INTRODUCTION

The identification of human herpesvirus (HHV) 6 (HHV-6) and 7 (HHV-7) was first reported in 1986 and 1990, respectively. HHV-6 was isolated initially from the blood of several immunodeficient adults, including some with acquired immunodeficiency syndrome (AIDS) (1). The existence of HHV-7 was first revealed in the blood of a healthy, human immunodeficiency virus (HIV)-negative adult (2). Extensive investigations since then have established that these viruses are ubiquitous in the general population and are responsible for the majority of cases of roseola, a common febrile rash illness of infants (3–5). This chapter provides a general overview of HHV-6 and HHV-7 and a summary of present knowledge related to the diagnosis of congenital and perinatal infections.

DESCRIPTION OF ORGANISMS

HHV-6 and HHV-7 are closely related to human cytomegalovirus (CMV). Like all herpesviruses, HHV-6 and HHV-7 possess a nucleocapsid containing deoxyribonucleic acid (DNA), surrounded by a dense tegument and a lipid envelope (6). Although HHV-6 and HHV-7 DNAs possess a high degree of homology, there are distinct antigenic differences (7). Two subtypes of HHV-6 (A and B) have been described (8). Most cases of roseola are caused by subtype B (9,10), and the few congenital HHV-6 infections evaluated thus far have also been caused by subtype B. No disease has been consistently associated with subtype A, although it may be found more frequently in African children (11). HHV-7 subtypes have not been reported.

EPIDEMIOLOGY

Seroprevalence

HHV-6 and HHV-7 infections occur early in life. Almost all newborns possess maternally derived antibodies, but by age 6 months, most have lost maternal antibodies and are susceptible to infection. Acquisition of HHV-6 occurs rapidly, with 50–60% of children becoming HHV-6 seropositive by age 12 months and essentially all children infected by age 2–3 years (12). HHV-7 infection typically occurs slightly later than HHV-6, with more than 90% prevalence reached by age 7–10 years (12).
Latency and Viral Shedding

HHV-6 and HHV-7, like all other herpesviruses, become latent following primary infection, with limited expression of viral genes. The primary sites of latency for HHV-6 and HHV-7 are the salivary glands and peripheral blood mononuclear cells (PBMCs). During primary infection, high concentrations of each virus can be found in PBMCs (13). Thereafter, viral DNA persists at very low concentrations in PBMCs. Other sites of viral latency probably include the central nervous system and the female genital tract.

Shedding of HHV-6 and HHV-7 reflects sites of viral latency. Approximately 50% of adults shed HHV-6 in saliva, and about 90% shed HHV-7. Another site of shedding is the female genital tract. HHV-6 was found in the genital tracts of 4% of healthy nonpregnant women (14) and 3% of women attending a sexually transmitted disease clinic (15). In early pregnancy, reported rates of genital HHV-6 shedding have ranged between 2 (14) and 26% (16), whereas 19% of women shed HHV-6 in late gestation (17). In the only published study of HHV-7, 3% of women in late pregnancy shed the virus in the genital tract (17). Women shedding HHV-6 or HHV-7 in the genital tract have no local clinical manifestations.

There is conflicting data on the presence of HHV-6 in breast milk. Dunne and Jevon (18), from the United States, examined 120 samples of breast milk by polymerase chain reaction (PCR) and found none positive. However, investigators from India, also using PCR, found HHV-6 in breast milk from 100% of healthy women and 89% of HIV-positive women (19).

Transmission

For children beyond the neonatal period, saliva is presumed to be the principal source for transmission of HHV-6 and HHV-7. This is based on the frequent shedding of virus in saliva among infected persons and limited molecular epidemiologic studies (20).

The relatively small number of in utero HHV-6 infections is presumably attributable to maternal viremia, yet documentation is lacking. Perinatal infections could occur as a result of exposure to contaminated breast milk, genital secretions, saliva, or other sources. Although one of two studies suggested the presence of HHV-6 within breast milk (19), there is no epidemiologic support for breast milk as an important source for HHV-6 acquisition in early infancy (21). Because HHV-6 and HHV-7 may be shed in the female genital tract, exposure to a contaminated birth canal during vaginal delivery or via ascending infection could account for perinatal HHV-6 and HHV-7 infections. One study evaluated the outcome of pregnant women with and without first trimester genital HHV-6 shedding; none of the newborns in either group was determined to have congenital infection based on PCR analysis of cord blood (16). Studies evaluating the association of late-term viral shedding with neonatal infection are lacking.

