CLINICAL MICROBIOLOGY REVIEWS, Jan. 2006, p. 63–79 Vol. 19, No. 1
0893-8512/06/$08.00+0 doi:10.1128/CMR.19.1.63–79.2006

Diagnosis of Hepatitis A Virus Infection: a Molecular Approach

Omana V. Nainan,[1]† Guoliang Xia,[1]* Gilberto Vaughan,[1,2] and Harold S. Margolis[1,3]

Division of Viral Hepatitis, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[1]; Institute of Diagnosis and Epidemiologic References, Mexico City 11340, Mexico[2]; and Pediatric Dengue Vaccine Initiative, International Vaccine Institute, Seoul, Korea[3]

INTRODUCTION

The disease described as "jaundice" in ancient Greek, Roman, and Chinese literature probably was viral hepatitis. A viral etiology was postulated as the cause of certain forms of jaundice as early as 1912, and the term "infectious hepatitis" was used because the disease often occurred in epidemics (43). Hepatitis A, a term first introduced by Krugman et al. in 1967 (138), is now known to be caused by infection with hepatitis A virus (HAV), one of five viruses, each belonging to a different family, whose primary site of replication is the liver.

Early epidemiological studies further characterized hepatitis into infectious and serum forms, based on patterns of disease transmission (17, 82, 102, 103, 104). Epidemiologic and transmission studies with humans showed that infectious hepatitis, or hepatitis A, was transmitted primarily by the fecal-oral route (136, 137, 138). In 1973, HAV was identified in the stools of infected persons (80), which eventually led to development of diagnostic tests, propagation in cell culture, molecular characterization, and development of a vaccine (80, 193).

HEPATITIS A VIRUS INFECTION

Clinical Features

Infection by HAV is generally self-limited and can produce effects that range from a lack of symptoms to death from

* Corresponding author. Mailing address: Centers for Disease Control and Prevention, 1600 Clifton Road, N.E., Mailstop A33, Atlanta, GA 30333. Phone: (404) 639-2339. Fax: (404) 639-1563. E-mail: ovn1@cdc.gov.
† Deceased.

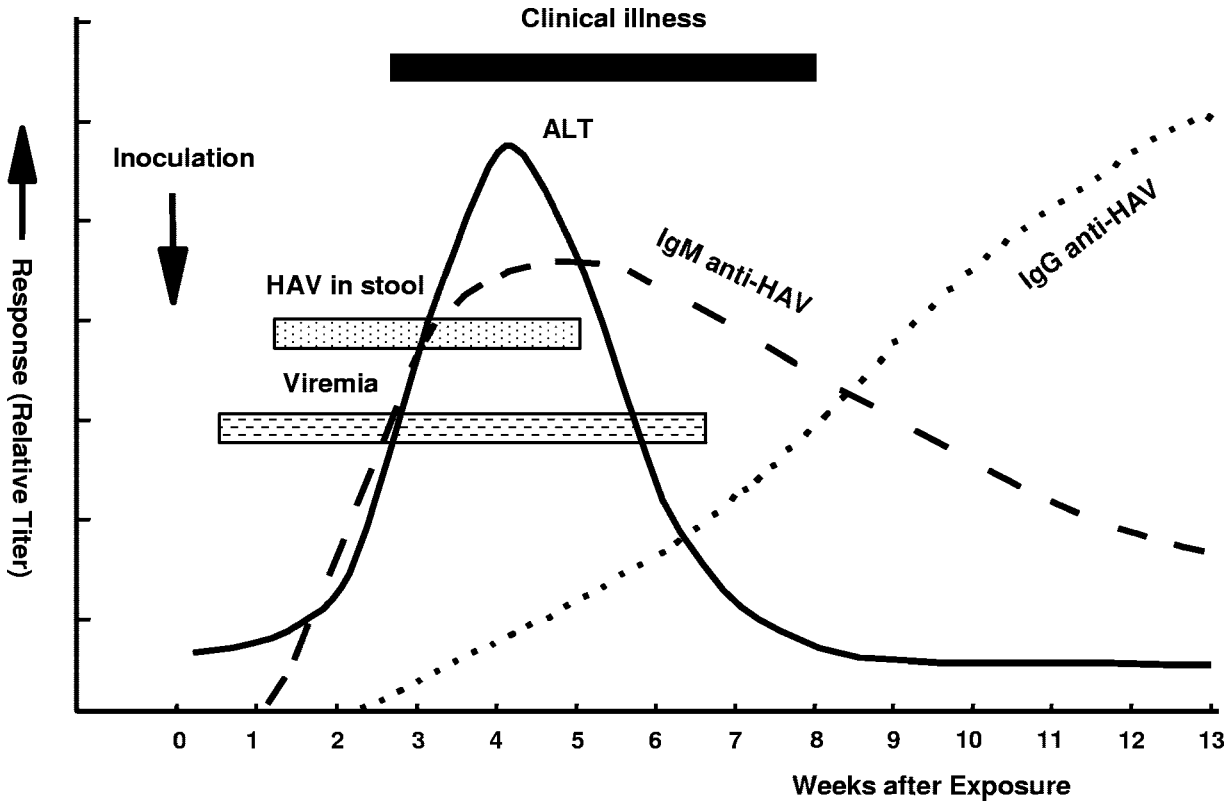

FIG. 1. Virologic, immunologic and biochemical events during the course of experimental hepatitis A virus infection in chimpanzees inoculated intravenously with human HAV, strain HLD2. ALT, alanine aminotransferase. (Adapted from reference 157 with permission of the publisher.)

fulminant hepatitis. The likelihood of clinically apparent disease associated with HAV infection increases with age. In children <6 years of age, most infections (70%) are asymptomatic (98), and if illness does occur, it is usually anicteric. Among older children and adults, infection is usually symptomatic, with jaundice occurring in >70% of patients (141). After an average incubation period of 28 days (range, 15 to 50 days), most HAV-infected persons developed nonspecific constitutional signs and symptoms followed by gastrointestinal symptoms. Typically, these include fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice, all of which usually last <2 months. There is no evidence of chronic liver disease or persistent infection following infection. However, 15 to 20% of the patients may have prolonged or relapsing disease lasting up to 6 months (93, 218), and HAV has been detected in serum for as long as 6 to 12 months after infection (19).

Fulminant hepatitis is a rare complication of hepatitis A. The risk of acute liver failure ranges from 0.015 to 0.5%, and the highest rates occur among young children and older adults with underlying chronic liver disease (2, 31). In a large epidemic in Shanghai, China, involving over 300,000 adolescents and young adults, 47 deaths (0.015%) were reported (100, 263). Other than older age and underlying chronic liver disease, no other host factors have been identified as predisposing patients to fulminant hepatitis A (250). Among persons referred to a tertiary care center with acute hepatic failure in the United States, 20 of 295 (6.8%) had hepatitis A (210). No single viral factor has been associated with fulminant disease. Reported findings among persons with fulminant disease include lower viral load (198) and nucleotide and/or amino acids substitutions in the 5′ untranslated region (5′UTR), P2 region, and P3 region of the HAV genome (86; CDC, unpublished data).

Pathogenesis and Natural History of HAV Infection

The pathogenetic events that occur during HAV infection have been determined in experimental infection of nonhuman primates and natural infection of humans (Fig. 1). HAV is primarily hepatotropic; it replicates in the liver, produces a viremia, and is excreted in bile and shed in the stools of infected persons. Feces can contain up to 10^9 infectious virions per gram and is the primary source of HAV infection (221, 241). Peak fecal excretion, and hence infectivity, occurs before the onset of jaundice, symptoms, or elevation of liver enzymes (221, 241) and declines after jaundice appears. Compared to adults, children and infants can shed HAV for longer periods, i.e., up to several months after the onset of clinical illness (35, 241). Fecal shedding of HAV has been shown to occur as late as 6 months after diagnosis of infection in premature infants (205).

Viremia occurs within 1 to 2 weeks after HAV exposure and persists through the period of liver enzyme elevation, based on studies in humans and experimentally infected chimpanzees (8, 48, 91, 137, 145, 157). Virus concentrations in serum are 2 to 3 $\log_{10}$ units lower than those in stool (19, 48, 145). An analysis of serum specimens collected prospectively during human and chimpanzee HAV infection showed that HAV RNA was

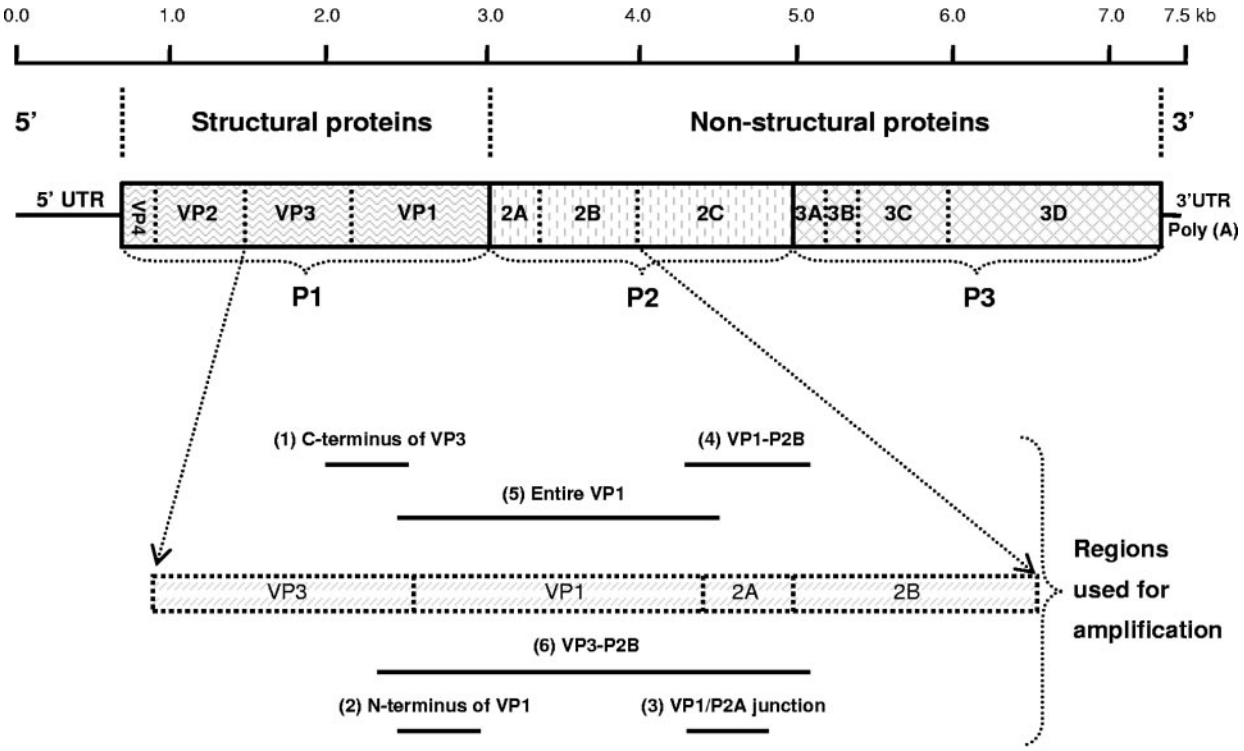

FIG. 2. Schematic representation of the HAV genome organization, translation products, and regions used for amplification. The area encoding the polyprotein is represented by solid box and the proposed cleavage sites by vertical lines. Regions commonly used for PCR amplifications are as follows: region 1, C terminus of VP3 region (nt 2,020 to nt 2,226); region 2, N terminus of VP1 region (nt 2,172 to nt 2,415); region 3, VP1/P2A junction region (nt 2,984 to nt 3,217); region 4, VP1-P2B region (nt 2,896 to nt 3,289); region 5, entire VP1 region (nt 2,172 to nt 3,125); and region 6, VP3-P2B region (nt 2,133 to nt 3,289). Nucleotide position numbering is according to the HM175 sequence (46).

present for ~3 to 4 weeks before the onset of jaundice and that virus concentrations were highest during the period that precedes onset of liver enzyme elevations (19). Viremia may be present for a much longer period during the convalescent phase of hepatitis A than was previously appreciated (19, 84, 264), although virus concentration is lowest during this period.

The virus is also shed in saliva in most hepatitis A patients. In experimentally infected marmosets (156, 187), the viral load appears to be 1 to 3 $\log_{10}$ units lower than that found in serum (156). However, no epidemiological data suggest that saliva is a significant source of HAV transmission.

A humoral immune response to HAV structural proteins occurs prior to onset of symptoms. Immunoglobulin M (IgM) antibodies to HAV (IgM anti-HAV) are detectable at or prior to onset of clinical illness, decline in about 3 to 6 months, and become undetectable by commercially available diagnostic tests (127). IgG antibodies to HAV (IgG anti-HAV) appear soon after IgM, persist for years after infection, and confer lifelong immunity (220). IgA is also produced during infection for a limited time (4, 152). The role of IgA antibodies in the response against HAV is still unknown. Unlike other *Picornaviridae* family members, HAV does not seem to elicit an effective intestinal immune response (229).

IgG and IgA anti-HAV are detected in serum, saliva, urine, and feces (39, 60, 115, 123, 151, 152, 177, 178, 183, 214). Saliva tests have been reported as an alternative to conventional serum testing for anti-HAV due to their simplicity of sample collection (115, 177, 178). Several studies have demonstrated the benefits of implementing saliva testing as screening tool in outbreak investigations and epidemiological studies (110, 115, 140). However, the sensitivity of detecting anti-HAV in saliva is 1 to 3 $\log_{10}$ units lower than that with serum (156, 177, 178; CDC, unpublished data).

Antibodies against nonstructural proteins are also produced, although their role in maintenance of immunity is probably less important than that of antibodies to capsid antigens due to their low concentration and lack of neutralization capacity. Antibodies to nonstructural proteins have been detected in humans and experimentally infected chimpanzees but are absent in vaccinated individuals (126, 202). However, because of what appears to be a variable host antibody response during HAV replication, a diagnostic test for these antibodies, which could be used to complement current anti-HAV testing and differentiate previously infected from vaccinated persons, has not been developed (201, 233).

HEPATITIS A VIRUS

HAV is a nonenveloped RNA virus 27 to 32 nm diameter in size, with an icosahedral symmetry, which belongs to the genus *Hepatovirus* of the *Picornaviridae* family. Unlike other members of the family, HAV requires a long adaptation period to grow in cell culture, replicates slowly, and rarely produces a cytopathic effect (57, 107, 146). HAV is stable in the environment for at least 1 month (162) and is more resistant to heating and chlorine inactivation than is poliovirus. Inactivation of HAV

TABLE 1. HAV genome organization and open reading frame[a]

HAV genome segment	Nucleotides	Polyprotein amino acids	Known function(s)
5′UTR	1–735 (741)[b]		Unclear, translation initiation
P1	735–3029	1–765	Capsid Proteins
1A	735–803	1–23	VP4
1B	804–1469	24–245	VP2
1C	1470–2207	246–491	VP3
1D	2208–3029	492–765	VP1
P2	3030–5000	766–1422	Nonstructural Proteins
2A	3030–3242	766–836	Morphogenesis
2B	3243–3995	837–1087	?
2C	3996–5000	1088–1422	RNA synthesis
P3	5001–7415	1423–2227	Nonstructural proteins and VPg
3A	5001–5222	1423–1496	Pre-VPg
3B	5223–5291	1497–1519	VPg
3C	5292–5948	1520–1738	Protease
3D	5949–7415	1739–2227	RNA polymerase
3′UTR	7416–7478		Unknown, translation terminator, poly(A)

[a] Reference HAV strain, HM175 (subgenotype IB, accession no. M14707) (46, 107).
[b] Downstream AUG codon at nt 741 to 743 preferred for translation initiation.

requires heating foods to >85°C for 1 min, and disinfection of surfaces requires 1 min of contact with a 1:100 dilution of sodium hypochlorite (i.e., household bleach), whereas poliovirus is inactivated at 72°C for 15 s and by treatment with a 1:125 dilution of bleach for 30 s (159, 234, 257).

