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Diagnosis of foodborne viral infections in patients

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Abstract

A significant global problem is the microbiological contamination of foods and water. The microorganisms associated with about half of the foodborne disease outbreaks still go unrecognized, primarily as a result of inadequate diagnostic methods and sampling. A significant amount of food- and waterborne diseases are associated with viruses, information that has been obtained only in recent years. Improved diagnostic methods have established that caliciviruses are the most important non-bacterial pathogens associated with food- and waterborne outbreaks, and are the major cause of seafood-associated gastroenteritis.

Keywords: Viruses; Food- and waterborne diseases; Microbiological contamination; Diagnosis; Calciviruses

1. Introduction

Viruses have emerged as significant causes of foodborne and waterborne diseases in recent years, with numerous of outbreaks associated with members of the calicivirus family. In the USA, investigators have reported that, between 1976 and 1980, 40% of non-bacterial cases of gastroenteritis were caused by caliciviruses (Kaplan et al., 1982). These viruses frequently cause large outbreaks of acute gastroenteritis in cruise ships, military camps, hotels, hospitals and nursing homes, and are frequently associated with food consumption. Recent epidemiological investigations also suggest that infections with calicivirus are increasing in communities.

Laboratory confirmation of viruses as the causes of foodborne and waterborne illness is usually based on demonstration of a specific immune response to the virus or detection of virus particles or antigen in the stool.

Until very recently detection of these viruses relied mainly on electron microscopy (EM); however, more recently molecular biology methods have been introduced that not only have increased the sensitivity but also the specificity of the diagnostic methods.

This review will discuss recent progress in diagnosis of foodborne viral infections in patients.

2. Virus infection

Viruses are obligate intracellular parasites and can

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thus only be transmitted by food and water, and do not replicate in these substrates. In contrast to bacteria, viruses contain either RNA or DNA, and their nucleic acid is protected from the environment by a protein capsid.

3. Epidemiology

Virtually all foodborne viruses are transmitted through the fecal–oral route, with man as the only known reservoir for calicivirus and hepatitis A, the two most important viruses associated with foodborne outbreaks. The incubation time for calicivirus is short, which makes it possible to associate a particular food product with a given outbreak. As the incubation time for hepatitis A is weeks, an epidemiological association of a particular food product to the outbreak is more difficult.

Viruses associated with acute gastroenteritis show two distinct patterns of epidemiology. Endemic diarrhea is associated with viruses, such as rotavirus, astrovirus, enteric adenovirus and calicivirus. These viruses infect nearly every child within the first years of life. A typical feature is that children acquire immunity early in life and then remain protected against symptomatic re-infections throughout adulthood. It is reasonable to assume, however, that adults are repeatedly re-infected without symptoms. Epidemic viral outbreaks often occur in schools, restaurants, cruise ships, etc., and are frequently associated with calicivirus. It should be pointed out that the distinction between endemic and epidemic viruses is not absolute; nonetheless, it permits a frame-work for the disease pattern and epidemiology of viral agents associated with acute diarrhea.

4. Poliovirus: the first virus associated with a foodborne outbreak

The only foodborne virus known before the second world war was poliovirus, which was transmitted through water and unpasteurized milk (Anonymous, 1988). Poliovirus is host-restricted to humans and thus cannot infect cows, but inadequate milk handling and lack of pasteurization sometimes allows infection to occur. Through a successful polio vaccination program and pasteurization of milk, poliovirus is not a problem nowadays. During the 1940s reports were presented indicating that hepatitis A could be transmitted via food, and during the 1950s hepatitis A was associated with an oyster-related outbreak in Sweden for the first time (Gard, 1957). Table 1 shows a summary of the viruses that are and could be associated with foodborne diseases

Table 1
Viruses associated with foodborne outbreaks

Major importance
Calicivirus
Hepatitis A
Viruses that occasionally can be transmitted via foods
Rotavirus
Picornavirus (echovirus)
TBE
Astrovirus
Hepatitis E

5. Viruses transmitted via food

5.1. Calicivirus

Calicivirus is the best studied group of the so-called small round structured viruses that produce diarrhea and vomiting in humans (Kapikian, 1994). Caliciviruses are non-enveloped viruses of about 30 nm in diameter with a single-stranded RNA genome (Figs. 1 and 2).

