Another possible food-borne outbreak of hepatitis A in the Netherlands indicated by two closely related molecular sequences, July to October 2011

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In November 2011, a cluster of five cases of hepatitis A infection with closely related strains was identified in the Netherlands. England reported possibly related cases. Strains with identical sequences had been involved in previous outbreaks linked to semi-dried tomatoes. Investigation of the Dutch cluster suggested a link with ready-to-eat salads including those containing semi-dried tomatoes. Despite traceback, a source was not identified. Vigilance is needed, and rapid sharing of data may help source tracing.

HAV surveillance in the Netherlands
HAV infections are reportable in the Netherlands and recorded to the National Institute for Public Health and the Environment (RIVM) according to standardised criteria [7]. Sera from confirmed hepatitis A cases, if available, are sent to the RIVM by the laboratories. Viral RNA is extracted from IgM positive sera, and the 460 nt VP1/2A region is sequenced and compared to sequences recorded in the Dutch [8] and an international HAV sequence database of the Food-Borne Viruses in Europe (FBVE) network [9].

Outbreak investigation
Confirmed cases were defined as laboratory-confirmed HAV cases who had no travel history to endemic countries, no male-to-male sexual contacts and was therefore considered as exposed to an unknown source in the Netherlands, who had a date of symptom onset from July to November 2011 and who was infected with the specific genotype IB strain identical to strains that
caused previous outbreaks in Europe and Australia in 2009–10 [1-5], i.e. Hu/Netherlands/RIVM-006/2010, closely related to Genbank accession number FJ687511, or Hu/Netherlands/RIVM-077/2010, closely related to strain FR-2010-LOUR, (GenBank Acc. No. GU646039). Cases related to primary cases and with onset of illness two weeks or more after the primary case were considered secondary cases. A probable case followed the same criteria but without laboratory confirmation.

Cases were contacted by the Dutch municipal health services and were requested to complete a questionnaire. The questionnaire included personal information like date of birth, sex, place of residence, vaccination status, symptoms, date of symptom onset and food history in the period two to six weeks before symptom onset. As recommended by the NVWA, questions on food consumption included a wide range of food products imported from the Middle East because of the similarity of the outbreak strain to strains in travellers from Middle East. The NVWA performed source tracing on the basis of questionnaire information, and tested the indicated food product for presence of hepatitis A RNA using methods described elsewhere [10].

The FBVE network [11] was alerted and asked to share sequences in the international database.

**Description of the cases**

By November, seven confirmed cases and one probable case had been identified in the Netherlands: two women and six men aged between 20 and 75 years. The date of onset of symptoms was between 29 July and 24 October 2011 (Figure). One of the seven confirmed cases was probably a secondary case (date of onset on 24 October 2011) because she was epidemiologically linked to the probable case with date of onset on 20 September 2011. Unfortunately, the serum sample of this probable case did not yield a PCR product, which may be explained by the fact that it was obtained on 4 November, i.e. 45 days after onset of disease. In a study by Tjon et al. sera were HAV RNA-positive for a median period of 42 days after onset of illness [12].

The HAV strain Hu/Netherlands/RIVM-006/2010 was found in the first four confirmed cases and the Hu/Netherlands/RIVM-077/2010 strain was found in the following three confirmed cases (Figure).

Six of seven confirmed cases completed the questionnaire, including the probable secondary confirmed case. All of them indicated the consumption of ready-to-eat (RTE) packaged salads obtained from two supermarket chains, however they did not, or not fully, specify the type of salad they had consumed. On the basis of initial reports from the Dutch municipal health services, 75% of the mentioned RTE packaged salads contained semi-dried tomatoes or dried tomatoes (tomato granulates). Nevertheless, only three of six cases specifically indicated to have consumed dried or semi-dried tomatoes. In November 2011, the NVWA systematically sampled stocks of semi-dried and dried tomatoes to be used as ingredients in RTE salads and present in store houses of the suppliers of the two supermarket chains involved. A total of 114 food samples were tested, but in none of the samples HAV RNA could be detected. It remained unclear whether the tested batches were the same as those consumed by the patients. The NVWA worked in close collaboration with the Food Standards Agency (FSA) in England, but a common link in the supply chain to the Dutch and English supermarket chains could not be identified.

The international alert and the sharing of sequences in the international database resulted in a case reported from Australia who locally acquired an infection with an HAV strain identical to Hu/Netherlands/RIVM-006/2010 in a 311 nt sequence in the VP1/2A genomic region. For this case source tracing is currently ongoing.

**Conclusion and recommendations**

Seven confirmed hepatitis A cases and one probable case were identified in the Netherlands with HAV genotype IB strains identical or closely related to the ones found recently in England and Australia and in previous food-borne outbreaks in the Netherlands, Australia and France. All cases that completed the questionnaire indicated consumption of RTE salads, including those containing semi-dried tomatoes, during their incubation period. However, only three cases specifically indicated to have consumed semi-dried tomatoes. Because of the low number of cases, the long and variable incubation period, the long time between dates of onset of the first and of the last cases, and the complexity of the traceback, no common source could be identified. These were also the reasons for not including controls in our study. This might be considered a limitation,

**Figure**

Confirmed and probable cases of hepatitis A, indicated by two closely related molecular sequences (10-006a and 10-077b), notified in the Netherlands from March to December 2011 (n=8)

![Figure](https://www.eurosurveillance.org)

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since we were not able to find an association to a single food product.

Until the time of publication of this report, no new cases have been reported. Nevertheless, we cannot exclude contaminated food items like RTE salads or semi-dried tomatoes being in circulation. Given the difficulty of identifying and tracing an international common food-borne source, combining information on all infected cases is of utmost importance. We are therefore interested in all cases infected with the HAV IB strains described here. The HAV database of the FBVE network can be used to compare sequences. Information on linked cases and requests for access to the database or assistance in source tracing activities can be addressed by email to fbve@rivm.nl.

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References