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# Joint analysis by the Nordic countries of a hepatitis A outbreak, October 2012 to June 2013: frozen strawberries suspected

Nordic outbreak investigation team<sup>a</sup>

1. The members of the group are listed at the end of the article

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The Nordic countries faced a food-borne outbreak of hepatitis A that started in October 2012 and was ongoing with 103 reported cases as of 27 June 2013. A case-control study in Denmark, Finland, Norway and Sweden, combined with trace-back investigations, has identified frozen strawberries as the likely cause of the outbreak. The origin of the berries is still being investigated.

Hepatitis A seroprevalence is under 10% in Nordic countries [1] where endemicity is very low [2]. In February 2013, Denmark noticed an increase in the number of notified hepatitis A virus (HAV) infections among individuals with no travel history. On 1 March 2013, following an urgent enquiry posted through the European Epidemic Intelligence Information System for food- and waterborne diseases (EPIS-FWD), Finland, Norway and Sweden reported a similar increase and identified cases that were infected with the same 1B genotype and sequence (KC876797) as the Danish cases, as well as cases with closely related sequences [3]. In March 2013, a case-control study conducted in Denmark identified frozen berries, particularly frozen strawberries, as the likely vehicle of the outbreak, but could not exclude other frozen berries [3]. As a result of this finding, the four Nordic countries recommended boiling all frozen berries before consumption [3].

While public health institutes in the four country coordinated their data collection methods to pool the analysis of the country-specific case-control studies to identify the vehicle of the outbreak more precisely, investigators compared the outbreak strains with the HAV network (HAVNET) database [4] to gain information on the probable phylogenetic origin of the outbreak strains, and food agencies analysed product distribution and tested fruit specimens.

## Methods

### Case definitions

A *probable* case was defined as a person living in Denmark, Finland, Norway or Sweden who developed

clinical illness compatible with HAV infection on or after\* 1 October 2012 (1 December 2012 for Sweden) and was positive for IgM antibodies against HAV. We excluded cases who (i) reported travel outside of Western European countries two to six weeks before onset of symptoms, (ii) were living in the same household as a patient with HAV infection typed with a genotype or sequence not belonging to the outbreak and (iii) reported other risk factors for hepatitis A exposure including injection drug use, homelessness or male-to-male sexual contact (the exposures under (iii) were not ascertained in Swedish and Finnish cases).

A *confirmed* case was defined as a probable case infected with HAV genotype 1B with the sequence identified by GenBank number KC876797 (hereafter called sequence 1) or a sequence that differed by no more than 2% from sequence KC876797 [3], and was isolated in at least two of the four affected countries.

A *secondary* case was defined as a probable or confirmed case with symptom onset two to six weeks after close contact with a primary probable or confirmed case.

### Descriptive epidemiology

We described the distribution of cases by age, sex, country of residence, disease status (confirmed, probable, secondary) and HAV sequence.