In 1990 the cloning and sequencing of a number of ‘Norwalk-like’ viruses led to the development of new, more sensitive detection methods, the application of which has expanded our understanding of the role that these viruses play in human disease. In the US, Sweden (unpublished) and other European countries, caliciviruses are the principal agents of non-bacterial gastroenteritis responsible for $>80\%$ of outbreaks (Vinje et al., 1997; Rebecca et al., 1998). Calicivirus is considered to be spread predominantly person-to-person via the fecal–oral route, often via a vehicle such as food or water.

Norwalk virus is the prototype strain of calicivirus, and was first described in Ohio, USA (Kapikian et al., 1972). Infection with calicivirus results, within 24–48 h, in symptoms including nausea, diarrhea, vomiting, abdominal cramps, fever and malaise (Kapikian, 1994). Caliciviruses are

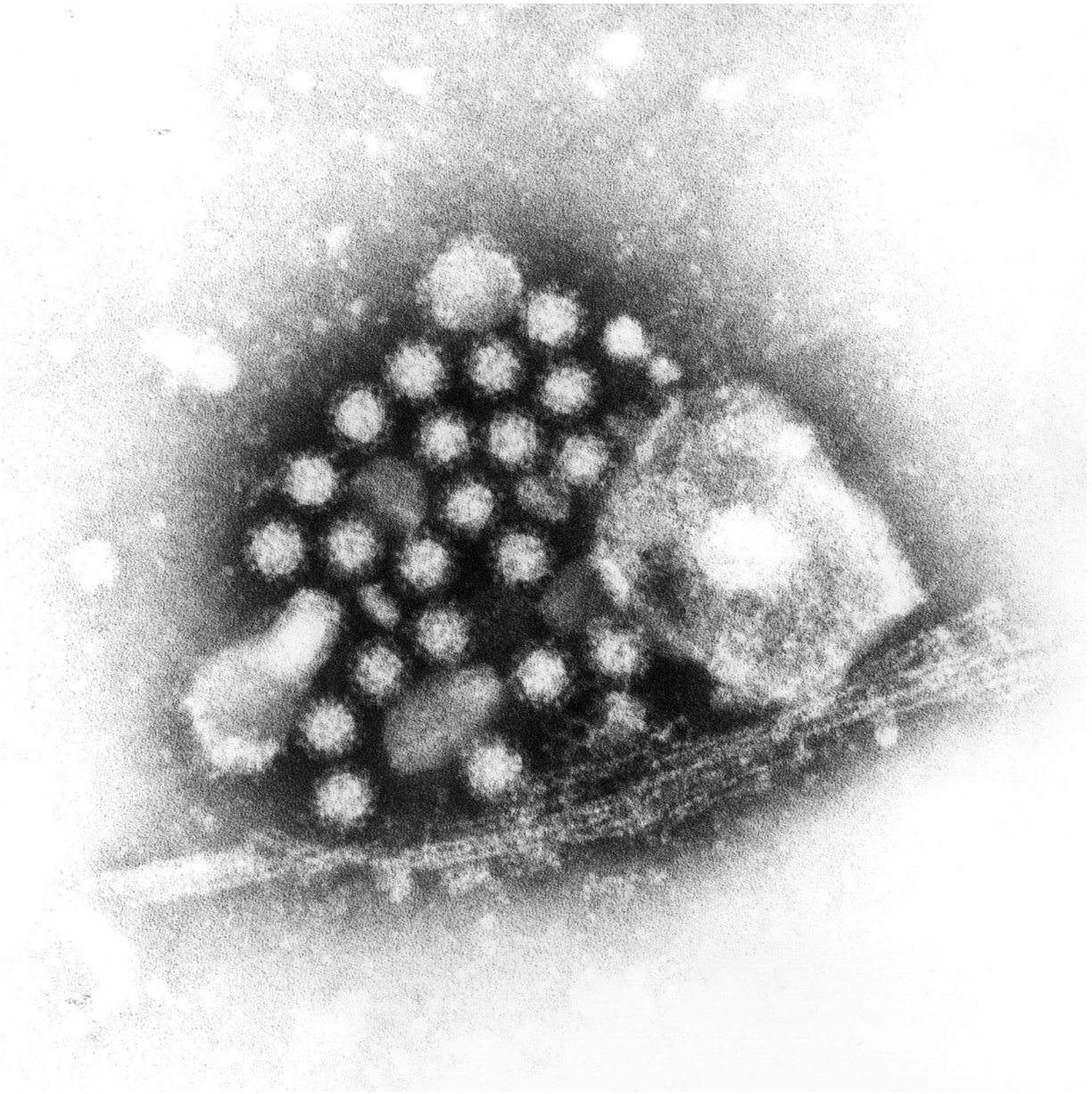

Fig. 1. Electron micrograph of calicivirus in a clinical specimen. Original magnification, × 300 000.

