Prevalence and control of hepatitis A virus in fresh produce

Nigel Cook and Martin D'Agostino
Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, United Kingdom; nigel.cook@fera.gsi.gov.uk

Summary

Hepatitis A virus (HAV) is a major cause of enteric disease. It is transmitted through the faecal/oral route, and has a low infectious dose. Fresh produce such as berry fruit and leafy vegetables are excellent vehicles for transmission of HAV, as they are often eaten raw or after minimal processing. Several outbreaks of hepatitis transmitted by consumption of contaminated fruit or vegetables have been described. HAV can contaminate foods via handling by infected persons, and fresh produce can be contaminated during production through irrigation with sewage-contaminated water. Efficient methods for detection of HAV on fresh produce exist, and some studies have found HAV contamination of fresh produce in the field or at retail, or contamination of waters used in primary production. HAV is relatively resistant to chlorination, but alternative disinfection procedures can be effective. The risk of HAV contamination of berry fruit and leafy vegetables can be reduced by good agricultural and manufacturing practice in primary production and good hygienic practice during food preparation. Further research work is necessary to understand fully the extent of the threat that this pathogen poses to food safety.

Keywords: berry fruit, control, detection prevalence, HAV, leafy vegetables, outbreaks

1. Introduction

Next to noroviruses, hepatitis A virus is currently the most significant viral agent of foodborne disease. This is due to the severity of the symptoms, including liver disease, which infection can produce. Hepatitis A virus (HAV) belongs to the genus *Hepatovirus*, in which it is the only species; the genus itself lies within the family *Picornaviridae*. HAV particles are approximately 28 nm, possessing an icosahedral capsid enclosing the RNA genome. The HAV genome is 7.5 kb in length, and contains four structural and seven non-structural genes. There are seven genotypes of HAV (Hollinger and Emerson, 2001; Robertson, 2001). However, there is only one known serotype of HAV (Banker, 2003; Hollinger and Emerson, 2001). Four genotypes have been isolated from human cases of hepatitis A and three genotypes are found in Old World monkeys (Robertson, 2001). The strains belonging to each genotype have at least 85% genetic identity, and most human strains belong to either genotype I or III (Cuthbert, 2001; Hollinger and Emerson, 2001).

The infectious dose of HAV particles may be very low, perhaps in some cases even single particles (Cliver, 1985). After ingestion, the virus passes through the gastrointestinal tract

and is carried to the liver via the hepatic portal vein (O'Connor, 2000). Replication of HAV in the liver is not believed to result in immediate cell damage; this may result primarily from the immune response to the virus-infected cells (Hollinger and Emerson, 2001). Released virions enter the bile duct, pass into the gastrointestinal tract, and are then excreted in faeces. During active virus replication infected persons remain generally asymptomatic. Peak virus shedding occurs approximately 4-6 weeks after ingestion of the agent, just before the onset of acute symptoms, and infected persons can have virus in their stools before becoming aware of any illness. Virus excretion can continue generally up to 8 days after onset of symptoms (Cliver, 1985; Hollinger and Emerson, 2001). In infants and young children, symptoms are generally milder, but faecal excretion persists for longer compared to adults (Hollinger and Emerson, 2001). The fatality rate is approximately 0.3% (Issa and Mourad, 2001), with serious complications being more likely to occur in persons over 40 years of age. Infection with HAV confers lifelong immunity (Melnick, 1995; Ryder and Beckingham, 2001).

In developing countries, most HAV infections occur among children, and adults are generally immune (Cuthbert, 2001; WHO, 2000). In North America and Western Europe, overall population immunity to HAV is declining (Banker, 2003; Jacobsen and Koopman, 2004), most likely due to increased standards of public health in recent decades. This creates a risk of the occurrence of large outbreaks (Advisory Committee on the Microbiological Safety of Food, 1998), with contaminated foods imported from countries of high endemicity being one of the potential hazards (Pebody *et al.*, 1998).

The types of foodstuff most often implicated in outbreaks of hepatitis A are those which are eaten raw or only slightly cooked, such as soft fruit, salad vegetables, and shellfish, or handled extensively prior to consumption, such as prepared salad (Fiore, 2004). These foodstuffs can become contaminated with HAV through several routes, but ultimately contamination originates from the intestines of an infected person. Foods may acquire viral contamination by contact with infected persons during harvesting or preparation, when viruses are transferred or shed on to foodstuffs via faecally contaminated hands. Faecal contamination by no means needs to be gross. Bidawid *et al.* (2000) estimated that the amount of infectious HAV in 1 mg faeces could be as high as 13,000 infectious particles, so microscopic quantities of faeces could harbour sufficient virus particles to constitute a hazard. Fresh produce such as leafy green vegetables or berry fruit can require extensive handling during harvesting. During harvesting of berry fruit such as strawberries and raspberries, the fruit is often picked by hand. Extensive handling post-harvest may also occur; for instance with green onions at least three workers can be required to peel, trim and bundle them (Dentinger *et al.*, 2001). Such practices increase the chances of contamination by infected persons. Contamination can also occur through contact with sewage-polluted waters, used for irrigation or washing.

