This report describes an outbreak investigation starting with two closely related suspected food-borne clusters of Dutch hepatitis A cases, nine primary cases in total, with an unknown source in the Netherlands. The hepatitis A virus (HAV) genotype IA sequences of both clusters were highly similar (459/460 nt) and were not reported earlier. Food questionnaires and a case–control study revealed an association with consumption of mussels. Analysis of mussel supply chains identified the most likely production area. International enquiries led to identification of a cluster of patients near this production area with identical HAV sequences with onsets predating the first Dutch cluster of cases. The most likely source for this cluster was a case who returned from an endemic area in Central America, and a subsequent household cluster from which treated domestic sewage was discharged into the suspected mussel production area. Notably, mussels from this area were also consumed by a separate case in the United Kingdom sharing an identical strain with the second Dutch cluster. In conclusion, a small number of patients in a non-endemic area led to geographically dispersed hepatitis A outbreaks with food as vehicle. This link would have gone unnoticed without sequence analyses and international collaboration.

Introduction

Hepatitis A virus (HAV) is a faecal-oral transmitted pathogen causing acute self-limiting hepatitis. Risk factors for infection include exposure to infected persons, contact with faecally contaminated surfaces, food or water. The incidence of hepatitis A in industrialised countries has decreased due to improved sanitary and living conditions. The decreasing immunity in younger age groups has led to increasing age and severity of first infection [1]. Hepatitis A could therefore re-emerge in regions such as North America or western Europe, where it is not endemic, affecting mostly adults, with more severe course of infection [1]. In the Netherlands the incidence rate for hepatitis A in 2010 was 1.3 cases per 100,000 population [2]. Hepatitis A is a notifiable disease in the Netherlands. Cases, including routine demographic and epidemiological data, are reported according to standardised national criteria [3] by regional public health services using Osiris, a national electronic registration system for infectious diseases hosted by the National Institute for Public Health and the Environment (RIVM). Surveillance is intensified for cases with no travel history to endemic countries and an unknown source of infection. For these cases, sequences are actively collected and additional hypothesis-generating (trawler) questionnaires are routinely administered, aiming to identify a potential common (food-borne) source of infection [2-4].

Recently, food-borne transmission of HAV has been implicated in several multinational outbreaks or involving multiple states in the United States (US), occurring in rapid succession in 2013 and 2014 [5-8]. Such diffuse outbreaks that are characterised by cases that are geographically or temporally dispersed benefit from combining epidemiological data with viral sequence data. Finding the source is challenging due to the long incubation period of two to six weeks [9]. Recall can be assisted by the use of structured questionnaires including known risk factors or risk products [3,4]. Absolute confirmation of a food source is rare, in part due to difficulties with detection of the viral RNA in
food or the absence of leftovers, while international traceback of food can be complex [8]. The high numbers of notifications in the Rapid Alert System for Food and Feed (RASFF) Portal website related to the presence of norovirus or hepatitis A virus in food in 2013 and 2014 compared with previous years indicate an increased awareness of the importance of food-borne viruses.

In this study we describe a thorough outbreak investigation by multiple institutes in two European Union (EU) Member States that yielded evidence for a common source of infection for nine Dutch hepatitis A patients and one patient in the United Kingdom (UK). The investigation was initiated after identification of a cluster consisting of four seemingly unrelated Dutch hepatitis A cases with an identical but unique HAV sequence (genotype IA) in August 2012. In November 2012, another cluster of seemingly unrelated hepatitis A cases, again with an identical HAV IA sequence, was identified. The HAV IA sequence of the second cluster was closely related (459/460 nt similarity) to the strain that caused the first cluster. The source was assumed to be food-borne, as hepatitis A is not endemic in the Netherlands, there was no known direct contact between the cases, and detailed case histories were negative for other common risk factors of hepatitis A. The onset of illness in each cluster occurred within a short period of two weeks with no recognised cases in the two to three month period in between these clusters. The final linkage between the Dutch and UK cases could only be conclusively made after combining traceback results of the suspected food items for each of the clusters and information provided by international partners.

