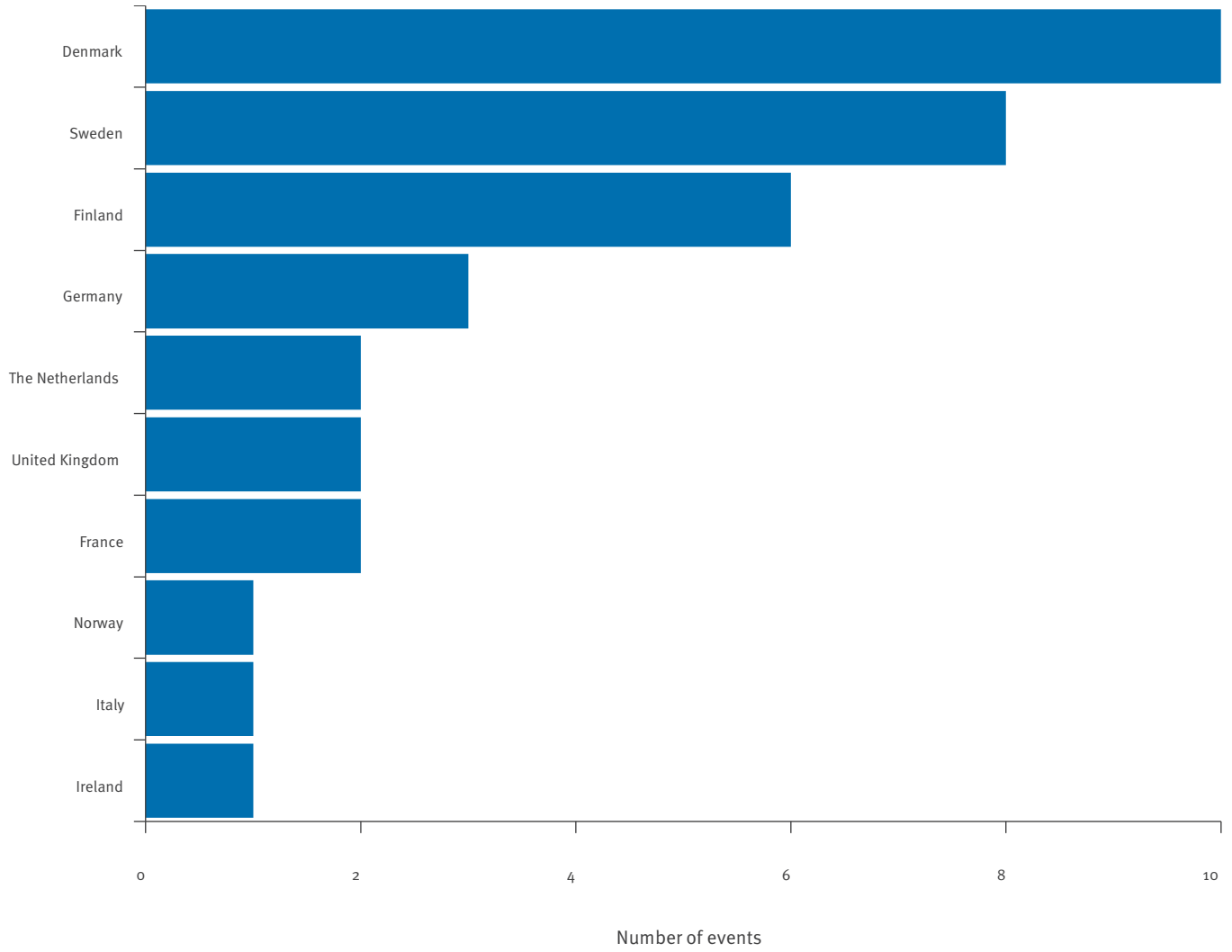
Frozen raspberries were the implicated food in 24 of the 32 events. For 23 of these 24 outbreaks, the isolated pathogen was NoV. According to RASFF notifications, contaminated raspberries are produced in several different countries. However, frozen strawberries produced in China were implicated in four of the five events associated with frozen strawberries.

Capture re-capture study

A capture–recapture analysis was performed including literature, RASFF and EFSA in the period from 2005 to 2013. These three independent sources identified a cumulative total of 26 unique events. The log-linear model used gave an estimate of 42 (95% confidence interval: 20–64) independent events occurring in the period from 2005 to 2013. The completeness of reported independent events can be estimated at 21.4% for the literature, 23.8% for the source in EFSA, and 42.9% for RASFF. The ascertainment of events, defined as reported outbreaks and contamination incidents, can be estimated at 61.9% in the period from 2005 to 2013.

