

**Mini-Review****Hepatitis A virus related to foods.**

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ARTICLE INFO**Keywords***Disease and Infection**Foods**Personal Hygiene**Properties of HAV**Transmission***Received** 27/08/2019**Accepted** 25/09/2020**Available On-Line**

01/04/2021

ABSTRACT

Hepatitis disease is caused by hepatitis A virus (HAV), Positive single stranded RNA virus, belongs to the family of Picornaviridae. Hepatitis A virus infection occurs globally and is causing a public health concern, primarily in developing countries due to its persistent circulation in the environment. The improved sanitary condition and increase in awareness of personal hygiene have led to the marked reduction of HAV prevalence in industrialized countries during childhood and to a shift of the infection towards adulthood. HAV is an environmentally stable, that is primarily transmitted by the fecal-oral route, person to person contact or ingestion of contaminated food and drink. One of the main causes leading to HAV infection is epidemiologically linked to the consumption of raw or undercooked shellfish particularly oysters and clams. Due to their filter-feeding style, these shellfishes readily concentrate viruses from the surrounding water containing municipal sewage, and as a consequence pose a health threat to consumers. Therefore, development of detection techniques possessing the requisite sensitivity and specificity for the practical routine monitoring purposes is of great importance necessary for the protection of shellfish-consuming public. Nucleic acid-based method such as reverse transcription PCR has emerged as the popular method of choice in view of rapidity, accuracy, and sensitivity in contrary of the time-consuming conventional cell culture and hybridization techniques. The low concentration of viral genome present in the environmental sample which requires effective isolation and concentration of virions and the labor-extensive purification, removal of PCR inhibitors will be unfavorable.

1. INTRODUCTION

Hepatitis A, a term first introduced in 1967, is known to be a liver infection caused by hepatitis A virus (HAV) whose primary replication site is in the hepatocytes (Krugman *et al.*, 1967).

HAV is a positive- sense, single stranded RNA virus that belongs to the family of *Picornaviridae* and is the unique member of the genus *Hepatovirus*. Unlike the other members of the family, HAV requires a long adaptation period to grow in cell culture, replicates slowly and rarely produces a cytopathic effect (Cromeans *et al.*, 1987; Lemon, 1992).

African green monkey kidney cells or fetal rhesus kidney cells are commonly used for culturing the virus, although many different cell types are suitable. The genome of the HAV is approximately 7.5kb, consists of a highly conserved 5' end non-translated region (NTR) and has a covalently linked virus specific protein (VPg) instead of a cap structure (Weitz *et al.*, 1986). Translation occurs in a cap-independent pattern under control of an internal ribosome entry segment within the 5'NTR (Brown *et al.*, 1991). The 3' end NTR has a poly-A tail (Cohen *et al.*, 1987), and the remainder of the genome is composed of a single open reading frame that will be translated to single large polyprotein.

The polyprotein will be subsequently cleaved by a viral protease which results in production of four capsid proteins and some non-structural proteins (Schultheiss *et al.*, 1994).

Infection by HAV confers life-long immunity and can produce effects that range from asymptomatic to fulminant hepatic failure, which in some cases can cause death (Ross and Anderson, 1991). However, the fatality rate in HAV infections is lower than 0.1%, and the higher risk is usually exposed to young children and older adults with underlying chronic liver disease (Akriviadis and Redeker, 1989).

The likelihood of clinically apparent disease associated with HAV infection increases with age (Hadler *et al.*, 1980). More than 70% of cases of HAV infection occur in children less than 6 years old are asymptomatic, or, if illness occurs, it is not accompanied by jaundice (Hadler *et al.*, 1980). However, in older children and adults, HAV infection causes more-severe clinical illness, including jaundice malaise, fever, and dark urine, in 70% of cases (Lednar *et al.*, 1985). In persons without jaundice, peak infectivity likely occurs as (SALT) concentrations increase.

2. SCIENTIFIC BACKGROUND

2.1. Morphology and physicochemical properties of Hepatitis A virus

Hepatitis A virus was first identified in 1973 by electron microscope and is one of the smallest and structurally simplest RNA animal viruses.