**Methods**

**Active case finding by laboratory surveillance network**

For cases with no travel history to endemic countries and an unknown source, additional routine hypothesis-generating questionnaires were administered. This questionnaire addresses a broad list of over 70 food products, 15 occupations and six health conditions, and is available upon request [3,4]. IgM positive sera or faecal samples from ca 70% of confirmed hepatitis A cases are sent by diagnostic laboratories to RIVM.
Outcomes of traceback activities for potentially implicated mussels, food-borne hepatitis A clusters, the Netherlands, 2012

**Figure 2**

<table>
<thead>
<tr>
<th>Supplier 1</th>
<th>Summer cluster</th>
<th>Autumn cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 batches</td>
<td>4 primary cases</td>
<td>5 primary cases</td>
</tr>
<tr>
<td>6 areas</td>
<td>Onset of disease</td>
<td>Onset of disease</td>
</tr>
<tr>
<td>2 countries</td>
<td>Aug 2012</td>
<td>Nov 2012</td>
</tr>
<tr>
<td>Seq: RIVM-HAV12–070</td>
<td>Seq: RIVM-HAV12–124</td>
<td></td>
</tr>
</tbody>
</table>

**Traceback period**
- **Supermarket 1**
  - 3 patients
- **Supermarket 2**
  - 2 patients
  - 15 Sep–30 Oct 2012

**Supplier 1**
- 30 batches
- 6 areas
- 2 countries

**Supplier 2**
- 96 batches
- 8 areas
- 3 countries

**Supplier 3**
- 122 batches
- 14 areas
- 4 countries

Mussels from Area 1 and Area 2 only were common to both summer and autumn HAV clusters

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for genotyping [3]. Subsequently, a 460 nt fragment of the VP1/2A region [10,11] is compared with sequences recorded in an international HAV sequence database of HAVNET and GenBank. HAVNET is a global network of scientists working in hepatitis A reference laboratories. The network shares molecular and epidemiological data on hepatitis A.

**Case definition** Hepatitis A cases in the Netherlands registered between 1 August 2012 and 18 February 2013 with an unknown source in the Netherlands were classified as follows: (i) summer cluster cases were cases infected by HAV strain RIVM-HAV12–070; (ii) autumn cluster cases were cases infected by HAV strain RIVM-HAV12–124; (iii) unrelated cases were unvaccinated persons infected by a HAV strain dissimilar from RIVM-HAV12–070 and RIVM-HAV12–124 with difference of at least 7/460 nt; (iv) Other cases: cases for which no HAV sequence is available (not included in the analysis).

**Statistical analyses of risk factors from questionnaire data**

The food items addressed in the hypothesis-generating questionnaire data were further investigated using data on consumption patterns in healthy people unrelated to any outbreak [12]. For this, data were collected in the flexible Dutch population control group over a four-year period from 2008 to 2011 (n = 1,402).

**Traceback investigation**

Fresh mussel suppliers to specific supermarket chains, as derived from questionnaire data, were identified as well as the harvesting areas of mussels potentially involved, and the shipping dates to the Netherlands, when applicable. Information on shipments was requested from the Dutch Fish Product Board for the period six weeks before disease onset in the first patient until two weeks before disease onset in the last patient for both clusters. The registration documents of the mussel consignments revealed the number of batches, harvesting areas and countries for each supplier during these two periods.

**International enquiry**

Enquiries for any information on possible matches with the unique patient sequences were sent out to international contact points within the HAV reference laboratory network (HAVNET sent out enquiries on 15 January 2013 and 7 June 2013) and the international network Epidemic Intelligence Information System of Food and Waterborne Diseases (EPIS-FWD, 15 January 2013 and 13 December 2013) of the European Centre for Disease Prevention and Control (ECDC). Additional enquiries for any information on possible matches with the unique patient sequences or on any information on HAV monitoring in shellfish were sent out to national contact points (3 June 2013 and 31 October 2013) and shellfish reference laboratories (26 February 2013) in countries identified to be possibly involved. Furthermore, the RASFF portal database was checked for HAV notifications in 2012.

