and young infants.[14,15] Differential cell counts of the CSF often first reveal a high proportion of neutrophils, but the differential typically shifts to a predominance of lymphocytes during the initial 1 to 2 days of illness.[16–18] In general, the CSF glucose concentration is normal, and the CSF protein concentration is normal or slightly elevated. However, the glucose content may be lower than normal in 18% to 33% of cases,[19–21] and values less than 40 mg/dL may occur.[6,20] Uncommonly, it may be difficult to exclude bacterial meningitis on the basis of the CSF profile alone. In some cases the CSF findings may closely mimic those of tuberculous meningitis.[22]

NAATs (e.g., reverse-transcriptase polymerase chain reaction [RT-PCR] and nucleic acid sequence-based amplification [NASBA]) have replaced cell culture as the primary means of detection of EVs in CSF and other specimens.[23,24] Most PCR protocols amplify a highly conserved portion of the 5′ nontranslated region of the genome, which enables the detection of the majority of EVs. For confirmed or suspected enteroviral meningitis cases, PCR sensitivity ranges from approximately 70% to greater than 90%.[2] The overall sensitivity of virus isolation from the CSF of patients with viral meningitis is typically 30% to 35%,[2,25–28] although higher figures have been reported during some E outbreaks.[12,29] Concomitant testing of serum, upper respiratory secretions, urine, and stool enhances the likelihood of virus detection by either PCR or cell culture.[2,30,31]

Differential Diagnosis

Bacterial meningitis is the most important disease to be distinguished from EV aseptic meningitis. Although some clinical features of bacterial meningitis that is incompletely treated with antibiotics may overlap those of EV aseptic meningitis, when therapy has been instituted before lumbar puncture, several studies have demonstrated that pretreatment of bacterial meningitis alters the CSF minimally. Even when some laboratory indicators are altered by therapy (i.e., change from polymorphonuclear to lymphocytic pleocytosis), others continue to indicate bacterial disease (i.e., low glucose or high protein concentration).[32–34] Arboviruses, lymphocytic choriomeningitis virus, leptospirosis, Lyme borreliosis, and acute human immunodeficiency virus infection account for most of the remaining cases of infectious aseptic meningitis. Mumps virus infection was a common cause of aseptic meningitis before the introduction of mumps vaccine in the United States. Aseptic meningitis also occurs with other infectious and noninfectious diseases (see Chapter 88), but the etiology is usually suggested by other clinical features.

Management and Prognosis

Although hospitalization is not necessary for all cases in adolescents and adults and, indeed, may not be feasible during summer epidemics of EV infections, it is advisable when disturbances in consciousness, muscle weakness, or a petechial or purpuric rash suggest the possibility of a more serious illness. Pyogenic bacterial meningitis should be excluded by lumbar puncture. When bacterial meningitis cannot be excluded because of prior antibiotic treatment, administration of appropriate antibiotics is advisable after performing Gram stains and bacterial cultures. CSF EV NAAT may be useful in deciding whether to continue administration of antibiotics and hospitalization if the test can be reported within 1 to 2 days.[35,36]

In most cases treatment consists only of relief of symptoms. Analgesics are usually given to older children and adults to alleviate headache and lassitude, and easy fatigability may be present for weeks after acute illness.[8] Pleconaril, an experimental orally administered EV capsid-stabilizing drug, modestly reduced the duration of headache and other symptoms in clinical trials but has not been further developed for this purpose.[11] Treatment studies of infants and young children, who generally experience a shorter duration of symptoms, have been inconclusive.[37]

In one large study of EV aseptic meningitis, subtle disturbances in motor function (limitation of passive motion, muscle spasm, and poor coordination) were observed during convalescence.[38] These abnormalities slowly resolve and are rarely detectable 1 year after infection. In young children fever and signs of meningeal irritation subside in a few days to 1 week. Infants younger than 3 months may have fewer symptoms of illness and fewer complications than older infants.[6] Although some investigators have suggested that EV meningitis in the first year of life may result in permanent neurologic sequelae,[39,40] studies of larger numbers of children using more rigorous methods indicate that the long-term prognosis for the youngest infants is also excellent.[41,42]