The viral particle is non-enveloped, therefore resistant to ether, chloroform, and alcohol. Morphologically, HAV is an isometric particle with a diameter of 27-32nm and composed entirely of 70% viral protein and 30% ribonucleic acid (Lemon, 1994) and it appears as a featureless sphere under the electron microscope. The buoyant density of the full viral particles is 1.32-1.34g/cm³ in CsCl and a sedimentation coefficient of 156-160 S in neutral sucrose solutions. During early infection, empty capsids, collected in feces, band at 1.20 and 1.29-1.31g/cm³, with sedimentation coefficient ranging from 50 S to 90 S, predominantly 70 S (Koff, 1998).

In common with all enteric viruses, hepatitis A is acid stable and able to retain infectivity below pH3. HAV remains infectious after refrigeration and freezing and is resistant to heating at 60°C for 30 minutes. Besides, chlorine a component commonly used as a disinfection agent is partially effective in removing the virus where conflicting reports on the efficacy for inactivation of HAV, which may be due to the different experimental condition used, have been reported. However, it is readily inactivated by ionizing radiation, phenol, and formaldehyde (Siegl et al. 1984).

2.2. Growth and biological properties

HAV can be cultured in several different primate cell lines including African green monkey kidney cells (BSC-1), fetal rhesus monkey kidney cells (FRhK-4 and FRhK-6), and human fibroblasts (HF), but wild-type strains are difficult to culture and generally do not produce CPE in cell cultures. Immunofluorescence is often used for detection of HAV antigen in infected cells because of the lack of CPE.

The virus is usually slow-growing and the yield in cell cultures is lower as compared with most other picornaviruses. Consequently, it is difficult to identify the virus in clinical, food, or environmental sources by culture alone. Under normal conditions, the virus requires 3 weeks for *in vitro* growth.

Laboratory-adapted strains such as HM 175 are able to produce CPE and so have been used extensively in research studies. These viruses require less time for *in vitro* growth and produce visible CPE or plaques. Molecular techniques, including culture-PCR, have become the method of choice for detection of virus in nonhuman samples, whereas clinical diagnosis is usually based on the patient's immune response. HAV antigens are conserved, and antibodies are generated against a single antigenic site composed of amino acid residues of VP3 and VP1 proteins on the virus surface. HAV is very stable:

- HAV Shows high resistance to chemical and physical agents such as drying, heat, low pH, and solvents, and has been shown to survive in the environment, including seawater and marine

sediments, for more than 3 months (Sobsey *et al.*, 1988). It is resistant to several preservatives and solvents including chloroform, Freon, Arklone, and 20% ether and (300 mg/L perchloroacetic acid) or 1 g/L chloramine at 20°C for 15 min (Hollinger and Emerson, 2001). HAV is resistant to storage at 20°C for years. It is also resistant to drying, remaining infectious for more than 1 month at 25°C and 42% humidity, and shows even greater resistance at low humidity and low temperatures. The heat resistance of HAV is reported to be greater in foods and shellfish. After heating in a can for 19 min at 60°C, HAV inoculated into oysters was not fully inactivated. The virus retains integrity and infectivity after 60-min incubation at 60°C and is only partially inactivated after 10–12 hrs. at 56°C. Under refrigeration and freezing conditions, the virus remains intact and infectious for several years.

- Although HAV infectivity decreased by 2 to 5 log₁₀ after exposure to 70% alcohol for 3 min and 60 min at 25°C. Overall HAV exhibits greater resistance to stressors than other picornaviruses. HAV is resistant to detergent but (survives at 37°C for 30min in 1% SDS). The virus is stable at pH 1.0 and survives acid marination at pH 3.75 in mussels for at least 4 weeks (Hollinger and Emerson, 2001) Autoclaving (121°C for 20 min). Chlorine-containing compounds (3 to10 mg/l sodium hypochlorite at 20°C for 5 to 15 min). Chlorine free residual (Conc. 2.0 to 2.5 mg/l for 15 min) Iodine (3 mg/l for 5 min) â. Propiolactone (0.03% for 72 hrs. at 4°C) Gamma irradiation is not Effective for inactivation of HAV on fresh fruits and vegetables,, now used as an isothermal preservation method for perishable foods.
- HAV is inactivated by high hydrostatic pressure, after 5-min exposure at 450 MPa (Kingsley et al., 2002), ultraviolet radiation (1.1 w at a depth of 0.9 cm for 1 min), formalin (8% for 1min at 25°C), and heating to 85°C for 1 min)