**Results**

**Descriptive epidemiology**

From 1 August 2012 to 18 February 2013, 89 hepatitis A cases were reported to the RIVM (Figure 1). Of these, 24 cases acquired their infection from an unknown source in the Netherlands as no travel history had been reported. The remaining 65 cases were reported with travel history to endemic regions, or with an epidemiological link to a confirmed case with recent travel history to an endemic region. In the same period, the RIVM received 79 samples from notified cases for typing. In total, 62 strains could be successfully typed, and 15 of these were from hepatitis A cases with an unknown infection source within the Netherlands. These 15 cases diverged into the summer and autumn clusters, each with an identical sequence which was further investigated in this study, and six unrelated cases with dissimilar sequences (i.e. unrelated cases).

The summer cluster of cases infected with RIVM-HAV12–070 consisted of three male and one female primary cases, with ages ranging from under 10 years to under 70 years of age, and onset of illness within 10 days in August 2012. One primary case needed hospitalisation. In addition, two secondary cases were reported, both relatives of primary cases.
**Figure 3**
Timeline of disease onset with cases and relevant harvesting periods, food-borne hepatitis A clusters, the Netherlands, 2012

**Table 1**
Food items consumed six weeks before the onset of disease by confirmed hepatitis A cluster (n=9) and unrelated (n=6) cases, the Netherlands, 2012

<table>
<thead>
<tr>
<th>Food item</th>
<th>OR (95% CI)</th>
<th>P value</th>
<th>Unrelated cases</th>
<th>Cluster cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Food questionnaire</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mussels</td>
<td>1.00 (0.0–999)</td>
<td>p = 0.99</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Spring onions, raw</td>
<td>1.00 (0.0–999)</td>
<td>p = 0.99</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Onions, raw</td>
<td>1.00 (0.0–999)</td>
<td>p = 0.99</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Prawns</td>
<td>8.0 (0.3–256)</td>
<td>p = 0.24</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Iceberg lettuce</td>
<td>8.0 (0.3–256)</td>
<td>p = 0.24</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Leafy green lettuce</td>
<td>7.0 (0.2–226)</td>
<td>p = 0.27</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Osiris notification</td>
<td>1.00 (0.0–999)</td>
<td>p = 0.99</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Combined food questionnaire and Osiris</td>
<td>1.00 (0.0–999)</td>
<td>p = 0.99</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

CI: confidence interval; OR: odds ratio.
Osiris is the electronic registration system for infectious diseases hosted by the Dutch National Institute for Public Health and the Environment.

*a* Leafy lettuce data missing for one of the cluster cases.
The autumn cluster cases were infected with RIVM-HAV12–124 and consisted of two male and three female primary cases, with ages ranging from over 40 years to under 70 years of age, and onset of illness within 12 days in November 2012.

Six unrelated cases (four males and two females) with ages ranging between 20 and 60 years-old were infected by HAV strains dissimilar from RIVM-HAV12–070 and RIVM-HAV12–124. It is assumed that they are primary cases, since their sequences were not seen in previous cases. Dates of onset of disease were between early August and late December 2012.

Phylogenetics and international enquiry

The RIVM-HAV12–070 and -124 were both unique in the international HAV sequence database of HAVNET and the origin of the virus could not be mapped to a specific geographic region [3, and data not shown]. Only four EU Member States out of 38 EPIS-FWD contact points responded to international enquiries reporting non-related cases or not to have matching sequences. Furthermore, three RASFF notifications on HAV were identified in 2012. One notification was related to mussels from New Zealand that had only been distributed to Italy.

Two others were related to the same batch of frozen strawberry cubes from China. Traceback activities to find a possible link to the cluster cases indicated that strawberries from this batch had been on the Dutch market partly in the same period of the incubation period of the patients. The strawberries had been used as decoration for consumer-ready packed ice cream sold by one of the specific supermarket chains identified by two of the six cases. Unfortunately, no sequence information for the HAV strain detected on the frozen sliced strawberries was available.