Figure 3

Distribution of number of events[a] by country of occurrence, EU/EEA, 1983–2013 (n=32 events)



EEA: European Economic Area; EU: European Union.

[a] One event may be associated with more than one country of occurrence (see Table 2).

Discussion

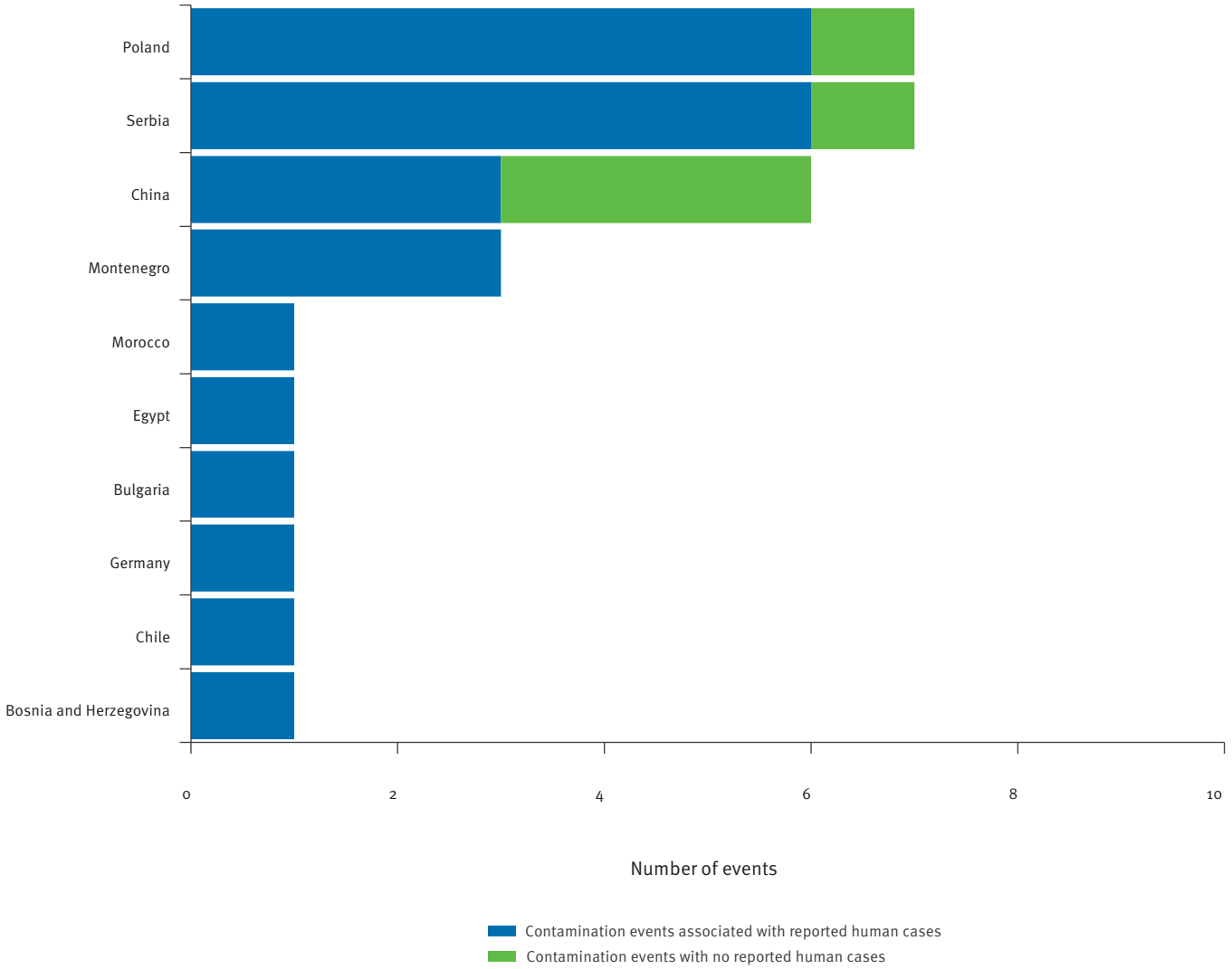
We reviewed contamination events of food-borne pathogens in frozen berries. The review combined different sources such as scientific literature and restricted access EU platforms. With the exclusion of one event associated with contamination with *Shigella sonnei*, events were due to contamination with NoV (27 events) and HAV (four events).