2.3. Infection and Disease

HAV infects the epithelial cells of small intestine and hepatocytes, causing elevation of liver enzymes and inflammation of the liver. The cytotoxic T-cell immune response destroys infected liver cells, releasing the virus particles into the bile duct from where they are excreted in the feces.

The virus is believed to initially enter the liver via the bloodstream, and it is not clear if intestinal replication occurs.

The virus has an incubation period of 2 to 6 weeks with an average of 28 days.

Initially the symptoms are nonspecific and include fever, headache, fatigue, anorexia, dark urine, lights tools, and nausea and vomiting with occasional diarrhea.

One to 2 weeks later, characteristic symptoms of hepatitis such as Viremia and jaundice appear. Peak infectivity occurs in 2 weeks preceding the onset of jaundice, and the virus is present in the blood at 2 to 4 weeks. The HAV is shed in large numbers (> 10⁶ particles/g) in feces from the latter 2 weeks of the incubation period for up to 5 weeks.

Jaundice is usually evident from week 4 to 7 and virus shedding generally continues throughout this period. Diagnosis is based on the detection of anti-HAV Ig M antibody, which can be detected before the onset of symptoms and becomes undetectable within 6 months of recovery. Acute hepatitis is usually self-limiting, but overall debility lasting several weeks is common and relapses may occur.

The HAV has not been associated with development of chronic liver disease, but on rare occasions fulminant disease that results in death may occur. Because the onset of symptoms occurs several weeks after infection, it is rare to have the suspected food available for analysis. A killed vaccine that provides long-lasting immunity has been commercially available since 1995 and is commonly given to travelers a thigh risk. This vaccine could be used in the food industry to immunize food workers to reduce the risk of food contamination by workers.

2.4. Epidemiology of hepatitis A virus

Approximately 1.4 million clinical cases of HAV reported annually worldwide and four major patterns of HAV infection can be described based on the age-specific prevalence of antibodies to HAV, which result in characteristic features of hepatitis A epidemiology including disease rates and predominant transmission (Bell, 2002). These range from high endemicity to very low, where the levels correlate with hygienic and social economy status of each geographic area. In the high endemicity areas such as parts of Africa, Asia and central and South America, where poor sanitary and unhygienic conditions are found, infection is acquired during early childhood and most infections are asymptomatic, and if symptoms occur, they are mild and non-specific. Reported disease rates in such as are therefore low and outbreak of disease is rare. However, in such high endemic areas disease rates may be high due to the high level of circulation virus. For example, a population-based study conducted in the Amazon basin of Brazil found the incidence of clinical disease among children to be over 100/100000 population (Bensabath *et al.*, 1987).

The route of transmission is mainly from person to person, and also contaminated food and water source. Developing countries with translational economies and some regions of industrialized countries where sanitary and socio-economic conditions are improved are defined as intermediate endemicity.

Southern and eastern Europe and some regions in Middle East are the examples where the reductions in exposure to the HAV in childhood have been reported. However, the disease rates are high in older children, adolescents, and young adults because of the high level of circulating HAV via the food and waterborne transmission that lead to the outbreak.

In the low and very low endemicity, majority of the population remains susceptible throughout adulthood whenever the virus is introduced, but the less opportunity for the exposure of virus contributes to the lower cases of hepatitis A infection. In North America, hepatitis A infection mainly occurs in the community as a whole, mostly affecting young adults and children in lower social-economic classes.