Risk factors from questionnaire data

From the questionnaire data available, mussels, raw spring onions, raw onions, prawns, iceberg lettuce and leafy green lettuce were recalled by at least eight of the nine cases as having being consumed within the incubation period. Univariate logistic regression analyses showed clear association for mussels, prawns and iceberg lettuce (Table 1). All of the cluster cases had consumed pre-packed fresh mussels (all of which were heat-treated in the home before consumption), raw spring onions and raw onions, compared with none of the unrelated cases, not allowing statistical calculations.

In the period before the onset of illness, hepatitis A cluster cases were found to have consumed mussels significantly more often than individuals in the flexible control group (Table 2). In the two to six weeks before the onset of illness in August and November 2012, all nine hepatitis A cluster cases consumed mussels, compared with 32 of 125 (26%) and 13 of 143 (9%) for control groups during the 4 weeks before August and November 2012, respectively. Prawns were also consumed more often by cluster cases i.e. 8/9 (89%) compared with 51/126 (40%) and 44/145 (30%) for control groups during the 4 weeks before August and November 2012, respectively. Lettuce, however, was consumed by over 80% of the control group throughout all seasons (Table 2). Lettuce seemed therefore less likely to be the source of infection for these cases. Although other food items could not be excluded here, the known association of shellfish with HAV outbreaks informed the initiation of mussel traceback investigations.

Traceback investigation

All cluster cases had bought mussels at local supermarkets, but no batch numbers or original package labels were available for traceback activities. Therefore the traceback was targeted at suppliers within the incubation periods of the patients. Within this period the suppliers had sourced from a Dutch growing area and from other countries within the EU. No plausible sewage pollution-related link between any of the cluster cases and the Dutch growing area was found, nor could an epidemiological linkage be identified within the Netherlands due to uniqueness of the strains despite intensive surveillance. The registration documents from the mussel consignments to the Netherlands revealed the number of batches, harvesting areas and countries for each supplier in these two periods (Figure 2). All the identified areas were in countries non-endemic for HAV. Shellfish reference laboratories in these countries enquired for information reported that no routine surveillance for HAV in shellfish was performed. Archival mussel samples from July to November 2012 (n = 28), randomly collected by the Dutch Food and Consumer

<table>
<thead>
<tr>
<th>Month of report</th>
<th>Mussels n/N %a</th>
<th>Prawns n/N %a</th>
<th>Lettuce n/N %a</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>2/9 (22)</td>
<td>4/9 (44)</td>
<td>8/8 (100)</td>
</tr>
<tr>
<td>February</td>
<td>12/82 (15)</td>
<td>22/81 (28)</td>
<td>71/86 (83)</td>
</tr>
<tr>
<td>March</td>
<td>14/129 (11)</td>
<td>36/132 (27)</td>
<td>116/139 (83)</td>
</tr>
<tr>
<td>April</td>
<td>17/108 (16)</td>
<td>36/113 (32)</td>
<td>100/116 (86)</td>
</tr>
<tr>
<td>May</td>
<td>0/11 (0)</td>
<td>6/11 (55)</td>
<td>10/11 (91)</td>
</tr>
<tr>
<td>June</td>
<td>12/201 (6)</td>
<td>74/202 (37)</td>
<td>178/208 (86)</td>
</tr>
<tr>
<td>July</td>
<td>13/156 (8)</td>
<td>37/162 (23)</td>
<td>154/167 (92)</td>
</tr>
<tr>
<td>Augustb</td>
<td>32/125 (26)</td>
<td>51/126 (40)</td>
<td>111/121 (92)</td>
</tr>
<tr>
<td>September</td>
<td>5/23 (22)</td>
<td>14/23 (61)</td>
<td>19/23 (83)</td>
</tr>
<tr>
<td>October</td>
<td>29/184 (16)</td>
<td>59/183 (32)</td>
<td>165/189 (88)</td>
</tr>
<tr>
<td>Novemberb</td>
<td>13/143 (9)</td>
<td>44/145 (30)</td>
<td>121/151 (80)</td>
</tr>
<tr>
<td>December</td>
<td>14/138 (10)</td>
<td>43/139 (30)</td>
<td>108/131 (82)</td>
</tr>
</tbody>
</table>

a Data missing for mussels (n=93), prawns (n=76) and lettuce (n=52).
b Data reported for August and November (i.e. consumption during past four weeks) to be compared with data reported for cases during their incubation period.
Table 3
Linking mussels consumed by patients to a specific harvester, food-borne hepatitis A clusters, the Netherlands, 2012