Genomic Organization

HAV has a positive-polarity, single-stranded 7.5-kb genome (45, 46, 171) that is organized similarly to those of the other picornaviruses (Fig. 2). The 5′UTR is 734 to 740 nucleotides (nt) long and has a covalently linked virus-specific protein (VPg) rather than a cap structure (259). Translation occurs in a cap-independent fashion under control of an internal ribosome entry site located within the 5′UTR (21). The translation terminator sequence, the 3′UTR of 40 to 80 nucleotides, has a poly(A) tract (46) (Table 1). The remainder of the genome is composed of a single open reading frame with three distinct regions (P1, P2, and P3) and is translated as a single polyprotein of 2,225 to 2,227 amino acids (10, 46). This polyprotein subsequently undergoes cleavage mediated by a viral protease (3Cpro) (211), which results in production of four capsid and several nonstructural proteins (Fig. 2; Table 1).

HAV Genetic Diversity

HAV displays a high degree of antigenic (amino acid) and genetic (nucleotide) conservation throughout the genome (45, 46, 147, 200, 207). However, enough genetic diversity exists to define several HAV genotypes and subgenotypes (200). The entire nucleic acid sequences of several HAV strains have been determined by molecular cloning (10, 45, 46, 96, 171, 182, 243, 244), and a large number of HAV isolates have been characterized by sequencing of short genome segments. The genomic regions most commonly used to define HAV genotypes include (i) the C terminus of the VP3 region (116), (ii) the N terminus of the VP1 region (5, 112, 199, 203), (iii) the 168-bp junction of the VP1/P2A regions (116, 200, 203), (iv) the 390-bp region of the VP1-P2B regions (112, 170), and (v) the entire VP1 region (52, 167) (Fig. 2; Table 2).

When sequence variation within the VP1/P2A junction is used to define genotypes and subgenotypes, genotypes have >15% nucleotide variation between isolates and subgenotypes have 7 to 7.5% nucleotide variation (200). Seven HAV genotypes have been identified; four genotypes (I, II, III, and VII) are of human origin, and three (IV, V, VI) are of simian origin. Genotypes II and VII were initially defined based on a single isolate for each (34, 200). However, further investigations have reclassified genotype VII as a subgenotype of genotype II (52, 154). Genotypes I and III are the most prevalent genotypes isolated from humans.

The three simian genotypes were each defined by unique nucleotide sequences from the P1 regions of HAV strains recovered from species of Old World monkeys. In addition, all simian HAVs have a distinct signature sequence at the VP3/VP1 junction which distinguishes these strains from human HAVs (20, 167, 248). Genotype IV was recovered from a cynomolgus macaque (*Macaca fasicularis*) imported from the Philippines (167). The prototype strain of genotype V, AGM27, was isolated from an African green monkey (*Cercopithecus aethiops*) imported from Kenya (248). Genotype VI was also isolated from a cynomolgus macaque (*M. fasicularis*) imported from Indonesia (167, 200).

HAV Proteins

Although HAV was successfully adapted to cell culture 25 years ago (193), its protein components have not been completely defined (107, 146). Infected cells contain low titers of virus, and consequently protein chemistry has been limited. The P1 region encodes the three major proteins of the viral capsid, i.e., VP1, VP2, and VP3 (90). A fourth viral capsid protein (VP4), which is essential for virion formation, is not detected in mature viral particles. Capsid proteins are primarily cleaved from the precursor polyprotein by the viral protease 3C (211), which is encoded in the P3 region. However, it remains unclear how the VP2/VP4 cleavage occurs. The native conformation of the VP1 and VP3 capsid proteins forms a single, dominant, antigenic epitope on the viral surface, which elicits a neutralizing antibody response. The predicted functions of nonstructural proteins encoded by the P2 and P3 regions are RNA synthesis and virion formation. VPg is also encoded in the P3 region (259) (Table 1; Fig. 2).

Antigenicity and Serotype

Only a single serotype of HAV exists, despite genetic heterogeneity at the nucleotide level. Individuals infected by HAV in one part of the world are protected from reinfection by HAV from other parts of the world. Immune globulin preparations containing anti-HAV, irrespective of their geographic origin, appear to provide protection from disease, and vaccines

TABLE 2. Oligonucleotide primers used for PCR amplification of HAV RNA from clinical specimens

Region (reference[s])	Primer type[a]	Primer[b]	Nucleotide sequence (5′→3′)	Position[c]
C terminus of VP3 (116)		2020P	ACAGGTATACAAAGTCAG	2020–2037
		2226N	CTCCAGAATCATCTCC	2226–2211
N terminus of VP1 (22, 112, 199, 202)	External	2133P	GTGAATGTTTATCTTTCAGCAAT	2133–2155
		2451N	GATCTGATGTATGTCTGGATTCT	2451–2429
	Internal	2172P	GCTCCTCTTTATCATGCTATGGAT	2172–2195
		2415N	CAGGAAATGTCTCAGGTACTTTCT	2415–2392
VP1/P2A junction (22, 116, 200)	External	2950P	TTGTCTGTCACAGAACAATCAG	2950–2972
		3308N	AGTCACACCTCTCCAGGAAAACTT	3308–3285
	Internal	2984P	TCCCAGAGCTCCATTGAA	2984–3001
		3217N	AGGGGGTGGAAGTACTTCATTTGA	3217–3193
VP1-P2B (112, 170)	External	2870P	GACAGATTCTACATTTGGATTGGT	2870–2893
		3381N	CCATTTCAAGAGTCCACACACT	3381–3360
	Internal	2896P	CTATTCAGATTGCAAATACAAT	2896–2918
		3289N	AACTTCATTATTTCATGCTCCT	3289–3268
Entire VP1 (52, 167)	External	2167P	GTTTTGCTCCTCTTTATCATGCTATG	2167–2192
		3308N	AGTCACACCTCTCCAGGAAAACTT	3308–3285
	Internal	2172P	GCTCCTCTTTATCATGCTATGGAT	2172–2195
		3125N	CCTGCATTCTATATGACTCT	3125–3106
VP3-P2B (CDC, unpublished data)	External	2020P	ACAGGTATACAAAGTCAG	2020–2037
		3381N	CCATTTCAAGAGTCCACACACT	3381–3360
	Internal	2133P	GTGAATGTTTATCTTTCAGCAAT	2133–2155
		3289N	AACTTCATTATTTCATGCTCCT	3289–3268

[a] External primers were used for first-round amplification, and internal primers were used for second-round nested amplification.
[b] P, positive-sense primer; N, negative-sense primer.
[c] Positions relative to the genome of HAV strain HM175 (accession no. M14707).

prepared from virus isolates originating in Australia or Costa Rica protect from infection worldwide (113, 172).

The antigenic structure of the virus is relatively simple, with a restricted number of overlapping epitopes combining to form a single dominant antigenic site that interacts with virus-neutralizing antibodies. These epitopes are highly conformational and are formed by amino acid residues located on more than one capsid protein (168, 185, 186, 228). Convalescent-phase sera obtained from hepatitis A patents are reactive primarily to VP1 and to a lesser extent to VP0 and VP3 (254). Recombinant VP1 fusion protein expressed in *Escherichia coli* reacted with rabbit anti-HAV serum (180). However, chimpanzees immunized with this recombinant protein produced antibodies that reacted with VP1 of only denatured and not intact virus, and the animals were not protected when they were challenged with wild-type HAV (CDC, unpublished data). Empty particles appear to be antigenically indistinguishable from infectious, RNA-containing virions, suggesting that antigenicity may depend on assembly of the major capsid proteins or smaller capsid precursors. An accurately processed and assembled recombinant HAV polyprotein has been produced, which was able to elicit neutralizing antibodies detected by commercial assays (139, 262).

Naturally occurring antigenic variants of HAV have been observed only among strains isolated from Old World monkeys (167, 248). These viruses are genetically distinct from human HAV isolates and are not recognized by certain monoclonal antibodies produced against human HAV (128, 167). However, simian HAV binds human polyclonal anti-HAV, and chimpanzees immunized with these viruses had an antibody response that was protective against infection with human HAV challenge (78; CDC, unpublished data). Recently, human HAV isolates with capsid amino acid substitutions and deletions in the immunodominant antigenic site were reported (52, 206). However, it is not clear if capsid antigenicity or virus neutralization was affected by these changes.

DIAGNOSTIC APPROACHES TO HAV DETECTION

Detection of HAV-Specific Antibodies

The humoral immune response plays the pivotal role in the diagnosis of HAV infection and the differentiation of hepatitis A from other types of viral hepatitis. There are a number of commercially available assays for the detection of IgM and total anti-HAV (71, 122, 188). IgM, IgA, and IgG anti-HAV are usually present at the onset of symptoms (Fig. 1). Since hepatitis due to HAV infection is clinically indistinguishable from disease caused by other hepatitis viruses (i.e., HBV, HCV, HDV, and HEV), serologic testing is required to make the diagnosis (230).

Antibodies to structural proteins. Diagnostically, IgM anti-HAV has been used as the primary marker of acute infection (58); it is comprised mainly of antibodies against capsid proteins. A number of methods have been used to detect this virus-specific antibody class, including radioimmunoassay (194), immunochemical staining (109), enzyme-linked immunosorbent assay (61), immunoblotting (254), and dot blot immunogold filtration (214). IgM anti-HAV enzyme immunoassays are available commercially (188) The commercially available diag-

nostic assays are configured in such a manner that although IgM antibodies may be present for long periods of time, the lower concentrations found 4 to 6 months after the onset of infection do not produce a positive test result (230). However, the current commercially available IgM assays detect antibody for a short period of time in persons recently administered hepatitis A vaccine (261).

Previous (resolved) HAV infection is diagnosed by detection of IgG anti-HAV. However, commercially available assays detect total anti-HAV (both IgG and IgM antibodies). The presence of total anti-HAV and the absence of IgM anti-HAV can be used to differentiate between past and current infections.

Antibodies to structural proteins are produced following immunization with hepatitis A vaccine. A small proportion (8 to 20%) of vaccinated persons have a transient IgM anti-HAV response (148, 216, 219). IgG anti-HAV is produced by all successfully immunized persons (148, 216, 219). However, unless they are modified, commercially available tests for total anti-HAV are not sensitive enough to detect antibody concentrations in a significant proportion of immunized persons, especially several years after immunization (28, 148).

Antigen Detection

Cell culture propagation. HAV has been grown in several cell types of human and nonhuman origins, including primary and secondary African green monkey kidney cells (59, 253) and fetal rhesus monkey kidney cells (83). In contrast to most picornaviruses, HAV of human origin requires an extensive adaptation period before it grows in cell culture, and once adapted, HAV produces a persistent infection and becomes attenuated, as shown by not producing disease in experimentally inoculated nonhuman primates (81). In addition, relatively low concentrations of virus and viral antigen are produced compared to other picornaviruses. Mutations in viral nucleic acid may play a major role in the adaptation of HAV in cell culture (47, 76, 77, 88) and attenuation (79, 81). HAV replicates in cell culture without cytopathic signs of infection and without apparent host cell damage. Because of the lack of a cytopathic effect in cell culture, immunological assays are required to detect HAV antigen (217). Methods commonly used to quantitate infectivity include radioimmunofocus assay (144), fluorescent focus assay, in situ radioimmunoassay (217), and in situ hybridization (121).

Cytopathic variants of HAV have been observed in selected cell culture systems. These cytopathic viruses produce an acute rather than persistent infection (55, 173). The replication cycle of these variants is shorter (2 to 3 days) than that observed for noncytopathic HAV, and they produce a much higher viral yield (55).

Detection in clinical and environmental samples. HAV was first visualized in fecal extracts by electron microscopy using homologous antiserum (80), and similar virus-like particles were observed in the sera and livers of marmosets experimentally infected with human HAV (192). HAV antigen has been detected in stool, cell culture, and environmental samples by using radioimmunoassays and enzyme immunoassays (107, 165). Viremia during HAV infection has been documented both by transmission studies (138) and as a result of outbreaks of posttransfusion hepatitis A (176). However, detection of antigen in blood has been difficult (106) because fibronectin can bind to HAV and mask antigenic determinants required for immunological detection (213). HAV capsid polypeptides and viral RNA have been detected in IgM circulating immune complexes isolated from experimentally infected chimpanzees (158).

Molecular Detection Methods

Nucleic acid detection techniques are more sensitive than immunoassays for viral antigen to detect HAV in samples of different origins (e.g., clinical specimens, environmental samples, or food). HAV has been detected with techniques such as restriction fragment length polymorphism (94), single-strand conformational polymorphism (85, 94), Southern blotting (25, 27, 169, 208), nucleic acid sequencing-based amplification (118, 119, 120), nucleic acid hybridization (266), and reverse transcription-PCR (RT-PCR) and antigen capture RT-PCR (56, 57, 116, 189,199, 200). Amplification of viral RNA by RT-PCR is currently the most sensitive and widely used method for detection of HAV RNA.

Purification of viral RNA from clinical and environmental samples is the first step in RT-PCR, and different extraction platforms are often required, depending on the source of the specimen. Early protocols for RNA extraction from serum or stool included proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation (167, 199). Subsequently, products which used guanidinium thiocyanate-phenol-chloroform (41) became commercially available for extraction of RNA and total nucleic acids (40) and increased the sensitivity and specificity of HAV detection (65, 112, 170). Antigen capture RT-PCR (116) and magnetic beads coated with anti-HAV (124) have been used to separate virus from potential inhibitors of reverse transcription and PCRs that are often found in environmental and stool samples. Automated RNA extraction protocols have been applied to HAV detection. These have increased specimen throughput, are reproducible and reliable, and have detection sensitivity similar to that of manual extraction methods (142; CDC, unpublished data).

The efficiency of reverse transcription of RNA to cDNA can be reduced by the presence of inhibitors in the source material. Engineered reverse transcriptase with no RNase activity (RNase H) is believed to increase transcription efficiency, although naturally occurring enzymes continue to be used for identification of a variety of targets, including HAV, with high specificity and sensitivity (169, 226, 227). Although thermostable enzymes may improve efficiency of the RT reaction by reducing secondary structure and improving priming, their effect on HAV detection has not been evaluated. Specific or random primers can be used for the reverse transcription reaction (226, 227). Random primers along with specific primers have been routinely used in our laboratory for the detection of HAV because of the increased sensitivity and specificity (CDC, unpublished data).

Primer pairs (Table 2; Fig. 2) spanning the desired genomic region are used to amplify cDNA. Nested PCR, where products obtained from first-round PCR are used as a template for a second round of PCR, has been used to amplify HAV from clinical and environmental samples where the viral load is

expected to be low (19, 72, 92, 149, 169). Analysis of the PCR product by probe hybridization also has been shown to increase the sensitivity of detection (62, 95, 116).

The development of single-step RT-PCR methods, in which reverse transcription and PCR are performed together, has considerably reduced the time and handling during cDNA synthesis (72, 149). However, this method appears to reduce detection sensitivity by up to 1 log unit compared to the two-step RT-PCR method (CDC, unpublished data).

Multiplex RT-PCR, where genome sequences of more than one organism are amplified simultaneously, provides the most efficient way to detect multiple agents in clinical and environmental samples compared to conventional RT-PCR amplification for each agent. This method has been developed for simultaneous detection of HAV and HEV (125) and of HAV, rotavirus, and poliovirus (92, 247).