Outbreaks have also been reported among men who

have sex with men and intravenous drug users, as well as isolated community. In the region of Europe where there is a low local prevalence of HAV, disease is only usually occurs among specific risk group like the international travelers and HAV-infected migrants (Arankalle *et al.*, 1995; Beutels *et al.*, 1997)

3.5. Prevention

3.5.1. Hygiene practices

The minimum infectious dose required for HAV infection in humans is unknown. In primate studies, HAV can remain infectious after 1 month on environmental surfaces at ambient temperatures and it is more resistant than poliovirus (another picornavirus) to degradation over time while on environmental surfaces (Mbithi *et al.*, 1991). Heating foods to 85°C (185°F) for 1 minor disinfection with a 1:100 dilution of house-hold bleach in water or cleaning solutions containing quaternary ammonium and/or HCl (including concentrations found in many toilet cleaners) is effective in inactivating HAV. HAV is resistant to disinfection by some organic solvents and by a pH as low as 3 (Favero *et al.*, 1998). No specific food handler hygiene practice has been shown to reduce the likelihood of transmission. Experimental deposition of fecally suspended HAV onto hands indicates that infectious HAV remains present for 4 h after application (Mbithi *et al.*, 1992). In experimental settings, water rinsing alone reduces the amount of HAV that is transferred to lettuce by 10- to 100-folds (Bidawid *et al.*, 2000)

Hygiene training for food handlers should include practical advice about the techniques of hand washing and education about the need to seek medical attention for post exposure prophylaxis after contact with a person with hepatitis A. Reducing bare hand contact with foods that are not subsequently cooked is also a reasonable preventative measure. Employers should provide access to hand washing stations and encourage ill food handlers to seek medical attention and to stay out of the work- place.

Exclusion from duties that involve contact with food for atleast 1–2 weeks after the onset of jaundice or until symptoms resolve is reasonable.

A symptomatic food handlers who are IgM anti-HAV positive are sometimes identified during investigations and measurements of ALT levels, in combination with likely dates of exposure, might be used to estimate whether the food handler has had recent infection and is potentially still capable of transmission. However, the validity of this approach is unknown.

Providing sanitary facilities for field workers and discouraging the presence of children in areas where food is harvested reduces the potential for contamination of food during harvesting or processing. Chlorinated water or water from a source not likely to be contaminated by sewage should be used for rinsing produce or ice used for packing.

3.5.2. Disinfection of potentially contaminated foods

Development of disinfection procedures for produce or shell- fish has been hampered by the technical difficulties involved with detection of infectious HAV in food. Cell culture assays can indicate the presence of infectious HAV, but they are ex- pensive and require several days to perform. Wild-type virus is not

easily detectable, because it usually is not cytopathic. RT-PCR protocols can detect viral particles more rapidly but cannot readily distinguish infectious virus from noninfectious HAV RNA, and the variety of PCR inhibitors present in foods requires the development of food-specific protocols. Specific methods to detect enteric viruses, such as HAV, are necessary, because water and shellfish with low coliform counts (commonly used as a measure of fecal contamination) have been shown to contain viable HAV and outbreaks of hepatitis A associated with shellfish harvested from waters where fecal coliform counts were within accepted limits have been reported (Portnoy *et al.*, 1975)

Despite these challenges, methods are being developed to detect HAV on some types of produce (Crocini *et al.*, 2002), and in water (De Serres *et al.*, 1999)

The effectiveness of various disinfection methods in reducing HAV contamination of fresh fruits and vegetables is an area of active investigation. Preliminary results indicate that disinfection modalities that are potentially applicable to produce, including chlorinated water (Li *et al.*, 2003), hydrostatic pressure (Kingsley *et al.*, 2002), and heat (Millard *et al.*, 1987), are effective in reducing or eliminating HAV infectivity; however, adapting these techniques for use on commercially distributed produce will require further refinement. Other than thorough cooking, no reliable disinfection method for shellfish exists. Shellfish are typically cooked until they open, which may occur at temperatures as low as 70°C (Koff *et al.*, 1967)

Steaming or boiling shell fish still in the shell for 2 min may not fully inactivate HAV (Crocini *et al.*, 1999) Shellfish have HAV concentrations as much as 100-fold that of surrounding water (Enriquez *et al.*, 1992) and HAV has been detected in clams, mussels, and oysters harvested from areas linked to hepatitis A outbreaks. Depuration (placing harvested live shellfish in clean water to promote purging of gastrointestinal contents) for up to 1 week reduces but does not eliminate HAV that has been taken up by shellfish (Enriquez *et al.*, 1992). If HAV-contaminated water is used during depuration, it may even introduce HAV into previously uncontaminated shellfish.