<table>
<thead>
<tr>
<th>Dutch supplier</th>
<th>Harvesting area</th>
<th>Harvesting company</th>
<th>Date of shipments in 2012</th>
<th>Number of batches</th>
<th>Number of associated cases</th>
<th>Location of associated cases</th>
<th>Month of onset of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Area 1</td>
<td>N.a.</td>
<td>17 Jul–9 Aug</td>
<td>2</td>
<td>3</td>
<td>Netherlands</td>
<td>August 2012</td>
</tr>
<tr>
<td>2</td>
<td>Area 1</td>
<td>N.a.</td>
<td>18 Jul–16 Aug</td>
<td>13</td>
<td>2</td>
<td>Netherlands</td>
<td>August 2012</td>
</tr>
<tr>
<td>3</td>
<td>Area 1</td>
<td>N.a.</td>
<td>25 Sep–29 Oct</td>
<td>17</td>
<td>3</td>
<td>Netherlands</td>
<td>November 2012</td>
</tr>
<tr>
<td>1</td>
<td>Area 2</td>
<td>Harvester 1</td>
<td>2 Jul–20 Jul</td>
<td>20</td>
<td>3*</td>
<td>Netherlands</td>
<td>August 2012</td>
</tr>
<tr>
<td>2</td>
<td>Area 2</td>
<td>Harvester 2</td>
<td>21 Jun–8 Aug</td>
<td>32</td>
<td>2*</td>
<td>Netherlands</td>
<td>August 2012</td>
</tr>
<tr>
<td>3</td>
<td>Area 2</td>
<td>Harvester 1</td>
<td>17 Sep–30 Sep</td>
<td>2</td>
<td>5</td>
<td>Netherlands</td>
<td>November 2012</td>
</tr>
<tr>
<td>4</td>
<td>Area 2</td>
<td>Harvester 1</td>
<td>29 Sep</td>
<td>1</td>
<td>1</td>
<td>United Kingdom</td>
<td>November 2012</td>
</tr>
</tbody>
</table>

N.a.: not applicable.
* One of these patients consumed mussels from either Supplier 1 or 2.

Product Safety Authority for microbiological assessment of bivalves on the Dutch market, tested negative for HAV RNA using the method described in ISO/TS 15216–2 [13].

Combining the traceback results for both clusters revealed that two harvesting areas, Area 1 and Area 2, were common to both summer and autumn clusters (Figure 2). The dates of consignment for both areas were compared with a known date of mussel consumption by one of the cases. As shipments of mussels from Area 1 only began five days after this consumption date, whereas mussel shipments from Area 2 predated this consumption date, the mussels from Area 2 were identified as the most likely source.

International case identification

It was reported that mussels in harvest Area 2 in the UK were all locally produced. Therefore, the Virus Reference Department of Public Health England was asked to check for similarities in their HAV database with the RIVM-HAV12–070 or RIVM-HAV12–124 sequences that could belong to local cases. For this, epidemiological and sequence data from different departments were combined, revealing two patients with identical sequences. The first patient identified, (Figure 3, UK case number 4) was a child of school age with onset of symptoms in early August 2012, infected with RIVM-HAV12–070 (100%), who lived at a distance less than 10 km from Area 2. The child was a household member of two other confirmed HAV cases infected with RIVM-HAV12–070 strain (100%): another child and a middle-aged adult with onset of illness on in early July 2012 and early August 2012, respectively (Figure 3, UK cases number 2 and 3). The household did not report recent history of mussel consumption. Searching the database for earlier local cases revealed a new archival sample that matched 100% with RIVM-HAV12–070 strain. This middle-aged adult (Figure 3, UK case number 1) had a travel history to a hepatitis A-endemic country in Central America while unvaccinated in April 2012. In May, the patient made a short visit to an area near the coast of Area 2, where the patient fell ill and was admitted to the local hospital in early June. Although direct contact between UK cases number 1 and 2 could not be demonstrated, the places of residence of these cases were within a few km of each other during the infectious period. The environmental and water authorities showed that biologically treated sewage from the residences and the hospital was discharged into rivers at a fluvial distances of less than 10 km from the nearest boundary of the designated mussel-harvesting Area 2. All sewage treatment plants were reported to be functioning normally during the relevant periods when contamination might have occurred.