2. Outbreaks of hepatitis A implicated to contaminated fruit and vegetables

There have been several outbreaks of hepatitis A globally in which consumption of contaminated fresh produce items was implicated (Table 1).

Table 1. Some outbreaks of hepatitis A in which consumption of fresh produce items was implicated.

Year	Country	Foodstuff implicated	Origin of foodstuff	No. of cases	Reference
2010	the Netherlands	semi-dried tomatoes	not identified	13	Petrignani *et al.*, 2010
2010	France	semi-dried tomatoes	not identified	59	Gallot *et al.*, 2011
2009	Australia	semi-dried tomatoes	Australia	144	Donnan *et al.*, 2012
2002	New Zealand	raw blueberries	New Zealand	19	Calder *et al.*, 2003
1998	USA	salad onions	USA / Mexico	43	Dentinger *et al.*, 2001
1997	USA	frozen strawberries	Mexico	258	Hutin *et al.*, 1999
1988	Scotland	fresh raspberries	Scotland	5	Ramsay and Upton, 1989
1983	Scotland	frozen raspberries	Scotland	24	Reid and Robinson, 1987

Some features of these outbreaks are interesting in that they reveal the challenges associated with identification of the mode and routes of contamination, but some features can occasionally be discerned.

Beginning in March 2009, a large hepatitis A outbreak involving over 500 cases occurred in several Australian states (Donnan *et al.*, 2012). The results of the consequent epidemiological investigation revealed a strong correlation with acquisition of the illness and consumption of dried semidried tomatoes. HAV genotype IB was detected in some batches of semidried tomatoes; sequences were identical to HAV sequences obtained from the sera of infected persons. No cases of hepatitis A were reported in workers in the Australian semidried tomato industry, and it was considered likely that imported produce was responsible, although precise identification of the source was complicated by the complexity of the supply chain and lack of product traceability. Between December 2009 and January 2010, 13 patients with acute hepatitis caused by an identical strain of HAV were detected in the Netherlands (Petrignani *et al.*, 2010). The HAV strain was the same as that implicated in the Australian outbreak, therefore semi-dried tomatoes were implicated from the beginning of the epidemiological investigation, and a case-control study indentified semidried tomatoes in oil as the source of the outbreak. A further cluster of cases infected with genotype IB strains occurred in November 2011 (Fournet *et al.*, 2012); the cases were attributed to consumption of semidried tomatoes. Trace back was hindered by the complex supply routes. Meanwhile in England 7 cases of hepatitis A occurred from July to November 2011, involving HAV strains related to those detected in the Netherlands outbreaks; consumption of semidried tomatoes was considered but could not be demonstrated. In January 2010, 49 cases of hepatitis A were reported in south-western France (Gallot *et al.*, 2011). The HAV strain isolated was genotype IB, and again consumption of semidried tomatoes was implicated. The HAV strain was highly similar to strains isolated from patients returning from Turkey. Trace-back investigations revealed that the implicated batches of semidried tomatoes had been imported frozen from Turkey by a supplier in France. In France, the frozen semidried tomatoes were defrosted and processed with oil and herbs before distribution to the outlets from which they were purchased and consumed. No heat treatment, disinfection, or washing was conducted after

defrosting. No samples of the foodstuff were available for analysis. To date, no information on how the produce came to be contaminated has come to light.

An outbreak of hepatitis A in which HAV was detected in implicated soft fruit was described by Calder *et al.* (2003). In Auckland, New Zealand in the first three months of 2002, there was a marked increase in the number of reported hepatitis A cases. HAV was detected, by reverse transcription polymerase chain reaction (RT-PCR), in stool specimens from several cases. An epidemiological investigation revealed that a majority of the cases studied had consumed raw blueberries, and there was a significant association of this with the illness. A trace back investigation through retailers and wholesalers implicated a single orchard as the probable source of the outbreak. When frozen blueberries stored at the orchard were analysed, HAV was detected by RT-PCR and confirmed by nucleic acid hybridization. Sequencing of the PCR products revealed a close identity between the HAV in the blueberries and that in the stool specimens derived from patients. It was not possible to identify exactly how the blueberries had become contaminated with HAV but it was considered that contamination at the orchard by infected food handlers or by polluted ground water were among the likely causes. This strongly indicates widespread contamination of a large amount of the original foodstuff batch, especially since viruses cannot replicate in foods.