### Case-control studies

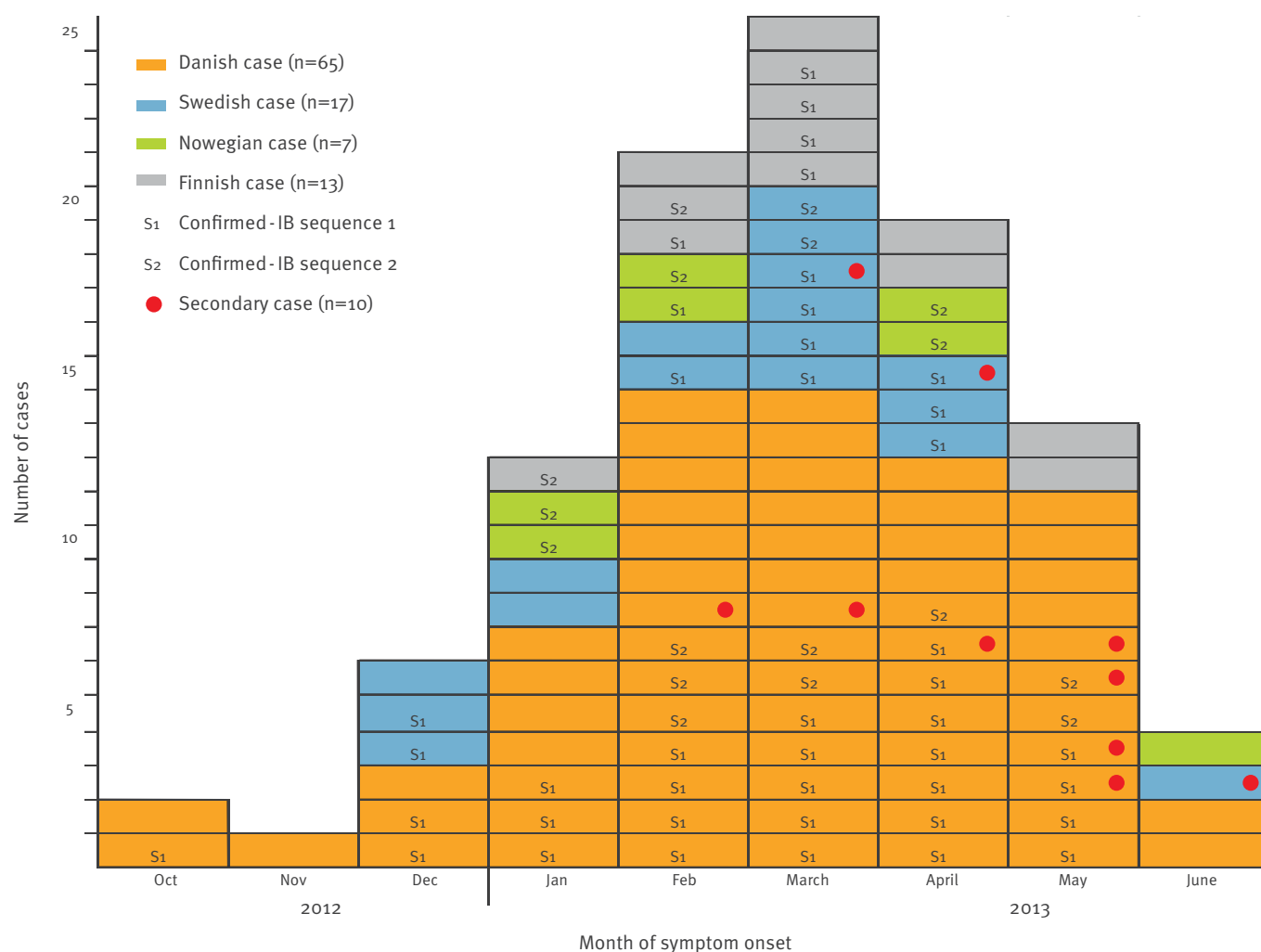
Each country conducted a matched case-control study based on the Danish case-control study protocol, modified according to findings from their own trawling questionnaires. Early and regular communication by email and teleconferences, as well as sharing of study plans and questionnaires, ensured that data could be used for joint analysis.

### Control selection and invitation

Each country randomly selected controls using national population registries, matched on age, sex and place of residence (municipality in Denmark, Norway and

**FIGURE 1**

Distribution of hepatitis A cases over time, Denmark, Finland, Norway and Sweden, October 2012–June 2013 (n=103)



Finland; county in Sweden). Controls were excluded if they were vaccinated against HAV, reported a previous HAV infection, or if they had been travelling for more than two weeks in western Europe or any length of time outside of western Europe in the six weeks prior to recruitment.

#### Data collection

In Denmark, investigators telephoned potential controls until two controls per case had been recruited. In Norway, the same procedure was followed for two to three controls per case. Sweden invited six controls per case by post and Finland invited 30 controls per case, by phone. The latter two countries included all controls who accepted the invitation.

