Hepatitis A outbreak in Italy, 2013: a matched case–control study

C Montaño-Remacha (cmontanno@gmail.com)[1,2], L Ricotta[1,3], V Alfonsi[1], A Bella[1], M E Tosti[1], A R Ciccaglione[1], R Bruni[1], S Taffon[1], M Equestre[1], M N Losio[4], V Carraro[5], S Franchini[5], B Natter[6], M Augschiller[6], A Foppa[6], C Gualanduzzi[7], E Massimiliani[7], A C Finarelli[7], B M Borrini[7], T Gallo[8], V Cozza[2,9], M Chironna[10], R Prato[9], C Rizzo[1], Central Task Force on Hepatitis[11]

1. Istituto Superiore di Sanità (ISS), Rome, Italy
2. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control, Stockholm, Sweden
3. Dept. of Biomedical and Neuromotor Sciences, University of Bologna, Italy
4. Institute Experimental Zooprophylactic of Lombardy and Emilia Romagna, Italy
5. Prevention Dept., Province of Trento, Italy
6. Prevention Dept., Province of Bolzano, Italy
7. Public Health Service - Directorate General for Health and Social Policy - Emilia-Romagna Region, Italy
8. Prevention Dept. - ASS4 Medio Friuli, Udine, Italy
9. Dept. of Medical and Surgical Sciences, University of Foggia, Italy
10. Dept. of Biomedical Sciences and Human Oncology, University of Bari, Italy
11. The members of the Central Task Force on Hepatitis A are listed at the end of the article

Between January and May 2013 a hepatitis A (HA) incidence increase was detected in Italy, signalling an outbreak. A retrospective matched case–control study was conducted to identify the source of infection. A case was defined as a resident of any of five regions (Apulia, autonomous province of Bolzano, Emilia-Romagna, Friuli–Venezia-Giulia and autonomous province of Trento), who had symptom onset between 1 January and 31 May2013 as well a positive test for anti-HA virus IgM. We compared each case with four age-and neighbourhood-matched controls. Overall 119 cases and 419 controls were enrolled. Berries were found as the main risk factor for HA (adjusted odds ratio (ORadj): 4.2; 95% confidence interval (CI): 2.5–7.0) followed by raw seafood (ORadj: 3.8; 95% CI: 2.2–6.8; PAF: 26%). Sequencing the virion protein (VP)1-2a region from 24 cases yielded a common sequence (GenBank number: KF182323). The same sequence was amplified from frozen mixed berries consumed by some cases as well as from isolates from Dutch and German HA patients, who had visited some of the affected Italian provinces during the outbreak. These findings suggested berries as the main source of the Italian outbreak. Control measures included voluntary recall of the confirmed frozen mixed berry batches and a trace-back investigation was initiated. The Ministry of Health website recommends frozen berries to be cooked for two minutes before eating.

Introduction

Hepatitis A virus (HAV) is highly transmissible and one of the most frequent causes of foodborne infections. It occurs worldwide, both sporadically and in the form of epidemics, with a tendency for cyclic recurrences in time. Every year an estimated 1.4 million cases of hepatitis A occur worldwide [1].

The incubation period of hepatitis A (HA) is usually between 28 and 30 days (maximum range 15–50 days) [2], and while the disease is often asymptomatic in children under five years-old, its severity increases with age in adults over 18 years-old [2]. HAV, which can be stable in the environment for several months is able to survive freezing [3], and can be transmitted through contaminated water and food as well as from person to person, by the faecal–oral route [1,4]. Other relevant risk factors associated with HAV are travel to highly endemic areas [5] as well as individual high-risk behaviours (such as for people who inject drugs or use other illicit drugs, for men who have sex with men and for people using contaminated blood products [2]).

In Italy, HA is mandatorily reported to the Italian National Surveillance System (Ministerial Decree (DM) of 15/12/90) [6]. A sentinel surveillance system for acute viral hepatitis (SEIEVA - Sistema Epidemiologico Integrato Epatiti Virali Acute), which was implemented in 1984 [7] is also in place to strengthen the surveillance and to promote the investigation and control of acute viral hepatitis.