In November 2003 in Pennsylvania, a large hepatitis A outbreak was linked to consumption of green onions in a restaurant (Wheeler *et al.*, 2005). 601 cases occurred, at least 124 were hospitalized and 3 died. The HAV sequence was similar to sequences found among persons who had been in Mexico during the two to six weeks before illness. An United States Food and Drug Administration trace-back investigation found that the green onions came from two farms in northern Mexico between September and early October 2003. At harvest, the outer layer of the onions was stripped off, before they were bundled by hand, packed on ice in boxes at the farms, and shipped to the United States. No repacking occurred between the packing sheds on the farms and delivery to the restaurant. Green onions from the implicated farms were probably delivered to other restaurants, but no other simultaneous restaurant-associated outbreaks of hepatitis A were identified. Therefore it may have been possible that HAV contamination was confined to a small portion of the harvested onions, but was nonetheless sufficient in scale to result in the infection of many people.

An indication of the potential scale of contamination can be derived from an outbreak of hepatitis which occurred in Scotland in 1983 (Reid and Robinson, 1987). Here, a ~1.5 kg batch of raspberries was implicated as the vehicle of transmission. One of the people who became ill was a caterer who prepared a dessert from the fruit, and reported that they had merely tasted it, probably by dipping the edge of a spoon into the pureed fruit and touching it to their tongue. If that tiny quantity of contaminated food, that would have been consumed in that action, contained an infectious dose of HAV (>10 particles), the quantity of HAV contaminating the raspberry batch may have been massive.

3. Detection methods for hepatitis A virus in fresh produce

In most outbreaks of hepatitis implicated to fresh produce, the virus has seldom been detected in the foodstuff. This has been primarily due to the lack of efficient methods to detect viruses in foods.

Several methods for detection of HAV in foods have been published in the scientific literature (Table 2), but few have actually been used in analysis of foodstuffs during outbreak investigations or for routine monitoring. Calder *et al.* (2003) used a method devised for calicivirus and based on washing of fruit followed by virus precipitation by acidification and centrifugation (Gulati *et al.*, 2001) to confirm the presence of HAV in the New Zealand outbreak described above. Detection of HAV in the semidried tomato samples implicated in

Table 2. Examples of published methods for the detection of hepatitis A in produce.

Reference	Methods used	Produce used
Dubois *et al.*, 2002	chloroform-butanol extraction / RT-PCR	fresh berries (raspberries and strawberries), frozen berries (raspberries and mixtures of raspberry, bilberry, blackberry, currant, blackcurrant, and cherry), mashed raspberries, or vegetables (lettuce, radishes, and tomatoes)
Rzeżutka *et al.*, 2006	ultracentrifugation / RT-PCR	raspberries and strawberries
Guévremont *et al.*, 2006	PEG precipitation / RT-PCR	green onions
Butot *et al.*, 2007	elution / centrifugation / RT-qPCR	berries (strawberries, raspberries, blueberries, blackberries, and black currants), vegetables/herbs (lettuce, green onions, mint, parsley, and basil) and a sugared berry mix (consisting of raspberries, blueberries and 10% sugar)
Dubois *et al.*, 2007	PEG precipitation / RT-qPCR	butter lettuce, iceberg lettuce, red oak leaf lettuce, Belgian chicory and curly endive, fresh strawberries, fresh and frozen raspberries, frozen bilberries and frozen red currants.
Papafragkou *et al.*, 2008	cationically charged magnetic particles / PFU	lettuce, strawberries and green onions
Love *et al.*, 2008	AEC / RT-qPCR	canned tomato sauce (with or without meat) and frozen, halved strawberries in sauce
Morales-Rayas *et al.*, 2010	cationically charged filtration / RT-qPCR	Strawberries, raspberries, green onions and lettuce
Hyeon *et al.*, 2011	positively charged membrane / ICC / RT-qPCR	Iceberg lettuce

AEC = acid-adsorption elution concentration; ICC = integrated cell culture; PEG = polyethylene glycol; RT-PCR = reverse transcription polymerase chain reaction; RT-qPCR = real-time RT-PCR.

the Australian outbreak was performed by CEERAM Laboratories in France, presumably using the forthcoming European Committee for Standardisation (CEN) method, described below.

The CEN procedures 'Microbiology of food and animal feeding stuffs - Horizontal method for detection of hepatitis A virus and *Norovirus* in food using real-time RT-PCR - Part 1: method for quantitative determination; Part 2: method for qualitative detection' have been prepared and are currently being led through standardisation by the working group CEN/TC275/WG6/TAG4. They describe a method for liberation, concentration and detection of HAV (as well as *Norovirus*) from foodstuffs. RNA extraction is achieved by lysis using guanidine thiocyanate and subsequent adsorption of the nucleic acids to silica. The viral RNA can then be amplified and detected using real-time RT-PCR. Removal of viruses from foods is performed in different ways depending on the matrix. For food surfaces and food preparation surfaces, a swabbing method is used. For soft fruits and salad vegetables, elution with agitation of the viruses is performed, followed by polyethylene glycol/NaCl precipitation. Real-time RT-PCR is then performed. Comprehensive suites of controls are included, due to the complex nature of the methods, to provide means of qualitative and/or quantitative detection of virus RNA in the test sample. When applying the methods, standards regarding general requirements for conventional (Anonymous, 2005) and real time PCR (Anonymous, 2011a), and PCR performance characteristics (Anonymous, 2011b) are recommended. This standard procedure for detection of HAV applied to fresh produce will become widely used in routine monitoring and outbreak investigations.