Cases and controls were asked about the consumption of a range of food items, including berries, during the six weeks before onset of illness (cases) or recruitment (controls). Norway asked controls about exposures

during the period corresponding to the exposure period of the cases (January to February 2013).

#### Data analysis

The pooled analysis regrouped primary confirmed cases included in the national studies with at least one matched control (Denmark, Norway, Sweden) or before 24 May (Finland). The strength of the association between HAV infection and consumption of food items present in at least three country questionnaires was estimated using matched odds ratio (mOR) and 95% confidence intervals (CI) using conditional logistic regression. Statistically significant exposures at the  $\alpha=0.05$  level were fitted in a multivariable conditional logistic regression model to adjust for confounding. We stratified the analysis by HAV sequence isolated in cases [3] and compared cases with HAV sequence 1 and cases with HAV sequence 2 in terms of consumption of berries using Fisher's exact test.

TABLE

Frequency of selected exposures among confirmed hepatitis A cases (n=26) and controls (n=56) in a matched analysis by the Nordic countries<sup>a</sup> and an unmatched analysis in Sweden, October 2012–June 2013

Exposure	Multicountry investigation										National investigation	
	Cases			Controls			Crude mOR	95% CI	Adjusted mOR	95% CI	Sweden <sup>b</sup>	
	Total	Exposed	%	Total	Exposed	%					Odds Ratio	95% CI
Frozen strawberries	26	22	84.6	53	19	35.9	8.8	2.5–30.5	11.4	1.9–69.9	24.5	1.9–1179.7
Frozen raspberries	25	16	64.0	54	16	29.6	7.3	2.1–26.0	5.1	0.9–26.4	2.1	0.3–18.4
Berries in smoothie	21	15	71.4	44	13	29.5	8.3	1.8–37.8	-	-	6.7	0.7–69.7
Frozen mixed berries	25	8	32.0	51	5	9.8	10.6	1.3–86.4	11.4	0.9–132.7	0.8	0.0–13.2
Berries in other forms	19	9	47.4	45	8	17.8	2.4	0.5–7.2	-	-	2.7	0.3–23.7
Other frozen berries	21	6	28.6	41	5	12.2	3.6	0.9–15.1	-	-	1.5	0.0–34.5
Frozen blueberries	24	11	45.8	53	15	28.3	2.6	0.9–7.6	-	-	0.7	0.0–9.9
Berries in desert	22	11	50.0	45	16	35.6	3.7	0.9–14.5	-	-	1	0.1–7.3
Nuts	13	10	76.9	34	25	73.5	1.6	0.3–9.4	-	-	0.85	0.1–7.7
Grapes	13	11	84.6	33	27	81.8	1.8	0.3–10.1	-	-	3.5	0.3–188.8

CI: confidence interval; mOR: matched odds ratio.

<sup>a</sup> Denmark, Finland, Norway, Sweden.

<sup>b</sup> Unmatched study, eight cases (confirmed), 18 controls.

The Swedish case–control study (including confirmed cases only) was also analysed individually. As controls could not be recruited for every case, the match was broken (after checking that matched and unmatched ORs were of the same magnitude) and odds ratios (OR) with 95% CI were calculated using logistic regression, adjusting for age.

### Identification of strain origin

Laboratory confirmation of the cases has been described previously [3]. To look for indications of the geographical origin of the outbreak strains, we analysed the phylogeny using the HAVNET database that contains sequence data of viruses from patients from non-endemic countries, many of whom contracted the infection in a foreign country (67% of sequences in HAVNET are from isolates from Dutch patients). A total of 442 nucleotides in the VP1–P2A region and 466 nucleotides in the VP1 region of the genome were analysed separately. The probable origin of the cluster of sequences including the outbreak strains was ascertained as previously described [5].

### Product distribution analysis

In Norway and Denmark sales receipts obtained from the cases were used to ascertain the types and brands of berries purchased before symptom onset. Supermarkets chains assisted in tracing suppliers and countries of origin of the berries.

