



Hepatitis Viruses—Prevention and Control in the Laboratory Setting

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Abstract

At least five human hepatitis viruses have been identified worldwide. Within the United States, hepatitis A, B, and C are endemic and account for over 97% of acute viral hepatitis infections. Infection with hepatitis E virus (HEV) is rarely reported in the United States but causes large waterborne outbreaks in developing countries. Mounting evidence indicates that HEV is a zoonotic agent that may result in asymptomatic infections. Both hepatitis A and E are transmitted by the fecal-oral route, while hepatitis B, C, and D are blood-borne diseases. This review summarizes the physical properties of these viruses and describes the timing and location of virus during an infection as background information for intelligent handling of these agents in the laboratory. The approaches and methods that should be used for control and prevention are summarized based upon the mode of transmission and the availability of vaccine prevention.

Background

Currently, there are five recognized agents of viral hepatitis: hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis delta virus (HDV), and hepatitis E virus (HEV). Scientific classifications that distribute these viruses into five different families are discussed in more detail below. However, from a public health perspective, there are multiple ways to categorize this collection of viral agents. Hepatitis A and hepatitis B infections are grouped as vaccine-preventable diseases. Classifica-

tion can also be based upon the primary mode of transmission: HBV, HDV, and HCV are blood-borne, while HAV and HEV are primarily enterically transmitted hepatitis viruses. A third perspective that can be used to categorize hepatitis viruses is whether or not they cause chronic disease. HBV, HDV, and HCV infections can persist for years and are associated with chronic liver disease, whereas HAV and HEV will not persist and cause only acute, self-limited infections. From a biosafety perspective, knowledge of the structure of the viruses and how they are transmitted, as well as an understanding of the epidemiology of these agents, provides a background for informed decisions. This article focuses on these five viral agents and their primary modes of transmission.

Enterically Transmitted Hepatitis Viruses: Hepatitis A and E Virus

What Are the Physical Properties of Hepatitis A and E Virus?

Both of these viruses are nonenveloped, icosahedral capsids surrounding a positive sense RNA genome (Table 1, Figures 1A and 1B). However, they differ in a number of other features. Hepatitis A virus (HAV) is a 27-30nm encapsidated member of the picornavirus family. The HAV positive-sense RNA genome contains a single open-reading frame, and individual viral proteins are generated by proteolytic cleavage from the long polyprotein (Hollinger & Ticehurst, 1996; Robertson & Lemon, 1998). Hepatitis E virus, however, is an unclassified, nonen-

veloped virus that shares some physical properties with caliciviruses. The HEV capsid (30-35nm) is formed using the single capsid polypeptide, and the positive-stranded RNA genome has three overlapping reading frames (Purcell, 1996; Robertson & Lemon, 1998).

These viruses have epidemiologic features that distinguish them from each other. Hepatitis A disease is common in the United States and occurs in all age groups, with young children playing a predominate role in disease transmission. Unlike HAV, HEV is not known to occur endemically in the U.S., but causes large, often waterborne outbreaks in developing countries of the Americas, Asia, and Africa. In addition, HEV disease occurs primarily among young adults, not infants and young children as commonly seen with HAV and other enteroviruses. This fact reflects features of the virus-host interaction that differ between these two agents. A building body of literature suggests that HEV is a naturally occurring virus in all parts of the world for which domestic animals may be a reservoir (Drobeniuc et al., 2001; Favorov et al., 1996; Meng et al., 2002).

The dramatic outbreaks in developing countries, resulting from massive fecal contamination (from humans and various domestic animals) of the drinking water, may represent a zoonotic infection due to virus found in animal stools. Human-to-human transmission has not been documented but has been discussed in the literature. Reports have documented the presence of virus in the feces of patients with acute HEV infection (Aggarwal et al., 2000); however, the levels were found to be low and short-lived, which makes them not a likely reservoir. Others have examined families in endemic areas for evidence of intrafamilial spread and none has been found (Arankalle et al., 2000). On the other hand, mother-to-infant transmission has been confirmed in a report from the United Arab Emirates (Kumar et al., 2001) and transfusion transmission has been implicated in others (Arankalle & Chobe, 2000).

Where and When is the Virus Present During Infection?