Real-time PCR, which has revolutionized nucleic acid detection by its high speed, sensitivity, and reproducibility and minimization of contamination, has been applied to the detection and quantification of HAV (53). Real-time PCR has been introduced in our laboratory for rapid analysis of specimens in outbreak situations, with <36 h required from amplification to nucleic acid sequence results. The increased speed of real-time PCR is mainly due to reductions in amplification cycles, elimination of post-PCR detection procedures, and availability of devices for sensitive detection of amplified products. Small amplicons are recommended, and this may also play a role in the speed; however, it has been shown that decreasing the size of the PCR fragment does not necessarily improve PCR performance (174). Molecular beacons (1, 155, 240) and TaqMan-based assays (53) are a few of the chemistries that have been used for real-time PCR detection of HAV. Real-time PCR chemistries use primers and probes with separate fluorogenic labels; the generation of a fluorescent signal depends on the interaction between PCR products and/or probes (24, 53, 155, 240). A DNA-binding fluorophore, SYBR green, has been widely used because of its simplicity (129, 131, 166). The advantage of SYBR green over labeled probe-based detection platforms is the detection of highly variable genome regions for which probe design is often difficult (134). SYBR green interference with nucleic acid sequencing can be eliminated by performing a nested PCR without the fluorophore.

Nucleic Acid Sequencing

Nucleic acid sequencing is performed on PCR products to confirm their specificity and provide the ultimate means to identify and characterize the organism. Nucleic acid sequencing of selected genomic regions of HAV has been used to determine the genetic relatedness of isolates (112, 116, 170, 199, 200). The original nucleic acid sequencing methodology described by Sanger et al. (209), which required independent labeling reactions for each nucleotide and conventional gel electrophoresis, has been replaced by high-throughput methods, including fluorescent dyes for label terminators and capillary arrays for electrophoresis, which have improved sequencing speed and accuracy (160, 196).

Molecular Detection from Water and Food

HAV is stable in the environment, especially when associated with organic matter, and is resistant to low pH and heating (107). These characteristics facilitate the likelihood of transmission by contaminated food and water and also improve the likelihood of detection in environmental samples, including water and sewage (15).

Because HAV grows so slowly in cell culture and because of the generally low levels of contamination in environmental samples, virus detection became feasible only with the availability of sensitive nucleic acid detection methods. However, HAV detection in food has not been included as a part of routine analysis of these outbreaks in most parts of the world (9). The primary reason is that because of the long disease incubation period, implicated foods usually have been consumed or discarded by the time the outbreak is recognized (135). The same holds true for detection of HAV in waterborne outbreaks, unless there is ongoing contamination.

Detection in food. HAV has been successfully isolated from bivalve mollusks (72, 73, 206), oysters (44, 67), mussels (36), and clams (18, 132, 236, 237). Methods for extraction of HAV RNA from food samples have included the use of guanidine isothiocyanate followed by several precipitation steps with polyethylene glycol (PEG) or ultracentrifugation (72, 73, 237). The addition of viral concentration steps (e.g., high pH or PEG precipitation) followed by RNA extraction and purification using poly(dT) magnetic beads has been shown to increase the sensitivity of HAV detection from bivalve mollusk samples (133).

Detection in water. Concentration by filtration is the classic approach for viral detection from water and wastewater, including the use of beef extracts to elute virus from filters and PEG for concentration (223). However, these components can interfere with RT-PCR (212). Ultrafiltration has been used as an alternate method for virus concentration, and vortex flow filtration followed by microcentrifugation has been used to overcome the limitations of conventional concentration methods (246).

Nucleic acid hybridization assays using labeled probes were initially used to detect HAV in contaminated water (121). However, this method had low sensitivity and required several logs of virus for detection. Today, PCR is widely used for HAV detection in environmental samples (62, 95, 124, 212, 246).

MOLECULAR EPIDEMIOLOGY OF HEPATITIS A

Molecular biomarkers have been used to determine the genetic relatedness of organisms, both to identify and track modes and chains of transmission and to characterize the evolution of organisms in host populations. New approaches from disciplines such as bioinformatics and molecular evolution have added to the understanding of both the epidemiology of agents within host populations and the dynamics of genetic changes within the agent itself. Molecular epidemiology has played an important role in improving the understanding of HAV infection by identifying sources of infection and the dynamics of virus evolution.

Overview of Hepatitis A Epidemiology

Worldwide, the endemicity of HAV infection varies according to regional hygienic standards; the highest prevalence of infection occurs in regions with the lowest socioeconomic levels (12, 99). The United States generally has a low endemicity of HAV infection, although high rates of infection occur in certain populations (11, 28, 32). Approximately every decade, epidemics of hepatitis A have occurred in the United States; the last occurred in 1995. Although the incidence of hepatitis A has declined over the past decade, primarily because of hepatitis A immunization (13, 14, 23, 163, 252, 255), it remains a frequently reported disease. In 2003, 7,653 cases of hepatitis A were reported to CDC (30), which, when corrected for underreporting and asymptomatic infections (7, 255), represents an estimated 36,700 cases and 79,600 infections.

Modes of Transmission and Sources of HAV Infection

The most common reported source of infection is household or other close contact with an infected person (11, 28, 32). Other potential sources of infection include men having sex with men (MSM), travel to countries where HAV is endemic, and illicit drug use. Contaminated food and water are an infrequent source of infection, although they have been associated with outbreaks. On rare occasions, HAV infection has been transmitted by transfusion of blood or blood products (145, 224).

Personal contact. Most transmission occurs among close contacts in households and extended-family settings (225); this mode accounts for at least 25% of infections. No identifiable source for infection is reported for 40 to 50% of cases, and personal contact with an unidentified source shedding HAV is likely to explain most of these cases. A study in Salt Lake City, Utah, showed that 98 of 390 (25%) household contacts of 167 infected cases without an identified infection source had serologic evidence of recent HAV infection (225). Children have the highest incidence of infection, and infected young children with their less scrupulous hygiene probably serve as a major source of transmission (98, 222). Children appear to excrete virus longer than adults (114, 205, 218, 265), which may facilitate transmission, although good epidemiologic studies are lacking in this area.

MSM. Although early studies did not show a higher prevalence of HAV infection among MSM (238), prospective studies showed a high incidence of infection (49, 54). In addition, hepatitis A outbreaks among MSM have been reported in the United States and Europe (22, 105, 170, 235). HAV isolates with nucleotide sequence identity have been observed within several outbreaks among MSM and between outbreaks which occurred in different geographic locations (22, 105, 170, 235).

Illicit drug use. Outbreaks of HAV infection among injection drug users (IDUs) have been reported in North America and Scandinavia (97, 101, 143). Several routes of transmission are likely to occur, and these include a combination of person-to-person and percutaneous spread. Injection of drugs is often a group activity, and poor personal hygiene among illicit drug users may be a source of HAV contamination of drugs or drug paraphernalia, as well as direct person-to-person transmission. Fecal contamination of drugs by rectal transportation has been reported but is probably a rare source of infection (143). Percutaneous transmission of HAV by needle sharing can also occur (97).

The complexity of hepatitis A transmission among injection and noninjection drug users is illustrated by an outbreak among methamphetamine users and their contacts in Polk County, Florida, during 2001 and 2002 (252). The significance of IDUs as the source of the outbreak was generally underestimated, since case patients were not willing to admit illicit drug use. However, HAV sequence analysis showed identity or near identity among cases, including cases with no direct links to each other and whose disease onsets varied over time. These findings indicated a limited number of HAV introductions with subsequent person-to-person transmission among IDU networks and their contacts (97, 170). Similar studies in Scandinavia have shown that HAV strains among IDUs involved in outbreaks were significantly different from those of the infected non-drug-using population (97, 143, 232).

International travel. Persons from regions of low endemicity traveling to regions of high HAV endemicity are at substantial risk for acquiring infection (28, 231). In the United States, about 10% of hepatitis A patients have reported recent travel outside the United States as their only possible source of infection (11, 32, 170). HAV sequences from case patients with history of travel are closely related to the sequence patterns of isolates from countries where the travel occurred (170, 232; CDC, unpublished data).

Food and water. HAV transmission has been associated with contaminated food and water (63, 112, 175, 263). Outbreaks associated with consumption of mussels (70), clams (18, 100), contaminated lettuce (204), ice slush beverages, raw oysters (67), frozen strawberries (112, 175), blueberries (27), raspberries (195, 197), green onions (3, 63, 260), and other salad items (108, 153, 179) have been reported. The potential for extensive disease transmission is illustrated by the largest recorded hepatitis A outbreak, which occurred from consumption of sewage-contaminated clams and caused illness in 300,000 persons in Shanghai, China (263), and by outbreaks that extended to multiple states in the United States through widespread distribution of HAV-contaminated food (3, 112, 260). Although food- and waterborne outbreaks are often newsworthy, they account for only 2% of the total reported cases in the United States (28, 32).

Waterborne transmission of hepatitis A appears to be less common than transmission by food, and during the past 10 to 15 years contaminated water has not been identified as a source for any cases of hepatitis A in the United States (28, 58). Inadequate sewage management has played an important role in most waterborne outbreaks, which have generally involved contaminated groundwater obtained from wells or rivers (16, 68, 164, 181) and drinking water (125, 190).

Unfortunately, because of the long incubation period of HAV infection, virus detection in food is difficult, unless some of the food was kept or contamination is ongoing. Even more difficult to detect are sporadic cases associated with contaminated food. Only recently have such transmission chains been identified, and then only through the use of molecular epidemiologic investigations (3, 18, 112, 206, 260).

TABLE 3. Geographic distribution of hepatitis A virus genotypes

Genotype	Host	Distribution of isolates	Reference(s)
IA	Human	United States, Mexico, Canada, Brazil, Argentina, Cuba, Chile, Uruguay, Costa Rica, El Salvador, Spain, former USSR, Russia, Central Asia countries, Estonia, Germany, Greece, The Netherlands, Italy, Denmark, England, Switzerland, Czechoslovakia, Sweden, Norway, France, Albanian, Kosovo, Moldova, China, Korea, Thailand, Israel, Malaysia, India, Japan, North Africa, Tunisia, South Africa	6, 22, 26, 33, 37, 42, 50, 51, 52, 64, 66, 69, 74, 86, 87, 89, 111, 112, 150, 161, 170, 184, 191, 199, 200, 203, 206, 232, 239, 242, 245, 249, 251, 256; CDC, unpublished data
IB	Human	United States, Brazil, Spain, Germany, Greece, The Netherlands, Italy, Sweden, Norway, France, China, Thailand, Israel, Egypt, Jordan, Iraq, Japan, North Africa, Tunisia, South Africa, Sierra Leone, Nigeria, Morocco, Australia	37, 38, 46, 51, 52, 64, 170, 200, 206, 242, 245, 249, 251, 256, 262; CDC, unpublished data
II	Human	The Netherlands, France, Sierra Leone	34, 154, 200, 245
III	Human	United States, Panama, Spain, Central Asia counties, Estonia, The Netherlands, Sweden, Denmark, Norway, Malaysia, India, Sri Lanka, Nepal, Japan	20, 87, 111, 117, 130, 170, 184, 199, 200, 232, 239, 245, 249; CDC, unpublished data
IV	Nonhuman primate	Philippines	167
V	Nonhuman primate	Kenya	248
VI	Nonhuman primate	Indonesia	200

Methods of Molecular Epidemiology

The determination and analysis of molecular biomarkers (e.g., nucleic acid sequencing or phylogenetic analysis) have been used to determine the relatedness of strains from two or more infected persons. These analyses have been greatly facilitated by (i) the ability to easily recover HAV from the serum, rather than stool, of infected persons and (ii) molecular methods (e.g., PCR, DNA sequencing, and sequence analysis) which have been adapted to achieve the rapid throughput of a large number of specimens. These recent advances have resulted in molecular epidemiology becoming an essential tool in the investigation of certain types of hepatitis A outbreaks and in epidemiologic studies of HAV transmission.

The key elements of a molecular epidemiologic analysis include determination of the genotypes and genetic relatedness of HAV isolates obtained from infected persons and a rigorous epidemiologic investigation. To facilitate the molecular analyses of HAV, RNA amplification and nucleic sequencing should be performed on one of the genome regions for which large amounts of comparative data exist (e.g., the VP1-P2B region) (Table 2; Fig. 2).

Distribution of HAV genotypes. Nucleotide sequence data indicate that HAV genotypes have unique geographic distributions (Table 3; Fig. 3). Genotype I is most prevalent worldwide, and subgenotype IA is more common than IB. Because genotype I is so common, genotyping alone rarely can be used to identify the source of an HAV outbreak or chain of transmission.

Countries and their commonly found genotypes are shown in Table 3. Subgenotypes IA and IB are most often found in North and South America, Europe, China, and Japan (200). For subgenotype IIIA, the prototype strain, PA21 (20), was originally isolated from captured Panamanian owl monkeys and thought to be a simian virus. However, once nucleotide sequence analysis was performed many years later, the unique nucleotide and amino acid sequence patterns which differentiate human from simian HAV (i.e., "simian signature") (167, 200) were not found in this strain. Over the ensuing years, subgenotype IIIA isolates have been recovered only from humans in many parts of the world (Table 3).

Cocirculation of multiple genotypes or subgenotypes has been reported in some regions of the world. Cocirculation of subgenotypes IA and IB is reported in South Africa (242), Brazil (251), and Israel (CDC, unpublished data), and subgenotypes IA and IIIA are reported in India (111) and the Central Asian Republics of the former Soviet Union (CDC, unpublished data). Both subgenotypes IA and IB have been isolated from the United States; however, most IB isolates were found among infected travelers returning from other countries (170; CDC, unpublished data).

Genetic relatedness of HAV. Hepatitis A virus displays a high degree of antigenic and genetic conservation (146, 147, 200, 207) and does not appear to accumulate the high frequency of genetic changes seen in many RNA viruses. Although precise data on the genome mutation rate are not available, it appears to be lower than those of other RNA viruses (10^{-4} to 10^{-5} substitution per base per round of copy) (75). Even in extended common-source outbreaks, viruses isolated from the first cases are genetically the same as those isolated from the last cases (63, 112, 170, 175). However, enough genetic heterogeneity exists in several HAV genome regions to differentiate the relatedness of isolates circulating within and between communities over time, including within subgenotypes (50, 51, 52, 161, 170).