HA in Italy arises both in sporadic and endemic-epidemic forms, with some regions in the south (such as Apulia and in Campania) permanently affected by the disease [8-10]. Since the early 1990s, in line with a global trend and as the result of improved sanitation

and socio-economic conditions, the epidemiology of HAV has greatly changed in the country, with a clear decline in the numbers of cases over the years, whereby the incidence of HAV dropped from 4 per 100,000 in 1991 to 1.4 per 100,000 in 2006 [11]. Over the last decade, the disease incidence further declined and was below 1 per 100,000 in the last two years before the outbreak reported here.

The outbreak

A new outbreak has been ongoing in Italy since 2013. Epidemiological data showed a clear increase in the incidence of HA from 1 January to 31 May 2013 compared to the same period of the previous three years [8]. The highest increase in numbers of patients affected by HA was observed in northern Italy, in particular in the regions of Emilia-Romagna, Friuli–Venezia-Giulia, Lombardy, Piedmont, Trento and Bolzano, and Veneto. Apulia (south Italy) also showed an increase in the number of cases in 2013. In May 2013, some European Union Member States (Germany, Netherlands, Poland) reported cases of HA linked to a ski holiday in northern Italy (autonomous provinces of Trento and Bolzano). After this notification, a retrospective epidemiological investigation started in the provinces of Trento and Bolzano, whereby cases notified to the local health units were contacted. Sequencing of the virion protein (VP)1-2a region of the virus derived from five cases residing in these provinces, one Dutch, and two German cases revealed 100% sequence similarity between all isolates. Thus the outbreak strain was characterised and submitted to GenBank (GenBank accession number: KF182323). The preliminary epidemiological investigation in the autonomous provinces (AP) of Trento and Bolzano showed that the only common food consumed by different cases had been mixed berries. Frozen mixed berries eaten by cases in another region, Veneto, were found positive for HAV and the VP1-2a region of the viral sequence derived from the berries showed 100% sequence similarity with that of the cases' isolates, so more samples of frozen mixed berries were collected throughout the country and to date a total of 15 frozen mixed berry samples in Italy have been found positive for HAV [8,12].

As soon as the outbreak was detected. a series of actions and control measures were undertaken by the Italian health authorities including enhanced of surveillance and awareness of HAV, the collection of additional epidemiological information on associated risk factors, and the characterisation of the virus RNA by genotyping and sequencing of the VP1-2a region from all new cases [13,14], since normally in Italy genotyping and sequencing are not performed on a routine basis.

Moreover, the voluntary recall of the confirmed mixed frozen berries batches was performed and advice to the population regarding the use of frozen mixed berries was given (i.e. advice in supermarket and shops to cook frozen berries for two minutes). In addition risk communication concerning the consumption of

frozen berries (i.e. cook frozen berries for two minutes) was provided to the general public through Ministry of Health (MoH) and Istituto Superiore di Sanità (ISS) websites.

Through a Ministerial Directive on 23 May 2013, the Italian MoH designated a task force of experts from different professions to participate in the management of the HAV outbreak in Italy in 2013. In this framework, an analytic epidemiological study was planned in some of the Italian regions (Apulia, AP of Bolzano, Emilia-Romagna, Friuli–Venezia-Giulia and AP of Trento) that experienced the highest increase of HAV cases.

Methods

Study design and objectives

A retrospective matched case–control study was performed to identify risk factors for HAV infection acquired among the population, from 1 January to 31 May 2013, in some of the regions where the largest increase in the number of cases was observed (Apulia, AP of Bolzano, Emilia-Romagna, Friuli–Venezia-Giulia and AP of Trento). The potential risk factors explored were the consumption of berries, other food items described as potential sources of HAV infection [2,5] and history of travel [15,16].

Case definition and control selection

The study population consisted of all residents in the four selected regions of northern Italy (AP of Bolzano, Emilia-Romagna, Friuli–Venezia-Giulia and AP of Trento) and from one of the southern regions of Italy (Apulia Region).