As viruses that are transmitted via the fecal-oral route, the primary source of HAV and HEV is stool

Table 1
Properties of Hepatitis Viruses

Family	HAV Picornavirus	HBV Hepadnavirus	HDV Viroid/Satellite	HCV Flavivirus	HEV Unclassified
Genome Characteristics					
Length	7.5Kb	3.2Kb	1.7Kb	9.4Kb	7.5Kb
Features	single strand (+) RNA	(ds) circular DNA	closed circular (-) RNA	single strand (+) RNA	single strand (+) RNA
Lipid Bilayer Coat	No	Yes	Yes	Yes	No
Dimensions	27-32nm	42nm	35-37nm	60-70nm	32-34nm
Primary Transmission Mode	fecal-oral	blood/body fluids	blood/body fluids	blood/body fluids	fecal-oral
Serum Titers (ml ⁻¹)	10 ⁴ -10 ⁵	up to 10 ⁹	up to 10 ¹¹	up to 10 ⁷	10 ³ -10 ⁴
Stool Titers (g ⁻¹)	10 ⁷ -10 ⁹	not infectious	not infectious	not infectious	10 ⁶ -10 ⁷
Acute/Chronic	acute	chronic	chronic	chronic	acute
Vaccine Available	yes	yes	yes (HBV)	no	no
Stability					
Heat	yes - 60C, 12hr	no - 60C, 10hr	see HBV	no - 60C, 4hr	unknown
Acid	yes	no	no	unknown	unknown
Organic Solvents	yes	no	no	no	yes

specimens (Table 1). After infection, the incubation period for HAV and HEV ranges from 14 to 45 days. The majority of virus shedding in feces occurs during the time period before clinical symptoms (fever, malaise, myalgia, nausea, anorexia and vomiting, upper right quadrant abdominal pain, jaundice, dark urine, clay-colored stools) develop and liver enzymes become elevated; however, virus excretion can be detected for approximately 80 days after the peak of live enzyme elevation (Bower et al., 2000). It is important to note that viremia associated with hepatitis A or E infections also brackets the clinical symptoms; however, the titer of HAV in the blood is 2-4 logs lower than that present in feces (Table 1, Purcell et al., 1984).

Hepatitis A is rarely reported to be transmitted by blood donated during the incubation period. Epidemiologic studies examining modes of transmission in humans have not found evidence that HAV is transmitted through saliva; however, the virus has been detected in the saliva of an experimentally infected chimpanzee (Cohen et al., 1989). Titers for HEV viremia are also 2-4 logs lower than that found in stools (Table 1, Tsarev et al., 1994), but to date there are no epidemiologic data that implicate HEV infection resulting from exposure to blood or blood products. However, it should be noted that the case fatality rate in pregnant women is much higher than that seen in the general population (15%-25% vs. 1%-3%). With this information, pregnant women should take care when with working with feces, blood, or blood products.

Control and Prevention

General approaches for the control and prevention of hepatitis A and E infections include sanitation and personal hygiene. The simplest and most efficient method to prevent the transmission of these two viruses within the laboratory setting is frequent hand-washing with soap and water when handling any specimen. From a practical perspective, wearing gloves during specimen preparation/set-up and when performing any assays directly on a sample, followed by their removal upon completion and with hand-washing is an effective means of preventing laboratory transmission. If accidental skin contact should occur, wash the area thoroughly

with soap and water. Should contact occur through a glove break, remove the gloves immediately and wash the hands thoroughly. Strict enforcement of rules that prohibit mouth pipetting, smoking, eating, and drinking in the laboratory must be maintained. All laboratory personnel should wear long-sleeved laboratory coats that are buttoned in the front or tied in the back to provide adequate protection. These are to be changed if they are grossly contaminated with blood or other body fluids. In situations where droplets of stool suspension or blood products could be splashed in the face, face protection (masks and goggles/face shields) are to be worn or the work is to be conducted in a fume hood or biosafety cabinet.

If inadvertent exposure to HAV occurs, immune globulin has been shown to be effective in prevention of disease if given within 2 weeks of the exposure. Currently, a vaccine that is highly protective against HAV is available and recommended for research laboratory workers handling HAV (Advisory Committee on Immunization Practices, 1999). With regard to HEV, at this time no immune globulin treatment is available for known exposures to infected samples or sources, and vaccine development is still underway.

Hepatitis Viruses Transmitted by Blood and Body Fluids: Hepatitis B/D Virus and Hepatitis C Virus

What are the Physical Properties of the Virus?