Reducing HAV contamination of foods should be possible using approaches, such as Hazard Analysis and Critical Control Point (HACCP) systems, similar to those recommended for reducing contamination by other foodborne pathogens (CDC, 2003)

3. Findings

- Hepatitis A virus (HAV) is an important pathogen which has been responsible for many food-borne outbreaks.
- HAV-excreting food handlers, especially those with poor hygienic practices, can contaminate the foods which they handle.
- Consumption of such foods without further processing has been known to result in cases of infectious hepatitis.
- Touching the lettuce with artificially contaminated finger pads for 10 s at a pressure of 0.2 to 0.4 kg/cm² resulted in transfer of 9.2±0.9 % of the infectious virus.
- Hepatitis A is a common form of acute viral hepatitis in many parts of the world. It is responsible for significant world-wide morbidity and occasional mortality (Koff, 1998)
- Outbreaks of hepatitis A occur periodically throughout the world, and fecal contaminated food and water are the main vehicles (Atmaret *et al.*, 1993).
- Less than 10% of the cases of hepatitis A in the United States are associated with food-borne outbreaks (CDC, 1994).
- Substantial costs are incurred by both society and the food industry as a result of hepatitis A food-borne outbreaks (Dalton *et al.*, 1996).
- The foods implicated in hepatitis A outbreaks include shellfish (Desenclos *et al.*, 1991), sandwiches, dairy products, baked products, salads, fruits, and vegetables (Cliver *et al.*, 1997)
- In 1992, more than 5,000 individuals were exposed to hepatitis A virus (HAV) due to consumption of a variety of gourmet foods prepared by an infected food handler (Dalton *et al.*, 1996).
- A recent outbreak in Michigan, which resulted in more than 200 cases of infectious hepatitis in school children, occurred due to the consumption of imported contaminated strawberries (CDC, 1997).
- In nearly 50% of cases, the mode and vehicle(s) of virus spread remain unidentified (Hadler *et al.*, 1989).
- A number of reports have suggested that infected food handlers may play an important role in food contamination in many cases (Cliver *et al.*, 1985).
- The ability of hands to transfer viruses such as HAV to foods is limited, and this in turn hampers the institution of proper hand hygiene measures to reduce the risk of food contamination.
- HAV titers were measured by performing plaque assays (Mbithi *et al.*, 1991). In particular, water and topical agents seemed to be the most effective agents for reducing virus titers on fingerpads. Consequently, the risk of virus spread and infection through foods can be significantly ($P < 0.001$) reduced.
- Cliver and Kostenbader (Cliver *et al.*, 1984) demonstrated that common disposable plastic gloves provided the best protection since no virus could penetrate the plastic either from within or from without. In view of our results, emphasis should be placed on proper hand washing by food handlers.
- followed by water rinsing significantly ($P < 0.05$) reduced the amount of virus remaining on the finger pads and resulted in a significant ($P < 0.05$) reduction in the amount of virus transferred to the lettuce (Š0.64%).
- Hollinger and Ticehurst (Hollinger *et al.*, 1996) found that the amount of HAV excreted in feces of infected individuals ranged from 10⁶ to 10⁹ virus particles per g. However, the actual minimal infectious dose of HAV required to cause human infection is unknown, although one infectious unit might be sufficient to cause infection. The highest level of virus transfer from contaminated finger pads to lettuce (approximately 9%) occurred after the lettuce was touched with soiled finger pads without any prior treatment of the finger pads with

interruption agent that common disposable plastic gloves provided the best protection since no virus could penetrate the plastic either from within or from without. In view of our results, emphasis should be placed on proper and washing by food handlers. In addition, the use of disposable gloves should be encouraged, particularly when preparers are handling foods such as fresh-cut produce that require no processing before they are served to consumers.

