

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

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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

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) $\geq 99.8\%$ 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

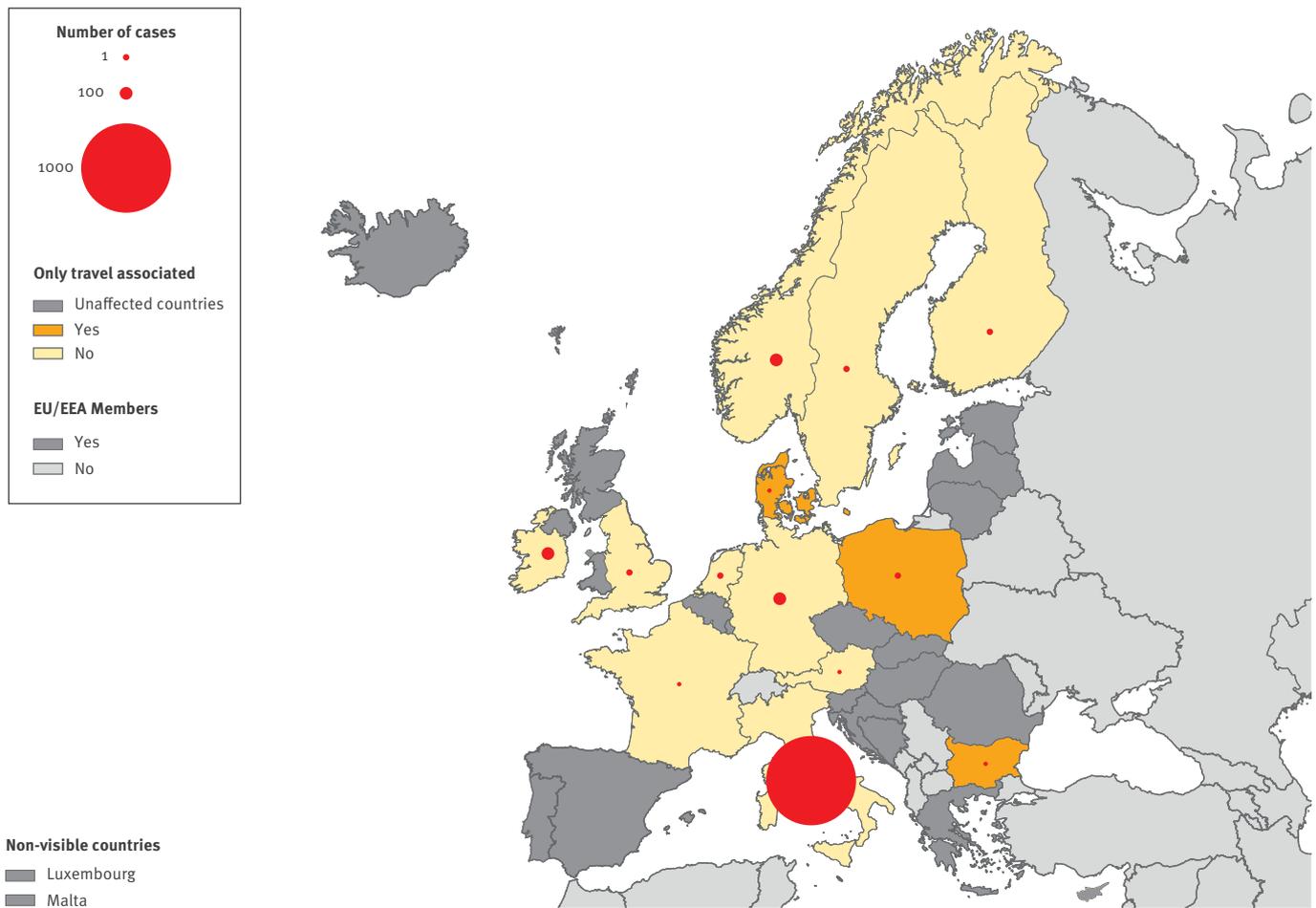
From 1 January 2013 to 31 August 2014, a total of 1,589 HA cases were reported as associated with this outbreak from 13 EU/EEA countries (Figure 1, Figure 2); most of the cases ($n=1,438$; 90%) were reported in Italy. Germany, Ireland and Norway each reported around 30 cases and all other countries reported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