highly infectious and thus frequently cause outbreaks in schools, cruise ships, restaurants and recreational camps (Kuritsky et al., 1984; Herwaldt et al., 1994; Dowell et al., 1995; Kohn et al., 1995). Transmission of the viruses has been documented by contaminated food (Kuritsky et al., 1984; Stevenson et al., 1994), especially oysters (Murphy et al., 1979; Dowell et al., 1995; Kohn et al., 1995), raspberries (Pönkä et al., 1999) and water (Lawson et al., 1991; Gray et al., 1997). Extensive efforts have been made to grow human calicivirus in cell culture, but all attempts have failed and many basic features of these viruses are thus unresolved. Animals that have been challenged with calicivirus include rabbits, mice, monkeys, cats, calves, guinea pigs, marmosets, baboons and chimpanzees. Of the animals tested, only chim-

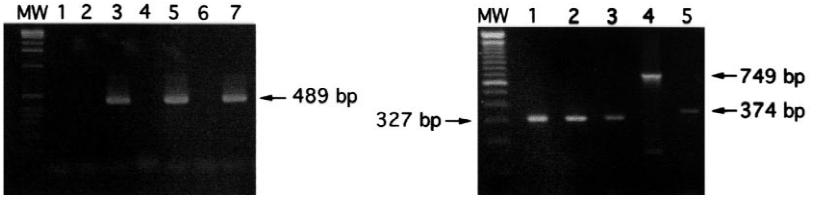

Fig. 2. Detection of calicivirus, hepatitis A and rotavirus group A in clinical specimens by PCR. (A) Detection of hepatitis A and (B) detection of caliciviruses (327 bp) and group A rotaviruses, serotype 1 (749 bp) and serotype 3 (374 bp).

panzees have shown evidence for infection with Norwalk virus, as determined by the demonstration of a serological response to the virus.

5.2. Classification of human caliciviruses by genome and serotype

A major obstacle in the characterization of human caliciviruses has been the difficulty to establish serotype specificities by viral neutralization assays, which is entirely due to the lack of in vitro systems. Alternative approaches not requiring cell cultures have therefore been explored for identification of antigenic differences. Early studies included human volunteer cross-challenge studies. Such studies revealed that the Norwalk virus and Hawaii viruses are antigenically distinct, as infection with one virus did not induce protective immunity against the other. Immune electron microscopy (IEM) using pre- and post-infection sera from volunteer studies have also been used to study antigenic relationships. In this technique, specific antibodies are mixed with a virus which results in virus coated with antibodies. Due to the requirement of an EM and the limited amount of reagents from volunteers, this method has its restricted use.

Due to the fastidious nature of Norwalk virus and other human caliciviruses, characterization has focused mainly on nucleotide sequence comparisons and less on antigenic characteristics. Antigenic grouping of caliciviruses has so far been based on studies with immune electron microscopy, solid-phase IEM and cross-challenge studies in volunteers. There are four recognized serotypes of human calicivirus: (i) Norwalk virus; (ii) Hawaii virus; (iii) Snow Mountain agent; and (iv) Taunton virus. More recently, four antigenic types have also been described in the UK represented by Norwalk virus and the prototype strains Bristol, Southampton and Melksham viruses.