Encephalitis

Encephalitis is a well-documented manifestation of nonpolio EV central nervous system (CNS) infection. In industrialized nations the EVs account for less than 5% of all encephalitis cases but may contribute to a larger proportion in developing nations.[25,43–46] EVs account for 11% to 22% of encephalitis cases that are proved to be viral.[25,43–46] Numerous serotypes have been implicated as causes of encephalitis; CV-A9 and CV-A16, CV-B2 and CV-B5, E-6 and E-9, and EV-A71 are the serotypes reported most often, but the evidence linking each of these serotypes to encephalitis is highly variable. A notable exception is that of EV-A71, where it has unambiguously been linked to encephalitis. In a minority of cases a specific etiology has been proved by isolating virus or detecting its genome in brain tissue or CSF; in others the cause of encephalitis has been inferred by isolating virus from a nonneurologic site or by serology.

In perinatally acquired EV infection, encephalitis is often only one manifestation of generalized viral disease, but beyond the neonatal period, signs and symptoms are generally limited to the CNS. Children and young adults are most frequently affected. CNS disease varies from relatively mild encephalopathic symptoms in patients with EV meningitis to severe generalized encephalitis with seizures, paresis, and coma. Children with focal encephalitis present with partial motor seizures, hemichorea, and acute cerebellar ataxia,[47–49] features that in some cases have suggested a diagnosis of herpes simplex virus (HSV) encephalitis.[50,51]

EV-A71 and, rarely, other EV serotypes are the cause of a severe, often fatal form of brainstem encephalitis (rhombencephalitis) with secondary cardiopulmonary manifestations, including noncardiogenic pulmonary edema.[51–54] EV-A71 encephalitis has a strikingly high prevalence in countries of the Asia–Pacific Rim. Multiple large outbreaks have occurred in this region of the world in the last 10 to 15 years.[55] The disease affects principally infants and toddlers. CNS disease is usually preceded by hand-foot-and-mouth disease (HFMD) or herpangina.[51,53] In addition to the neurologic signs of encephalitis mentioned previously, myoclonus occurs frequently.[55] The use of glucocorticoids and/or pyrazolones may be risk factors for the development of life-threatening disease.[56]

The CSF findings in EV encephalitis are similar to those in aseptic meningitis. Magnetic resonance imaging (MRI) of the brain and electroencephalography may demonstrate either generalized or localized abnormal signals, reflecting the extent and severity of brain involvement. Most patients with CV and E encephalitis beyond the neonatal period recover fully, although permanent neurologic sequelae and rare deaths occur.[25,54,57–59] EV-A71 encephalitis may be associated with such sequelae as limb atrophy and weakness, as well as long-term behavior problems in children.[52,60]

Acute Flaccid Paralysis/Myelitis and Other Neurologic Complications

The clinical presentation of acute flaccid paralysis (AFP) mimics that of poliomyelitis. For some EV serotypes the link with AFP has been established through detection of the virus or its nucleic acid in the CSF or CNS tissue of affected individuals.[61,62] In the majority, however, the link is inferential by viral detection in stool, respiratory tract, or by an increase in virus-specific antibodies.[63–66] The detection of AFP for monitoring the progress of polio eradication has led to an increase in the identification of serotypes inferentially associated with this syndrome. EV-A71 has been associated with large outbreaks of AFP in Russia, Eastern Europe, Thailand, and Taiwan.[52,67,68] CV-A7, which is neuropathogenic in monkeys, has also been associated with outbreaks of AFP.[69,70] Sporadic cases of AFP have been associated with multiple serotypes from EV-A, EV-B, and EV-C spp., in decreasing order of frequency.