19. The method described in this paper provides a model for future studies in which workers investigate the transfer of viruses (or other microorganisms) from food handlers to foods and vice versa. Although the many steps in this method were meant to simulate a real-life scenario of food contamination by infected food handlers, certain aspects of the method may need to be modified and/or refined. Future studies may include examinations of the role of greater pressure and friction on virus transfer to foods, the effectiveness of lathering and rubbing on virus removal from hands, and the manner in which different topical agents affect virus removal and/or inactivation.

4. CONCLUSION

Hepatitis A is an acute, usually self-limiting disease of the liver caused by hepatitis A virus (HAV). HAV is transmitted from person to person, primarily by the fecal-oral route. The incidence of hepatitis A is closely related to socioeconomic development, and sero-epidemiological studies show that prevalence of anti-HAV antibodies in the general population varies from 15% to close to 100% in different parts of the world. An estimated 1.5 million clinical cases of hepatitis A occur each year.

In young children HAV infection is usually asymptomatic whereas symptomatic disease occurs more commonly among adults. Infection with HAV induces lifelong immunity.

In areas of low endemicity, hepatitis A usually occurs as single cases among persons in high-risk groups or as outbreaks involving a small number of persons. In areas of high endemicity most persons are infected with HAV without symptoms during childhood.

This explains why clinical hepatitis A is uncommon. In countries of low and intermediate disease endemicity, adult disease is seen more often. Hepatitis A may represent a substantial medical and economic burden. Currently, four inactivated vaccines against HAV are internationally available.

All four vaccines are safe and effective, with long-lasting protection. None of the vaccines are licensed for children less than one year of age.

The virus has a worldwide distribution and causes about 1.5 million cases of clinical hepatitis each year. Humans are the only reservoir of the organism. It is closely associated with poor sanitary conditions. The most common modes of transmission include close personal contact with an infected person and ingestion of contaminated food and water.

The virus is shed in the feces of persons with both asymptomatic and symptomatic infection. Under favorable conditions HAV may survive in the environment for months. Bloodborne transmission of

HAV occurs, but is much less common. The average incubation period is 28 days, but may vary from 15–50 days. Approximately 10–12 days after infection the virus can be detected in blood and feces. In general, a person is most infectious from 14–21 days before the onset of symptoms, through to 7 days after the onset of symptoms. Antibodies against HAV develop in response to infection and seroprevalence can be used as a marker of viral transmission in a community. The lowest seroprevalence is found in the Nordic countries (about 15%). In Australia, other parts of Europe, Japan and in the United States, 40%–70% of the adult population has demonstrable antibodies to HAV. Practically all adults living in developing areas of the world have serological evidence of past infection. The risk of developing symptomatic illness following HAV infection is directly correlated to age. In children below six years of age, HAV infection is usually asymptomatic, with only 10% developing jaundice. Among older children and adults, infection usually causes clinical disease, with jaundice occurring in more than 70% of cases. Therefore, highly HAV-endemic regions are characterized by asymptomatic childhood infection, with only the occasional occurrence the clinical course of acute hepatitis A is indistinguishable from other types of acute viral hepatitis.

Symptoms typically include fever, malaise, anorexia, nausea, and abdominal discomfort, followed by dark urine and jaundice. The severity of disease and mortality increases in older age groups. The convalescence following hepatitis A may be slow, and is characterized by fatigue, nausea, and lack of appetite. Complications of hepatitis A include relapsing hepatitis, cholestatic hepatitis and fulminant hepatitis. Fulminant hepatitis occurs in approximately 0.01% of clinical infections and is characterized by rapid deterioration in liver function and a very high fatality rate. Chronic infection with HAV does not occur. No specific antiviral therapy is currently available.

Diagnosis is made by the demonstration of IgM antibodies to HAV (IgM anti-HAV) in serum. Detection of the virus or viral antigens in the stool is of limited value for routine diagnosis.