Genetic analysis of a region of the genome encoding the RNA polymerase, and in a few cases the capsid protein, has resulted in a molecular classification based on nucleotide sequence homology. Based on these data, viruses can be phylogenetically divided into three genetic groups with genogroup I and II viruses representing the Norwalk-like viruses (NLV), or small round structured viruses) and genogroup III or Sapporo-like viruses (SLV) representing the group of typical human caliciviruses (Table 2)

5.3. Hepatitis A

When hepatitis transmission via food and contaminated water first was recorded it was not known that

Table 2
Classification of caliciviruses

Genera	Species
Vesivirus	Feline calicivirus
	Vesicular exanthema of swine virus
Lagovirus	Rabbit hemorrhagic disease virus
	European brown hare syndrome virus
Norwalk-like viruses (NLV)	*Human*
	Norwalk virus (genogroup I)
	Southampton virus (genogroup I)
	Desert Shield virus (genogroup I)
	Hawaii virus (genogroup II)
	Snow Mountain virus (genogroup II)
	Animal
	Bovine Newbury agent (genogroup I)
Sapporo-like viruses (SLV)	*Human*
	Sapporo virus (genogroup III)
	Manchester virus (genogroup III)
	Animal
	Porcine enteric calicivirus

multiple types of viral hepatitis existed, nor how many of these could be transmitted by the fecal–oral route. Hepatitis A is a positive-stranded RNA virus belonging to the hepatovirus group of picornaviruses, and is one of the most serious illnesses associated with shellfish disease. The first documented outbreak of shellfish-borne hepatitis occurred in Sweden in 1956 with 629 cases associated with consumption of raw oyster (Roos, 1956). Approximately 7% of the reported cases of infectious hepatitis are attributed to the consumption of raw or inadequately cooked shellfish (Gerba and Goyal, 1978; Cliver et al., 1983). It should be noted that hepatitis A illness has also been associated with consumption of raspberries (Reid and Robinson, 1987; Ramsay and Upton, 1989) and strawberries (Niu et al., 1992; Anonymous, 1997a,b; Hutin et al., 1999).

Most hepatitis A infections result from contact with infected persons, but a few percent are associated with a food- and waterborne mode of transmission. A common source of foodborne hepatitis A outbreaks is contamination of food by an infected food handler. Outbreaks due to contamination of food before retail distribution are less common and have mainly been associated with shellfish.

6. Viruses that can occasionally be transmitted via food

6.1. Astrovirus

Astroviruses comprise a distinct group of viruses that is responsible for a few percent of all cases of acute gastroenteritis. The virus is assigned to a newly established virus family, *Astroviridae*. Astrovirus was first identified in 1975 in the stool of an infant with diarrhea, and features of the disease include short incubation time resulting in usually mild diarrhea effecting children more often than adults. Epidemiological evidence on transmission via food is limited.

6.2. Rotavirus

Rotaviruses are the most important etiological agents of severe gastroenteritis causing approximately 870 000 deaths every year in developing countries. Virtually all children world-wide have experienced a clinical infection with rotavirus by the age of 3 years, resulting in significant morbidity in developed countries. Mature rotavirus particles measure about 75 nm in diameter and possess a triple-layered protein capsid. The virus genome comprises 11 segments of double-stranded RNA, each of which encodes a single protein. Rotaviruses are classified into six serogroups (A–F) based on genomic and antigenic properties. Only groups A–C have been found in humans and, among them, group A rotavirus is by far the clinically most important. While group A rotavirus is the most important, they are rarely associated with food- or waterborne outbreaks. Group B rotavirus, a virus that in humans has only been found in China, is frequently associated with waterborne outbreaks, sometimes with significant mortality (Hung et al., 1984; Fang et al., 1989). Group C rotavirus is rarely detected due to inadequate or lack of diagnostic methods (Bonsdorf and Svensson, 1988; Maunula et al., 1992). The virus appears to infect older children and adults more frequently than young children, a feature different from group A rotavirus which mainly causes disease in children between 6 and 24 months. Group C rotavirus has been associated with a large foodborne outbreak in Japan affecting schoolchildren (Matsumoto et al., 1989).

6.3. Hepatitis E

Hepatitis E has a single (+)-stranded RNA surrounded by a protein capsid. Infection with hepatitis E occurs frequently in Latin America, Africa and Asia, but rarely in Europe. Hepatitis E is known to be transmitted by the fecal–oral route. While waterborne outbreaks occur, foodborne outbreaks have not yet been documented.