The other reported case (Figure 3, UK case number 5) matching the RIVM-HAV12–124 strain had no links to the identified Area 2, but reported having consumed mussels in a pub in London within the incubation period for the illness. Local food safety officers reported that the consumed mussels were bought by the pub from a UK wholesaler that had received mussels from a Dutch supplier. This Dutch supplier, Supplier 4, had packed mussels in early October 2012 that had been harvested from Area 2 by Harvester 1 (Table 3). Registration documents demonstrated that Dutch Suppliers 1 and 3 had also bought mussels from this harvester. The mussel beds of Harvester 1 were those located closest to the implicated sewage treatment plant inputs of harvest Area 2.

Sequence analyses for UK case 4 showed a strong signal for the RIVM-HAV12–070, but also a weak signal for the subspecies RIVM-HAV12–124 suggestive of a subspecies within this patient which may subsequently have become the dominant strain in the autumn cluster of cases. Further Dutch cases and a UK case in autumn 2012 came after a period of heavy rainfall (23–25 September 2012) in Area 2. Heavy rain would have led to the activation of combined sewage overflow systems and thus the bypass of the sewage treatment works and potential release of untreated sewage into Area 2. This rainfall occurred one week before delivery of mussels to Supplier 4 that were consumed by UK case number 5 and before delivery of mussels to...
Supplier 3 consumed by at least four of the five Dutch patients (Figure 3).

A second request to EPIS-FWD in December 2013 was posed specifically to five countries that might have imported mussels, according the Dutch company’s website, in order to identify additional international related cases. None were reported by Austria, Belgium, Germany, or Switzerland. Previously (January and October 2013) none had been reported by France via HAVNET.

Discussion
This report describes a unique investigation of two closely related food-borne clusters of hepatitis A cases. Investigations following the traceback of the implicated mussels led to international matching of the nine primary and two secondary Dutch cases to three primary and two secondary cases in the UK with identical HAV sequences within the same time period. Consumption of a common source of mussels by Dutch cases and a UK case confirmed our findings. The investigation convincingly points towards the case with a travel history to Central America being the source of the HAV cluster, who then infected a subsequent household cluster, all of whom lived close to the mussel production area. This unique multinational outbreak investigation demonstrates that a small number of patients in a non-endemic area led to geographically dispersed HAV outbreaks via food as a vehicle. Such a link would have gone unnoticed without sequence analyses and the combined forces of diverse institutes.

The number of patients in this investigation was small compared with other HAV outbreaks recently reported [5–8], which limited the available epidemiological data. Notably, the identification of the clusters was not based on an increase in the total number of patients, but was the result of the existing intensive surveillance with molecular typing of HAV in patients in the Netherlands [3]. The rising numbers of reported food-borne HAV outbreaks and RASFFs are likely to promote interest in diagnostic sample typing. This would assist source tracing in outbreak investigation and may result in an improved estimate of the impact of contaminated food on public health.

Bivalve molluscs (e.g. oysters and mussels) have long been associated with viral food-borne disease. Several large outbreaks associated with consumption of HAV contaminated bivalve molluscs have been described [14–16], often traced back to harvesting areas within endemic areas. HAV infections through consumption of bivalve molluscs may be anticipated with global trade from endemic to less endemic HAV areas [17]. The implicated bivalve molluscs in this report were however harvested from a non-endemic area with no prior indication of recent local HAV outbreaks, as occurred in 1997 although the 1997 outbreak had many more cases [18]. Generally, mussels from non-endemic areas are considered to be at low risk for HAV infection, especially as they are usually consumed after domestic heat treatment, like the Dutch cases described here. Domestic cooking procedures usually involve heating by steaming until the shells open, rather than immersion for a defined period in boiling water. Internal temperatures reached during steaming have been shown to be ineffective for complete inactivation of infectious HAV in mussels [19,20], but the outcome depends on initial contamination levels. The degree of cooking to reach an internal temperature of 90 °C for 90 seconds as required by EU legislation for commercial heat treatment [21], which has been shown to reliably inactivate HAV [22], may however render bivalve molluscs unpalatable to consumers when applied in the domestic setting.