A case was defined as a symptomatic person, positive for HAV IgM with onset of symptoms (or date of testing if onset date not available) between 1 January 2013 and 31 May 2013. For Apulia, where HAV is endemic and where molecular typing of the viruses isolated from cases is a standard procedure [17], only cases presenting a sequence identical to the outbreak strain (GenBank number: KF182323) and of sub-genotype 1A were included in the study.

Potential controls, that had not presented with hepatitis A symptoms during the period from 1 January to 31 May 2013, were selected from the general population residing in the five Italian regions and matched with each case by age (±3 years) and place of residence (individual matching). The exclusion criteria for controls were: had been diagnosed with hepatitis A in the past, had previously presented symptoms consistent with a diagnosis of hepatitis A (i.e. jaundice + dark urine) or had been vaccinated against hepatitis A.

Three analyses were conducted. The first analysis included all cases and controls as defined above, while a second analysis was restricted to a group of cases, for which the virus had been sequenced and shown to harbour the 'outbreak' sequence. As in the first

analysis, the respective controls for the second analysis were individually matched by age (± 3 years) and place of residence. Because all cases from the Apulia region reported having consumed raw seafood, a third analysis based on cases with the outbreak sequence but excluding the Apulia cases was undertaken. For this, controls were also individually matched by age and place of residence.

Sample size

We selected up to four matched controls for each case (assuming 5% exposure among controls, 80% power to detect a minimum odds ratio (OR) of 3, alpha error of 5%). The minimum sample size necessary was estimated to be of 595 people (476 controls and 119 cases) using STATA 13.

Data collection

The data were collected via telephone interviews using an ad hoc questionnaire. Cases and controls were contacted by health professionals and epidemiologists specifically instructed and involved in the study, and gave verbal informed consent before enrolment. The data were anonymous and used only by the investigation team for the purpose of the study.

Data included socio-demographic features, contact with a household member or sex partner who has HA, food history and travel history that occurred in the 15 to 50 days before symptom onset.

Laboratory investigations

Regions were requested to provide leftover sera and/or faecal samples from IgM-positive cases included in the case–control study. Sera and/or faecal samples were analysed by the Institute Experimental Zooprophylactic of Lombardy and Emilia Romagna (IZSLER) and the national reference laboratory at ISS for further characterisation by genotyping and sequencing. The VP1/2a region of the HAV genome was amplified [18].

Moreover, retail frozen berry samples were analysed directly from food business operators. A reverse transcription-polymerase chain reaction (RT-PCR) was used to detect HAV. In order to further characterise the genotype of detected HAV strains, a nested PCR was performed with degenerate primers targeting the VP1-2a genomic region. Following purification, the VP1-2A region amplicons were subjected to double strand sequencing and the sequence was checked by basic local alignment search tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in Genbank.

Statistical analysis

A descriptive analysis was made by time, place and person. A univariate analysis was conducted to estimate the association between various potential risk factors and HAV infection, crude ORs were calculated with 95% confidence intervals (CI) and p-values using conditional logistic regression. A multivariate analysis was done by using a conditional logistic regression model to test for

Number of cases of hepatitis A by week of symptom onset in five regions, Italy, 1 January–31 May 2013 (n=127)
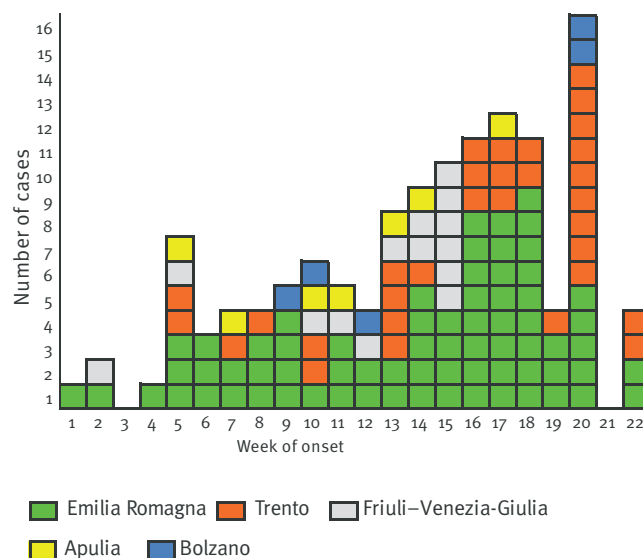
Cases are presented in different colours according to the region where they were notified.