A potential limitation of procedures such as RTPCR is that detection of nucleic acid by itself may not indicate whether the virus is infectious (Richards, 1999). Bhattacharya *et al.* (2004) have however reported a correlation between HAV infectivity and RTPCR signal, when using a reverse transcription primer directed against the 3' poly (A) tail of the viral genome, and PCR primers amplifying a region from the 5' end; damage to the RNA at any point in the genome will affect reverse transcription and subsequent PCR.

4. Prevalence of hepatitis A virus in fresh produce

There is little information on prevalence of any enteric virus in fresh produce. HAV was detected on lettuce sold in a Costa Rican market (Hernández *et al.*, 1997); it was considered that it had been contaminated by irrigation or washing with sewage-contaminated water.

Kokkinos *et al.* (2012) analysed the lettuce supply chain in three European countries, and obtained HAV-positive samples from toilet facilities at primary production, and from harvesters' hands. The level of contamination, as estimated by PCR-detectable units, was 280 and 19-24 virus particles respectively. This revealed vulnerability to contamination within this food supply chain, a finding which was reinforced by the detection of other human enteric viruses such as adenovirus and *Norovirus* at other points within the supply chain, including produce at point of sale. A parallel analysis of the berry fruit supply chain found similar evidence of vulnerability there (Maunula, unpublished data).

5. Survival of hepatitis A virus on fresh produce

A major potential route of virus contamination of produce is via the use of contaminated water used for irrigation. HAV has been demonstrated to survive under pre-harvest conditions on bell pepper, cantaloupe, and lettuce (Stine *et al.*, 2005); it was calculated that it could take 822 days in pre-harvest conditions for HAV to decline by 99.9%. Post-harvest, HAV has been found to display similarly robust survival potential. On fresh carrots and fennel Croci *et al.* (2002) found a less than 2-log reduction of HAV after 9 days at 4 °C, indicating that the virus can persist under normal storage conditions over the periods usually elapsing between purchase and consumption. HAV survived on spinach leaves over days at 5 °C, with a decimal reduction (D-) value of 28.6 days (Shieh *et al.*, 2009). Sun *et al.* (2012) examined survival of HAV on green onions at various temperatures from 3 to 23 °C. It was found that survival was lowest at the higher temperature; even so the indication was that HAV would lose only 1 log infectivity after 5 days at 23 °C. Bidawid *et al.* (2001) found little decline in HAV infectivity on lettuce kept under modified atmosphere packaging conditions for 12 days.

The evidence from several outbreaks of hepatitis A implicated to frozen fruit (Hutin *et al.*, 1999; Ramsay and Upton, 1989) strongly indicates that HAV can survive, i.e. persist in an infectious state, for several months in frozen foods. Butot *et al.* (2007) examined the survival of HAV in berries (blueberries, raspberries and strawberries) and herbs (basil and parsley), and found no decline over 90 days of storage at -20 °C.

It is clear from the above studies that HAV can survive from a contamination event to point of consumption.

6. Control measures

The prevention and control of foodborne viral disease may be attempted by various means, including virus elimination procedures, and good hygienic and agricultural practice.

Physical control measures can involve washing, disinfection, or heating of foodstuffs. However items such as salad vegetables and berries are if processed at all only minimally, generally involving brief washing in a dilute chlorine solution. This may not be completely effective in removing HAV: treatment of strawberries, tomatoes and lettuce with 20 mg/kg chlorine resulted in less than 2 log reduction (Casteel *et al.*, 2008), and Butot *et al.* (2008) found less than two log reductions after washing HAV-contaminated berry fruits and herbs in cold and warm chlorinated water. HAV is not resistant to thorough cooking, but this is seldom performed on fresh produce items. The high sugar content in berry fruits may have a protective effect on HAV under mild heat treatment (Deboosere *et al.*, 2004). High pressure processing could be used effectively against HAV contaminating fresh produce items: greater than 4 log reduction in infectious virus in mashed raspberries and sliced green onions was obtained after 5 min at 375 MPa (Kingsley *et al.*, 2005).

The key measure in the prevention of foodborne transmission of viral disease in any setting, either domestic or industrial, is good hygienic practice. The Codex Committee on Food

Hygiene has produced a code of hygienic practice for the control of viruses in food, entitled 'Guidelines on the application of general principles of food hygiene to the control of viruses in food' (FAO/WHO, 2012). These guidelines follow the format of the Codex Recommended International Code of Practice - general principles of food hygiene (FAO/WHO, 2003) and define hygienic practices during the production, processing, manufacturing, transport and storage of foods which are considered essential to ensure the safety and suitability of food for consumption. The Guidelines contain Annexes which are relevant to the soft fruit, salad vegetable and shellfish supply chains; these give specific mention to HAV and *Norovirus*. The European Commission project 'Integrated monitoring and control of foodborne viruses in European food supply chains (VITAL)' produced guidance sheets for preventing contamination of berry fruits and leafy green vegetables by viruses. These are intended for use in conjunction with the Codex guidelines, and are available at http://www.eurovital.org.