Recently, a cluster of acute flaccid myelitis (AFM) temporally associated with an outbreak of EV-D68 respiratory disease characterized by asymmetrical limb weakness/paralysis, bulbar weakness, and cranial nerve dysfunction drew an inferential link between the two.[71] Nationwide

surveillance for cases of AFP further pointed to a possible association between EV-D68 and AFP.[72] Although EV-D68 has rarely been identified from the CSF, evidence for the causality of EV-D68 and AFP was further supported by the Bradford Hill criteria.[72] As a result of the bulbar weakness and paralysis of the muscles of respiration, inability to protect the airway and respiratory failure secondary are frequently seen. EV-D68 is rarely isolated from the CSF of cases of AFM but can be found in the nasopharynx and stool of infected individuals. Cytochemical analysis of the CSF demonstrates a lymphocytic pleocytosis in the majority of cases.[72] CSF protein and glucose concentrations are generally increased and normal, respectively. MRI findings included longitudinally extensive nonenhancing lesions of the gray matter in the spinal cord or brainstem,[71–73] the cervical spinal cord being the most commonly involved segment. Electrodiagnostic studies of affected limbs demonstrate motor neuropathy or neuronopathy without sensory abnormalities.[74] The majority of patients with AFM will experience persistent sequelae.[71,72,74] A national survey of AFP documented that only 5% of respondents reported complete recovery of strength, and only 18% were fully functional.

Treatment of EV-D68 associated AFM is supportive. Antiviral agents that inhibit EVs, such as pleconaril, pocapavir, and vapendavir, do not have in vitro antiviral activity against EV-D68. Fluoxetine, a selective serotonin reuptake inhibitor, has been shown to be active in vitro against EV-B and EV-D spp., including EV-D68, failed to show activity against EV-D68 in a murine model[75,76] (also see Chapter 48).

Paralytic disease caused by the nonpolio EVs, other than EV-A71 and EV-D68, is characteristically less severe than poliovirus-associated paralysis.[77,78] Prodromal fever or presence of fever at the time of onset of paralysis and residual paralysis or atrophy are less frequently encountered in paralytic disease due to the nonpolio EVs. Muscle weakness is more common than flaccid paralysis.

Cranial nerve involvement has occasionally resulted in complete unilateral oculomotor palsy.[79,80] Guillain-Barré syndrome has been reported in a small number of patients in association with CV-A2, CV-A5, and CV-A9 and with E-6 and E-22.[26,81,82] In a few cases the implicated virus has been isolated from CSF or the brainstem.[82] Transverse myelitis caused by CV-B4, CV-A9, and E-18, and has been reported in patients who had rises in neutralizing antibody and in patients who had E-5 and CV-B5 recovered from CSF.[83,84] Systemic CV-B2 disease has been reported with many of the clinical features of Reye syndrome.[85] Furthermore, several children with well-documented Reye syndrome have had a variety of EVs isolated concurrently from multiple sites, including the brain and CSF.[86,87] However, a clear etiologic or epidemiologic link between EV infection and Reye syndrome has not been established. Opsoclonus-myoclonus, or the "dancing eyes" syndrome, has been reported in two children with concurrent CV-B3 infection and in an adult with EV-A71 infection.[88,89]

EXANTHEMS

CVs and Es cause a variety of exanthems, which are sometimes associated with enanthems. With the exception of HFMD, these rashes are not sufficiently distinctive to permit a reliable etiologic diagnosis on clinical grounds alone. Virus can be isolated from the vesicular lesions of patients with HFMD, and therefore these lesions appear to be a direct result of viral invasion of the skin after viremia. No attempts at isolation of virus from the skin in cases of maculopapular and petechial exanthems have been reported; in consequence, it is not known whether these lesions are also caused by the virus directly or by immunopathologic mechanisms.

EV exanthems themselves cause little morbidity. They are important as sentinels of the prevalence of CVs and Es in the community and because they are often confused with other infective exanthems, some of which have more serious implications. Rashes caused by EVs may be grouped according to the type of exanthem that they mimic: rubelliform or morbilliform, roseoliform, vesicular, or petechial. Some overlap between these types of exanthems may be observed in different patients infected with the same EV or even among different morphologic lesion types in the same patient.