The three hepatitis agents (HBV, HDV, HCV) known to be transmitted by blood or body fluids all have a lipid bilayer as part of their external coat, and this is the feature that makes them susceptible to inactivation by detergents and organic solvents. HBV, the first recognized blood-borne hepatitis agent, is a member of the orthohepadnavirus family and contains a partially double-stranded DNA genome within a protein shell composed of the core protein. This core particle is further enclosed within a lipid bilayer containing three molecular weight forms of the viral glycoprotein commonly referred to as surface antigen (HBsAg) (Hollinger, 1996; Kann

& Gerlich, 1998). The infectious form of HBV is about 42nm in diameter as shown in Figure 1C.

The second agent to be discovered was HDV, or delta agent. This incomplete virus requires the presence of HBV to cause infection. HDV is composed of a covalently closed, circular, negative-sense RNA surrounded by a capsid composed of the delta antigen. The particle is enclosed within the HBV lipid bilayer made up entirely of the HBV surface antigen (HBsAg) (Purcell & Gerin 1983; Taylor, 1996). Negatively stained HDV is slightly smaller (35nm) than the infectious form of HBV.

Hepatitis C virus, a recently recognized agent of viral hepatitis, is a member of the flavivirus family and is composed of a single-stranded, positive-sense RNA genome approximately 9 kb in length (Simmonds et al., 1998). This RNA genome is enclosed within a nucleocapsid structure composed of the core protein. In a relationship similar to that seen for HBV and HDV, the nucleocapsid is covered in a lipid bilayer containing the viral surface glycoproteins, E1 and E2. Figure 1D is a graphic illustration of a virus particle (-70nm) with the nucleocapsid and surrounding lipid bilayer derived from the serum.

Where and When is the Virus Present?

HBV, HDV, and HCV are present in the blood and body fluids of acute or chronically infected persons and can be transmitted by percutaneous and permucosal (sexual and perinatal) exposures. Sero-prevalence studies indicate that 1.26% of the U.S. population (2.7 million) is chronically infected with HCV while about 0.3% are chronic HBV carriers. The concentration of HBV in various body fluids (Davison et al., 1987; Karayiannis et al., 1985; Kutsun et al., 2000; Mitsuda et al., 1989; Su et al., 1994) varies from high to not detectable during infection, as illustrated in Table 2. The concentration found in blood or blood fluids can be up to 10^9 per ml (Zyzik et al., 1986), making HBV the most readily transmitted blood-borne virus. Titers of HDV in blood during acute infection are also high, up to 10^{11} per ml (Ponzetto et al., 1987). Efficient transmission can be achieved by sexual contact and exposure to blood and blood products, as well as by close personal contact in crowded household settings. However, specific studies on HDV detection and titers have not been performed on the same spectrum of body fluids as has been done for HBV and HCV, thus limiting our ability to recognize all possi-

Table 2

Detection or Titers of Blood-borne Hepatitis Viruses in Different Body Fluids

Body Fluid/Secretions	HBV	HDV	HCV
Blood	up to 10^9 /ml	up to 10^{11} /ml	up to 10^7 /ml
Semen	10^6 - 10^7 /ml	ND	neg
Vaginal Fluid	pos	ND	neg
Saliva	10^5 - 10^7 /ml	ND	neg
Urine	up to 10^6	ND	neg
Tears	pos	ND	pos
Breastmilk	PCR pos/Epi neg	ND	neg
Feces	(neg) infectivity	ND	neg

ND: not determined

pos: positive

neg: negative

ble routes of transmission. Data have shown that perinatal HDV transmission is rare; this may reflect the need to have active replication of HBV present for efficient transmission.

HCV titers of up to 10^7 per ml in the blood of infected individuals have been measured (Table 2, Hsu et al., 1991). HCV has not been detected by sensitive methods (RT-PCR) in other body fluids such as semen, vaginal fluid, and saliva (Hsu et al., 1991), while there is a single positive report of HCV RNA detected in tears (Feucht et al., 1995). These data collectively indicate that transmission efficiency for HCV (aside from blood-borne) differs from that of HBV and HIV. Sexual transmission of HCV occurs but is much less efficient than with HBV or HIV based upon epidemiologic data that indicate that promiscuous sexual activity is a weak risk factor for infection (Alter, 1999). Perinatal transmission is

rare (~5%) and generally correlates with the virus titer in the mother.

