Ongoing multi-strain food-borne hepatitis A outbreak with frozen berries as suspected vehicle: four Nordic countries affected, October 2012 to April 2013

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A food-borne outbreak of hepatitis A in Denmark was notified to other countries on 1 March 2013. A case–control study identified frozen berries eaten in smoothies as potential vehicle. In the following weeks, Finland, Norway and Sweden also identified an increased number of hepatitis A patients without travel history. Most cases reported having eaten frozen berries at the time of exposure. By 17 April, 71 cases were notified in the four countries. No specific type of berry, brand or origin of berries has yet been identified.

Identification of the outbreak

In February 2013, Denmark registered a higher than usual number of notified patients with hepatitis A virus (HAV) infection who had no travel history 2–6 weeks before symptom onset or other known risk factors for HAV infection. Concurrently, viruses from six hepatitis A patients who had been notified since October 2012 were shown to be genotype IB with the same sequence across 1,231 nucleotides of the capsid protein VP1 gene, including the VP3/VP1 and VP1/2A junctions (GenBank accession number KC876797). An outbreak investigation was initiated and an urgent enquiry was posted through the European Epidemic Intelligence Information System for food- and waterborne diseases (EPIS-FWD) on 1 March 2013, asking if any other countries had also seen an increase in the number of domestic patients with HAV infection. The sequence was also shared within the International HAV laboratory network managed in the Netherlands [1].

Following the urgent enquiry, Finland, Norway and Sweden also reported an increase in the number of patients with HAV infection who had no history of foreign travel (Table 1). Each country identified one or more cases with HAV genotype 1B that had identical sequences to the HAV of the Danish cases. The outbreak is still ongoing.

The following outbreak case definition was defined in Denmark and applied in all four countries, except that Sweden only includes cases from 1 December 2012 onwards and Finland is not excluding cases with other potential risk factors.

- A *probable* case is defined as a person living in Denmark, Finland, Norway or Sweden with clinical illness compatible with HAV infection and positive for HAV IgM antibodies, no travel history outside of Nordic countries two to six weeks before onset of symptoms or having other known HAV risk factors, such as intravenous drug use, homelessness or male-to-male sexual contact and symptom onset on 1 October 2012 or later.
- A *confirmed* case is defined as a probable case typed with HAV genotype IB with a sequence that differs by no more than 2% from sequence KC876797.
- We *exclude* all patients with HAV genotypes other than IB, or patients with an HAV genotype IB sequence that differs by more than 2% from sequence KC876797, or patients with an untyped HAV from the household of an excluded patient with HAV infection.
- A *secondary* case is defined as a probable or confirmed case with close contact to a probable or confirmed case and having symptom onset two or more weeks after that of the primary case.

Table 1

Hepatitis A patients by travel history, virus genotype and outbreak case type, Denmark, Sweden, Norway and Finland, 1 October 2011–17 April 2012 (n=53) and 1 October 2012–17 April 2013 (n=180)

Description	Number of patients							
	Denmark		Sweden		Norway		Finland	
	1 Oct 2011–17 Apr 2012	1 Oct 2012–17 Apr 2013	1 Dec 2011–17 Apr 2012	1 Dec 2012–17 Apr 2013	1 Oct 2011–17 Apr 2012	1 Oct 2012–17 Apr 2013	1 Oct 2011–17 Apr 2012	1 Oct-2012–17 Apr 2013
Total	**11**	**64**	**16**	**68**	**23**	**31**	**3**	**17**
Travel history								
Travel in foreign country	4	20	8	27	15	15	2	7
No foreign travel	7	43	7	34	7	16	1	10
Unknown	0	1	1	7	1	0	0	0
Genotype								
1A	0	2	4	3	0	6	0	0
1B	6	24	1	8	0	10	0	3
3A	3	2	0	1	0	3	0	0
Unknown[a]	2	36	11	56	23	12	3	14
Outbreak cases[b]								
Confirmed	NA	13	NA	8	NA	4	NA	3
Probable	NA	22	NA	12	NA	2	NA	7

NA: not applicable.

[a] Typing is still ongoing on some of the patients for whom the viral genotype is currently unknown.

[b] The number of outbreak cases is a subset of the total number of hepatitis A patients in each country.

Outbreak investigation in Denmark

HAV disease is notifiable in Denmark: physicians report patients directly to Statens Serum Institut (SSI). Diagnostic testing is performed at local laboratories and SSI using serology. Virus typing is carried out only at the SSI Microbiological Diagnostics and Virology Department, which receives IgM-positive diagnostic samples for confirmatory identification of virus RNA by PCR and further characterisation by genotyping and sequencing. Viral RNA is extracted, typed and sequenced at the VP1 region using a protocol supplied by H. Norder (personal communication, 21 December 2006), including some previously published primer sequences [2].