REFERENCES

1. AcadSci U. S. A. (2007): 104:7253–7258. Age-specific prevalence of antibodies to hepatitis A and E viruses in Pune, India, 1982 and 1992. *Journal of Infectious Disease* 171:447–450.
2. American Academy of Pediatrics Committee on Infectious Diseases. Hepatitis A vaccine recommendations. *Pediatrics*. (2007) 120(1):189–199.
3. Anonymous. (1996). Hepatitis A among persons with hemophilia who received clotting factor concentrate—United States, September–December 1995. *Morb. Mortal. Wkly. Rep.* 45:29–32.
4. Ansari, A. S.; Springthorpe, V.S. (1991): Comparison of cloth, paper, and warm air drying in eliminating viruses and bacteria from washed hands. *Am. J. Infect. Control* 19: 243–249.
5. Atmar, R. L.; Matcalf, T.G.; Neill, F.H. and Estes, M.K. (1993): Detection of enteric viruses in oyster by using the polymerase chain reaction. *Appl. Environ. Microbiol.* 59:631–635.

6. B. Liver-derived cytotoxic T cells in hepatitis A virus infection. *J Infect Dis*(1989): 160:209–217.
7. Boggs, J. D.; Melnick, J.L.; Conrad, M.E. and Felsler, B.F. (1970): Viral hepatitis: clinical and tissue culture studies. *JAMA* 214:1041–1046.
8. CDC. Updated Recommendations from the Advisory Committee on Immunization Practices (ACIP) for Use of Hepatitis A Vaccine in Close Contacts of Newly Arriving International Adoptees. *MMWR* (2009) 58: 1006–7.
9. Centers for Disease Control and Prevention. Hepatitis A FAQs for health professionals. <http://www.cdc.gov/hepatitis/HAV/HAVfaq.htm>. Accessed June 1, 2012.
10. Barbuti, S. (2002): Detection of hepatitis A virus in mussels from different sources marketed in Puglia region (South Italy). *International Journal of Food Microbiology* 75:11-18.
11. Choi, Y.S.; Lee, J.; Lee, H.W, (2014): Liver injury in acute hepatitis A is associated with decreased frequency of regulatory T cells caused by Fas-mediated apoptosis.
12. Cliver, D. O. (1997): Virus transmission via food. *World Health Stat. Q.* 50:91–104.
13. Dienstag, J. L.; Feinstone, S.M.; Kapikian, A.Z.; Purcell, R.H.; Boggs, and, G.D.; Conrad, M.A. (1975): Faecal shedding of hepatitis-A antigen. *Lancet* ii:765–76
14. Divizia, M (1999): Concomitant poliovirus infection during an outbreak of hepatitis A. *Journal of Infection* 39: 227-230..
15. Enriquez, R., Frosner, G.G. (1992): Accumulation and persistence of hepatitis A virus in Brazil's Amazon Basin. *Bulletin of Pan American Health Organization* 21: 16-27.
16. Feinstone, S. M. (1996): Hepatitis A: epidemiology and prevention. *Eur. J. Gastroenterol. Hepatol.*8:300–305.
17. Gerba, C.P. and Goyal, S. M. (1978): Detection and occurrence of enteric viruses in shellfish: a review. *Journal of Food Protection* 41: 743-754.
18. Hadler, S.C. (1991): Global impact of hepatitis A infection: changing patterns .
19. Keeffe E.B. (2006) Hepatitis A and B superimposed on chronic liver disease: vaccine-preventable diseases. *Trans Am Clin. Climatol Assoc.*; 117:227-237
20. Kingsley, D.H.; Meade, G. K. and Richards, G. P. (2002): Detection of both Hepatitis A Virus and Norwalk-Like Virus in Imported Clams Associated with Food-Borne Illness. *Applied and Environmental Microbiology* 68: 3914-3918.
21. Koff, R.S.; (1998) Hepatitis A. *Lancet*: 351: 1643-1649.
22. Krugman, S.; Giles, J.P. and Hammond, J. (1967): Infectious hepatitis. Evidence for two distinctive clinical, epidemiological, and immunological types of infection. *JAMA* 200:365–373.