Vaccination of all food handlers, from primary production through to point of sale or catering, would be an effective means of preventing foodborne hepatitis A (Cliver, 1997a) and the WHO has recommended (WHO, 1993) that it should be considered if resources are available. Vaccination is available for HAV, and in the US the use of hepatitis A vaccine has resulted in an overall decline in the number of cases being reported annually (Acheson and Fiore, 2004). However, the number of reported handler-associated outbreaks of hepatitis A may not be sufficient to justify the routine vaccination of food handlers (Anonymous, 1996; Jacobs *et al.*, 2000), although it could be worthwhile to keep this issue under consideration (Advisory Committee on the Microbiological Safety of Food, 1998; Keeffe, 2004). It would however be expensive (Jacobs *et al.*, 2000; Meltzer *at el.*, 2001), and potentially, it might introduce a level of complacency in adherence to hygienic standards. It would be useful during an outbreak, where immunization of at-risk individuals can be used to control the spread of infection (D'Argenio *et al.*, 2003; Sanchez *et al.*, 2002).

7. Conclusions

7.1 What has been achieved?

Hepatitis A virus has been recognised as the most significant viral agent of foodborne disease next to *Norovirus*. It is known that in the developed world, immunity to HAV in the population is declining, and this creates the risk of the occurrence of outbreaks, if contaminated foods are consumed. Foodborne outbreaks of hepatitis A have been associated with minimally processed or raw foodstuffs such as fresh produce which can become contaminated at various points of the supply chain, primarily through contact by infected persons or by contaminated water. There is now an increasing body of information related to outbreak data which may shed some light on how foodstuffs can become contaminated with HAV. Full outbreak investigation has hitherto been hindered by the lack of efficient methods to detect viruses in foods, but this is now being rectified by the development of international standards. It is known that HAV has robust survival characteristics outside a host and information from various studies has shown that the virus is capable of surviving from point of contamination within the food supply chain to point of consumption. Elimination studies are also revealing that except for cooking, the physical control measures employed in food

production, e.g. chlorination, may not be sufficient to inactivate infectious HAV and thus completely remove the risk it poses when contaminating produce. With the concern of virus contamination of foodstuffs, international guidelines have been produced; mainly focussing on procedural controls such as good hygienic practice, these should help to reduce the risk of viruses such as HAV contaminating food supply chains.

7.2 What has been neglected?

Several knowledge gaps regarding HAV exist. For example, what is the infectious dose? It is not known precisely how many infectious particles are needed to cause infection in a susceptible person. We have insufficient prevalence data to fully determine what the scale of the issue of HAV contamination of produce might be. We do not know what the scale of HAV contamination is in fresh produce such as berry fruits and leafy green vegetables sold at retail and at other points within their supply chains. Thus, it is not known what the exposure potential is within the population to HAV in contaminated produce. Consequently, necessary virological risk assessments have not been performed. We still do not have an efficient, rapid infectivity assay for HAV and the forthcoming standard methods based on PCR will not indicate whether the detected virus is infectious or not. The threat of foodborne contamination with HAV could be completely eradicated through a global immunisation program, as efficient vaccines exist, however the disease is not felt to be severe enough to warrant the expense.

7.3 What needs to be done?

More details on survival of infectious virus in foods and food supply environments, and which disinfection measures are thoroughly effective, would be beneficial especially in outbreak situations where disinfection of e.g. implicated farms or restaurants is required. Detailed assessments of the risk of acquiring hepatitis from consumption of HAV-contaminated products would allow regulators to consider if specific food safety criteria would be appropriate. Especially, exposure assessments are required, but these in turn require knowledge of HAV prevalence in the fresh produce supply chain. Thorough surveillance using standardised methodology is necessary to gather sufficient data to clearly discern the amount of HAV contamination within fresh produce supply chains. But as this contamination may be sporadic and inconsistent, a more valuable approach (Kokkinos *et al.*, 2012) is to perform monitoring for a set of enteric viruses including adenovirus which can indicate that a route of contamination exists from infected humans to points of vulnerability in the food supply chains, which HAV would be able to follow. Various studies have demonstrated the potential for viruses to enter the berry fruit supply chain via contaminated food handlers' hands and water at crop production. It would be advantageous to have virus-aware revisions of existing food safety management systems for implementation in the fresh produce industry. It has been observed that more non-compliance with prerequisite food safety programs in fresh produce can occur during production than during processing and at point-of-sale, indicating that most virus contamination is likely to occur at that early phase (Willems, unpublished data). Compliance with prerequisite programmes, such as the Codex guidelines is essential to reduce the risk of contamination of food supply chains with viruses. It will also be beneficial to have a harmonised integration of monitoring and control, to be able

to routinely monitor that compliance measures are being undertaken effectively. With clear recommendations on regaining control through compliance with prerequisite programs, and appropriate monitoring procedures, fresh produce supply chains can be safeguarded from HAV contamination, with a consequent protection of public health.