As of 17 April 2013, a total of 35 cases, including 13 confirmed, and two secondary cases have been identified in Denmark: 21 were female and the median age was 22 years (range: 4–66 years). Date of symptom onset ranged from 1 October 2012 to 27 March 2013 (Figure). Two families with two and three cases respectively, as well as a group of four friends exposed at the same time, were identified.

The HAV strain with GenBank accession number KC876797 (sequence 1) was identified in 10 cases. The HAV strain with GenBank accession numbers KC876798 and KC876799 (sequence 2), which differs by 1.7% over 847 base pairs from sequence 1, was identified in three of the four friends.

On 4 March 2013, the Netherlands and France reported via the EPIS-FWD and HAV laboratory network that sequence 1 resembles HAV sequences from hepatitis A cases returning from Egypt. Sequence 1 also showed 98.7% identity with HAV sequences from a Canadian outbreak associated with pomegranate seeds imported from Egypt in 2012 (Dr Anton Andonov, personal communication, 19 March 2013). Furthermore, the outbreak strains are closely related to the strain presently seen in European tourists returning from Egypt [1]. Sequence 1 also shows 99% homology with GenBank accession number HQ401265 from Spain in 2010 and 98% homology with EF190998 from Hungary in 2006.

Epidemiological investigation

A total of 11 initial cases were interviewed using a trawling questionnaire to identify possible common exposures, such as common events or consumption of foods implicated in other food-borne hepatitis A outbreaks (e.g. shellfish, semi-dried tomatoes, dried fruit, berries and edamame beans). Based on the information obtained and the temporal distribution of cases, a case–control study was set up to test the hypothesis of an item on a list of long shelf-life food items being the source of infection.

The case–control study included 25 probable and confirmed cases and 50 controls and was carried out with questionnaires in telephone interviews from 6 to 13 March 2013. Secondary cases were not included. Controls were identified using the Danish population

registry, matched 1:2 by age, sex and municipality. Controls with a history of hepatitis A infection or vaccination were excluded at the interview phase. Cases were asked about exposures in the six weeks before onset of symptoms and controls six weeks before the interview. One case was later excluded from the analysis, as the person was found to have HAV genotype IA.

Of the 24 cases, 18 had eaten frozen berries used in freshly prepared smoothies (matched odds ratio (MOR): 12.5; 95% CI: 2.8–55) and 20 had eaten frozen strawberries (MOR: 15.8; 95% CI: 3.6–69). Statistically significant associations were found univariably with seven food items: frozen strawberries, frozen raspberries, frozen blueberries, other frozen berries, dates, figs and sun-dried tomatoes eaten in a dish other than salad or a sandwich (Table 2). Only six cases reported consuming sun-dried tomatoes, dates or figs. For the last two food items, a biased association may have arisen from the fact that the exposure period of cases – but not of controls – in a few instances included Christmas, at which dried dates and figs are traditionally eaten in Denmark. No specific supermarket chain was associated with illness of cases.

The results were communicated directly to the Danish Food and Veterinary Administration and to the public through a news item on the SSI website. The international community was informed through the European Early Warning and Response System (EWRS) on 14 March 2013. Concurrently, the Danish Veterinary and Food Administration recommended boiling all frozen berries for at least one minute before consumption.