possible confounding factors and interaction. All factors identified as disease-associated with p-value <0.15 and with OR>1 in the univariate analysis were included in the model. In order to quantify the contribution of risk factors to the disease, the population attributable fraction (PAF) was calculated for those factors with the biggest OR. P-values<0.05 were considered as statistically significant. The same statistical analysis was conducted on the restricted samples containing only the cases with an outbreak sequence (second and third analyses described earlier). Moreover, the PAF was estimated in order to define the contribution of the risk factors to HA.

EpiInfo version 5.3.4 was used to enter questionnaire responses and to perform the whole statistical analysis.

Results

The matched case–control study included 119 sets of cases and controls. A total of 588 subjects were interviewed (127 cases and 461 controls). In Figure 1 the epidemic curve is reported. The number of cases increased progressively, peaking at week 20. As of June 2013 the outbreak was still ongoing. Two cases were excluded due to lack of appropriately matched controls within the specified time frame; another six cases that were part of a family cluster due to a common source of infection were also excluded. An additional, 42 controls were excluded due to at least one of the exclusion criteria being present.

Regions selected for the case–control study in an outbreak of hepatitis A, Italy, 1 January–31 May 2013 (n=5 regions)

The name of each participating region is indicated, followed by the numbers of respective cases and controls selected.

Thus, the study included a total of 538 subjects, 119 cases (22%) and 419 controls (78%). The median age was 37.0 years (range: 3–70) for HAV cases and 38.0 years (range: 1–72) for controls (p=0.6384). The majority of study participants in both groups were male. No significant difference in the sex of cases and controls was observed. As a result a median of 3.5 controls for each case were included in the study.

Most of the HAV cases involved in the study, 68/119 (57%), were from the region of Emilia Romagna (Figure 2).

Univariate and multivariate analysis

In the univariate analysis, compared with the 419 controls, cases were more likely to have eaten berries (OR: 4.42; 95% CI: 2.70–7.27), raw seafood (OR: 4.65; 95% CI: 2.70–8.00), or travelled (OR: 2.34; 95% CI: 1.45–3.77) (Table 1). In the multivariate analysis, the highest association with illness was for people who had consumed berries (adjusted odds ratio (ORadj): 4.2; 95% CI: 2.54–7.02).

Among those who reported berry consumption, the majority had eaten berries at home (70%, 169/240)

or at a restaurant (15%, 36/240). The food types consumed that included berries were: yogurt (36%, 86/240), cakes (28%, 67/240), ice cream (21%, 51/240), panna cotta (8%, 19/240), cheesecake (5%, 13/240), cornflakes (5%, 11/240) and fruit juices (1%, 2/240). Other subjects (5%, 12/240) consumed only berries. The types of berries consumed were: blueberries (7%, 16/240), strawberries (3%, 7/240), raspberries (3%, 6/240), blackberries (3%, 6/240), red currants (2%, 4/240) and mixed berries (82%, 197/240).

Raw seafood was found to have the second highest significant association with HAV (ORadj: 3.83; 95% CI: 2.54–7.02) with the third highest factor being history of travel (ORadj: 1.98; 95% CI: 1.15–3.41). The majority of the cases (60%) referring to having travelled during the incubation period indicated Italy as the destination (distributed as 61% in the north of Italy, 21% in the centre of Italy and 17% in the south). The 30% of the subjects who travelled abroad indicated a country outside of Europe. The remaining 10% indicated a travel history in European countries other than Italy.