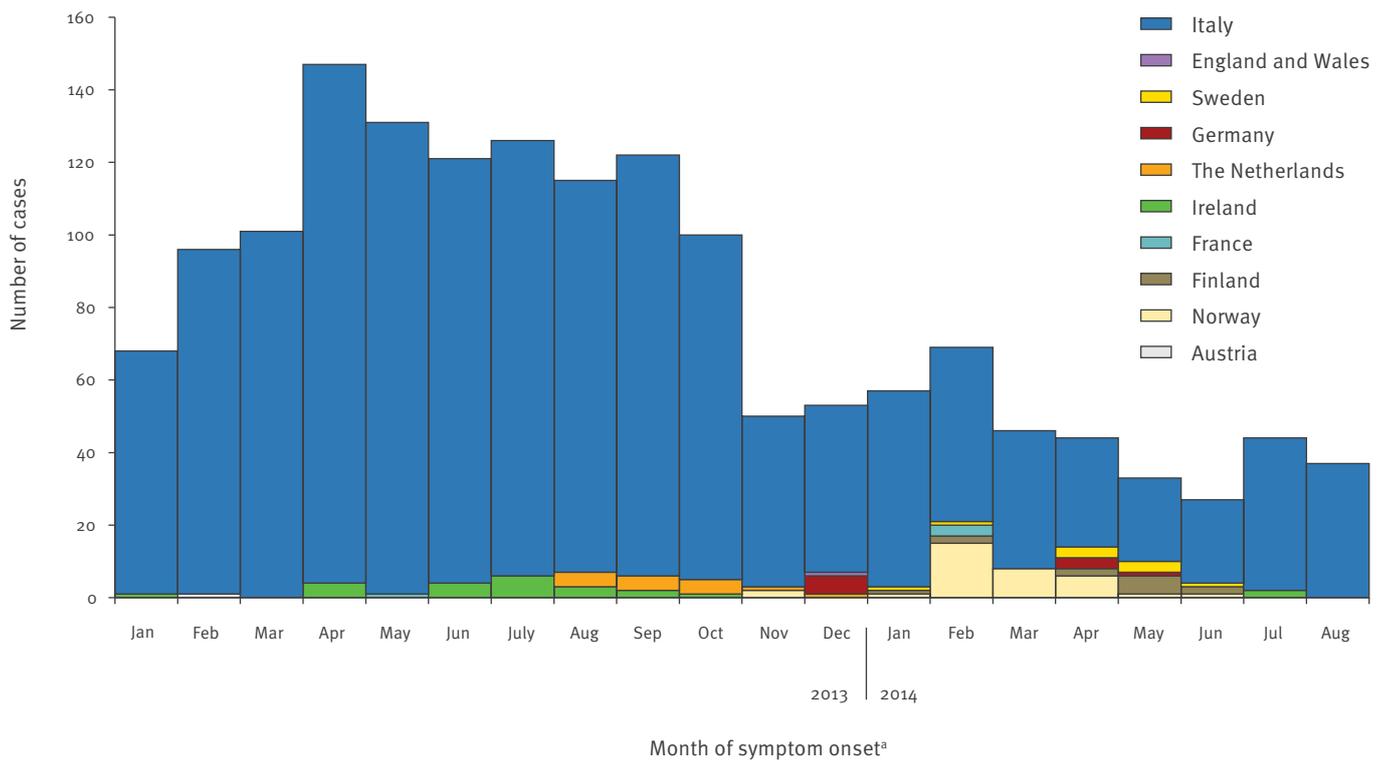
country experiencing the HAV outbreak (Box), 42 to Italy and one to Norway. Apart from Bulgaria, Denmark and Poland, where all cases had a travel history to Italy, in all other countries there was evidence of local transmission, with all or part of the cases not reporting any history of travel.

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.

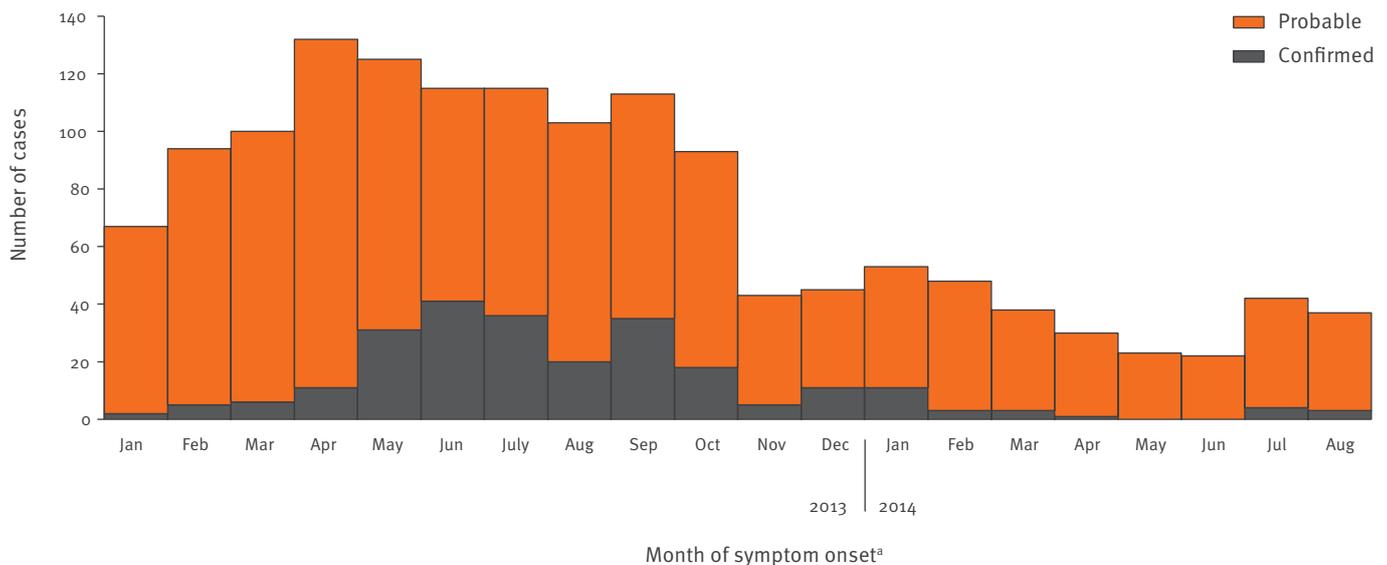
As only some EU/EEA countries perform molecular typing, and often only on a subset of cases, it is likely that additional cases associated with this outbreak were missed in those countries not performing routine sequencing of isolates from HA patients. In support of this, a confirmed case reported by 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)



^aOr 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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