Initially, a 168-nucleotide fragment of the VP1/P2A junction was used for genotype analysis (116, 200). However, this sized fragment was shown not to sufficiently differentiate genotype or relatedness among HAV isolates (52, 206). Currently, the majority of molecular epidemiologic studies conducted at CDC use a 390-nucleotide-long fragment from the VP1-P2B region which includes 2A, one of the most variable regions of the genome (112, 170; CDC, unpublished data) (Table 2; Fig. 2). In a comparative analysis of 240 HAV isolates from a number of communities, using the 350-nucleotide fragment of the VP1-

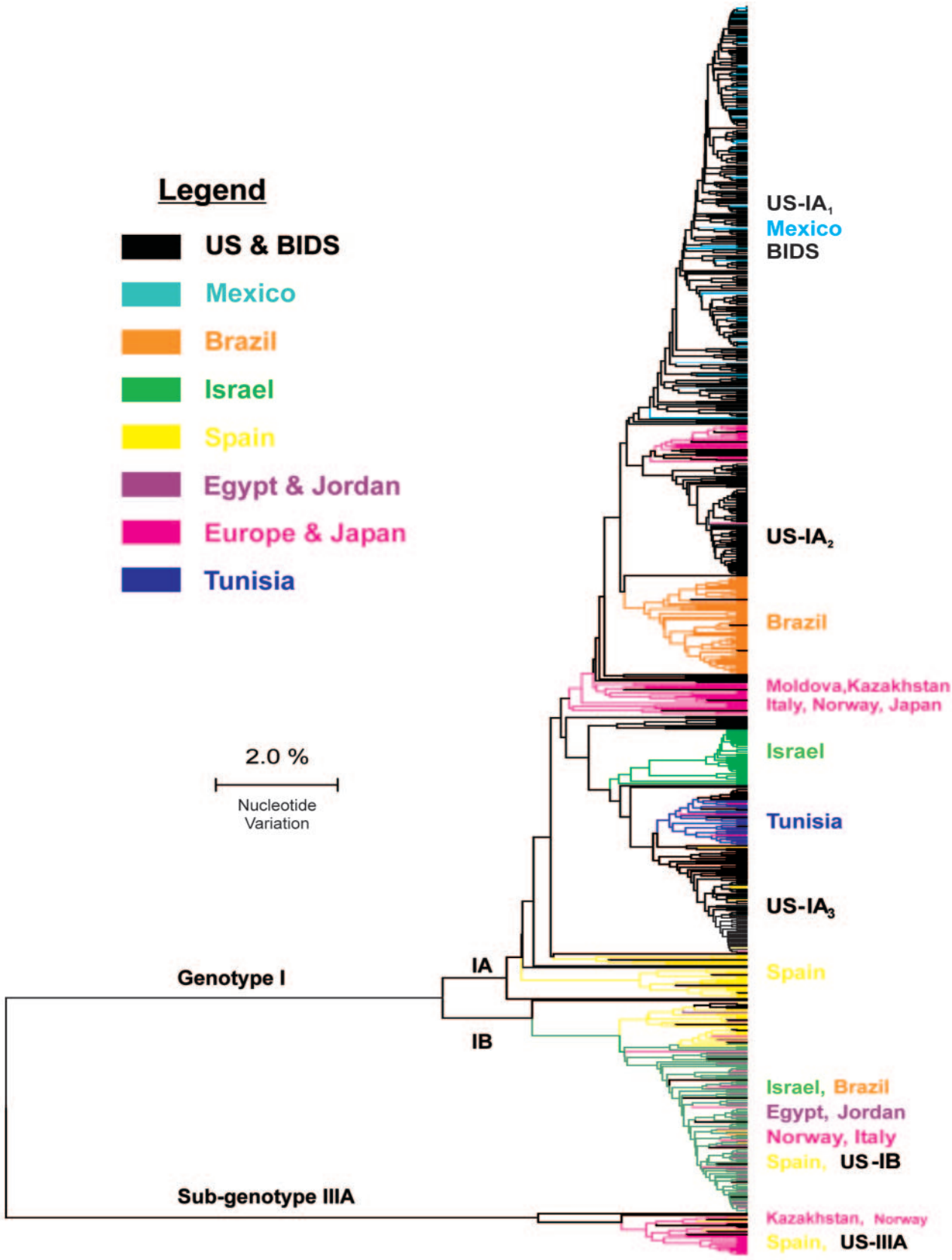

FIG. 3. Phylogenetic analysis of 3,582 HAV isolates in the VP1/P2B region (315-bp fragment). The dendrogram (unweighted-pair group method using average linkages) was created by using Kimura's two-parameter model. Isolates were obtained from the United States (n = 2,190) and other parts of the world. The U.S. specimens included specimens from hepatitis A cases from CDC's Sentinel Counties Study of Acute Viral

P2B region and a 900-nucleotide fragment from the entire VP1 region, genotype assignment was concordant in all instances and the longer fragment increased the likelihood of identifying a difference between two isolates by only 3% (CDC, unpublished data).

A phylogenetic analysis, restricted to a 315-nucleotide fragment of VP1-P2B, of over 3,000 HAV isolates from the United States and 12 other countries is shown in Fig. 3. The majority of isolates (85%) were obtained from investigations conducted by CDC's Division of Viral Hepatitis; the remaining sequences were obtained from GenBank (accession numbers AB020564 to AB020569, AF050223 to AF050238, AF386846 to AF386888, AF396391 to AF396408, AJ296172, AJ299460 to AJ299467, AJ505561 to AJ505625, AY294047 to AY294049, AY322842 to AY323047, AY875649 to AY875672, and AY753408 to AY753530). Most isolates (80%) belong to subgenotype IA, 17% belong to subgenotype IB, and 3% belong to genotype IIIA. However, these genotype proportions probably do not represent the true worldwide distribution of HAV genotypes, since this is essentially a convenience sample which contains a disproportionate number of isolates from North America. Within a genotype or subgenotype, there was a clustering of HAV isolates by location or geographical regions. For example, isolates from North America, South America, Europe, and Middle East tended to cluster together. Phylogenetic analysis also showed distinct clusters of HAV isolates within subgenotype IA and defined three major "clusters," i.e., US-IA_1, US-IA_2, and US-IA_3 (Fig. 3). Nucleic acid sequences obtained from HAV isolates from the interior of Mexico and the U.S.-Mexican border were closely related to isolates in US-IA_1, while US-IA_2 isolates were predominantly from IDUs and US-IA_3 isolates were from MSM.

Applications of Molecular Epidemiologic Investigations

The long incubation period of hepatitis A, the epidemic but often sporadic nature of disease transmission, and the often unapparent links between persons involved in outbreaks have made molecular markers important tools for epidemiologic investigations of this infection. The use of nucleotide sequence patterns allows the investigator to determine whether viruses from the same or different locations are related to each other. Case patients with identical sequence patterns and similar epidemiologic characteristics usually suggest a common-source exposure (112, 170). However, there have been instances where sequence analysis suggested that temporally related common-source outbreaks might have been related, when in fact they represented different transmission incidents. This occurred because of the somewhat limited variability of the HAV nucleic acid sequence and the inability of the relatively short sequenced fragments to precisely differentiate the genetic relatedness of HAV isolates. In these instances, outbreak strains had identical or nearly identical sequences which represented similar geographical sources of the infecting virus. However, the epidemiologic investigation showed that the outbreaks were not related (3, 63; CDC, unpublished data).

Molecular epidemiologic investigations have to be planned and conducted with equal attention being paid to the methodological requirements of both disciplines: epidemiology and molecular biology. The use of molecular biomarkers alone will not fix a flawed epidemiologic investigation, and patterns of transmission suggested by patterns of HAV genetic relatedness may not be identified if the epidemiologic information was not collected at the time of the initial investigation.

Population-based molecular epidemiologic studies have shown that HAV is often transmitted within networks of persons with similar risk factors for infection (97, 143, 170, 232, 252). Certain sequence patterns appear to cluster among persons with particular risk factors and may be used as an indicator of the source of infection. For instance, in the United States, outbreaks among MSM have often shared one sequence pattern in subgenotype IA, and most cases associated with injection drug use have shared certain "signature" patterns (170, 249).

Molecular epidemiology has become a particularly powerful tool for the investigation of food-borne outbreaks of disease. The close or identical genetic relatedness of isolates from cases in different locales has provided the link to what previously would have been considered sporadic, yet independent, outbreaks. In addition, the availability of a large and ever-growing database of HAV sequences has allowed the identification of the geographic source of virus contamination (3, 112, 260). Recognition that geographically separated outbreaks may be linked by a food source and identification of the potential geographic origin of the contamination have accelerated the public health response to these outbreaks.

PREVENTION OF HEPATITIS A

The availability of vaccines to provide long-term immunity against HAV infection has the potential to significantly reduce disease incidence and possibly eliminate infection transmission (13, 28, 107, 159). A dramatic effect of widespread childhood hepatitis A vaccination has been observed in the United States. Significant disease reductions have occurred in vaccinated populations that historically had the highest disease incidence, namely, American Indians, Alaskan Natives, and persons living in the western United States (14, 23, 30, 32, 163, 215, 255).

The significant reduction in hepatitis A incidence in the United States due to immunization has also changed the epidemiology of this infection. Prior to childhood hepatitis A vaccination, the highest disease incidence occurred among children (28, 29, 159, 215). Now the highest incidence of dis-

Hepatitis (11, 170), published and unpublished outbreak investigations (30, 63, 112), and others submitted to CDC for investigation. Specimens from the U.S.-Mexico Border Infectious Disease Surveillance (BIDS) project (258) were also included and represented cases primarily from the United States. Specimens from other countries include those from Mexico, Brazil, Israel, Jordan, Egypt, Moldova, and Kazakhstan and were submitted to CDC as part of outbreak or epidemiologic investigations. Sequences from GenBank (Italy, Norway, Spain, Tunisia, and Japan) were also included in the analysis. Only unique sequence patterns are shown in the tree. Genotypes and subgenotypes are indicated on the branches, and the geographical locations of case patients are indicated in different colors.

ease is among adults, a substantial proportion of whom have risk factors for infection, such as IDUs and MSM (11, 28, 32, 170, 252). In addition, childhood immunization appears to have blunted or possibly even eliminated the cyclical nature of hepatitis A in the United States (13, 29, 163). However, at this time, hepatitis A immunization appears not to have changed the epidemiology of sporadic cases of the disease.

What might become the role of molecular diagnostics in the era of hepatitis A immunization? One role would be the continued use of molecular epidemiology to identify chains of transmission, including the extent of outbreaks among IDUs and MSM. Another would be the expanded use of molecular epidemiology to investigate small outbreaks or sporadic cases in different locales and determine if they are related, such as through the distribution of contaminated food. In addition, most food-borne outbreaks should be investigated to determine the genetic relatedness of HAV isolates to improve the identification of the source of contamination and the extent of the distribution of contaminated food. The use of molecular epidemiology to investigate hepatitis A outbreaks has provided a powerful tool which has improved the public health response to these situations. However, it has also meant that forensic practices (e.g., a documented chain of specimen custody) must be used during the investigation, because these data have eventually been entered into evidence in court cases.

Molecular diagnostics should become more widely used to assess the effectiveness of hepatitis A vaccination as disease incidence declines to very low levels. Because of the high proportion of asymptomatic HAV infections, nucleic acid amplification techniques will be required to determine the extent to which unidentified infection occurs. In addition, it will be very important to characterize the genetic makeup of HAV from immunized persons who may subsequently become infected. Such investigations would identify the possible establishment of antibody-resistant mutants, which may have a selective transmission advantage in immunized populations with continued exposure to HAV.

ACKNOWLEDGMENTS

The following people or groups provided specimens or sequences that have not been previously published: Daniel Shouval, Nili Daudi (Liver Unit, Hadassah University Hospital, Jerusalem, Israel), Ala Toukan (University of Jordan, Amman, Jordan), Hend El Sherbini (Cairo University, Cairo, Egypt), Michael O. Favorov (CDC-CAR, Almaty, Kazakhstan), and the following members of the Epidemiology Branch, Division of Viral Hepatitis, CDC: Gregory L. Armstrong, Anthony Fiore, Ian Williams, and Beth P. Bell. We also thank our many other colleagues whose past efforts have made this work possible.

We regretfully report that author Omana V. Nainan passed away on 3 September 2005, and we dedicate this article to her memory and her exceptional contributions on molecular epidemiology of viral hepatitis.

The entire study was funded by the intramural budget of the Division of Viral Hepatitis, Centers for Disease Control and Prevention (CDC). The authors of this paper do not have any commercial association that might pose a conflict of interest.

Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

REFERENCES

1. **Abd El Galil, K. H., M. A. El Sokkary, S. M. Kheira, A. M. Salazar, M. V. Yates, W. Chen, and A. Mulchandani.** 2004. Combined immunomagnetic separation-molecular beacon-reverse transcription-PCR assay for detection of hepatitis A virus from environmental samples. Appl. Environ. Microbiol. **70:**4371–4374.
2. **Akriviadis, E. A., and A. G. Redeker.** 1989. Fulminant hepatitis A in intravenous drug users with chronic liver disease. Ann. Intern. Med. **110:**838–839.
3. **Amon, J. J., R. Devasia, G. Xia, O. V. Nainan, S. Hall, B. Lawson, J. Wolthius, P. D. M. MacDonald, C. Shepard, I. T. Williams, G. Armstrong, J. A. Gabel, P. Erwin, L. Sheeler, W. Kuhnert, P. Patel, G. Vaughan, A. Weltman, A. Craig, B. P. Bell, and A. Fiore.** 2005. Molecular epidemiology of foodborne hepatitis A outbreaks in the United States, 2003. J. Infect. Dis. **192:**1323–1330.
4. **Angarano, G., F. Trotta, L. Monno, T. Santantonio, and G. Pastore.** 1985. Serum IgA anti-hepatitis A virus as detected by enzyme-linked immunosorbent assay: diagnostic significance in patients with acute and protracted hepatitis A. Diagn. Microbiol. Infect. Dis. **3:**521–523.
5. **Apaire-Marchais, V., B. H. Robertson, V. Aubineau-Ferre, M. G. LeRoux, F. Leveque, L. Schwartzbrod, and S. Billaudel.** 1995. Direct sequencing of hepatitis A virus strains isolated during an epidemic in France. Appl. Environ. Microbiol. **61:**3977–3980.
6. **Arauz-Ruiz, P., L. Sundqvist, Z. Garcia, L. Taylor, K. Visona, H. Norder, and L. O. Magnius.** 2001. Presumed common source outbreaks of hepatitis A in an endemic area confirmed by limited sequencing within the VP1 region. J. Med. Virol. **65:**449–456.
7. **Armstrong, G. L., and B. P. Bell.** 2002. Hepatitis A virus infections in the United States: model-based estimates and implications for childhood immunization. Pediatrics **109:**839–845.
8. **Asher, L. V. S., L. N. Binn, T. L. Mensing, R. H. Marchwicki, R. A. Vassell, and G. D. Young.** 1995. Pathogenesis of hepatitis A in orally inoculated owl monkeys (*Aotus trivirgatus*). J. Med. Virol. **47:**260–268.
9. **Atmar, R. L., T. G. Metcalf, F. H. Neill, and M. K. Estes.** 1993. Detection of enteric viruses in oysters by using the polymerase chain reaction. Appl. Environ. Microbiol. **59:**631–635.
10. **Baroudy, B. M., J. R. Ticehurst, T. A. Miele, J. V. Maizel, R. H. Purcell, and S. M. Feinstone.** 1985. Sequence analysis of hepatitis A virus cDNA coding for capsid proteins and RNA polymerase. Proc. Natl. Acad. Sci. USA **82:**2143–2147.
11. **Bell, B. P., C. N. Shapiro, M. J. Alter, L. A. Moyer, F. N. Judson, K. Mottram, M. Fleenor, P. L. Ryder, and H. S. Margolis.** 1998. The diverse patterns of hepatitis A epidemiology in the United States—implications for vaccination strategies. J. Infect. Dis. **178:**1579–1584.
12. **Bell, B. P.** 2002. Global epidemiology of hepatitis A: implications for control strategies, p. 9–14. *In* H. S. Margolis, M. J. Alter, J. T. Liang, and J. L. Dienstag (ed.), Viral hepatitis and liver disease. International Medical Press, London, United Kingdom.
13. **Bell, B. P., and S. M. Feinstone.** 2004. Hepatitis A vaccine, p. 269–297. *In* S. A. Plotkin, W. A. Orenstein, and P. A. Offit (ed.), Vaccine, 4th ed. Saunders, Philadelphia, Pa.
14. **Bialek, S. R., D. A. Thoroughman, D. Hu, E. P. Simard, J. Chattin, J. Cheek, and B. P. Bell.** 2004. Hepatitis A incidence and hepatitis a vaccination among American Indians and Alaska Natives, 1990–2001. Am. J. Public Health **94:**996–1001.
15. **Biziagos, E., J. Passagot, J. M. Crance, and R. Deloince.** 1988. Long-term survival of hepatitis A virus and poliovirus type 1 in mineral water. Appl. Environ. Microbiol. **54:**2705–2710.
16. **Bloch, A. B., S. L. Stramer, J. D. Smith, H. S. Margolis, H. A. Fields, T. W. McKinley, C. P. Gerba, J. E. Maynard, and R. K. Sikes.** 1990. Recovery of hepatitis A virus from a water supply responsible for a common source outbreak of hepatitis A. Am. J. Public Health **80:**428–430.
17. **Blumer, G.** 1923. Infectious jaundice in the United States. JAMA **81:**353–358.
18. **Bosch, A., G. Sanchez, F. Le Guyader, H. Vanaclocha, L. Haugarreau, and R. M. Pinto.** 2001. Human enteric viruses in Coquina clams associated with a large hepatitis A outbreak. Water Sci. Technol. **43:**61–65.
19. **Bower, W. A., O. V. Nainan, X. Han, and H. S. Margolis.** 2000. Duration of viremia in hepatitis A virus infection. J. Infect. Dis. **182:**12–17.
20. **Brown, E. A., R. W. Jansen, and S. M. Lemon.** 1989. Characterization of a simian hepatitis A virus (HAV): antigenic and genetic comparison with human HAV. J. Virol. **63:**4932–4937.
21. **Brown, E. A., S. P. Day, R. W. Jansen, and S. M. Lemon.** 1991. The 5′ nontranslated region of hepatitis A virus: secondary atructure and elements required for translation in vitro. J. Virol. **65:**5828–5838.
22. **Bruisten, S. M., J. E. Steenbergen, A. S. Pijl, H. G. Niesters, G. J. van Doornum, and R. A. Coutinho.** 2001. Molecular epidemiology of hepatitis A virus in Amsterdam, The Netherlands. J. Med. Virol. **63:**88–95.
23. **Bulkow, L. R., R. B. Wainwright, B. J. McMahon, J. P. Middaugh, S. A. Jenkerson, and H. S. Margolis.** 1993. Secular trends in hepatitis A virus infection among Alaska Natives. J. Infect. Dis. **168:**1017–1020.
24. **Bustin, S. A.** 2002. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. J. Mol. Endocrinol. **29:**23–39.
25. **Buti, M., R. Jardi, A. Bosch, F. Rodriguez, G. Sanchez, R. Pinto, X. Costa, J. F. Sanchez-Avila, M. Cotrina, R. Esteban, and J. Guardia.** 2001. Assessment of the PCR-Southern blot technique for the analysis of viremia in patients with acute hepatitis A. Gastroenterol. Hepatol. **24:**1–4.