TABLE 1

Factors positively associated with hepatitis A in univariate and multivariate analysis, Italy, 1 January–31 May 2013

Factor	Univariate analysis	Multivariate analysis	P-value
	Crude OR (95%CI)	Adjusted OR (95%CI)	
Fennel	1.03 (0.66–1.62)	NA[a]	–
Fresh salad	1.02 (0.57–1.73)	NA[a]	–
Bag salad	0.93 (0.59–1.45)	NA[a]	–
Radishes	0.77 (0.44–1.34)	NA[a]	–
Carrots	0.70 (0.43–1.12)	NA[a]	–
Celery	1.29 (0.80–2.07)	NA[a]	–
Raw seafood	4.65 (2.70–8.00)	3.83 (2.16–6.79)	<0.001
Milk products (non-packaged)	0.62 (0.37–1.02)	NA[a]	–
Untreated water	0.77 (0.39–1.50)	NA[a]	–
Berries	4.42 (2.70–7.27)	4.22 (2.54–7.02)	<0.001
Travel	2.34 (1.45–3.77)	1.98 (1.15–3.40)	0.014
Age	1.02 (0.93–1.13)	NA[a]	–
Sex	0.83 (0.55–1.26)	NA[a]	–

CI: confidence interval; NA: not applicable; OR: odds ratio.

[a] All factors identified as disease-associated with p-value <0.15 and with OR>1 in the univariate analysis were included in the multivariate analysis.

PAF was the same (26%) for berries and raw seafood, meaning that the impact of both risk factors was similar, while PAF for travel was lower (16%).

Of the 119 cases enrolled in the case–control study, 24 had respective viral sequence information within the time of the study. The sequenced viruses were identical or highly similar (nucleotide identity between 99.8% and 100%) to the outbreak strain (HAV genotype 1A), in an RNA sequence of 440 nucleotides overlapping the VP1/P2a region. The outbreak sequence had already been submitted to GenBank prior to this study [8].

A restricted statistical analysis was conducted on the 24 cases and respective 82 controls in order to confirm the above described results. Twelve of the 24 cases were from the AP of Trento, seven from Apulia, four from Emilia Romagna and one from the AP of Bolzano. Seventeen of the 24 cases (71%) ate berries and 10 (42%) ate raw seafood. All the 24 cases and 82 controls were included in the restricted analysis with no significant difference in the sex of cases and controls observed.

The statistical analysis (Table 2) identified berries as the highest associated risk factor for developing the disease (ORadj: 4.99; 95% CI: 1.32–18.92) followed by raw seafood (ORadj: 4.46; 95% CI: 1.10–18.04).

As all cases from the Apulia region reported to have eaten raw seafood, an analysis excluding cases from this region was undertaken resulting in berries being the unique risk factor for the disease with an OR of 7.29 (95% CI: 1.56–34.02).

Discussion

The analytical investigation conducted in Italy indicated that the consumption raw seafood or berries constituted risk factors for HA. The highest significant association with illness was however found for berries, which were the most likely source of the outbreak. Berries, as the most implicated risk factor in a HAV foodborne outbreak, is a new finding in Italy.

During the outbreak we were also able to demonstrate that the sequences derived from cases were 100% similar to the sequences isolated in frozen berry food samples [8]. The same sequence has also been isolated 2008 and 2009 in the Czech Republic and Slovakia as well as in Ireland among men who have sex with men (MSM) [19,20]. Sequence analysis of HAV RNA-positive samples, of some of the enrolled cases in the study, showed that all the cases tested had an identical IA genotype, supporting the hypothesis of a widespread, common source of infection.

Since HAV genotyping and sequencing is not performed on a routine basis in Italy we cannot compare the current outbreak genotype with previously identified Italian genotypes. The exception is for the Campania and Apulia regions, where HAV is endemic.

Factor	Univariate analysis	Multivariate analysis	P-value
	Crude OR (95%CI)	Adjusted OR (95%CI)	
Fennel	0.95 (0.32–2.89)	NA[a]	–
Fresh salad	0.31 (0.0–1.34)	NA[a]	–
Bag salad	1.78 (0.58–5.83)	NA[a]	–
Radishes	0.71 (0.17–2.92)	NA[a]	–
Carrots	0.41 (0.14–1.21)	NA[a]	–
Celery	0.58 (0.16–2.06)	NA[a]	–
Raw seafood	5.05 (1.38–18.49)	4.46 (1.10–18.04)	0.027
Milk products (non-packaged)	0.51 (0.10–2.69)	NA[a]	–
Untreated water	4.74 (1.49–15.15)	NA[a]	–
Berries	3.03 (1.00–9.17)	4.99 (1.32–18.92)	0.018
Travel	3.03 (1.00–10.81)	NA[a]	–
Age	–	–	–
Sex	0.61 (0.21–1.81)	NA[a]	–

CI: confidence interval; NA: not applicable; OR: odds ratio.