Danish, Finnish, Norwegian and Swedish, national food authorities collected soft fruit specimens (berries and mango) from confirmed cases' freezers and from shops selling suspected batches. Denmark, Norway and Sweden analysed the specimens applying the same standardised HAV detection method [6] based on reverse transcriptase polymerase chain reaction (RT-PCR). In addition, Denmark and Norway followed a protocol specifically developed for soft fruits [7]. Specimens from Finland were tested in Denmark.

### Results

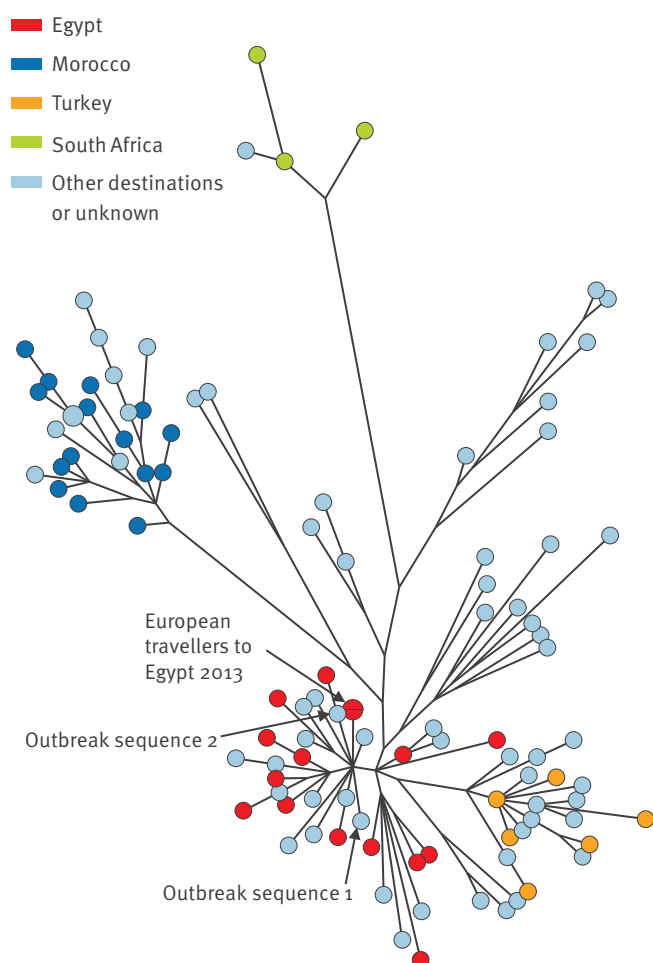
As of 27 June 2013, 103 cases (59 confirmed, 34 probable and 10 secondary) were reported, 66 in Denmark, 17 in Sweden, 13 in Finland and seven in Norway (Figure 1). The age range was 4–76 years (median: 24 years) and 61% were female. No cases with the outbreak sequences were excluded because of other hepatitis A risk factors. The most recent case (as of June 27) was reported in Norway (illness onset 14 June 2013). Sequence 1) was isolated from 42 of 59 confirmed cases, whereas a second sequence with 1.7% difference to sequence 1 over 847 bp (GenBank accession number KC876799, hereafter called sequence 2) was isolated from 17 of 59 cases.

### Case–control studies

After excluding cases that did not have a matched control, the multicountry analysis included 26 confirmed cases (Denmark: 12, Sweden: 6, Norway: 4, Finland:

**FIGURE 2**

Comparison of hepatitis A virus sequences included in the HAVNET database with the two sequences involved in the outbreak in the Nordic countries<sup>a</sup>, October 2012–June 2013



<sup>a</sup> Denmark, Finland, Norway, Sweden.