26. **Byun, K. S., J. H. Kim, K. J. Song, L. J. Baek, J. W. Song, S. H. Park, O. S. Kwon, J. E. Yeon, J. S. Kim, Y. T. Bak, and C. H. Lee.** 2001. Molecular epidemiology of hepatitis A virus in Korea. J. Gastroenterol. Hepatol. **16:**519–524.
27. **Calder, L., G. Simmons, C. Thornley, P. Taylor, K. Pritchard, G. Greening, and J. Bishop.** 2003. An outbreak of hepatitis A associated with consumption of raw blueberries. Epidemiol. Infect. **131:**745–751.
28. **Centers for Disease Control and Prevention.** 1999. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). Morb. Mortal. Wkly. Rep. **48**(RR-12)**:**1–37.
29. **Centers for Disease Control and Prevention.** 1997. Hepatitis A vaccination programs in communities with high rates of hepatitis A. Morb. Mortal. Wkly. Rep. **46:**600–603.
30. **Centers for Disease Control and Prevention.** 2004. Final 2003 reports of notifiable diseases. Morb. Mortal. Wkly. Rep. **53:**687–705.
31. **Centers for Disease Control and Prevention.** 1996. Hepatitis surveillance report no. 56. U.S. Department of Health and Human Services, Public Health Service, CDC, Atlanta, Ga. (Available at http://www.cdc.gov/nciod/diseases/hepatitis/h96surve.htm. Accessed 1 September 1998.)
32. **Centers for Disease Control and Prevention.** 2004. Hepatitis surveillance report no. 59. U.S. Department of Health and Human Services, Public Health Service, CDC., Atlanta, Ga. (Available at http://www.cdc.gov/ncidod/diseases/hepatitis/resource/PDFs/Hep_surveillance_59.pdf. Accessed 25 March 2005.)
33. **Chen, Y., J. Mao, Y. Hong, L. Yang, Z. Ling, and W. Yu.** 2001. Genetic analysis of wild-type hepatitis A virus strains. Chinese Med. J. **114:**422–423.
34. **Ching, K. Z., T. Nakano, L. E. Chapman, A. Demby, and B. H. Robertson.** 2002. Genetic characterization of wild-type genotype VII hepatitis A virus. J. Gen. Virol. **83:**53–60.
35. **Chiriaco, P., C. Gaudalupi, M. K. Armigliato, F. Bortolotti, and G. Realdi.** 1986. Polyphasic course of hepatitis type A in children. J. Infect. Dis. **154:**231.
36. **Chironna, M., C. Germinario, D. De Medici, A. Fiore, S. Di Pasquale, M. Quarto, and S. Barbuti.** 2002. Detection of hepatitis A virus in mussels from different sources marketed in Puglia region (South Italy). Int. J. Food Microbiol. **75:**11–18.
37. **Chironna, M., A. Grottola, C. Lanave, E. Villa, S. Barbuti, and M. Quarto.** 2003. Genetic analysis of HAV strains recovered from patients with acute hepatitis from Southern Italy. J. Med. Virol. **70:**343–349.
38. **Chironna, M., P. Lopalco, R. Prato, C. Germinario, S. Barbuti, and M. Quarto.** 2004. Outbreak of infection with hepatitis A virus (HAV) associated with a foodhandler and confirmed by sequence analysis reveals a new HAV genotype IB variant. J. Clin. Microbiol. **42:**2825–2828.
39. **Chitambar, S. D., and M. S. Chadha.** 2000. Use of filter paper disks for hepatitis A surveillance. Indian J. Gastroenterol. **19:**165–167.
40. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
41. **Chomczynski, P.** 1993. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. BioTechniques **15:**532–537.
42. **Chudy, M., I. Budek, B. Keller-Stanislawski, K. A. McCaustland, S. Neidhold, B. H. Robertson, C. M. Nubling, R. Seitz, and J. Lower.** 1999. A new cluster of hepatitis A infection in hemophiliacs traced to a contaminated plasma pool. J. Med. Virol. **57:**91–99.
43. **Cockayne, E. A.** 1912. Catarrhal jaundice, sporadic and epidemic, and its relation to acute yellow atrophy of the liver. Q. J. Med. **6:**1–29.
44. **Coelho, C., A. P. Heinert, C. M. Simoes, and C. R. Barardi.** 2003. Hepatitis A virus detection in oysters (*Crassostrea gigas*) in Santa Catarina State, Brazil, by reverse transcription-polymerase chain reaction. J. Food Prot. **66:**507–511.
45. **Cohen, J. I., B. Rosenblum, J. R. Ticehurst, R. J. Daemer, S. M. Feinstone, and R. H. Purcell.** 1987. complete nucleotide sequence of an attenuated hepatitis A virus: comparison with wild-type virus. Proc. Natl. Acad. Sci. USA **84:**2497–2501.
46. **Cohen, J. I., J. R. Ticehurst, R. H. Purcell, A. Buckler-White, and B. M. Baroudy.** 1987. Complete nucleotide sequence of wild-type hepatitis A virus: Comparison with different strains of hepatitis A virus and other picornaviruses. J. Virol. **61:**50–59.
47. **Cohen, J. I., B. Rosenblum, S. M. Feinstone, J. Ticehurst, and R. H. Purcell.** 1989. Attenuation and cell culture adaptation of hepatitis A virus (HAV): a genetic analysis with HAV cDNA. J. Virol. **63:**5364–5370.
48. **Cohen, J. I., S. Feinstone, and R. H. Purcell.** 1989. Hepatitis A virus infection in a chimpanzee: duration of viremia and detection of virus in saliva and throat swabs. J. Infect. Dis. **160:**887–890.
49. **Corey, L., and K. K. Holmes.** 1980. Sexual transmission of hepatitis A in homosexual men: incidence and mechanism. N. Engl. J. Med. **302:**435–438.
50. **Costa-Mattioli, M., V. Ferre, S. Monpoeho, L. Garcia, R. Colina, S. Billaudel, I. Vega, R. Perez-Bercoff, and J. Cristina.** 2001. Genetic variability of hepatitis A virus in South America reveals heterogeneity and co-circulation during epidemic outbreaks. J. Gen. Virol. **82:**2647–2652.
51. **Costa-Mattioli, M., S. Monpoeho, C. Schvoerer, B. Besse, M. H. Aleman, S. Billaudel, J. Cristina, and V. Ferre.** 2001. Genetic analysis of hepatitis A virus outbreak in France confirms the co-circulation of sub-genotypes Ia, Ib and reveals a new genetic lineage. J. Med. Virol. **65:**233–240.
52. **Costa-Mattioli, M., J. Cristina, H. Romero, R. Perez-Bercof, D. Casane, R. Colina, L. Garcia, I. Vega, G. Glikman, V. Romanowsky, A. Castello, E. Nicand, M. Gassin, S. Billaudel, and V. Ferre.** 2002. Molecular evolution of hepatitis A virus: a new classification based on the complete VP1 protein. J. Virol. **76:**9516–9525.
53. **Costa-Mattioli, M., S. Monpoeho, E. Nicand, M. H. Aleman, S. Billaudel, and V. Ferre.** 2002. Quantification and duration of viraemia during hepatitis A infection as determined by real-time RT-PCR. J. Viral Hepat. **9:**101–106.
54. **Coutinho, R. A., P. Albrecht-Van lent, T. Rijsdijk, N. Lelie, N. Nagelkerke, and H. Kuipers.** 1983. Prevalence and incidence of hepatitis A among male homosexuals. Br. Med. J. (Clin. Res. Ed.) **287:**1743–1745.
55. **Cromeans, T., M. D. Sobsey, and H. A. Fields.** 1987. Development of a plaque assay for a cytopathic, rapidly replicating isolate of hepatitis A virus. J. Med. Virol. **22:**45–56.
56. **Cromeans, T. L., O. V. Nainan, and H. S. Margolis.** 1997. Detection of hepatitis A virus RNA in oyster meat. Appl. Environ. Microbiol. **63:**2460–2463.
57. **Cromeans, T. L., M. O. Favarov, O. V. Nainan, and H. S. Margolis.** 2001. Hepatitis A and E viruses, p. 23–76. *In* Y. H. Hui, S. A. Sattar, K. D. Murrell, W.-K. Nip, and P. S. Stanfield (ed.), Foodborne disease handbook, 2nd ed., vol. 2. Viruses, parasite, pathogens, and HACCP. Marcel Dekker, New York, N.Y.
58. **Cuthbert, J. A.** 2001. Hepatitis A: old and new. Clin. Microbiol. Rev. **14:**38–58.
59. **Daemer, R. J., S. M. Feinstone, I. D. Gust, and R. H. Purcell.** 1981. Propagation of human hepatitis A virus in African green monkey kidney cell culture: primary isolation and serial passage. Infect. Immun. **32:**388–393.
60. **de Almeida, L. M., R. S. Azevedo, A. A. Guimaraes, S. Coutinho Eda, C. J. Struchiner, and E. Massad.** 1999. Detection of antibodies against hepatitis A virus in eluates of blood spotted on filter-paper: a pilot study in Rio de Janeiro, Brazil. Trans. R. Soc. Trop. Med. Hyg. **93:**401–404.
61. **Delem, A. D.** 1992. Comparison of modified HAVAB and ELISA for determination of vaccine-induced anti-HAV response. Biologicals **20:**289–291.
62. **Deng, M. Y., S. P. Day, and D. O. Cliver.** 1994. Detection of hepatitis A virus in environmental samples by antigen-capture PCR. Appl. Environ. Microbiol. **60:**1927–1933.
63. **Dentiger, C. M., W. A. Bower, O. V. Nainan, S. M. Cotter, G. Myers, L. M. Dubusky, S. Fowler, E. D. Salehi, and B. P. Bell.** 2001. An outbreak of hepatitis A associated with green onions. J. Infect. Dis. **183:**1273–1276.
64. **de Paula, V. S., M. L. Baptista, E. Lampe, C. Niel, and A. M. C. Gaspar.** 2002. Characterization of hepatitis A virus isolates from sub-genotypes IA and IB in Rio de Janeiro, Brazil. J. Med. Virol. **66:**22–27.
65. **de Paula, V. S., L. M. Villar, and A. M. C. Gaspar.** 2003. Comparison of four extraction methods to detect hepatitis A virus RNA in serum and stool samples. Braz. J. Infect. Dis. **7:**135–141.
66. **de Paula, V. S., L. Lu, C. Niel, A. M. C. Gaspar, and B. H. Robertson.** 2004. Genetic analysis of hepatitis A virus isolates from Brazil. J. Med. Virol. **73:**378–383.
67. **Desenclos, J. C., K. C. Klontz, M. H. Wilder, O. V. Nainan, H. S. Margolis, and R. A. Gunn.** 1991. A multistate outbreak of hepatitis A caused by the consumption of raw oysters. Am. J. Public Health **81:**1268–1272.
68. **De Serres, G., T. L. Cromeans, B. Levesque, N. Brassard, C. Barthe, M. Dionne, H. Prud'homme, D. Paradis, C. N. Shapiro, O. V. Nainan, and H. S. Margolis.** 1999. Molecular confirmation of hepatitis A virus from well water: epidemiology and public health implications. J. Infect. Dis. **179:**37–43.
69. **Diaz, B. I., C. A. Sariol, A. Normann, L. Rodriguez, and B. Flehmig.** 2001. Genetic relatedness of Cuban HAV wild-type isolates. J. Med. Virol. **64:**96–103.
70. **Dienstag, J. L., I. D. Gust, C. R. Lucas, D. C. Wong, and R. H. Purcell.** 1976. Mussel-associated viral hepatitis, type A: serological confirmation. Lancet **i:**561–564.
71. **Dignani, M. C., M. H. Miceli, C. M. Rosa, J. Gatica, J. Martinez-Rolon, and M. Pizzolato.** 2003. Loss of hepatitis A virus (HAV) antibodies after peripheral stem cell transplantation (PSCT). Bone Marrow Transplant. **31:**809–812.
72. **Di Pinto, A., V. T. Forte, G. M. Tantillo, V. Terio, and C. Buonavoglia.** 2003. Detection of hepatitis A virus in shellfish (Mytilus galloprovincialis) with RT-PCR. J. Food Prot. **66:**1681–1685.
73. **Di Pinto, A., M. C. Conversano, V. T. Forte, G. La Salandra, C. Montervino, and G. M. Tantillo.** 2004. A comparison of RT-PCR-based assays for the detection of HAV from shellfish. New Microbiol. **27:**119–124.
74. **Divizia, M., R. Gabrieli, A. Macaluso, B. Bagnato, L. Palombi, E. Buonomo, F. Cenko, L. Leno, S. Bino, A. Basha, and A. Pana.** 2005. Nucleotide correlation between HAV isolates from human patients and environmental samples. J. Med. Virol. **75:**8–12.