[a] All factors identified as disease-associated with p-value <0.15 and with OR>1 in the univariate analysis were included in the multivariate analysis.

The phylogenetic analysis conducted in Apulia between 2008 and 2009 revealed the co-circulation of subtypes IA (74%) and IB (26%), clustering with strains from Germany and France, and with those previously circulating in the region [17]. The sequence from the outbreak reported here was different from sequences of genotype IA found in Apulia in 2008 and 2009. In Campania, laboratory investigations conducted during an outbreak in 2004 showed the 1B HAV genotype as the most common circulating strain (90% of sera) [10].

Consumption of fresh or frozen produce is known to be associated with outbreaks of foodborne enteric viruses, particularly norovirus and HAV [21]. Produce (fruits and vegetables) can become contaminated during cultivation prior to harvest due to contact with inadequately treated sewage or sewage polluted water, or fomites. Contamination may also occur by infected food handlers, during harvesting, processing, storage, distribution or final preparation, with the virus likely to be found on the surface of the food. Fruits and vegetable are more prone to being the vehicle of

foodborne infections as they are more likely to be left uncooked before consumption [22]. Berries as a vehicle for transmission of HAV, have been described in several outbreaks with most of the infections connected with consumption of minimally processed frozen berries [22].

Furthermore, it has been shown that among berries (frozen raspberries in particular) and vegetables (i.e. parsley) those with uneven shapes are more likely to retain viruses on their surface. Raspberries, for example, have crevices and hair-like projections which may prevent the virus from being removed by rinsing [21].

Recently outbreaks connected to berry consumption have been reported in some Nordic European countries (Denmark, Finland, Norway and Sweden) [23] and in the United States [24]. The outbreak in Nordic countries was associated to frozen strawberries [25,26] while the outbreak in the United States was associated to Townsend Farms Organic Antioxidant Blend, a frozen blend containing a pomegranate seed mix [24]. However, the virus genotype 1A isolated to the Italian outbreak is different from the virus genotypes implicated in the above mentioned recent outbreaks (genotype 1B), which also have frozen strawberries and berries as the suspected vehicles [25,26].

The main limitation of this study is the recall bias: as the incubation period of hepatitis A occurs over a long period of time (range 15–50 days), the food consumption history in the weeks before symptom onset may be vague and better remembered by cases than by controls. Another possible limitation is due to some controls being not susceptible to HAV (having forgotten if they had been vaccinated or had asymptomatic infection); this underestimated the association between outcome and exposure of interest. Another limitation was that no other known hepatitis A exposures (e.g. persons who inject drugs, male same-sex sexual contact) were taken into consideration.

An additional limitation is that we were unable to compare cases and controls by exposure to different types of berries consumed, due to both cases and controls inability to discriminate between fresh or frozen berries and because most of them reported consuming a mix of berries.

Compared with international findings on viral foodborne outbreaks, what is new in this Italian outbreak associated to berries is that the epidemic involved a large number of people over a wide area. Because the batches of frozen berries consumed by some of the cases were composed of a mixture of berries respectively originating from different lots, it has been difficult to trace-back the origins of each type of berry.

Rapid and effective monitoring is critical for detecting outbreaks and new risk factors for infectious diseases. For this reason, as soon as the outbreak was confirmed [8], the MoH enhanced the national surveillance system and undertook appropriate control measures that are still in place: voluntary recall of the confirmed frozen mixed berries batches and advice to the population regarding the use of the frozen mixed berries already purchased or still available in retail (i.e. advice in supermarket and shops to cook frozen berries for 2 minutes). Moreover, risk communication to the general population through MoH and Istituto Superiore di Sanità (ISS) websites concerning the consumption of frozen berries (i.e. cook frozen berries for 2 minutes) was done. Frozen berries were also included as another potential risk factor in the epidemiological investigation and sequencing of the virus affecting HA cases was requested.