Incidence in Mothers

Studies among healthy adults, including pregnant women, indicate that past infections with HHV-6 and HHV-7 are extremely common, presumably as a result of viral exposure early in life (7,14,22,23). Thus, like Epstein-Barr virus, primary (initial) HHV-6/HHV-7 infections during adulthood are expected rarely. However, because latent virus exists in all infected persons, there is some risk for “reactivation.” Reactivation of HHV-6 or HHV-7 typically is identified by increasing rates of virus shedding or
increases in specific immunoglobulin (Ig) G antibody titers. One study indicated that HHV-6 reactivation may occur in approx 5–10% of pregnant women (22). Symptomatic illness in adults as a consequence of primary or reactivated HHV-6 or HHV-7 reactivation is not well described. Some authors have reported a mononucleosis-like illness in nonpregnant adults (24). No illnesses in pregnant women attributable to primary or reactivated HHV-6/HHV-7 infection have been reported.

**Incidence in Neonates**

Accumulating data from several different sociogeographic areas indicate that the incidence of congenital or perinatal HHV-6 infections is quite low (Table 1); there is no comparable information for HHV-7. Studies have typically measured HHV-6 DNA in cord blood mononuclear cells by qualitative PCR, or HHV-6 IgM antibodies in cord blood serum, or both. In newborns of healthy mothers, the rate of in utero HHV-6 infection is 0–3% (Table 1). Congenital HHV-6 infection occurred at slightly higher rates (7–19%) in infants born to HIV-infected mothers (Table 1). Only one study contains data on congenital HHV-7, with no infections identified (48).

**Incidence in Children Outside the Neonatal Period**

Like CMV, most HHV-6 and HHV-7 infections are clinically silent. The incidence of roseola, the most common symptomatic illness attributable to these agents, is estimated as 30–35% by age 3 years. Other illnesses possibly associated with HHV-6 are very rare.

**CONSEQUENCES OF CONGENITAL OR ACQUIRED HUMAN HERPESVIRUS 6 AND 7 INFECTIONS**

Although more than 60 newborns with congenital or perinatal HHV-6 infections have been reported in large series (Table 1), most apparently are healthy and have no

**Table 1**

Detection of HHV-6 DNA or HHV-6 IgM Antibodies in Cord Blood

<table>
<thead>
<tr>
<th>Population</th>
<th>Reference</th>
<th>Year</th>
<th>Country</th>
<th>No. PCR positive/ no. tested (%)</th>
<th>No. IgM positive/ no. tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy mothers</td>
<td>42</td>
<td>1990</td>
<td>US</td>
<td>5/305 (1.6)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>1992</td>
<td>US</td>
<td>ND</td>
<td>2/799 (0.3)</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>1992</td>
<td>Japan</td>
<td>ND</td>
<td>3/100 (3)</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>1998</td>
<td>UK</td>
<td>ND</td>
<td>0/235 (0)</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>1999</td>
<td>Sweden</td>
<td>2/211 (2.0)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>1999</td>
<td>Japan</td>
<td>0/58 (0)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>1999</td>
<td>Thailand</td>
<td>0/13</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>2004</td>
<td>US</td>
<td>57/5638 (1.0)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>64/6225 (1.0)</td>
<td>5/1134 (0.4)</td>
</tr>
<tr>
<td>HIV-positive mothers</td>
<td>47</td>
<td>1999</td>
<td>Thailand</td>
<td>3/41 (7.3)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>2000</td>
<td>India</td>
<td>7/36 (19.4)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>10/77 (13.0)</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not done.