6.4. Tick-borne encephalitis (TBE)

TBE virus belongs to the flavivirus family and is the only known foodborne virus that is not transmitted by a fecal–oral route (Gresíková, 1994). TBE is also the only enveloped virus known to be associated with foodborne infections. The virus infects dairy animals mainly in central Europe via bits of its ticks, *Ixodes riccinus* and *I. persulcatus*. Infected animals shed the virus in their milk, which if ingested without pasteurization may infect

humans. Products made from unpasteurized milk may also be vehicles. A recent outbreak of TBE presumably associated with milk consumption was reported in 1994 (WHO, 1994)

7. Food associated with virus transmission and disease

Numerous viral illnesses have been associated with consumption of contaminated shellfish. It is not surprising that mollusks, i.e., oysters, cockles, mussels and clams, which are filter feeders are frequently associated with non-bacterial foodborne gastroenteritis. Previously hepatitis A infection was the predominant disease, but today caliciviruses are emerging as the single most important pathogens associated with gastroenteritis and consumption of shellfish. The implicated food products are typically eaten raw, or cold without reheating. Food typically associated with foodborne outbreaks is shown in Table 3.

8. Diagnosis of foodborne viruses

In the past, investigations of foodborne viral gastroenteritis outbreaks relied on epidemiological methods to demonstrate the association of illness with the consumption of particular food. Diagnostic methods included examination of stool specimens by EM and sometimes the measurement of an antibody response. The lack of adequate diagnostic methods lead to difficulties in linking illness in the food handler or consumer to an outbreak strain, or in determining if patients had become ill from a virus originating from a common source.

The recent development of molecular methods for a variety of viruses associated with foodborne illness has allowed for a diagnosis of gastroenteritis and hepatitis with increased sensitivity and specificity, and provided tools to perform molecular epidemiology aimed to identify strains responsible for outbreaks.

Table 3
Food products frequently associated with viral transmission

Shellfish (oyster)
Raspberries
Strawberries
Bakery products
Salads
Sandwiches
Cold cooked meat

8.1. Calicivirus

A variety of methods have been used to identify caliciviruses in clinical samples from foodborne and waterborne outbreaks. In England electron microscopy has been used to identify viruses associated with consumption of seafood (Appleton and Pereira, 1977; Appleton et al., 1981). In Japan, Finland and Sweden EM has been used to identify Norwalk-like viruses from several foodborne outbreaks. During the 1970s IEM was frequently used not only to identify a specific virus in the stool (Kapikian et al., 1972), but also for virus classification (Lewis et al., 1988, 1995; Le Guyader et al., 1996). However, limited by poor sensitivity and short of immunological reagents IEM remained impractical for most public health investigations.

Recent advances in the cloning of the Norwalk virus genome and the expression of the capsid protein from Norwalk virus and related members of the calicivirus family (Jiang et al., 1990, 1992a,b) have provided a simplified and reproducible means for detection of calicivirus antibodies (Gray et al., 1993, 1994; Graham et al., 1994; Parker et al., 1994; Hinkula et al., 1995) and caliciviruses (Parker et al., 1993; Herrmann et al., 1995; Jiang et al., 1995) by ELISAs. However, due to the great immunological diversity of the members within the calicivirus family, current immunological assays are still not optimal.

8.2. RT-PCR

To overcome the obstacle with detection of only a narrow range of calicivirus strains by ELISA and EM, PCR methods have been established and evaluated by several groups (De Leon et al., 1992; Green et al., 1993, 1995; Ando et al., 1994; Moe et al., 1994). The application of RT-PCR techniques for detection of caliciviruses in stools have meet two major problems which have hindered the development of a generic RT-PCR recognizing all caliciviruses. The first problem concerns the presence of

PCR amplification inhibitors. A range of techniques have been employed in an attempt to remove inhibitory substances prior to RT-PCR and to optimize the recovery of viral RNA. A number of modifications to phenol–chloroform extraction and ethanol precipitation of viral RNA have been developed. CF-11 cellulose and cetyl trimethylammonium bromide (CTAB) (Jiang et al., 1992) have been tried to remove inhibitors. Guanidinium thiocyanate (GTC) combined with silica to bind viral RNA is another method which has been explored (Boom et al., 1990). Another method used is gel chromatography using spin column (De et al., 1992) and heat release of viral RNA (Schwab et al., 1997). In a recent comparison between four different methods GTC/silica proved to be the most efficient in removing inhibitory substances (Hale et al., 1996). In agreement with Hale et al. (1996), we have found that GTC/silica is the most efficient to remove inhibitory substances.