The Italian national reference laboratories (for human and food investigation) and the public health authorities are conducting epidemiological and laboratory investigations as well as trace-back and trace-forward investigations in parallel. The collaboration between the surveillance system for foodborne diseases and the molecular epidemiology has been essential to understand the risk factors associated with this outbreak.

In the future, attention should be paid to the prevention of contamination before or during food processing by implementation of good agricultural, hygienic and manufacturing practices, as well as hazard analysis critical control points (HACCP) systems. Continuous health education would also be helpful in the effective control of hepatitis A.

Conflict of interest

None declared.

Author contributions

CR contributed to the design the study, coordinated the activities, done the interviews, introduced and analysed the data and drafted the manuscript as the lead writer. LR and VA contributed to the design the study, done the interviews, introduced and analysed the data and drafted the manuscript. AB

References

1. World Health Organization (WHO). Global policy report on the prevention and control of viral hepatitis in WHO member states. Geneva:WHO; 2013.

2. Heymann DL, editor. Control of communicable diseases manual. Washington DC: American Public Health Association; 2008.

3. Siegl G, Weitz M, Kronauer G. Stability of hepatitis A virus. Intervirology. 1984;22(4):218-26. http://dx.doi.org/10.1159/000149554

4. European Food Safety Authority Panel on Biological Hazards (BIOHAZ); Scientific Opinion on An update on the present knowledge on the occurrence and control of foodborne viruses. EFSA Journal 2011;9(7):2190.

5. Koslap-Petraco MB, Shub M, Judelsohn R. Hepatitis A: disease burden and current childhood vaccination strategies in the United States. J Pediatr Health Care. 2008; 22(1):3-11. http://dx.doi.org/10.1016/j.pedhc.2006.12.011

6. Decreto Ministeriale 15 dicembre 1990. [Ministerial Decree of 15 December 1990]. Gazz. Uff. 8 gennaio 1991, n. 6. Rome: Ministero della Salute. Italian. Available from: http://www.salute.gov.it/imgs/C_17_normativa_1357_allegato.pdf

7. Mele A, Tosti ME, Spada E, Mariano A, Bianco E and SEIEVA collaborative group. Epidemiology of acute viral hepatitis: twenty years of surveillance through SEIEVA in Italy and a review of the literature. Rome: Istituto Superiore di Sanita; 2006: 12.

8. Rizzo C, Alfonsi V, Bruni R, Busani L, Ciccaglione AR, De Medici D et al. Ongoing outbreak of hepatitis A in Italy: preliminary report as of 31 May 2013. Euro Surveill. 2013;18(27):pii=20518.

9. Lopalco PL, Malfait P, Menniti-Ippolito F, Prato R, Germinario C, Chironna M, et al. Determinants of acquiring hepatitis A virus disease in a large Italian region in endemic and epidemic periods. J Viral Hepat. 2005;12(3):315-21. http://dx.doi.org/10.1111/j.1365-2893.2005.00593.x

10. Pontrelli G, Boccia D, Di Renzi M, Massari M, Giugliano F, Celentano LP, et al. Epidemiological and virological characterization of a large community-wide outbreak of hepatitis A in southern Italy. Epidemiol Infect. 2008;136(8):1027-34. http://dx.doi.org/10.1017/S095026880700951X

11. Tosti ME, Spada E, Romanò L, Zanetti A, Mele A; SEIEVA collaborating group. Acute hepatitis A in Italy: incidence, risk factors and preventive measures. J Viral Hepat. 2008;15 Suppl 2:26-32. http://dx.doi.org/10.1111/j.1365-2893.2008.01025.x

12. European Centre for Disease Prevention and Control (ECDC). Outbreak of hepatitis A in EU/EEA countries – Second update, 11 April 2014. Stockholm: ECDC; 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/ROA-Hepatitis%20A%20virus-Italy%20Ireland%20Netherlands%20Norway%20France%20Germany%20Sweden%20United%20Kingdom%20-%20final.pdf