Maximum parsimony tree of 442 nucleotides of the VP1/2A junction region of hepatitis A virus type IB, using 253 sequences deposited in the Dutch HAVNET database. This database contains 67% Dutch travellers. The two sequences involved in the outbreak in the Nordic countries are indicated by arrows

4) and 56 controls, with one to five controls per case. In the univariable analysis, eating frozen strawberries (mOR 8.8; 95% CI 2.5–30), frozen raspberries (mOR 7.3; 95% CI 2.1–26), berry-containing smoothies (mOR 8.3; 95% CI 1.8–38) and frozen mixed berries (mOR 11; 95% CI 1.3–86, Table) was associated with being a case. Of the 26 confirmed cases, 22 reported eating frozen strawberries, 16 reported eating frozen raspberries, and eight reported eating frozen mixed berries. When including strawberries, raspberries and mixed berries in a multivariable model, only strawberries remained significantly associated with being a confirmed case

(mOR 11.4; 95% CI 1.9–70, Table). When restricting the analysis to cases with sequence 1, eating strawberries and raspberries were both associated with HAV infection (crude mOR 6.1; 95% CI 1.7–22 and mOR 5.6; 95% CI 1.1–27, respectively). However, only eating strawberries was associated with being a confirmed case in multivariable analysis (mOR 5.8; 95% CI 1.2–27 for strawberries, mOR 5.3; 95% CI 0.77–36 for raspberries). When restricting the analysis to cases with sequence 2, eating frozen raspberries was also associated with being a confirmed case (crude mOR 11; 95% CI 1.3–92). Since all sequence 2 cases consumed frozen strawberries, an mOR for frozen strawberries could not be calculated, but the association was statistically significant. Cases with sequence 1 were as likely to have eaten strawberries and raspberries as cases with sequence 2 (Fisher's exact test:  $p=0.54$  and  $p=0.35$ , respectively).

The Swedish case-control study included eight confirmed cases and 18 controls. Frozen strawberries were the only exposure significantly associated with HAV infection (crude OR: 24; 95% CI 1.9–1,200) (Table), which remained significant after adjusting for age (OR: 82; 95% CI 1.7–3,929).

### Identification of strain origin

The most frequently represented countries of infection included in HAVNET were Egypt ( $n=30$ ), Morocco ( $n=21$ ) and Turkey ( $n=15$ ). All samples were from travellers. A comparison of the two investigated genomic regions indicated that the outbreak strains were associated with strains commonly isolated in travellers infected in Egypt ( $p<0.001$ , Figure 2). Sequence 1 and 2 differed by 1.22% over 1,233 bp and by 1.26% over 397 bp, respectively, from the strain causing a concurrent outbreak in travellers returning from Egypt [4].

### Product investigation analysis

As of 27 June, 54 soft fruit specimens (17 from Denmark, 14 from Finland, 11 from Sweden, 12 from Norway) were tested, 23 of which were strawberries. HAV was not detected in any of the specimens.

Trace-back analysis was ongoing as of 27 June 2013, pointing at strawberries from several countries.

### Public health actions

On 22 May 2013 Public Health and Food agencies in Denmark, Finland and Norway issued statements identifying strawberries as the likely vehicle of the outbreak, but maintained previous recommendations to boil all frozen berries before consumption due to the potential implication of other berries. Sweden restricted the boiling notice to strawberries only. On 30 May 2013, one supermarket chain in Denmark, Norway and Sweden voluntarily recalled frozen strawberries from Egypt and Morocco packed in Belgium [8,9].



## Discussion

After pooling data from the four affected countries, consumption of frozen strawberries, frozen raspberries and mixed frozen berries (which can contain both strawberries and raspberries) were significantly associated with being a confirmed case. Although the pooled analysis was restricted to earlier confirmed cases, this is unlikely to have introduced bias since there was no reason to believe later cases were different from earlier ones. Hepatitis A outbreaks have been previously linked to both strawberries [10,11] and raspberries [12,13], and frozen mixed berries are suspected in two hepatitis A outbreaks that were ongoing as of 27 June 2013 in Italy and the United States (US) [14,15]. Frozen strawberries were most strongly associated with being a confirmed case and were the product most commonly eaten by cases. Additional elements pointed to the strawberries as the vehicle of the outbreak: Firstly, strawberries were most strongly associated with being a case in the Swedish national case-control study as well as national case-control studies in Denmark and Norway [3]. Secondly, strawberries were the only exposure significantly associated with being a confirmed case in the multicountry multivariable analysis. Thirdly, the preliminary food trace-back investigations pointed towards strawberries. Finally, when restricting the analysis to individuals who did not consume raspberries, the association between being a confirmed case and frozen strawberries remained significant.