*a*HHV-6 PCR testing was negative on the two HHV-6 IgM-positive samples.
unusual features. Several case reports have described fetuses or infants with HHV-6 infection, yet most have no abnormalities. Aubin et al. (25) first described in utero infection with HHV-6. These investigators examined 52 electively aborted fetuses from HIV-infected women from France and noted one fetus (26 weeks) with HHV-6 DNA distributed throughout fetal tissues. No abnormalities were identified. One study from Japan identified HHV-6 antigens in tissues from 2 of 30 (7%) fetuses spontaneously aborted at 6–12 weeks; fetal abnormalities were not described (26). HHV-6 DNA has also been found in fetal tissues of two of eight cases (25%) of fetal hydrops (17 and 19 weeks of gestation) (27). However, both fetuses also had a chromosomal abnormality (Down syndrome and Turner’s syndrome) possibly contributing to the hydrops. Fulminant hepatitis in two neonates (aged 3 and 5 days) has purported to be linked to congenital HHV-6 infection based on HHV-6 viremia in mother and baby (28); further documentation of HHV-6 infection of liver tissue was not performed in these cases. Because HHV-6 viremia may occur in asymptomatic neonates (29), there is some question whether these cases are attributable to HHV-6 infection. Illness resembling roseola has also been reported in one neonate (age 3 weeks) despite the presence of maternal HHV-6 antibodies (30). These studies clearly demonstrate that HHV-6 can be transmitted congenitally as early as 6–12 weeks of gestation, However, there is insufficient evidence indicating a characteristic clinical syndrome; most newborns appear healthy. No reports of congenital or acquired HHV-7 infections have appeared.

CLINICAL EVALUATION

Prenatal

Based on current literature, there are few data suggesting the necessity of evaluating a mother or her fetus for HHV-6 or HHV-7 infection. HHV-6 (and probably HHV-7) may be reactivated during pregnancy, but no studies have clearly established serious consequences. Reports of spontaneous abortion (26) and fetal hydrops (27) purportedly associated with HHV-6 infection require further corroboration before routine diagnostic studies can be recommended.

Neonatal

Although most neonates with congenital or acquired HHV-6 infection appear normal, disease possibly linked to the virus has occurred in a few patients. One child had fever, rash, and aseptic meningitis (30). Two neonates (28) and two infants age younger than 3 months (31,32) have been described with liver dysfunction. A newborn with congenital HHV-6 infection has been described with seizures and neurological complications (32a).

VIRAL DIAGNOSTIC ASSAYS AND THEIR INTERPRETATION

General

Because of the rarity of congenital or perinatal HHV-6 and HHV-7 infections as well as the apparent absence of serious consequences in the majority of patients, no standards for diagnostic testing have yet been established. However, numerous tests have been developed for the diagnosis of HHV-6 and HHV-7 infection in other age categories and have been used in studies evaluating newborns for suspected congenital and acquired infections. Specific testing for HHV-6 or HHV-7 infection may include
serology, virus culture, PCR, immunohistochemistry, in situ hybridization, and antigen detection.

In considering performance of these viral tests, one must remember the latent nature of these herpesviruses and understand that tests differ in their ability to distinguish nonreplicating, latent virus from replicating, active virus (Table 2). The presence of HHV-6 or HHV-7 DNA in PBMCs or other cellular material indicates viral infection but does not necessarily imply viral disease because these viruses persist latently following primary infection. Assays that detect active viral infection are necessary. In cellular specimens, active infection is typically indicated by the isolation of virus, the presence of specific viral ribonucleic acid, or the expression of viral proteins on cell membranes. In cell-free specimens (e.g., serum, plasma, or cerebrospinal fluid [CSF]), viral replication is indicated by the presence of HHV-6 or HHV-7 DNA or antigens. Serologic methods indirectly measure virus infection and are of limited benefit in diagnosing congenital or perinatal HHV-6/HHV-7 infections.

**Serology**

The most common antibody tests are enzyme immunoassay (EIA) and indirect immunofluorescence assay (IFA); both assays are available commercially. Reference strains of HHV-6 and HHV-7 are typically grown in a susceptible lymphoblastoid cell line or human mononuclear cells, then harvested and attached to plates (for EIA) or slides (for IFA). Commercial EIA and IFA tests cannot distinguish HHV-6 subtypes. One group of investigators has described a serologic test identifying “low-avidity” IgG antibodies to HHV-6, which is claimed to distinguish primary from past infection in older infants (33); however, this test is not used widely, is not available commercially, and has not been evaluated in neonates.

Serologic profiles are best described for infants with HHV-6-associated roseola, in whom an HHV-6 IgM response typically develops by days 5–7 of illness, peaks at 2–3 weeks, and resolves within 2 months. However, false-positive and false-negative results can occur (34); therefore, IgM testing alone is not reliable. Although not timely,
seroconversion of IgG antibodies in serum samples collected 2–3 weeks apart is more reliable than a single IgM test for establishing primary infection. However, because most mothers are already infected with HHV-6 and HHV-7 and newborns acquire passive maternal antibodies, IgG seroconversion is expected to occur rarely in this population. Fourfold increases or decreases in IgG antibodies can also suggest infection. Because of the high seroprevalence of HHV-6 and HHV-7 in the general population, a single positive IgG test is of no diagnostic importance. Because HHV-6 and HHV-7 antibodies may cross-react with each other as well as with CMV, diagnosis of HHV-6 or HHV-7 infection by serologic means alone is insufficient and requires concurrent testing for CMV infection. Acute HHV-6 and HHV-7 infections are more reliably demonstrated by more direct viral assays, described next.