13. Individuazione di casi di epatite A in Italia. [Identification of cases of hepatitis A in Italy]. DGPRE 0011949-P. Rome: Ministero della Salute; 23 May 2013. [Accessed 18 Sep 2013]. Italian. Available from: http://www.trovanorme.salute.gov.it/norme/renderNormsanPdf;jsessionid=ijGa0kwJ7YDaNPYv-T5Z7A__.sgc3-prd-sal?anno=0&codLeg=46074&parte=1%20&serie=

14. Petrignani M, Verhoef L, Vennema H, van Hunen R, Baas D, van Steenbergen JE, et al. Underdiagnosis of foodborne hepatitis A, the Netherlands, 2008–2010. Emerg Infect Dis. 2014;20(4):596-602. http://dx.doi.org/10.3201/eid2004.130753

15. European Centre for Disease Prevention and Control (ECDC). Rapid risk assessment. Outbreak of hepatitis A virus infection in travellers returning from Egypt. Stockholm: ECDC; 30 Apr 2013. Available from: http://ecdc.europa.eu/en/publications/Publications/RRA-Outbreak-hepatitis-A-virus-infection-travellers-returning-from-Egypt.pdf

16. MacDonald E, Steens A, Stene-Johansen K, Gillesberg Lassen S, Midgley SE, Lawrence J, et al. Increase in hepatitis A in tourists from Denmark, England, Germany, the Netherlands, Norway and Sweden returning from Egypt, November 2012 to March 2013. Euro Surveill. 2013;18(17):pii=20468.

17. Chironna M, Prato R, Sallustio A, Martinelli D, Tafuri S, Quarto M, et al. Hepatitis A in Puglia (South Italy) after 10 years of universal vaccination: need for strict monitoring and catch-up vaccination. BMC Infect Dis. 2012;12:271. http://dx.doi.org/10.1186/1471-2334-12-271

18. Tallo T, Norder H, Tefanova V, Ott K, Ustina V, Prukk T, et al. Sequential changes in hepatitis A virus genotype distribution in Estonia during 1994 to 2001. J Med Virol. 2003;70(2):187-93. http://dx.doi.org/10.1002/jmv.10377

19. Cástková J, Beneš C. Increase in hepatitis A cases in the Czech Republic in 2008 – an update. Euro Surveill. 2009;14(3):pii=19091.

20. Sfetcu O, Irvine N, Ngui SL, Emerson C, McCaughey C, Donaghy P. Hepatitis A outbreak predominantly affecting men who have sex with men in Northern Ireland, October 2008 to July 2009. Euro Surveill. 2011;16(9):pii=19808.

21. Butot S, Putallaz T, Sanchez G. Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. Int J Food Microbiol. 2008;126(1-2):30-5. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.04.033

22. Butot S, Putallaz T, Amoroso R, Sanchez G. Inactivation of enteric viruses in minimally processed berries and herbs. Appl Environ Microbiol. 2009;75(12):4155-61. http://dx.doi.org/10.1128/AEM.00182-09

23. European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA). Outbreak of hepatitis A virus infection in four Nordic countries. Joint ECDC-EFSA rapid outbreak assessment. Stockholm: ECDC; 15 Apr 2013. Available from: http://www.ecdc.europa.eu/en/publications/publications/hepatitis-a-rapid-assessment-nordic-countries-april2013.pdf

24. Centers for Disease Control and Prevention (CDC). Fact Sheets: Multistate outbreak of hepatitis A virus infections linked to pomegranate seeds from Turkey. Atlanta: CDC; 23 Sep 2013. Available from: http://www.cdc.gov/hepatitis/outbreaks/2013/a1b-03-31/index.html

25. Nordic outbreak investigation team. Joint analysis by the Nordic countries of a hepatitis A outbreak, October 2012 to June 2013: frozen strawberries suspected. Euro Surveill. 2013;18(27):pii=20520.

26. Gillesberg Lassen S, Soborg B, Midgley SE, Steens A, Vold L, Stene-Johansen K, et al. Ongoing multi-strain food-borne hepatitis A outbreak with frozen berries as suspected vehicle: four Nordic countries affected, October 2012 to April 2013. Euro Surveill. 2013;18(17):pii=20467.