75. **Domingo, E., C. Escarmis, N. Sevilla, A. Moya, S. F. Elena, J. Quer, I. S. Novella, and J. J. Holland.** 1996. Basic concepts in RNA virus evolution. FASEB J. **10:**859–864.
76. **Emerson, S. U., S. A. Tsarev, and R. H. Purcell.** 1991. Biological and molecular comparisons of human (HM-175) and simian (AGM-27) hepatitis A viruses. J. Hepatol. **13**(Suppl. 4)**:**S144–S145.
77. **Emerson, S. U., Y. K. Huang, and R. H. Purcell.** 1993. 2B and 2C mutations are essential but mutations throughout the genome of HAV contribute to adaptation to cell culture. Virology **194:**475–480.
78. **Emerson, S. U., S. A. Tsarev, S. Govindarajan, M. Shapiro, and R. H. Purcell.** 1996. A simian strain of hepatitis A virus, AGM-27, functions as an attenuated vaccine for chimpanzees. J. Infect. Dis. **173:**592–597.
79. **Emerson, S. U., Y. K. Huang, H. Nguyen, A. Brockington, S. Govindarajan, M. S. Claire, M. Shapiro, and R. H. Purcell.** 2002. Identification of VP1/2A and 2C as virulence genes of hepatitis A virus and demonstration of genetic instability of 2C. J. Virol. **76:**8551–8559.
80. **Feinstone, S. M., A. Z. Kapikian, and R. H. Purcell.** 1973. Hepatitis A: detection by immune electron microscopy of a virus-like antigen associated with acute illness. Science **182:**1026–1028.
81. **Feinstone, S. M., R. J. Daemer, I. D. Gust, and R. H. Purcell.** 1983. Live attenuated vaccine for hepatitis A. Dev. Biol. Stand. **54:**429–432.
82. **Findlay, G. M., J. L. Dunlop, and H. C. Brown.** 1931. Observations on epidemic catarrhal jaundice. Br. Med. Hyg. **25:**7–24.
83. **Flehmig, B.** 1980. Hepatitis A-virus in cell culture. I. Propagation of different hepatitis A-virus isolates in a fetal rhesus monkey kidney cell line (FrhK-4). Med. Microbiol. Immunol. **168:**239–248.
84. **Fujiwara, K., O. Yokosuka, and T. Ehata.** 1997. Frequent detection of hepatitis A viral RNA in serum during early convalescent phase of acute hepatitis A. Hepatology **26:**1634–1639.
85. **Fujiwara, K., O. Yokosuka, T. Ehata, F. Imazeki, and H. Saisho.** 2000. PCR-SSCP analysis of 5′-nontranslated region of hepatitis A viral RNA: comparison with clinicopathological features of hepatitis A. Dig. Dis. Sci. **45:**2422–2427.
86. **Fujiwara, K., O. Yokosuka, K. Fukai, F. Imazeki, H. Saisho, and M. Omata.** 2001. Analysis of full-length hepatitis A virus genome in sera from patients with fulminant and self-limited acute type A hepatitis. J. Hepatol. **35:**112–119.
87. **Fujiwara, K., O. Yokosuka, F. Imazeki, H. Saisho, N. Saotome, K. Suzuki, K. Okita, E. Tanaka, and M. Omata.** 2003. Analysis of the genotype-determining region of hepatitis A viral RNA in relation to disease severities. Hepatol. Res. **25:**124–134.
88. **Funkhouser, A. W., R. H. Purcell, E. D'Hondt, and S. U. Emerson.** 1994. Attenuated hepatitis A virus: genetic determinants of adaptation to growth in MRC-5 cells. J. Virol. **68:**148–157.
89. **Gabrieli, R., G. Sanchez, A. Macaluso, F. Cenko, S. Bino, L. Palombi, E. Buonomo, R. M. Pinto, A. Bosch, and M. Divizia.** 2004. Hepatitis in Albanian children: molecular analysis of hepatitis A virus isolates. J. Med. Virol. **72:**533–537.
90. **Gauss-Muller, V., F. Lottspeich, and F. Deinhardt.** 1986. Characterization of hepatitis A virus structural proteins. Virology **155:**732–736.
91. **Giles, J. P., H. Liebhaber, S. Krugman, and C. Lattimer.** 1964. Early viremia and viruria in infectious hepatitis. Virology **24:**107–108.
92. **Gilgen, M., D. Germann, J. Luthy, and P. Hubner.** 1997. Three-step isolation method for sensitive detection of enterovirus, rotavirus, hepatitis A virus, and small round structured viruses in water samples. Int. J. Food Microbiol. **37:**189–199.
93. **Glikson, M., E. Galun, R. Oren, R. Tur-Kaspa, and D. Shouval.** 1992. Relapsing hepatitis A: review of 14 cases and literature survey. Medicine (Baltimore) **71:**14–23.
94. **Goswami, B. B., W. Burkhardt III, and T. A. Cebula.** 1997. Identification of genetic variants of hepatitis A virus. J. Virol. Methods **65:**95–103.
95. **Graff, J., J. Ticehurst, and B. Flehmig.** 1993. Detection of hepatitis A virus in sewage sludge by antigen capture polymerase chain reaction. Appl. Environ. Microbiol. **59:**3165–3170.
96. **Graff, J., A. Normann, S. M. Feinstone, and B. Flehmig.** 1994. Nucleotide sequence of wild-type hepatitis A virus GBM in comparison with two cell culture-adapted variants. J. Virol. **68:**548–554.
97. **Grinde, B., K. Stene-Johansen, B. Sharma, T. Hoel, M. Jensenius, and K. Skaug.** 1997. Characterisation of an epidemic of hepatitis A virus involving intravenous drug abusers—infection by needle sharing? J. Med. Virol. **53:**69–75.
98. **Hadler, S. C., H. M. Webster, J. J. Erben, J. E. Swanson, and J. E. Maynard.** 1980. Hepatitis A in day-care centers: a community-wide assessment. N. Engl. J. Med. **302:**1222–1227.
99. **Hadler, S. C.** 1991. Global impact of hepatitis A virus infection: changing patterns, p. 14–20. *In* F. B. Hollinger, S. M. Lemon, and H. S. Margolis (ed.), Viral hepatitis and liver disease, Williams & Wilkins, Baltimore, Md.
100. **Halliday, M. L., L. Y. Kang, T. K. Zhou, M. D. Hu, Q. C. Pan, T. Y. Fu, Y. S. Huang, and S. L. Hu.** 1991. An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. J. Infect. Dis. **164:**852–859.
101. **Harkess, J., B. Gildon, and G. R. Istre.** 1989. Outbreaks of hepatitis A among illicit drug users, Oklahoma, 1984–87. Am. J. Public Health **79:**463–466.
102. **Havens, W. P., Jr., and J. R. Paul.** 1945. Prevention of infectious hepatitis with gamma globulin. JAMA **129:**270–272.
103. **Havens, W. P., and R. E. Marck, Jr.** 1946. The leukocytic response of patients with experimentally induced infectious hepatitis. Am. J. Med. Sci. **212:**129–138.
104. **Havens, W. P.** 1947. The etiology of infectious hepatitis. JAMA **134:**653–655.
105. **Henning, K. J., E. Bell, J. Braun, and N. D. Barker.** 1995. A communitywide outbreak of hepatitis A: risk factors for infection among homosexual and bisexual men. Am. J. Med. **99:**132–136.
106. **Hollinger, F. B., D. W. Bradley, J. E. Maynard, G. R. Dreesman, and J. L. Melnick.** 1975. Detection of hepatitis A viral antigen by radioimmunoassay. J. Immunol. **115:**1464–1466.
107. **Hollinger, F. B., and S. U. Emerson.** 2001. Hepatitis A virus, p. 799–840. *In* D. M. Knipe and P. M. Howley (ed.), Fields virology, 4th ed. Lippincott Williams & Wilkins, New York, N.Y.
108. **Hooper, R. R., C. W. Juels, J. A. Routenberg, W. O. Harrison, M. E. Kilpatrick, S. J. Kendra, and J. L. Dienstag.** 1977. An outbreak of type A viral hepatitis at the Naval Training Center, San Diego: epidemiologic evaluation. Am. J. Epidemiol. **105:**148–155.
109. **Huang, S. N., D. Lorenz, and R. J. Gerety.** 1979. Electron and immunoelectron microscopic study on liver tissues of marmosets infected with hepatitis A virus. Lab. Investig. **41:**63–71.
110. **Hurni, W. M., D. Laufer, W. J. Miller, J. Ryan, and B. Watson.** 1993. Anti-hepatitis A in the general population and in hepatitis A vaccinees using saliva and serum as diagnostic media. Ann. N.Y. Acad. Sci. **694:**289–292.
111. **Hussain, Z., B. C. Dasb, S. A. Husain, M. Asima, S. Chattopadhyay, A. Malik, Y. Poovorawan, A. Theamboonlers, and P. Kar.** 2005. Hepatitis A viral genotypes and clinical relevance: clinical and molecular characterization of hepatitis A virus isolates from northern India. Hepatol. Res. **32:**16–24.
112. **Hutin, Y. J., V. Pool, E. H. Cramer, O. V. Nainan, J. Weth, I. T. Williams, S. T. Goldstein, K. F. Gensheimer, B. P. Bell, C. N. Shapiro, M. J. Alter, H. S. Margolis, et al.** 1999. A multistate, foodborne outbreak of hepatitis A. N. Engl. J. Med. **340:**595–602.
113. **Innis, B. L., R. Snitbhan, P. Kunasol, T. Laorakpongse, W. Poopatanakool, C. A. Kozik, S. Suntayakorn, T. Suknuntapong, A. Safary, D. B. Tang, and J. W. Boslego.** 1994. Protection against hepatitis A by an inactivated vaccine. JAMA **271:**1328–1334.
114. **Inoue, K., M. Yoshiba, H. Yotsuyanagi, T. Otsuka, K. Sekiyama, and R. Fujita.** 1996. Chronic hepatitis A with persistent viral replication. J. Med. Virol. **50:**322–324.
115. **Jacobson, S. K., R. Buttery, J. V. Parry, K. R. Perry, and T. G. Wreghitt.** 1995. Investigation of a hepatitis A outbreak in a primary school by sequential saliva sampling. Clin. Diagn. Virol. **3:**173–180.
116. **Jansen, R. W., G. Siegl, and S. M. Lemon.** 1990. Molecular epidemiology of human hepatitis A virus defined by an antigen-capture polymerase chain reaction method. Proc. Natl. Acad. Sci. USA **87:**2867–2871.
117. **Jansen, R. W., G. Siegl, and S. M. Lemon.** 1991. Molecular epidemiology of human hepatitis A virus (HAV), p. 58–62. *In* B. N. Hollinger, S. M. Lemon, and H. S. Margolis (ed.), Viral hepatitis and liver disease. Williams & Wilkins, Baltimore, Md.
118. **Jean, J., B. Blais, A. Darveau, and I. Fliss.** 2001. Detection of hepatitis A virus by the nucleic acid sequence-based amplification technique and comparison with reverse transcription-PCR. Appl. Environ. Microbiol. **67:**5593–5600.
119. **Jean, J., B. Blais, A. Darveau, and I. Fliss.** 2002. Simultaneous detection and identification of hepatitis A virus and rotavirus by multiplex nucleic acid sequence-based amplification (NASBA) and microtiter plate hybridization system. J. Virol. Methods **105:**123–132.
120. **Jean, J., D. H. D'Souza, and L. A. Jaykus.** 2004. Multiplex nucleic acid sequence-based amplification for simultaneous detection of several enteric viruses in model ready-to-eat foods. Appl. Environ. Microbiol. **70:**6603–6610.
121. **Jiang, X., M. K. Estes, and T. G. Metcalf.** 1987. Detection of hepatitis A virus by hybridization with single-stranded RNA probes. Appl. Environ. Microbiol. **53:**2487–2495.
122. **Jindal, M., S. S. Rana, R. K. Gupta, K. Das, and P. Kar.** 2002. Serological study of hepatitis A virus infection amongst the students of a medical college in Delhi and evaluation of the need of vaccination. Indian J. Med. Res. **115:**1–4.
123. **Joshi, M. S., S. D. Chitambar, V. A. Arankalle, and M. S. Chadha.** 2002. Evaluation of urine as a clinical specimen for diagnosis of hepatitis A. Clin. Diagn. Lab. Immunol. **9:**840–845.
124. **Jothikumar, N., D. O. Cliver, and T. W. Mariam.** 1998. Immunomagnetic captures PCR for rapid concentration and detection of hepatitis A virus from environmental samples. Appl. Environ. Microbiol. **64:**504–508.
125. **Jothikumar, N., R. Paulmurugan, P. Padmanabhan, R. B. Sundari, S. Kamatchiammal, and K. S. Rao.** 2000. Duplex RT-PCR for simultaneous