Outbreak investigation in Finland, Norway and Sweden

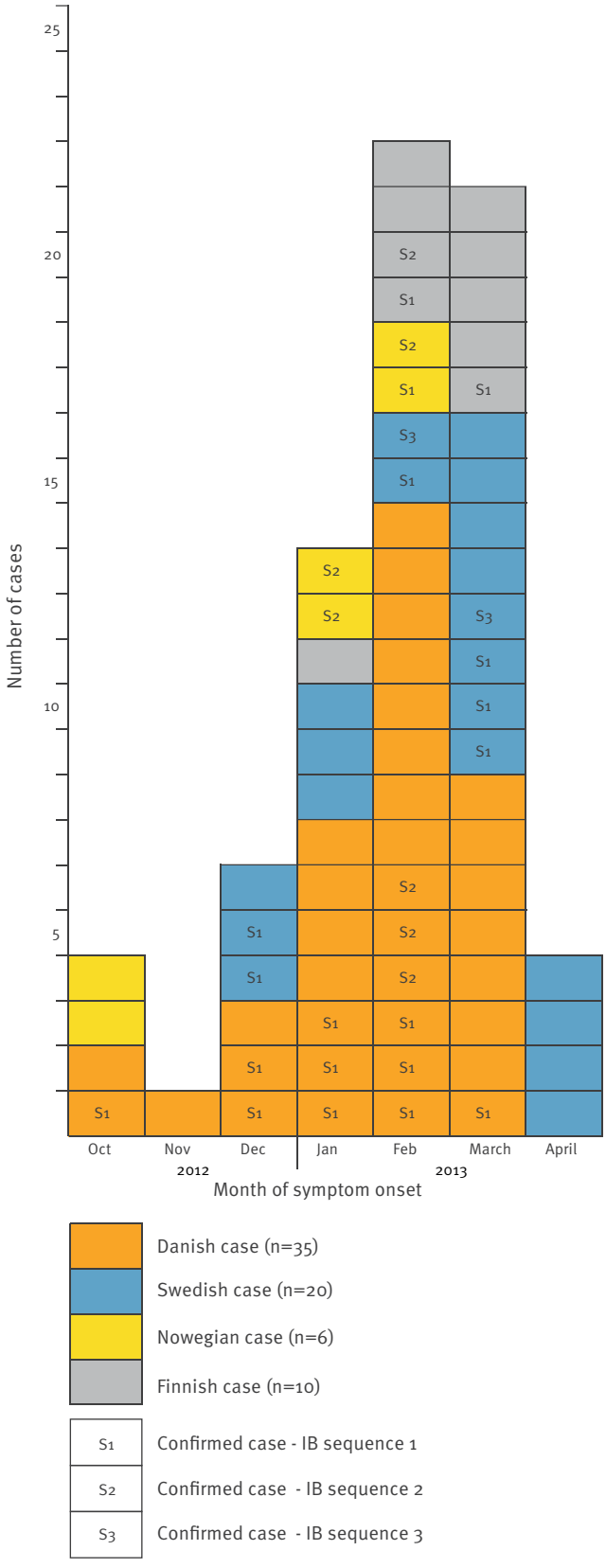
As of 17 April 2013, 36 cases, of whom 15 were confirmed, have been identified in Finland, Norway and Sweden, giving a total of 71 cases in the four countries (Table 1). Finland and Norway have reported confirmed cases with sequence 1 and 2. In Sweden, two of the eight confirmed cases have an HAV IB sequence with 2% difference to sequence 1 and 1% difference to sequence 2 (called sequence 3).

The overall median age for cases and the median age for confirmed cases is 25 years (range: 3–78 years); 43 cases are female. In Norway and Sweden (but not Finland), more women are affected than men. The distribution of cases by month and HAV sequence type is shown in Figure 1. As of 17 April 2013, Sweden is the only country with cases with symptom onset in April. An increased number of travel-related hepatitis A patients in the same time period (Table 1) may be explained in part by patients infected in Egypt [1].

Outbreak investigations are ongoing in Sweden, Finland and Norway. In Sweden, the Danish case questionnaire was sent to all cases. Of 12 cases, who answered the questionnaire as of 15 April, nine reported eating frozen berries at the possible time of infection. Among the seven confirmed cases who replied to the

Figure

Probable and confirmed hepatitis A cases by month of symptom onset[a], country and sequence type, in Denmark, Sweden[b], Norway and Finland, 1 October 2012–17 April 2013 (n=71)



[a] When date of symptom onset was unknown, date of diagnosis or date of sampling was used.

[b] Cases in Sweden notified from 1 December 2012 to 17 April 2013.

Table 2

Univariable matched analysis of association of hepatitis A and food consumption[a] in Denmark, 6–13 March 2013 (24 cases, 48 controls)

Exposure	Matched odds ratio	95% confidence interval	Number (%) of cases with exposure
Frozen berries in freshly prepared smoothie	12.5	2.8–55	18 (75)
Frozen berries eaten in dessert or ice cream	3.2	1.0–10	8 (33)
Frozen berries eaten in a different way	10.0	1.2–86	5 (21)
Frozen berry type			
Strawberry	15.8	3.6–69	20 (83)
Raspberry	5.6	1.8–17	14 (58)
Blueberry	4.3	1.3–14	11 (46)
Mixed berries[b]	–	–	11 (46)
Other berries	13.9	1.7–110	7 (29)
Other exposures			
Dates	12.8	1.5–110	6 (25)
Figs	4.0	1.0–16	6 (25)
Sun-dried tomatoes[c]	10.0	1.2–86	6 (25)

[a] Only statistically significantly associated food items are shown.
[b] No controls ate mixed berries.
[c] Eaten in dishes other than salads and sandwiches.

questionnaire, five reported having eaten frozen berries. Finland and Norway conducted trawling interviews with all patients with HAV infection. In Finland, seven cases had eaten frozen berries at the possible time of infection. All three confirmed cases in Finland had all eaten frozen strawberries; two had also eaten other types of berries (raspberries, blueberries and mixed berries). In Norway, all six cases reported having eaten frozen berries at the possible time of infection and all four confirmed cases reported having eaten frozen strawberries.