We identified 32 contamination events in a 30-year period, of which 26 occurred after 2004. This rise in number of reported outbreaks of NoV and HAV associated with consumption of frozen berries could be due to several concomitant factors. Increased likelihood of reporting over time and development of appropriate online platforms need to be considered alongside possible boosted interest in the scientific community. Technical developments in the detection of pathogens in food have resulted in the identification of implicated food vehicles with an increased accuracy over time [26-29]. The evolving molecular typing techniques have allowed matching food and human isolates and the identification of large multinational outbreaks sharing a common source [1,2,23]. The increase of RASFF notifications for contaminated frozen berries provides additional evidence on the rise in large contamination events. In 2013, several RASFF notifications were linked to a large multinational HAV outbreak associated with mixed frozen berry consumption in Italy and 12 additional EU/EEA countries [23-25]. This indicates the extensive environmental and trace back investigations performed by the affected countries when experiencing a large food-borne outbreak.

Liberalisation of markets and increased consumption of 'healthy' and raw food, such as berries, has increased the production and subsequently the risk of exposure to NoV and HAV for the EU population [6]. In 2013, three RASFF notifications for NoV contaminated berries were issued following border or other routine

Figure 4

Distribution of number of events in EU/EEA, by place of origin of the implicated berries, 1996–2013 (n=32 events[a])



EEA: European Economic Area; EU: European Union.

[a] Ten events missing information on the place of origin of the implicated berries.

Data source: European Food Safety Authority (EFSA); Epidemic Intelligence System (EPIS); Literature review; Rapid Alert System for Food and Feed (RASFF); Threat Tracking Tool (TTT).

food safety controls. This might have been related to the EU regulation [30] issued by the EC in 2012 to intensify the level of official controls on imports of specific food items of non-animal origin, including checks for NoV and HAV in strawberries from China.

Our review highlights the higher frequency of NoV outbreaks compared with HAV in the EU/EEA in the past decades. Although the two viruses share some common features, such as low dose infectivity and ability for long survival in the environment, HAV appears to be associated with a lower number of reported cases. This may be due in part to prevalence of individuals with long-lasting immunity after infection or vaccination, a high proportion of asymptomatic HAV infections or, alternatively, to challenging recognition of outbreaks and their association with a particular food item due to the long HAV incubation period and recall bias [31].

Our review identified one single event associated with Shigella in 1996. Although this does not appear to be a common health risk associated with the consumption of frozen berries, prevention and control measures should also include this pathogen.

There were some limitations in our study approach. We did not perform a systematic review of the available evidence. Hence some records may have not been retrieved. The manual record-linkage based on multiple variables may have resulted in imprecise estimates of the number of independent events reported in the study period. In addition, the number of human cases associated with each event may be affected by considerable under-reporting, typical of self-limiting gastrointestinal diseases and of asymptomatic manifestation of disease.

The different periods during which the sources have been operating and the different reporting practices may have impacted on the information retrieval, especially in the earlier decades. Comparison was also limited, as some of the EU-based platforms were introduced from 2005 onwards. Moreover, while reporting to the RASFF or EFSA database is mandatory for EU Member States, this is not the case for the other sources included in the analysis, resulting in reporting or publication bias. A more in-depth analysis of the grey literature such as national public health institutes reports, may have allowed more events to be retrieved, including a larger fraction of outbreaks with a national dimension, particularly from earlier years covered by our review.

As shown in the capture–recapture study, as many as 38% (16/42) of the estimated independent events, which occurred during the period 2005–2013, were not reported by any of the study sources. Although the accuracy of the capture-recapture study relies on an assumption of independence between the different sources, as well as on the correct identification of independent events, it suggests an appreciable level of incompleteness. The reasons why the investigated data sources were incomplete could not be disclosed by the present study, and was not one of the study objectives. Food-borne outbreak reporting systems at the national level are not harmonised among EU/EEA countries. The differences in the number and type of reported outbreaks may indicate differences in the sensitivity of the national systems in identifying and investigating food-borne outbreaks. These differences may not necessarily reflect the level of food safety in Member States.