detection of hepatitis A and hepatitis E virus isolated from drinking water samples. J. Environ. Monit. **2:**587–590.
126. **Kabrane-Lazizi, Y., S. U. Emerson, C. Herzog, and R. H. Purcell.** 2001. Detection of antibodies to HAV 3C proteinase in experimentally infected chimpanzees and in naturally infected children. Vaccine **19:**2878–2883.
127. **Kao, H. W., M. Ashcavai, and A. G. Redeker.** 1984. The persistence of hepatitis A IgM antibody after acute clinical hepatitis A. Hepatology **4:**933–936.
128. **Karetnyi, I., A. G. Andzhaparidze, T. M. Orlova, and M. S. Balaian.** 1989. Study of human and simian hepatitis A virus isolated by an immunoenzyme method using polyclonal and monoclonal antibodies. Vopr. Virusol. **34:**50–53. (In Russian.)
129. **Karlsen, F., H. B. Steen, and J. M. Nesland.** 1995. SYBR green I DNA staining increases the detection sensitivity of viruses by polymerase chain reaction. J. Virol. Methods **55:**153–156.
130. **Khanna, B., J. E. Spelbring, B. L. Innis, and B. H. Robertson.** 1992. Characterization of a genetic variant of human hepatitis A virus. J. Med. Virol. **36:**118–124.
131. **Kiltie, A. E., and A. J. Ryan.** 1997. SYBR Green I staining of pulsed field agarose gels is a sensitive and inexpensive way of quantitating DNA double-strand breaks in mammalian cells. Nucleic Acids Res. **25:**2945–2946.
132. **Kingsley, D. H., G. K. Meade, and G. P. Richards.** 2002. Detection of both hepatitis A virus and Norwalk-like virus in imported clams associated with food-borne illness. Appl. Environ. Microbiol. **68:**3914–3918.
133. **Kingsley, D. H., and G. P. Richards.** 2001. Rapid and efficient extraction method for reverse transcription-PCR detection of hepatitis A and Norwalk-like viruses in shellfish. Appl. Environ. Microbiol. **67:**4152–4157.
134. **Komurian-Pradel, F., G. Paranhos-Baccala, M. Sodoyer, P. Chevallier, B. Mandrand, V. Lotteau, and P. Andre.** 2001. Quantitation of HCV RNA using real-time PCR and fluorimetry. J. Virol. Methods **95:**111–119.
135. **Koopmans, M., C. H. von Bonsdorff, J. Vinje, D. de Medici, and S. Monroe.** 2002. Foodborne viruses. FEMS Microbiol. Rev. **26:**187–205.
136. **Krugman, S., R. Ward, J. P. Giles, D. Bodansky, and A. M. Jacobs.** 1959. Infectious hepatitis: detection of virus during the incubation period and in clinically inapparent infection. N. Engl. J. Med. **261:**729–734.
137. **Krugman, S., R. Ward, and J. P. Giles.** 1962. The natural history of infectious hepatitis. Am. J. Med. **32:**717–728.
138. **Krugman, S., J. P. Giles, and J. Hammond.** 1967. Infectious hepatitis. Evidence for two distinctive clinical, epidemiological, and immunological types of infection. JAMA **200:**365–373.
139. **LaBrecque, F. D., D. R. LaBrecque, D. Klinzman, S. Perlman, J. B. Cederna, P. L. Winokur, J. Q. Han, and J. T. Stapleton.** 1998. Recombinant hepatitis A virus antigen: improved production and utility in diagnostic immunoassays. J. Clin. Microbiol. **36:**2014–2018.
140. **Laufer, D. S., W. Hurni, B. Watson, W. Miller, J. Ryan, D. Nalin, and L. Brown.** 1995. Saliva and serum as diagnostic media for antibody to hepatitis A virus in adults and in individuals who have received an inactivated hepatitis A vaccine. Clin. Infect. Dis. **20:**868–871.
141. **Lednar, W. M., S. M. Lemon, J. W. Kirkpatrick, R. R. Redfield, M. L. Fields, and P. W. Kelley.** 1985. Frequency of illness associated with epidemic hepatitis A virus infections in adults. Am. J. Epidemiol. **122:**226–233.
142. **Lee, D. H., and A. M. Prince.** 2001. Automation of nucleic acid extraction for NAT screening of individual blood units. Transfusion **41:**483–487.
143. **Leino, T., P. Leinikki, T. Hyypia, M. Ristola, J. Suni, J. Sutinen, A. Holopainen, O. Haikala, M. Valle, and T. Rostila.** 1997. Hepatitis A outbreak amongst intravenous amphetamine abusers in Finland. Scand. J. Infect. Dis. **29:**213–216.
144. **Lemon, S. M., L. N. Binn, and R. H. Marchwicki.** 1983. Radioimmunofocus assay for quantitation of hepatitis A virus in cell cultures. J. Clin. Microbiol. **17:**834–839.
145. **Lemon, S. M.** 1994. The natural history of hepatitis A: the potential for transmission by transfusion of blood or blood products. Vox Sang **67**(Suppl 4)**:**19–23.
146. **Lemon, S. M.** 1992. HAV: current concepts of the molecular virology, immunobiology and approaches to vaccine development. Rev. Med. Virol. **2:**73–87.
147. **Lemon, S. M., R. W. Jansen, and E. A. Brown.** 1992. Genetic, antigenic and biological differences between strains of hepatitis A virus. Vaccine **10**(Suppl. 1)**:**S40–S44.
148. **Lemon, S. M.** 1993. Immunologic approaches to assessing the response to inactivated hepatitis A vaccine. J. Hepatol. **18**(Suppl 2)**:**S15–S19.
149. **Li, J. W., T. X. Zhong, W. W. Xin, L. Z. Jin, and H. C. Fu.** 2004. Mechanisms of inactivation of hepatitis A virus in water by chlorine dioxide. Water Res. **38:**1514–1519.
150. **Liu, G., N. Hu, and Y. Hu.** 2003. Full-length genome of wild-type hepatitis A virus (DL3) isolated in China. World J. Gastroenterol. **9:**499–504.
151. **Locarnini, S. A., A. G. Coulepis, A. A. Ferris, N. I. Lehmann, and I. D. Gust.** 1978. Purification of hepatitis A virus from human feces. Intervirology **10:**300–308.
152. **Locarnini, S. A., A. G. Coulepis, J. Kaldor, and I. D. Gust.** 1980. Coproantibodies in hepatitis A: detection by enzyme-linked immunosorbent assay and immune electron microscopy. J. Clin. Microbiol. **11:**710–716.
153. **Lowry, P. W., R. Levine, D. F. Stroup, R. A. Gunn, M. H. Wilder, and C. Konigsberg, Jr.** 1989. Hepatitis A outbreak on a floating restaurant in Florida, 1986. Am. J. Epidemiol. **129:**155–164.
154. **Lu, L., K. Z. Ching, V. S. de Paula, T. Nakano, G. Siegl, M. Weitz, and B. H. Robertson.** 2004. Characterization of the complete genomic sequence of genotype II hepatitis A virus (CF53/Berne isolate). J. Gen. Virol. **85:**2943–2952.
155. **Mackay, I. M., K. E. Arden, and A. Nitsche.** 2002. Real-time PCR in virology. Nucleic Acids Res. **30:**1292–1305.
156. **Mackiewicz, V., E. Dussaix, M. F. Le Petitcorps, and A. M. Roque-Afonso.** 2004. Detection of hepatitis A virus RNA in saliva. J. Clin. Microbiol. **42:**4329–4331.
157. **Margolis, H. S., O. V. Nainan, K. Krawczynski, D. W. Bradley, J. W. Ebert, J. Spelbring, H. A. Fields, and J. E. Maynard.** 1988. Appearance of immune complexes during experimental hepatitis A infection in chimpanzees. J. Med. Virol. **26:**315–326.
158. **Margolis, H. S., and O. V. Nainan.** 1990. Identification of virus components in circulating immune complexes isolated during hepatitis A virus infection. Hepatology **11:**31–37.
159. **Margolis, H. S.** 2000. Viral hepatitis, p. 174–188. *In* R. B. Wallace and B. N. Doebbeling (ed.), Maxcy-Rosenau-Last Public Health and Preventive Medicine, 14th ed. Appleton & Lange, Stamford, Conn.
160. **Marziali, A., and M. Akeson.** 2001. New DNA sequencing methods. Annu. Rev. Biomed. Eng. **3:**195–223.
161. **Mbayed, V. A., S. Sookoian, V. Alfonso, and R. H. Campos.** 2002. Genetic characterization of hepatitis A virus isolates from Buenos Aires, Argentina. J. Med. Virol. **68:**168–174.
162. **McCaustland, K. A., W. W. Bond, D. W. Bradley, J. W. Ebert, and J. E. Maynard.** 1982. Survival of hepatitis A virus in feces after drying and storage for 1 month. J. Clin. Microbiol. **16:**957–958.
163. **McMahon, B. J., M. Beller, J. Williams, M. Schloss, H. Tanttila, and L. Bulkow.** 1996. A program to control an outbreak of hepatitis A in Alaska by using an inactivated hepatitis A vaccine. Arch. Pediatr. Adolesc. Med. **150:**733–739.
164. **Morace, G., G. Pisani, M. Divizia, and A. Pana.** 1993. Detection of hepatitis A virus in concentrated river water by polymerase chain reaction. Zentralbl. Hyg. Umweltmed. **193:**521–527.
165. **Moritsugu, Y., J. L. Dienstag, J. Valdesuso, D. C. Wong, J. Wagner, J. A. Routenberg, and R. H. Purcell.** 1976. Purification of hepatitis A antigen from feces and detection of antigen and antibody by immune adherence hemagglutination. Infect. Immun. **13:**898–908.
166. **Morrison, T. B., J. J. Weis, and C. T. Wittwer.** 1998. Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. BioTechniques **24:**954–958, 960, 962.
167. **Nainan, O. V., H. S. Margolis, B. H. Robertson, M. Balayan, and M. A. Brinton.** 1991. Sequence analysis of a new hepatitis A virus naturally infecting cynomolgus macaques (*Macacafascicularis*). J. Gen. Virol. **72:**1685–1689.
168. **Nainan, O. V., M. A. Brinton, and H. S. Margolis.** 1992. Identification of amino acids located in the antibody binding sites of human hepatitis A virus. Virology **191:**984–987.
169. **Nainan, O. V., T. L. Cromeans, and H. S. Margolis.** 1996. Sequence-specific, single-primer amplification and detection of PCR products for identification of hepatitis viruses. J. Virol. Methods **61:**127–134.
170. **Nainan, O. V., G. L. Armstrong, X. Han, I. T. Williams, B. P. Bell, and H. S. Margolis.** 2005. Hepatitis A molecular epidemiology in the United States, 1996–1997: sources of infection and implications of vaccination policy. J. Infect. Dis. **191:**957–963.
171. **Najarian, R., D. Caput, W. Gee, S. J. Potter, A. Renard, J. Merryweather, G. Van Nest, and D. Dina.** 1985. Primary structure and gene organization of human hepatitis A virus. Proc. Natl. Acad. Sci. USA **82:**2627–2631.
172. **Nalin, D. R., B. J. Kuter, L. Brown, C. Patterson, G. B. Calandra, A. Werzberger, D. Shouval, E. Ellerbeck, S. L. Block, and R. Bishop.** 1993. Worldwide experience with the CR326F-derived inactivated hepatitis A virus vaccine in pediatric and adult populations: an overview. J. Hepatol. **18**(Suppl 2)**:**s51–s55.
173. **Nasser, A. M., and T. G. Metcalf.** 1987. Production of cytopathology in FRhK-4 cells by BS-C-1-passaged hepatitis A virus. Appl. Environ. Microbiol. **53:**2967–2971.
174. **Nitsche, A., N. Steuer, C. A. Schmidt, O. Landt, H. Ellerbrok, G. Pauli, and W. Siegert.** 2000. Detection of human cytomegalovirus DNA by real-time quantitative PCR. J. Clin. Microbiol. **38:**2734–2737.
175. **Niu, M. T., L. B. Polish, B. H. Robertson, B. K. Khanna, B. A. Woodruff, C. N. Shapiro, M. A. Miller, J. D. Smith, J. K. Gedrose, M. J. Alter, H. S. Margolis, and the National Hepatitis A Investigation Team.** 1992. Multistate outbreak of hepatitis A associated with frozen strawberries. J. Infect. Dis. **166:**518–524.
176. **Noble, R. C., M. A. Kane, S. A. Reeves, and I. Roeckel.** 1984. Posttransfusion hepatitis A in a neonatal intensive care unit. JAMA **252:**2711–2715.
177. **Oba, I. T., A. M. Spina, C. P. Saraceni, M. F. Lemos, R. Senhoras, R. C. Moreira, and C. F. Granato.** 2000. Detection of hepatitis A antibodies by

ELISA using saliva as clinical samples. Rev. Inst. Med. Trop. Sao Paulo **42:**197–200.
178. **Ochnio, J. J., D. W. Scheifele, M. Ho, and L. A. Mitchell.** 1997. New, ultrasensitive enzyme immunoassay for detecting vaccine- and disease-induced hepatitis A virus-specific immunoglobulin G in saliva. J. Clin. Microbiol. **35:**98–101.
179. **Osterholm, M. T., R. J. Kantor, D. W. Bradley, W. N. Hall, D. P. Francis, H. C. Aaron, J. W. Washburn, and D. Velde.** 1980. Immunoglobulin M-specific serologic testing in an outbreak of foodborne viral hepatitis, type A. Am. J. Epidemiol. **112:**8–16.
180. **Ostermayr, R., K. Helm, V. Gauss-Muller, E. L. Winnacker, and F. Deinhardt.** 1987. Expression of hepatitis A virus cDNA in *Escherichia coli*: antigenic VP1 recombinant protein. J. Virol. **61:**3645–3647.
181. **Pana, A., M. Divizia, P. de Filippis, and A. di Napoli.** 1987. Isolation of hepatitis A virus from polluted river water on FRP/3 cells. Lancet **ii:**1328.
182. **Paul, A. V., H. Tada, K. von der Helm, T. Wissel, R. Kiehn, E. Wimmer, and F. Deinhardt.** 1987. The entire nucleotide sequence of the genome of human hepatitis A virus (isolate MBB). Virus Res. **8:**153–171.
183. **Perry, K. R., J. V. Parry, E. M. Vandervelde, and P. P. Mortimer.** 1992. The detection in urine specimens of IgG and IgM antibodies to hepatitis A and hepatitis B core antigens. J. Med. Virol. **38:**265–270.
184. **Pina, S., M. Buti, R. Jardi, P. Clemente-Casares, J. Jofre, and R. Girones.** 2001. Genetic analysis of hepatitis A virus strains recovered from the environment and from patients with acute hepatitis. J. Gen. Virol. **82:**2955–2963.
185. **Ping, L. H., R. W. Jansen, J. T. Stapleton, J. I. Cohen, and S. M. Lemon.** 1988. Identification of an immunodominant antigenic site involving the capsid protein VP3 of hepatitis A virus. Proc. Natl. Acad. Sci. USA **85:**8281–8285.
186. **Ping, L. H., and S. M. Lemon.** 1992. Antigenic structure of human hepatitis A virus defined by analysis of escape mutants selected against murine monoclonal antibodies. J. Virol. **66:**2208–2216.
187. **Pinto, M. A., R. S. Marchevsky, M. L. Baptista, M. A. de Lima, M. Pelajo-Machado, C. L. Vitral, C. F. Kubelka, J. W. Pissurno, M. S. Franca, H. G. Schatzmayr, and A. M. Gaspar.** 2002. Experimental hepatitis A virus (HAV) infection in Callithrix jacchus: early detection of HAV antigen and viral fate. Exp. Toxicol. Pathol. **53:**413–420.
188. **Poddar, U., B. R. Thapa, A. Prasad, and K. Singh.** 2002. Changing spectrum of sporadic acute viral hepatitis in Indian children. J. Trop. Pediatr. **48:**210–213.
189. **Polish, L. B., B. H. Robertson, B. Khanna, K. Krawczynski, J. Spelbring, F. Olson, and C. N. Shapiro.** 1999. Excretion of hepatitis A virus (HAV) in adults: comparison of immunologic and molecular detection methods and relationship between HAV positivity and infectivity in tamarins. J. Clin. Microbiol. **37:**3615–3617.
190. **Poonawagul, U., S. Warintrawat, R. Snitbhan, S. Kitisriwarapoj, V. Chaiyakunt, and H. M. Foy.** 1995. Outbreak of hepatitis A in a college traced to contaminated water reservoir in cafeteria. Southeast Asian J. Trop. Med. Public Health **26:**705–708.
191. **Poovorawan, Y., A. Theamboonlers, V. Chongsrisawat, P. Jantaradsamee, S. Chutsirimongkol, and P. Tangkijvanich.** 2005. Clinical features and molecular characterization of hepatitis A virus outbreak in a child care center in Thailand. J. Clin. Virol. **32:**24–28.
192. **Provost, P. J., B. S. Wolanski, W. J. Miller, O. L. Ittensohn, W. J. McAleer, and M. R. Hilleman.** 1975. Physical, chemical and morphologic dimensions of human hepatitis A virus strain CR326 (38578). Proc. Soc. Exp. Biol. Med. **148:**532–539.
193. **Provost, P. J., and M. R. Hilleman.** 1979. Propagation of human hepatitis A virus in cell culture in vitro. Proc. Soc. Exp. Biol. Med. **160:**213–221.
194. **Purcell, R. H., D. C. Wong, Y. Moritsugu, J. L. Dienstag, J. A. Routenberg, and J. D. Boggs.** 1976. A microtiter solid phase radioimmunoassay for hepatitis A antigen and antibody. J. Immunol. **116:**349–356.
195. **Ramsay, C. N., and P. A. Upton.** 1989. Hepatitis A and frozen raspberries. Lancet **i:**43–44.
196. **Randhawa, J. S., and A. J. Easton.** 1999. Demystified. DNA nucleotide sequencing. Mol. Pathol. **52:**117–124.
197. **Reid, T. M., and H. G. Robinson.** 1987. Frozen raspberries and hepatitis A. Epidemiol. Infect. **98:**109–112.
198. **Rezende, G., A. M. Roque-Afonso, D. Samuel, M. Gigou, E. Nicand, V. Ferre, E. Dussaix, H. Bismuth, and C. Feray.** 2003. Viral and clinical factors associated with the fulminant course of hepatitis A infection. Hepatology **38:**613–618.
199. **Robertson, B. H., B. Khanna, O. V. Nainan, and H. S. Margolis.** 1991. Epidemiologic patterns of wild-type hepatitis A virus determined by genetic variation. J. Infect. Dis. **163:**286–292.
200. **Robertson, B. H., R. W. Jansen, B. Khanna, A. Totsuka, O. V. Nainan, G. Siegl, A. Widell, H. S. Margolis, S. Isomura, K. Ito, T. Ishizu, Y. Moritsugu, and S. M. Lemon.** 1992. Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. J. Gen. Virol. **73:**1365–1377.
201. **Robertson, B. H., X. Y. Jia, H. Tian, H. S. Margolis, D. F. Summers, and E. Ehrenfeld.** 1992. Serological approaches to distinguish immune response to hepatitis A vaccine and natural infection. Vaccine **10**(Suppl. 1)**:**S106–S109.
202. **Robertson, B. H., X. Y. Jia, H. Tian, H. S. Margolis, D. F. Summers, and E. Ehrenfeld.** 1993. Antibody response to nonstructural proteins of hepatitis A virus following infection. J. Med. Virol. **40:**76–82.
203. **Robertson, B. H., F. Averhoff, T. L. Cromeans, X. H. Han, B. Khoprasert, O. V. Nainan, J. Rosenberg, L. Paikoff, E. DeBess, C. N. Shapiro, and H. S. Margolis.** 2000. Genetic relatedness of hepatitis A virus isolates during a community-wide outbreak. J. Med. Virol. **62:**144–150.
204. **Rosenblum, L. S., I. R. Mirkin, D. T. Allen, S. Safford, and S. C. Hadler.** 1990. A multifocal outbreak of hepatitis A traced to commercially distributed lettuce. Am. J. Public Health **80:**1075–1079.
205. **Rosenblum, L. S., M. E. Villarino, O. V. Nainan, M. E. Melish, S. C. Hadler, P. P. Pinsky, W. R. Jarvis, C. E. Ott, and H. S. Margolis.** 1991. Hepatitis A outbreak in a neonatal intensive care unit: risk factors for transmission and evidence of prolonged viral excretion among preterm infants. J. Infect. Dis. **164:**476–482.
206. **Sanchez, G., R. M. Pinto, H. Vanaclocha, and A. Bosch.** 2002. Molecular characterization of hepatitis A virus isolates from a transcontinental shellfish-borne outbreak. J. Clin. Microbiol. **40:**4148–4155.
207. **Sanchez, G., A. Bosch, and R. M. Pinto.** 2003. Genome variability and capsid structural constraints of hepatitis A virus. J. Virol. **77:**452–459.
208. **Sanchez, G., C. Villena, A. Bosch, and R. M. Pinto.** 2004. Hepatitis A virus: molecular detection and typing. Methods Mol. Biol. **268:**103–114.
209. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
210. **Schiodt, F. V., E. Atillasoy, A. Obaid Shakil, E. R. Schiff, C. Caldwell, K. V. Kowdley, R. Stribling, J. S. Crippen, S. Flamm, K. A. Somberg, H. Rosen, T. M. McCashland, J. E. Hay, W. M. Lee, and the Acute Liver Failure Study Group.** 1999. Etiology and outcome for 295 patients with acute liver failure in the United States. Liver Transplant. Surg. **5:**29–34.
211. **Schultheiss, T., Y. Y. Kusov, and V. Gauss-Muller.** 1994. Proteinase 3C of hepatitis A virus (HAV) cleaves the HAV polyprotein P2-P3 at all sites including VP1/2A and 2A/2B. Virology **198:**275–281.
212. **Schwab, K. J., R. De Leon, and M. D. Sobsey.** 1995. Concentration and purification of beef extract mock eluates from water samples for the detection of enteroviruses, hepatitis A virus, and Norwalk virus by reverse transcription-PCR. Appl. Environ. Microbiol. **61:**531–537.
213. **Seelig, R., G. Pott, H. P. Seelig, H. Liehr, P. Metzger, and R. Waldherr.** 1984. Virus-binding activity of fibronectin: masking of hepatitis A virus. J. Virol. Methods **8:**335–347.
214. **Shao, Z. J., D. Z. Xu, Y. P. Yan, J. H. Li, J. X. Zhang, Z. Y. Zhang, and B. R. Pan.** 2003. Detection of anti-HAV antibody with dot immunogold filtration assay. World J. Gastroenterol. **9:**1508–1511.
215. **Shapiro, C. N., P. J. Coleman, G. M. McQuillan, M. J. Alter, and H. S. Margolis.** 1992. Epidemiology of hepatitis A: seroepidemiology and risk groups in the USA. Vaccine **10**(Suppl. 1)**:**S59–S62.
216. **Shouval, D., Y. Ashur, R. Adler, J. A. Lewis, M. E. Armstrong, J. P. Davide, B. McGuire, B. Kuter, L. Brown, and W. Miller.** 1993. Single and booster responses to an inactivated hepatitis A vaccine: comparisons with immune globulin prophylaxis. Vaccine **11**(Suppl. 1):S9–S14.
217. **Siegl, G., J. deChastonay, and G. Kronauer.** 1984. Propagation and assay of hepatitis A virus *in vitro*. J. Virol. Methods **9:**53–67.
218. **Sjogren, M. H., H. Tanno, O. Fay, S. Sileoni, B. D. Cohen, D. S. Burke, and R. J. Feighny.** 1987. Hepatitis A virus in stool during clinical relapse. Ann. Intern. Med. **106:**221–226.
219. **Sjogren, M. H., C. H. Hoke, L. N. Binn, K. H. Eckels, D. R. Dubois, L. Lyde, A. Tsuchida, S. Oaks, Jr., R. Marchwicki, W. Lednar, R. Chloupek, J. Ticehurst, and W. H. Bancroft.** 1991. Immunogenicity of an inactivated hepatitis A vaccine. Ann. Intern. Med. **114:**470–471.
220. **Skinhoj, P., F. Mikkelsen, and F. B. Hollinger.** 1977. Hepatitis A in Greenland: importance of specific antibody testing in epidemiologic surveillance. Am. J. Epidemiol. **105:**140–147.
221. **Skinhoj, P., L. R. Mathiesen, P. Kiryger, and A. M. Moller.** 1981. Faeccel excretion of hepatitis A virus in patients with symptomatic hepatitis A infection. Scand. J. Gastroenterol. **16:**1057–1059.
222. **Smith, P. F., J. C. Grabau, A. Werzberger, R. A. Gunn, H. R. Rolka, S. F. Kondracki, R. J. Gallo, and D. L. Morse.** 1997. The role of young children in a community-wide outbreak of hepatitis A. Epidemiol. Infect. **118:**243–252.
223. **Sobsey, M. D., S. E. Oglesbee, and D. A. Wait.** 1985. Evaluation of methods for concentrating hepatitis A virus from drinking water. Appl. Environ. Microbiol. **50:**1457–1463.
224. **Soucie, J. M., B. H. Robertson, B. P. Bell, K. A. McCaustland, and B. L. Evatt.** 1998. Hepatitis A virus infections associated with clotting factor concentrate in the United States. Transfusion **38:**573–579.
225. **Staes, C. J., T. L. Schlenker, I. Risk, K. G. Cannon, H. Harris, A. T. Pavia, C. N. Shapiro, and B. P. Bell.** 2000. Sources of infection among persons with acute hepatitis A and no identified risk factors during a sustained community-wide outbreak. Pediatrics **106:**E54.
226. **Stahlberg, A., J. Hakansson, X. Xian, H. Semb, and M. Kubista.** 2004. Properties of the reverse transcription reaction in mRNA quantification. Clin. Chem. **50:**509–515.