From 18 to 21 March 2013, Norway conducted a matched case–control study including 10 patients and 25 controls. Cases were patients with HAV infection or persons epidemiologically linked to a confirmed HAV case, and having similar symptoms with no travel history in the two months before symptom onset, assumed primary infection and symptom onset from 1 November 2012 to 21 February 2013. Controls were selected from the Norwegian population register and matched by age, sex and municipality. No food items were found to be statistically significantly associated with illness; however, this study suffered from limited power due to low case numbers. Eating frozen berries had a MOR of 2.7 (95% CI: 0.5–16); six of the cases reported having eaten frozen berries. MORs were also increased for blackberries (MOR: 8.5; 95% CI: 0.9–78), frozen strawberries (MOR: 7.4; 95% CI: 0.8–64) and frozen raspberries (MOR: 3.1; 95% CI: 0.5–18). Typing later showed that only four of the 11 'cases' in the case–control study met the outbreak case definition. This may explain the lack of a significant association with frozen berries.

Trace-back investigations and laboratory testing of food samples

Currently, trace-back investigations are being performed with the aim of identifying specific types or brands of frozen berries consumed by cases. Trace-back analysis is performed based on the information on the berries that were present in the freezers of the cases' homes and in Denmark, use is also being made of purchase histories from supermarket till receipts. To date, laboratory testing of berries from the homes of seven confirmed cases from Denmark, Sweden and Norway has been negative for HAV. Results are still pending for berry samples from two confirmed cases in Finland.

Public health action taken

As of 11 April, food authorities in Denmark, Finland and Sweden have issued a recommendation to heat treat all frozen berries or berries of non-domestic origin before consumption. On 12 April, the Norwegian Food Authorities and the Norwegian Institute of Public Health informed the public that the risk of contracting HAV through frozen imported berries can be reduced by boiling the berries before consumption. The European Centre for Disease Prevention and Control (ECDC) published a rapid outbreak assessment on 16 April 2013, informing other European countries as well as the European Commission about the outbreak [3].

Discussion

This outbreak is the first Nordic food-borne outbreak of HAV infection. The results of the Danish case–control study clearly indicated frozen berries (particularly

strawberries) as the likely vehicle of the outbreak. Frozen berries have previously been identified as vehicles in outbreaks of HAV infection [4-6]. The epidemiological investigations in the three other Nordic countries, primarily case interviews, further support this notion. Sweden and Finland are considering conducting case–controls studies, to further test this main hypothesis.

As the available evidence has established consumption of frozen berries as the likely vehicle of the infections, the ongoing investigation is now mainly aimed at identifying the specific type and brand (or types and brands) of berries responsible for the outbreak. Comparison of information for trace-back on berries collected at cases' homes, as well as information obtained through purchase history from all four countries, is ongoing and the food authorities in the four countries are sharing information.

Three sequences of HAV genotype 1B with a maximum difference of 2% – corresponding to a difference of a few nucleotides – are implicated in the outbreak. One possible explanation for this would be that the berries are contaminated with several closely related strains. While outbreaks of HAV infection are often caused by a single viral genotype and sequence [7], food-borne outbreaks due to multiple HAV strains have been described previously [8,9] as has outbreaks of HAV infection due to food imported from HAV- endemic countries [10,11].

Given the long shelf life of frozen products, the long incubation time of HAV of 28–30 days (range: 15–50 days) [12] and the potential delay in notifications, more cases will probably still be notified in the four countries. The four national public health institutes and food authorities are therefore collaborating closely in order to confirm the source of the outbreak and stop further transmission. This is done in collaboration with ECDC and European Food Safety Authority (EFSA).

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Authors' contributions

SGL contributed to the study design collection and analysis of data and drafted the manuscript as the lead writer. BS contributed to the study design, collection and analysis of data and was lead in conducting the Danish case–control study. SEM and HTV contributed to the laboratory sequencing and analysis. AS and LV were in charge of the epidemiological investigations including the case–control study in Norway. KSJ were in charge of the laboratory typing and analysis in Norway. RRF was in charge of the epidemiological investigations in Finland. MK was in charge of the laboratory typing and analysis in Finland. ML and LE were in charge of the epidemiological investigations in Sweden. LS was in charge of the laboratory typing and analysis in Sweden. TKF contributed with interpretation of the virological data. TJ contributed to the trace-back. KM and SE contributed to the design of the Danish case–control study and to the epidemiological investigations. SE did the analysis of the Danish case–control study. BS, SEM, AS, LV, RRF, ML, ME, HTV, SE all critically reviewed the first draft of the paper. All authors approved the final version.

Conflict of interest

None declared.

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