Area 2 is a source of mussels imported into the Netherlands, and mussels from this area have been previously tested for norovirus for microbiological assessment of bivalves on the Dutch market. The frequent occurrence of samples testing positive for norovirus indicates potential human faecal contamination, which is consistent with its class B classification (4,600 Escherichia coli per 100 g shellfish flesh in 90% of samples) under EU food safety regulations. (EU Regulation number 854/2004). It is therefore feasible that sewage containing faeces from the sporadic cases of HAV patients may have entered the mussel growing water and led to contamination of parts of the mussel bed. It is interesting to reflect that over 1,000 tonnes of mussels were harvested from this area during the relevant time period, the majority was exported to other European countries following processing and packaging in the Netherlands, and yet only 10 associated HAV cases were identified. This could suggest a low, heterogeneous or temporary contamination, the risk of which may have been reduced through heat treatment. Otherwise, the low number of associated HAV cases could point towards low recovery from laboratory surveillance, as sequence analyses of patient material is not common practice in most countries or is performed for some cases only [5,8] or is not systematically reported. In this study, it was only after more direct questioning regarding the residence of HAV cases and their proximity to mussel beds that samples taken from these particular cases were forwarded for sequencing. The occurrence of the two clusters, with the second cluster potentially associated with heavy rainfall, suggests environmental factors played a part in increasing the risk. The guidelines for the control of viruses in food [23] recommend that after heavy rainfall, and/or after overflow from sewage treatment plants, harvesting of bivalve molluscs should cease for a period, until the water and/or bivalve mollusc quality in the harvesting area has been assessed and has returned to normal background levels for the area. Similarly, official EU guidelines to Regulation 854/2004 require investigation following a pollution or extreme weather event and, if necessary, additional controls to protect public health [24]. Area 2, like other similar areas affected by human faecal pollution, remains vulnerable to
sporadic HAV cases shedding virus within the drainage catchment area. Monitoring of shellfish or effluents of treated sewage for HAV contamination during periods of elevated risk, for example following identification of sporadic or outbreak cases within the catchment, could potentially assist public health protection. This would require real-time exchange of data on hepatitis A cases or virus detection between regional agencies dealing with public health, food safety and water quality, as even sporadic cases can shed a large amounts of virus over a period of time, and these viruses are only partially reduced by water treatment plants, and viruses in bivalve molluscs are bioaccumulated [1].

International sharing of HAV sequences proved helpful in this as well as in other recent outbreak investigations [4-8]. A database such as HAVNET (www.havnet.nl) when used in real time may result in a rapid traceback of the product involved, followed by a recall, if still possible, or other preventive measures to increase food safety. Moreover, finding related cases in other countries could give an insight into the true size of food-borne outbreaks. Access to HAVNET can be requested by e-mailing havnet@rivm.nl.

Acknowledgements

We would like to thank the officers of several regional public health services in the Netherlands and the United Kingdom for gathering epidemiological data, and the officers of the Food and Consumer Product Safety Authority in the Netherlands and Environmental Officers in United Kingdom for traceback activities.

Conflict of interest

None declared.

Authors' contributions

IB and LV coordinated the investigation, and collected, analysed and interpreted the data. IB coordinated the traceback investigation and international contacts concerning food. IB drafted the manuscript. LV coordinated the international contacts with public health institutes. HV and SLN coordinated genotyping part and interpretation of the results. IF and LV worked on the analyses of the epidemiological data. DL and CW collected and interpreted the environmental data. MK participated as advisor in investigation and drafting of the manuscript. All authors participated in editing the manuscript, and read and approved the final manuscript.

References


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