The strength of association between raspberries and being a confirmed case in the multicountry analysis was weaker than for strawberries. In addition, raspberries were not associated with illness in the Swedish study. Restricting the analysis to people who had not eaten strawberries was difficult because only four cases did not eat strawberries. Trace-back analysis found no evidence to implicate raspberries, but based on the epidemiological analyses, we cannot completely exclude that raspberries or frozen mixed berries may have played a role in the outbreak.

The outbreak included two distinct (less than 2% different) HAV sequences that were found in the four countries during the outbreak period. There was no evidence that one outbreak strain was more strongly associated with one vehicle than the other. The HAV mutation rate is low [16], suggesting the two sequences identified in the outbreak represented distinct strains rather than a sporadic mutation. Such multi-strain food-borne hepatitis A outbreaks have been reported previously [17,18]. At this stage we do not know where in the production chain or in which country the contamination occurred. While the comparison of strains in the HAVNET database indicated an association with strains from travellers infected in Egypt, we cannot exclude that these strains also circulate in other countries. As HAV is not genotyped routinely, the known genetic diversity is biased towards more densely sampled regions. Finally, the trace-back investigation has not yet pointed to a single country. The origin of the

contaminated strawberries or the point of contamination can therefore not be identified at this moment.

This outbreak occurred in the context of several hepatitis A outbreaks affecting Europe and EU residents [4,14,19] as well as another genotype 1B outbreak in the US related to frozen mixed berries [15]. The US and Nordic HAV strains are both genotype 1B and originate from the same geographic region but there is no evidence so far they are related [15].

HAV has as yet not been detected in the tested berries. Possible reasons could be that the HAV concentration in the samples collected may have been below the detection limit, or that other berries from the same batch contained HAV.

In conclusion, during this outbreak, combined evidence from case-control studies and the food trace-back contributed to implicate frozen strawberries as the source of the outbreak, leading one supermarket chain voluntary recall this product. Investigations of the source will continue in order to identify the producer and batch and to test berries in the laboratory. In view of the long incubation period of hepatitis A [20] and of notification delay, more cases can be expected to occur for at least another few months, and possibly even later, despite interventions, as frozen berries can be stored in freezers for up to two years.

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## Conflict of interest

None declared.

## Authors' contributions

ME contributed to the study design, collection and analysis of data for the joint case-control study, led the Swedish case-control study and drafted the manuscript as the lead writer. SGL contributed to the study design and was in charge of the joint descriptive epidemiology. BS contributed to the study design and led the Danish case-control study. SEM and HTV contributed to the laboratory sequencing and analysis in Denmark. TKF contributed to interpretation of the virological data in Denmark. TJ was in charge of the trace-back in Denmark and drafted the trace-back section of the manuscript. KM and SE contributed to the design of the Danish case-control study and to the epidemiological investigations. AS contributed to the study design. AS and LV were in charge of the epidemiological investigations including the case-control study in Norway. LJ was in charge of the trace-back investigation in Norway, with assistance from ML and TS. KSJ was 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. HL was in charge of the trace-back in Finland. ML were in charge of the epidemiological investigations in Sweden. LS was in charge of the laboratory typing and analysis in Sweden. MS was in charge of the trace back in Sweden. LV, HV and MK were in charge of

the phylogenetic analysis. SGL, KM, BS, TKF, TJ, AS, LV, LJ, RRF, HL, ST, ML, LS, AW, MS, LV, MK reviewed the first draft of the paper. All authors approved the final version.

## \*Authors' correction:

On request of the authors, the probable case definition was corrected from "after 1 October 2012" to "on or after 1 October 2012". This change was made on 4 July, 2015.

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