References

Acheson, D.W. and Fiore, A.E., 2004. Preventing foodborne disease - what clinicians can do. N. Engl. J. Med. 350(5), 437-440.

Advisory Committee on the Microbiological Safety of Food (ACMSF), 1998. Report on foodborne viral infections. The Stationery Office, London, UK.

Anonymous, 1996. Immunisation against infectious disease. The Stationery Office, London, UK.

Anonymous, 2005. ISO 22174:2005 - Microbiology of food and animal feeding stuffs. Polymerase chain reaction (PCR) for the detection of foodborne pathogens. General requirements and definitions. International Organization for Standardization, Geneva, Switzerland.

Anonymous, 2011a. ISO 22119:2011 - Microbiology of food and animal feeding stuffs. Real-time polymerase chain reaction (PCR) for the detection of foodborne pathogens. General requirements and definitions. International Organization for Standardization, Geneva, Switzerland.

Anonymous, 2011b. ISO 22118:2011 - Microbiology of food and animal feeding stuffs. Polymerase chain reaction (PCR) for the detection of foodborne pathogens. Performance characteristics. International Organization for Standardization, Geneva, Switzerland.

Banker, D.D., 2003. Viral hepatitis (Part-I). Indian J. Med. Sci. 57(8), 363-368.

Bhattacharya, S.S., Kulka, M., Lampel, K.A., Cebula, T.A. and Goswami, B.B., 2004. Use of reverse transcription and PCR to discriminate between infectious and non-infectious hepatitis A virus. J. Virol. Methods 116(2), 181-187.

Bidawid, S., Farber, J.M. and Sattar, S.A., 2000. Contamination of foods by food handlers: experiments on hepatitis A virus transferred to food and its interruption. Appl. Environ. Microbiol. 66(7), 2759-2763.

Bidawid, S., Farber, J.M. and Sattar, S.A., 2001. Survival of hepatitis A virus on modified atmosphere-packaged (MAP) lettuce. Food Microbiol. 18(1), 95-102.

Butot, S., Putallaz, T. and Sánchez, G., 2007. Procedure for rapid concentration and detection of enteric viruses from berries and vegetables. Appl. Environ. Microbiol. 73(1), 186-192.

Butot, S., Putallaz, T. and Sánchez, G., 2008. Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. Int. J. Food. Microbiol. 126, 30-35.

Calder, L., Simmons, G., Thornley, C., Taylor, P., Pritchard, K., Greening, G. and Bishop, J., 2003. An outbreak of hepatitis A associated with consumption of raw blueberries. Epidemiol. Infect. 131(1), 745-751.

Casteel, M.J., Schmidt, C.E. and Sobsey, M.D., 2008. Chlorine disinfection of produce to inactivate hepatitis A virus and coliphage MS2. Int. J. Food Microbiol. 125(3), 267-273.

Cliver, D.O., 1985. Vehicular transmission of hepatitis A. Public Health Rev. 13(3/4), 235-292.

Cliver, D.O., 1997. Hepatitis A from strawberries: who's to blame? Food Technol. 51(6), 132.

Croci, L., De Medici, D., Scalfaro, C., Fiore, A. and Toti, L., 2002. The survival of hepatitis A virus in fresh produce. Int. J. Food Microbiol. 73(1), 29-34.

Cuthbert, J.A., 2001. Hepatitis A: old and new. Clin. Microbiol. Rev. 14(1), 38-58.

D'Argenio, P., Adamo, B., Cirrincione, R. and Gallo. G., 2003. The role of vaccine in controlling hepatitis A epidemics. Vaccine 21(19/20), 2246-2249.

Deboosere, N., Legeay, O., Caudrelier, Y. and Lange, M., 2004. Modelling effect of physical and chemical parameters on heat inactivation kinetics of hepatitis A virus in a fruit model system. Int. J. Food Microbiol. 93, 73-85.

Dentinger, C.M., Bower, W.A., Nainan, O.V., Cotter, S.M., Myers, G., Dubusky, L.M., Fowlere, S., Salehi, E.D.P. and Bell, B., 2001. An outbreak of hepatitis A associated with green onions. J. Infect. Dis. 183(8), 1273-1276.

Donnan, E.J., Fielding, J.E., Gregory, J.E., Lalor, K., Rowe, S., Goldsmith, P., Antoniou, M., Fullerton, K.E., Knope, K., Copland, J.G., Bowden, D.S., Tracy, S.L., Hogg, G.G., Tan, A., Adamopoulos, J., Gaston, J. and Vally, H., 2012. A multistate outbreak of hepatitis A associated with semidried tomatoes in Australia, 2009. Clin. Infect. Dis. 54(6), 775-781.