The second major problem concerning the development of a generic RT-PCR is the genomic heterogeneity among caliciviruses. While current RT-PCRs have been proven to be more sensitive than electron microscopy for a given strain, it is limited by the narrow range of viruses that are detected. This is due to difficulties in identifying conserved regions within the genome, and thus the lack of broadly reactive primers. However, Vinje and co-workers (Vinje and Koopmans, 1996; Vinje et al., 1997) recently reported generic primers (Table 4) that appear to detect most genogroup I and II viruses in Europe. We have evaluated these primers and found them to recognize most of our EM-positive Norwalk-like viruses (unpublished).

8.3. Rotavirus

Detection of rotaviruses in clinical specimens has become routine and includes diagnostic methods such as EM, IEM, RNA gel electrophoresis and ELISA (Svensson et al., 1983, 1986), with ELISA methods being the most commonly used for detection of rotavirus. More recently a specific and sensitive PCR method was described (Gouvea et al., 1990) that allowed not only detection and serotyping (Table 4) of group A rotavirus in clinical specimens, but also detection of rotavirus in shellfish and water (Le Guyader et al., 1994; Gilgen et al., 1997). PCR

Table 4
Primers used for detection of viruses associated with food- and waterborne diseases

Virus	Primer-pair	Sequence $5' > 3'$	Refs.
Calicivirus	JV12	ATACCACTATAGTGCAGATTA	Vinje and Koopmans (1996)
(NV-like)	JV13	TCATCATCACCATAGAAAGAG	
Rotavirus	Beg9	GGCTTTAAAAGAGAGAATTTCCGTCTGG	Gouvea et al. (1990)
Group A	End9	GGTCACATCATACAATTCTAATCTAAG	
ST1	aBT1	CAAGTACTCAAATCAATGATGG	
ST2	aCT2	CAATGATATTAACACATTTTCTGTG	
ST3	aET3	CGTTTGAAGAAGTTGCAACAG	
ST4	aDT4	CGTTTCTGGTGAGGAGTTG	
ST8	aAT8	GTCACACCATTTGTAAATTCG	
ST9	aFT9	CTAGATGTAACTACAACTAC	
Group B	B1	CTATTCAGTGTGTCGTGAGAGG	Gouvea et al. (1991)
	B3	CGAAGCGGGCTAGCTTGTCTGC	
	B4	CGTGGCTTTGGAAAATTCTTG	
Group C	C1	CTCGATGCTACTACAGAATCAG	Gouvea et al., 1991
	C3	GGGATCATCCACGTCATGCG	
	C4	AGCCACATAGTTCACATTTCATCC	
Hepatitis A	H1	GGAAATGTCTCAGGTACTTTCTTTG	Le Guyader et al. (1994)
	H2	GTTTTGCTCCTCTTTATCATGCTATG	
	H3	TCCTCAATTGTTGTGATAGC	
	Hp	TCAACAACAGTTTCTACAGA	

methods for group B and C rotaviruses have also been established (Gouvea et al., 1991).

8.4. Hepatitis A (HAV)

In contrast to calicivirus and rotavirus where the primarily goal for a clinical diagnosis is to detect the antigen or detect specific nucleic acids in the stool, the clinical diagnosis of hepatitis A is primarily performed by detection of IgM anti-HAV antibodies in serum but also PCRs for detection of HAV RNA in stool (Niu et al., 1992) and serum (Hutin et al., 1999) have been reported.The clinical diagnosis of acute hepatitis is made by biochemical assessment of liver function, and should include testing of urine and serum for bilirubin. Apart from detection of HAV RNA in clinical samples, methods have also been established to detect HAV RNA in shellfish (Le Guyader et al., 1994; Atmar et al., 1995) and water (Gilgen et al., 1997).

9. Summary

While food- and waterborne virus outbreaks occur frequently, and have a major impact on our lives, identification and characterization of the responsible pathogens has been hampered by lack of adequate diagnostic methods. However, recent development in molecular biology methods for detection of these pathogens in clinical specimens, and in some cases even in food and water, have clearly shown that viruses, and in particular caliciviruses, play a significant role in foodborne diseases.

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