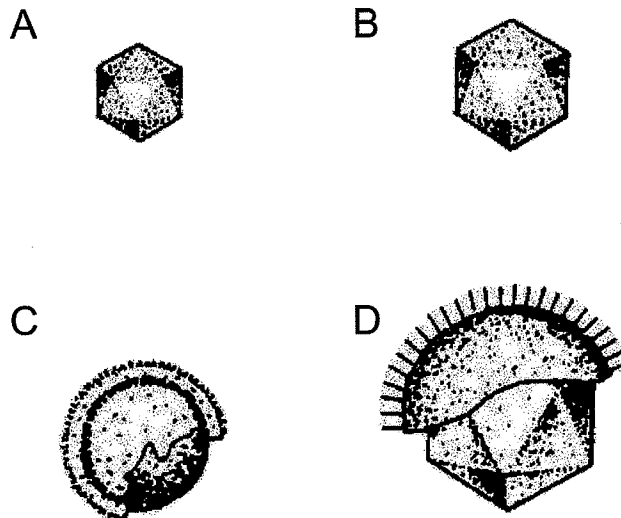
Control and Prevention

From a practical perspective in the United States, the use of universal precautions while working in the laboratory provides adequate barrier protection against all of the blood-borne virus diseases. These precautions include wearing gloves, face protection, and gowns when working with potentially infected material. All protective equipment should be removed when not working at the bench. In addition, careful handling, disposal, and disinfection of sharps that might be contaminated are also important. The available vaccine, which protects against HBV, is the most effective prevention strategy; OSHA mandates that hepatitis B vaccine be offered

Figure 1

Schematic representation of the hepatitis viruses. These figures are reproduced with permission of Arnold Limited from Figure 3.3 in the Virology volume of Topley and Wilson's *Microbiology and Microbial Infections* (1998, Arnold).

- A. Hepatitis A virus represented by a schematic of the picornavirus family.
- B. Hepatitis E virus represented by a schematic of the calicivirus family.
- C. Hepatitis B virus represented by a schematic of the hepadnavirus family.
- D. Hepatitis C virus represented by a schematic of the flavivirus family.



to all laboratory workers who are reasonably anticipated to have exposure to blood and body fluids. Due to the unique relationship between HBV and HDV resulting from their common surface antigen protein, vaccination against HBV protects against infection with HDV.

At this time, no vaccine is available that offers protection against HCV. Practicing universal precautions when working with blood or other body fluids is extremely important to prevent transmission of HCV that can result in chronic disease in 70%-85% of infections (Advisory Committee on Immunization Practices, 1998). Due to the lack of vaccine, treatment for HCV infection has been an important consideration. Jaekel et al. (2001) have demonstrated, in a small group of acutely infected HCV patients, that early intervention with therapy can prevent chronic infection. Additional data focusing on treatment for chronically infected patients are also available (http://consensus.nih.gov/cons/116/116cdc_intro.htm). However, it is important to note that for HCV, prevention is the most effective strategy.

Other Putative Hepatitis Agents

Since the cloning and the discovery of HCV and HEV, many investigators have searched for other elusive viral agents that may be responsible for non-ABCDE hepatitis. Using exquisitely sensitive molecular methods, several putative viruses have been identified from the sera of various high-risk groups as well as from chronic hepatitis patients. These include GBV-C/HGV, TT virus, and the SEN viruses. The work of many researchers has demonstrated that none of these agents is responsible for the remaining cases of acute hepatitis (< 2%-3% within the U.S.). All appear to be viruses that are not associated with liver disease but, rather, are ubiquitous within human populations.

Summary

A common theme for the prevention of laboratory-acquired infections with any of the hepatitis viruses is personal protective equipment. Wearing gloves, lab coats (and face protection when warranted) when working with contaminated material

and hand-washing following the removal of the equipment will prevent transmission and infection with all of the described hepatitis agents. In addition, vaccines that protect against HAV and HBV can provide protection against hepatitis A, B, and D infections.

Acknowledgements

The Division of Viral Hepatitis, Centers for Disease Control and Prevention has a slideset available for viewing at the following address: <http://www.cdc.gov/ncidod/diseases/hepatitis/slideset/>. It covers much of the data presented in this article. Readers are encouraged to visit this web site for additional information.

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