23. Le Guyader, F.; Neill, F. H.; Estes, M. K.; Monroe, S. S.; Ando, T. and Atmar, R.L. (1996): Detection and analysis of a small round-structured virus strain in oysters implicated in an outbreak of acute gastroenteritis. *Applied and Environmental Microbiology* 62: 4268-4272.
24. Lednar, W.M.; Lemon, S. M.; Kirkpatrick, J. W.; Redfield, Q.R; Fields, M.L. and Kelley, P.W. (1985): Frequency of illness associated with epidemic hepatitis A virus infections in adults. *American Journal of Epidemiology* 122: 226-33 .
25. Prevot, J. S.; Dubrou, S. and Marechal, J. (1993): Detection of human hepatitis A virus in environmental water by an antigen-capture polymerase chain reaction. *Water Sci. Technol.* 27:227–233.
26. Richards, G.P. (2006). Shellfish-associated viral disease outbreaks. Springer, New York.
27. Rippey, S.R. (1994). Infectious diseases associated with molluscan shellfish consumption. *Clinical Microbiology Review* 7:419-425.
28. Ririe, K.M.; Rasmussen, R. P. and Wittwer, C. T. (1997). Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Anal Biochemistry* 245: 154-160.
29. Robertson, B.H.; Jansen, R. W.; Khanna, B.; Totsuka, A.; Nainan, O. V.; Siegl, G.; Widell, A.; Margolis, H. S.; Isomura, S. and Ito, K. (1992). Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. *Journal of General Virology* 73: 1365-1377.
30. Salamina, G. (1998). Shellfish consumption and awareness of risk of acquiring hepatitis A among Neapolitan families - Italy 1997. *European Surveillance* 3: 97-98.
31. Sanchez, G.; Pinto, R. M.; Vanaclocha, H. and Bosch, A. (2002): Molecular characterization of hepatitis a virus isolates from a transcontinental shellfish-borne outbreak. *Journal of Clinical Microbiology* 40: 4148- 4155.
32. Sattar, S. A.; Springthorpe, V.S.; Karim, Y. and Loro, P. (1989): Chemical disinfection of nonporous inanimate surfaces experimentally contaminated with four human pathogenic viruses. *Epidemiol. Infect.* 102:493–505.
33. Schulman, A.N.; Dienstag, J.L.; Jackson, D.R. Hoofnagle, J. H.; Gerety, R. J.; Purcell, R. H. and Barker, L. F. (1976). Hepatitis A antigen particles in liver, bile, and stool of chimpanzees. *Journal of Infectious Diseases* 134: 80-84.
34. Schulte I, Hitziger T, Giugliano S. (2010): Characterization of CD8+ T-cell response in acute and resolved hepatitis A virus infection. *J Hepatol*; 54:201–208.
35. Siegl, G.; Weitz, M. and Kronauer, G. (1984b): Stability of hepatitis A virus. *Inter virology* 22: 218-226.
36. Sobsey, M. D.; Shields, P.A.; Hauchman, F.S.; Davis, A.L.; Rullman, V.A. and Bosch, A. (1988): Survival and persistence of hepatitis A virus in environmental samples, p. 121–124. In A. J. Zuckerman (ed.), *Viral hepatitis and liver disease*. Alan R. Liss, Inc., New York, N.Y.
37. Sockett, P.N.; Cowden, J. M.; Le Baigue, S.; Ross, D.; Adak, G. K. and Evans, H. (1993). Foodborne disease surveillance in England and Wales: 1989-1991. *Communicable Disease Report CDR Review* 3:R159- 73.
38. Sunen, E. and Sobsey, M.D. (1999): Recovery and detection of enterovirus, hepatitis A virus and Norwalk virus in hard-shell clams (*Mercenaria Mercenaria*) by RT-PCR methods. *Journal of Virology Methods* 77:179-187.
39. Villar, L.M.; DePaula, V.S.; Diniz-Mendes, L.; Lampe, E. and Gaspar, A. M. (2006). Evaluation of methods used to concentrate and detect hepatitis A virus in water samples. *Journal of Virology Methods* 137:169-176.