227. **Stahlberg, A., M. Kubista, and M. Pfaffl.** 2004. Comparison of reverse transcriptases in gene expression analysis. Clin. Chem. **50:**1678–1680.
228. **Stapleton, J. T., and S. M. Lemon.** 1987. Neutralization escape mutants define a dominant immunogenic neutralization site on hepatitis A virus. J. Virol. **61:**491–498.
229. **Stapleton, J. T., D. K. Lange, J. W. LeDuc, L. N. Binn, R. W. Jansen, and S. M. Lemon.** 1991. The role of secretory immunity in hepatitis A virus infection. J. Infect. Dis. **163:**7–11.
230. **Stapleton, J. T.** 1995. Host immune response to hepatitis A virus. J. Infect. Dis. **171:**S9–S14.
231. **Steffen, R., M. A. Kane, C. N. Shapiro, N. Billo, K. J. Schoellhorn, and P. van Damme.** 1994. Epidemiology and prevention of hepatitis A in travelers. JAMA **272:**885–889.
232. **Stene-Johansen, K., K. Skaug, H. Blystad, B. Grinde, et al.** 1998. A unique hepatitis A virus strain caused an epidemic in Norway associated with intravenous drug abuse. Scand. J. Infect. Dis. **30:**35–38.
233. **Stewart, D. R., T. S. Morris, R. H. Purcell, and S. U. Emerson.** 1997. Detection of antibodies to the nonstructural 3C proteinase of hepatitis A virus. J. Infect. Dis. **176:**593–601.
234. **Strazynski, M., J. Kramer, and B. Becker.** 2002. Thermal inactivation of poliovirus type 1 in water, milk and yoghurt. Int. J. Food Microbiol. **74:**73–78.
235. **Sundkvist, T., C. Aitken, G. Duckworth, and D. Jeffries.** 1997. Outbreak of acute hepatitis A among homosexual men in East London. Scand. J. Infect. Dis. **29:**211–212.
236. **Sunen, E., and M. D. Sobsey.** 1999. Recovery and detection of enterovirus, hepatitis A virus and Norwalk virus in hardshell clams (Mercenaria mercenaria) by RT-PCR methods. J. Virol. Methods **77:**179–187.
237. **Sunen, E., N. Casas, B. Moreno, and C. Zigorraga.** 2004. Comparison of two methods for the detection of hepatitis A virus in clam samples (Tapes spp.) by reverse transcription-nested PCR. Int. J. Food Microbiol. **91:**147–154.
238. **Szmuness, W., J. L. Dienstag, R. H. Purcell, E. J. Harley, C. E. Stevens, and D. C. Wong.** 1976. Distribution of antibody to hepatitis A antigen in urban adult populations. N. Engl. J. Med. **295:**755–759.
239. **Tallo, T., H. Norder, V. Tefanova, K. Ott, V. Ustina, T. Prukk, O. Solomonova, J. Schmidt, K. Zilmer, L. Priimagi, and T. Krispin.** 2003. Sequential changes in hepatitis A virus genotype distribution in Estonia during 1994 to 2001. J. Med. Virol. **70:**187–193.
240. **Tapp, I., L. Malmberg, E. Rennel, M. Wik, and A. C. Syvanen.** 2000. Homogeneous scoring of single-nucleotide polymorphisms: comparison of the 5′-nuclease TaqMan assay and Molecular Beacon probes. BioTechniques **28:**732–738.
241. **Tassopoulos, N. C., G. J. Papaevangelou, J. R. Ticehurst, and R. H. Purcell.** 1986. Fecal excretion of Greek strains of hepatitis A virus in patients with hepatitis A and in experimentally infected chimpanzees. J. Infect. Dis. **154:**231–237.
242. **Taylor, M. B.** 1997. Molecular epidemiology of South African strains of hepatitis A virus: 1982–1996. J. Med. Virol. **51:**273–279.
243. **Ticehurst, J. R., V. R. Racaniello, B. M. Baroudy, D. Baltimore, R. H. Purcell, and S. M. Feinstone.** 1983. Molecular cloning and characterization of hepatitis A virus cDNA. Proc. Natl. Acad. Sci. USA **80:**5885–5889.
244. **Ticehurst, J. C., J. I. Cohen, and R. H. Purcell.** 1988. Analysis of molecular sequences demonstrates that hepatitis A virus (HAV) is a unique picornavirus, p. 33–35. *In* A. J. Zuckerman (ed.), Viral hepatitis and liver disease. Alan R. Liss, New York, N.Y.
245. **Tjon, G. M. S., C. J. Wijkmans, R. A. Coutinho, A. G. Koek, J. A. R. van den Hoeka, A. C. A. P. Leenders, P. M. Schneeberger, and S. M. Bruisten.** 2005. Molecular epidemiology of hepatitis A in Noord-Brabant, The Netherlands. J. Clin. Virol. **32:**128–136.
246. **Tsai, Y. L., M. D. Sobsey, L. R. Sangermano, and C. J. Palmer.** 1993. Simple method of concentrating enteroviruses and hepatitis A virus from sewage and ocean water for rapid detection by reverse transcriptase-polymerase chain reaction. Appl. Environ. Microbiol. **59:**3488–3491.
247. **Tsai, Y. L., B. Tran, L. R. Sangermano, and C. J. Palmer.** 1994. Detection of poliovirus, hepatitis A virus, and rotavirus from sewage and ocean water by triplex reverse transcriptase PCR. Appl. Environ. Microbiol. **60:**2400–2407.
248. **Tsarev, S. A., S. U. Emerson, M. S. Balayan, J. Ticehurst, and R. H. Purcell.** 1991. Simian hepatitis A virus (HAV) strain AGM 27: comparison of genome structure and growth in cell culture with other HAV strains. J. Gen. Virol. **72:**1677–1683.
249. **Van Steenbergen, J. E., V. G. Tjon, A. v. d. Hoek, A. Koek, R. A. Coutinho, and S. M. Bruisten.** 2004. Two years' prospective collection of molecular and epidemiological data shows limited spread of hepatitis A virus outside risk groups in Amsterdam, 2000–2002. J. Infect. Dis. **189:**471–482.
250. **Vento, S., T. Garofano, C. Renzini, F. Cainelli, F. Casali, G. Ghironzi, T. Ferraro, and E. Concia.** 1998. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. N. Engl. J. Med. **338:**286–290.
251. **Villar, L. M., E. Lampe, A. Meyer, and A. M. Gaspar.** 2004. Genetic variability of hepatitis A virus isolates in Rio de Janeiro: implications for the vaccination of school children. Braz. J. Med. Biol. Res. **37:**1779–1787.
252. **Vong, S., A. E. Fiore, D. O. Haight, J. Li, N. Borgsmiller, W. Kuhnert, F. Pinero, K. Boaz, T. Badsgard, C. Mancini, O. V. Nainan, S. Wiersma, and B. P. Bell.** 2005. Vaccination in the county jail as a strategy to reach high risk adults during a community-based hepatitis A outbreak among methamphetamine drug users. Vaccine **23:**1021–1028.
253. **Wang, Z. Q., L. Ma, H. Gao, and X. G. Dong.** 1986. Propagation of hepatitis A virus in human diploid fibroblast cells. Acta Virol. **30:**463–467.
254. **Wang, C. H., S. Y. Tschen, U. Heinricy, M. Weber, and B. Flehmig.** 1996. Immune response to hepatitis A virus capsid proteins after infection. J. Clin. Microbiol. **34:**707–713.
255. **Wasley, A., T. Samandari, T., and B. P. Bell.** 2005. Incidence of hepatitis A in the United States in the era of vaccination. JAMA **294:**194–201.
256. **Wattanasri, N., K. Ruchusatsawat, and S. Wattanasri.** 2005. Phylogenetic analysis of hepatitis A virus in Thailand. J. Med. Virol. **75:**1–7.
257. **Weber, D. J., S. L. Barbee, M. D. Sobsey, and W. A. Rutala.** 1999. The effect of blood on the antiviral activity of sodium hypochlorite, a phenolic, and a quaternary ammonium compound. Infect. Control Hosp. Epidemiol. **20:**821–827.
258. **Weinberg, M., J. Hopkins, L. Farrington, L. Gresham, M. Ginsberg, and B. P. Bell.** 2004. Hepatitis A in Hispanic children who live along the United States-Mexico border: the role of international travel and food-borne exposures. Pediatrics **114:**e68–e73.
259. **Weitz, M., B. M. Baroudy, W. L. Maloy, J. R. Ticehurst, and R. H. Purcell.** 1986. Detection of a genome-linked protein (VPg) of hepatitis A virus and its comparison with other picornaviral VPgs. J. Virol. **60:**124–130.
260. **Wheeler, C., T. Vogt, G. L. Armstrong, G. Vaughan, A. Weltman, O. V. Nainan, V. Dato, G. Xia, K. Waller, J. Amon, T. M. Lee, A. Highbaugh-Battle, C. Hembree, S. Evenson, M. A. Ruta, I. T. Williams, A. Fiore, and B. P. Bell.** 2005. An outbreak of hepatitis A associated with green onions. N. Engl. J. Med. **353:**890–897.
261. **Wiedmann, M., S. Boehm, W. Schumacher, C. Swysen, and M. Zauke.** 2003. Evaluation of three commercial assays for the detection of hepatitis A virus. Eur. J. Clin. Microbiol. Infect. Dis. **22:**129–130.
262. **Xia, G., Y. Yi, K. Guo, and H. Tian.** 1997. Comparison of the Chinese LJ strain structural gene with HM175, MBB, LA strains and the expression of hepatitis A virus antigen by LJ/HM175 recombinant vaccinia virus. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi **11:**208–211.
263. **Yao, G.** 1991. Clinical spectrum and natural history of viral hepatitis A in a 1988 Shanghai epidemic, p. 76–78. *In* F. B. Hollinger, S. M. Lemon, and H. S. Margolis (ed.), Viral hepatitis and liver disease. Williams & Wilkins, Baltimore, Md.
264. **Yotsuyanagi, H., S. Iino, K. Koike, K. Yasuda, K. Hino, and K. Kurokawa.** 1993. Duration of viremia in human hepatitis A viral infection as determined by polymerase chain reaction. J. Med. Virol. **40:**35–38.
265. **Yotsuyanagi, H., K. Koike, K. Yasuda, K. Moriya, Y. Shintani, H. Fujie, K. Kurokawa, and S. Iino.** 1996. Prolonged fecal excretion of hepatitis A virus in adult patients with hepatitis A as determined by polymerase chain reaction. Hepatology **24:**10–13.
266. **Zhou, Y. J., M. K. Estes, X. Jiang, and T. G. Metcalf.** 1991. Concentration and detection of hepatitis A virus and rotavirus from shellfish by hybridization tests. Appl. Environ. Microbiol. **57:**2963–2968.