The findings from this review are in line with two recent publications from EFSA [7,32]. EFSA developed an ad hoc model to identify and rank specific food/pathogen combinations most often linked to human cases originating from food of non-animal origin (FoNAO) in the EU, using seven criteria: strength of associations between food and pathogen, incidence of illness, burden of disease, dose-response relationship, consumption, prevalence of contamination and pathogen growth potential during shelf-life [32]. The assessment is based on the analyses of EU food-borne outbreaks reported to EFSA and associated with FoNAO in 2007–2011. The highest number of food-borne outbreaks was reported for the combination of NoV and raspberries. According to the model, NoV and raspberries ranked fourth among top groups of food/pathogen combinations. NoV and other berries ranked fifth. The model, however, does not distinguish between fresh and frozen berries. The resulting EFSA's Biological Hazards Panel (BIOHAZ) opinion [7] focused on noroviruses in frozen berries, and specifically assessed risk factors in the production chain and proposes adequate mitigation measures. If properly implemented, many of these measures are likely to have a positive impact on prevention of HAV contamination. However, the best and simplest preventive measure at the farm level seems to be hand washing after using the toilet [33].

The route by which fresh and frozen berries become contaminated is not fully understood. The three pathogens causing the outbreaks are likely to come from human sources. Manually picked fruits such as soft berries are at great risk of viral contamination if the quality of farming practices, e.g. worker hygiene during farming and harvesting, is insufficient. Contamination may also derive from poor quality irrigation water and spraying of berry crops [3,7,17,34]. Frozen as compared with fresh berries, may lead to larger scale contamination due to processing routines such as mixing batches of different origin during freezing and before packaging. As contamination of raspberries with different NoV genotypes has been documented, large batches of product for export may come from different farms [2,3,14,17]. The consequently uneven distribution of contaminated berries may result in poor detection of viruses in food samples undergoing routine food safety checks. The detection of these viruses in contaminated food vehicles is further hampered by the absence of a robust, quantitative method for sampling, concentration and analysis, the low levels of contamination and the effect of inhibitory materials for RT-PCR detection [3,26,27,35-37]. These factors may have impacted on the capacity to identify, and subsequently report, outbreaks or contamination events involving frozen berries, particularly in the earlier decades of the study period.

Although the size of berry farms varies broadly across the EU, small-scale farming is common. Together with wild-picked berries, berries from these farms have been considered to be more vulnerable for HAV and NoV contamination. Nevertheless, there is no evidence of association between farm size and risk of contamination. In addition, small-scale production, as well as inadequate labelling of the site of production, impacts the traceability of batches from wholesalers to the farm level [3,38].

According to several studies, decontamination of berries proves difficult as the ability to survive of enteric viruses on frozen berries is quite long, with marginal reduction of the infectivity even after several months of storage [39,40]. Considering the long shelf-life and the wide distribution of these products, contaminated batches may result in a long-term source of outbreaks of a national or multinational size. In consideration of this, and due to repeated NoV and HAV outbreaks related to frozen berries, some countries have implemented risk communication interventions and recommended to heat-treat frozen berries before use, for one to three minutes [3].

We presented evidence for an increasing number of outbreaks of HAV and NoV associated with the consumption of frozen berries reported in the EU/EEA, predominately in the past 10 years. In consideration of the

increasing consumption of berries in the EU/EEA [6], and such an emerging public health risk [7], a review of the risks for NoV and HAV contaminations associated with farming, picking, processing and distribution of fresh and frozen berries is needed. In particular, such a risk assessment would provide the necessary evidence to assess the adequateness of existing food safety regulations on the production and handling of berries to ensure safe products are on the market (and trade) with respect to contamination with NoV and HAV. From a public health perspective, risk communication messages such as heat-treating frozen berries before consumption should be re-iterated. Recommendations for HAV vaccination for habitual consumers of frozen berries may also be considered, especially for high-risk individuals.

In addition, to enhance the detection, control and investigation of outbreaks due to contaminated berries, and to support the assessment of the health risks, a better integration of the available communication platforms and databases should be sought at EU/EEA level to improve coordinated data collection and reporting.

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Conflict of interest

None declared.

Authors' contributions

LT wrote the manuscript. ES contributed extensively to the manuscript drafting. TN, VR, FB and EL contributed with comments and revised the manuscript. ES and LT collected the data and performed the analysis. GN, TN, VR, FB and EL contributed to data collection. JGD performed the capture-recapture analysis. JT and DC reviewed the manuscript.

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