Dubois, E., Agier, C., Traore, O., Hennechart, C., Merle, G., Cruciere, C. and Laveran, H., 2002. Modified concentration method for the detection of enteric viruses on fruits vegetables by reverse transcriptase-polymerase chain reaction or cell culture. J. Food Prot. 65(12), 1962-1969.

Dubois, E., Hennechart, C., Merle, G., Burger, C., Hmila, N., Ruelle, S., Perelle, S. and Ferré, V., 2007. Detection and quantification by real-time RT-PCR of hepatitis A virus from inoculated tap waters, salad vegetables, and soft fruits: characterization of the method performances. Int. J. Food Microbiol. 117(2), 141-149.

Fiore, A.E., 2004. Hepatitis A transmitted by food. Clin. Infect. Dis. 38(5), 705-715.

Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO), 2003. General principles of food hygiene to the control of viruses in food (CAC/RCP-1).

Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO), 2012. Guidelines on the application of general principles of food hygiene to the control of viruses in food (CAC/GL 79 - 2012).

Fournet, N., Baas, D., Van Pelt, W., Swaan, C., Ober, H.J., Isken, L., Cremer, J., Friesema, I., Vennema, H., Boxman, I., Koopmans, M. and Verhoef, L., 2012. Another possible food-borne outbreak of hepatitis A in the Netherlands indicated by two closely related molecular sequences, July to October 2011. Euro Surveill. 17(6), pii: 20079.

Gallot, C., Grout, L., Roque-Afonso, A.-M., Couturier, E., Carrillo-Santisteve, P., Pouey, J., Letort, M.J., Hoppe, S., Capdepon, P., Saint-Martin, S., De Valk, H. and Vaillant, V., 2011. Hepatitis A associated with semidried tomatoes, France, 2010. Emerg. Infect. Dis. 17(3), 566-567.

Guévremont, E., Brassard, J., Houde, A., Simard, C. and Trottier, Y.L., 2006. Development of an extraction and concentration procedure and comparison of RT-PCR primer systems for the detection of hepatitis A virus and *Norovirus* GII in green onions. J. Virol. Methods 134(1-2), 130-135.

Gulati, B.R., Allwood, P.B., Hedberg, C.W. and Goyal, S.M., 2001. Efficacy of commonly used disinfectants for the inactivation of calicivirus on strawberry, lettuce, and a food-contact surface. J. Food Prot. 64(9), 1430-1434.

Hernández, F., Monge, R., Jiménez, C. and Taylor, L., 1997. *Rotavirus* and hepatitis A virus in market lettuce (*Latuca sativa*) in Costa Rica. Int. J. Food Microbiol. 37(2/3), 221-223.

Hollinger, F.B. and Emerson, S.U., 2001. Hepatitis A virus. In: Fields, B.N., Howley, P.M. and Griffin, D.E. (Eds.). Fields virology, 4th Ed. Lippincott Williams & Wilkins, New York, NY, USA, pp. 799-840.

Hutin, Y.J.F., Pool, V., Cramer, E.H., Nainan, O.V., Weth, J., Williams, I.T., Goldstein, S.T., Gensheimer, K.F., Pell, B.P., Shapiro, C.N., Alter, M.J. and Margolis, H.S., 1999. A multistate, foodborne outbreak of hepatitis A. N. Engl. J. Med. 340(8), 595-602.

Hyeon, J.Y., Chon, J.W., Park, C., Lee, J.B., Choi, I.S., Kim, M.S. and Seo, K.H., 2011. Rapid detection method for hepatitis A virus from lettuce by a combination of filtration and integrated cell culture-real-time reverse transcription PCR. J. Food Prot. 74(10), 1756-1761.

Issa, I.A. and Mourad, F.H., 2001. Hepatitis A: an updated overview. J. Med. Liban. 49(2), 61-65.

Jacobs, R.J., Grover, S.F., Meyerhoff, A.S. and Paivanas, T.A., 2000. Cost effectiveness of vaccinating food service workers against hepatitis an infection. J. Food Protect. 63(6), 768-774.

Jacobsen, K.H. and Koopman, J.S., 2004. Declining hepatitis A seroprevalence: a global review and analysis. Epidemiol. Infect. 133, 1005-1022.

Keeffe, E.B., 2004. Occupational risk for hepatitis A: a literature-based analysis. J. Clin. Gastroenterol. 38(5), 440-8.

Kingsley, D.H., Guan, D.S. and Hoover, D.G., 2005. Pressure inactivation of hepatitis A virus in strawberry puree and sliced green onions. Journal of Food Protection 68, 1748-1751.

Kokkinos, P., Kozyra, I., Lazic, S., Bouwknegt, M., Rutjes, S., Willems, K., Moloney, R., de Roda Husman, A., Kaupke, A., Legaki, E., D'Agostino, M., Cook, N., Rzeżutka, A., Petrovic, T. and Vantarakis, A., 2012. Harmonised investigation of the occurrence of human enteric viruses in the leafy green vegetable supply chain in three European countries. Food Environ. Virol. 4(4), 179-191. DOI 10.1007/s12560-012-9087-8.