**Virus Culture**

When incubated under suitable conditions, HHV-6 and HHV-7 may be grown in culture. Because no cell lines reliably sustain growth of virus, it is necessary to incubate specimens in the presence of fresh human mononuclear cells. Virus culture requires prolonged (1–3 weeks) incubation in the presence of fetal calf serum, interleukin 6, and other reagents. Rarely, virus has been identified in other biologic specimens. The presence of HHV-6 or HHV-7 in culture is suggested by ballooning and eventual lysis of infected cells; infection is confirmed by staining with commercially available monoclonal antibodies. Virus culture presently is available only in specialized research laboratories. A rapid shell vial culture for HHV-6 is available commercially but has not been evaluated extensively.

Identification of HHV-6 or HHV-7 in blood by virus culture firmly establishes the presence of active infection. No studies to date, however, have reported isolation of HHV-6 or HHV-7 from infants or fetuses. In fact, cord blood is frequently used for cocultivation with PBMCs from older patients with suspected HHV-6/7 infection (it is assumed that cord blood does not contain HHV-6 or HHV-7).

**Polymerase Chain Reaction**

Amplification of HHV-6 and HHV-7 DNA by PCR is becoming widely available, and has been utilized in most studies of congenital HHV-6 infection. However, it is important to reemphasize that active, replicating infection is demonstrated only if viral DNA is detected in noncellular specimens such as CSF, serum, or plasma. Detection in other specimens containing cellular material (e.g., PBMCs, tissues) does not necessarily indicate active infection because these viruses exist in latent form in these tissues following primary infection. Quantitative measurement of virus DNA concentration, such as quantitative PCR, may help distinguish active from latent infection and is available commercially. However, concentration thresholds indicating active infection have not been established for newborns or older children and adults; for this reason, interpretation of results is problematic.

Reverse transcriptase PCR (RT-PCR) is an assay that detects specific viral messenger ribonucleic acid transcripts, indicating viral replication. Data suggest that RT-PCR is a sensitive and specific method for identifying active HHV-6 infection and correlates well with virus culture. RT-PCR is typically performed on PBMCs, may be used on bone marrow samples, and is available from at least one commercial laboratory. There is no information on interpretation of results in newborns. Studies in infants
and children suggested that certain combinations of PCR tests are more accurate for diagnosis of primary HHV-6 or HHV-7 infections, such as positive PCR of whole blood with negative IgG antibody in serum (39) or positive PCR of whole blood with negative PCR of saliva (13). The accuracy of these combination tests in newborns is unknown.

**Other Assays**

Other diagnostic tests for HHV-6 and HHV-7 may be useful in selected circumstances.

**Immunohistochemistry**

Viral antigens, indicating expression of HHV-6/HHV-7 proteins, may be detected in infected tissues and have been utilized in studies of aborted fetuses (26) and other subjects (40). Monoclonal antibodies for performance of these tests are available commercially, including antibodies directed specifically against HHV-6 antigens, HHV-7 antigens, or common antigens shared by both viruses.

**In Situ PCR**

Tissues may also be evaluated for the presence of HHV-6 or HHV-7 by using *in situ* PCR. This test can detect DNA in tissues using appropriate genomic DNA probes. Typically, probes are labeled with radioactivity (or another tag) and then hybridized to cells or tissues immobilized on glass slides. The presence of intracellular viral DNA by *in situ* PCR indicates viral infection but does not provide evidence of viral replication because these viruses may exist latently in numerous tissues.

**Antigen Capture Assay**

An HHV-6 antigen capture assay is available for detection of HHV-6 antigens in serum, but this test has not been evaluated in neonates (41). In this test, anti-HHV-6 monoclonal antibodies directed at HHV-6 proteins are immobilized in plastic wells. When serum or another cell-free specimen containing HHV-6 antigen is added, these antibodies bind the viral antigens and are detected by a colorimetric reaction. The presence of viral antigen in noncellular specimens indicates active viral infection. No assays for HHV-7 antigen detection are available.

**REFERENCES**