Love, D.C., Casteel, M.J., Meschke, J.S. and Sobsey, M.D., 2008. Methods for recovery of hepatitis A virus (HAV) and other viruses from processed foods and detection of HAV by nested RT-PCR and TaqMan RT-PCR. Int. J. Food Microbiol. 126(1-2), 221-226.

Melnick, J.L., 1995. History and epidemiology of hepatitis A virus. J. Infect. Dis. 171(Suppl.1), 2-8.

Meltzer, M.I., Shapiro, C.N., Mast, E.E. and Arcari, C., 2001. The economics of vaccinating restaurant workers against hepatitis A. Vaccine 19(15/16), 2138-2145.

Morales-Rayas, R., Wolffs, P.F. and Griffiths, M.W., 2010. Simultaneous separation and detection of hepatitis A virus and norovirus in produce. Int. J. Food Microbiol. 139(1-2), 48-55.

O'Connor, J.A., 2000. Acute and chronic viral hepatitis. Adolesc. Med. 11(2), 279-92.

Papafragkou, E., Plante, M., Mattison, K., Bidawid, S., Karthikeyan, K., Farber, J.M. and Jaykus, L.A., 2008. Rapid and sensitive detection of hepatitis A virus in representative food matrices. J. Virol. Methods 147, 177-187.

Pebody, R.G., Leino, T., Ruutu, P., Kinnunen, L., Davidkin, I., Nohynek, H. and Leinikki, P., 1998. Foodborne outbreaks of hepatitis A in a low endemic country: an emerging problem? Epidemiol. Infect. 120(1), 55-59.

Petrignani, M., Harms, M., Verhoef, L., Van Hunen, R., Swaan, C., Van Steenbergen, J., Boxman, I., Peran i Sala, R., Ober, H.J., Vennema, H., Koopmans, M. and Van Pelt, M., 2010. Update: A food-borne outbreak of hepatitis A in the Netherlands related to semi-dried tomatoes in oil, January-February 2010. Euro Surveill. 15(20), pii: 19572.

Ramsay, C.N. and Upton, P.A., 1989. Hepatitis A and frozen raspberries. Lancet 1(8628), 43-44.

Reid, T.M.S. and Robinson, H.G., 1987. Frozen raspberries and hepatitis A. Epidemiol. Inf. 98, 109-112.

Richards, G.P., 1999. Limitations of molecular biological techniques for assessing the virological safety of foods. J. Food Prot. 62(6), 691-697.

Robertson, B.H., 2001: Viral hepatitis and primates: historical and molecular analysis of human and nonhuman primate hepatitis A, B, and the GB-related viruses. J. Viral. Hepat. 8(4), 233-242.

Ryder, S.D. and Beckingham, I.J., 2001. ABC of diseases of liver, pancreas, and biliary system: acute hepatitis. BMJ 322(7279), 251-253.

Rzeżutka, A., D'Agostino, M. and Cook, N., 2006. An ultracentrifugation-based approach to the detection of hepatitis A virus in soft fruits. Int. J. Food Microbiol 108, 315-320.

Sanchez, G., Pinto, R., Vanaclocha, H. and Bosch, A., 2002. Molecular characterization of hepatitis A isolates from a transcontinental shellfish-borne outbreak. J. Clin. Microbiol. 40(11), 4148-4155.

Shieh, Y.C., Stewart, D.S. and Laird, D.T., 2009. Survival of hepatitis A virus in spinach during low temperature storage. J. Food Prot. 72, 2390-2393.

Stine, S.W., Song, I., Choi, C.Y. and Gerba, C.P., 2005. Effect of relative humidity on preharvest survival of bacterial and viral pathogens on the surface of cantaloupe, lettuce, and bell peppers. J. Food Prot. 68(7), 1352-1358.

Sun, Y., Laird, D.T. and Shieh, Y.C., 2012. Temperature-dependent survival of hepatitis A virus during storage of contaminated onions. Appl. Environ. Microbiol. 78(14), 4976-4983.

Wheeler, C., Vogt, T.M., Armstrong, G.L., Vaughan, G., Weltman, A., Nainan, O.V., Dato, V., Xia, G., Waller, K., Amon, J., Lee, T.M., Highbaugh-Battle, A., Hembree, C., Evenson, S., Ruta, M.A., Williams, I.T., Fiore, A.E. and Bell, B.P., 2005. An outbreak of hepatitis A associated with green onions. N. Engl. J. Med. 353(9), 890-897.

World Health Organization (WHO), 1993. Prevention of foodborne hepatitis A. Weekly Epidemiol. Record 68(22), 157-158.

World Health Organization (WHO), 2000. WHO/CDS/CSR/EDC/2000.7. Hepatitis A. World Health Organization Department of Communicable Disease Surveillance and Response. Available at: www.who.int/emc.