Rubelliform and Morbilliform Exanthems

Overall, EVs account for about 5% of acute morbilliform exanthems that occur in populations with high measles and rubella vaccine coverage.[90] Maculopapular rashes resembling rubella commonly occur during summer E epidemics. High attack rates have been noted with E-9, the most common serotype associated with rubelliform rash. In one epidemic 57% of persons younger than 5 years with illness caused by E-9 had rash, 41% of those 5 to 9 years of age had rash, but rash affected only 6% of those older than 10 years.[29] The rash, which characteristically appears simultaneously with fever, begins on the face and then spreads to the neck, chest, and extremities. The illness may be distinguished from rubella by the absence of pruritus and posterior cervical lymphadenopathy.[91,92] In occasional patients with an enanthem resembling Koplik spots and a blotchy eruption, the disease may be confused with measles, but the coryza and conjunctivitis characteristic of that disease are absent.[93]

Roseoliform Exanthems

These EV exanthems are distinctive, not in their appearance but in their timing; as in roseola, the rash does not appear until defervescence. The prototype is the "Boston exanthem," the first of the EV exanthems to be recognized and now known to be caused by E-16.[94,95] Multiple cases often occur sequentially in families, with rash developing in as many as one-fourth of the children in a household who are mildly ill with low-grade fever and pharyngitis. The fever lasts 24 to 36 hours and then declines simultaneously with the appearance of discrete, nonpruritic, salmon-pink macules and papules about 1 cm in diameter on the face and upper part of the chest. The extremities are less commonly involved. The duration of the rash is 1 to 5 days. Other EV serotypes (CV-B1 and CV-B5, E-11 and E-25) have also been associated with roseola-like illness.[94,96,97] Exanthem subitum (roseola infantum), a common nonseasonal exanthem in which the rash typically develops as the fever declines, is caused by human herpesvirus 6 (see Chapter 139).

Herpetiform Exanthems

Hand-Foot-and-Mouth Disease

HFMD is primarily associated with EV-A serotypes (CV-A4, -A5 to -A7, -A10, -A16, -A24; EV-A71), although EV-B serotypes (CV-A9, CV-B2 to CV-B5; E-4, E-18, E-19) are also associated with sporadic cases.[51,53,98–101] CV-A16 and EV-A71 are the most common causes of this distinctive vesicular eruption, also known as vesicular stomatitis with exanthema (Fig. 172.1A–C). In Southeast Asia the latter serotype has caused large outbreaks associated with severe CNS disease and deaths.[51–53] In recent years reports of outbreaks of HFMD caused by CV-A6 and CV-A10 have come from Europe, Asia, and the United States.[102–105]

Children younger than 10 years are often affected, and spread to other family members occurs commonly. Most patients complain of

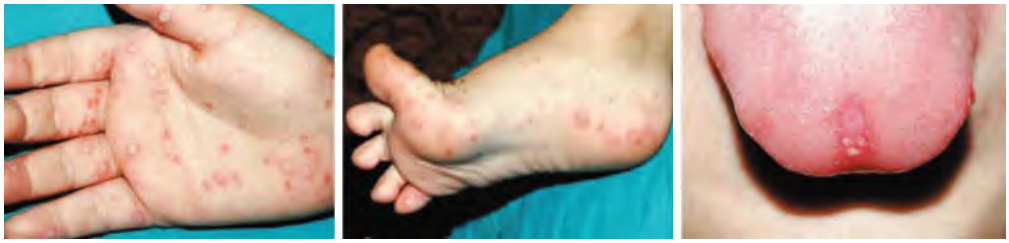

FIG. 172.1 Hand-foot-and-mouth disease caused by enterovirus A71 in a young child. *(From Goksugur N, Goksugur S. Hand, foot, and mouth disease.* N Engl J Med. *2010;362:e49.)*

sore throat or sore mouth, and affected young children may refuse to eat. Temperatures of 38°C to 39°C last 1 to 2 days and are accompanied in essentially all cases by vesicles in the oral cavity, occurring chiefly on the buccal mucosa and tongue. Several lesions may coalesce to form bullae and ulcerate. Peripherally distributed cutaneous lesions occur in roughly 75% of patients, commonly on the extensor surfaces of the hands and feet and sometimes on the buttocks or genitalia.[106] The lesions are tender and consist of mixed papules and clear vesicles with a surrounding zone of erythema. Skin biopsy demonstrates subepidermal lesions with a mixed lymphocytic and polymorphonuclear inflammatory response and acantholysis of the overlying epidermis.[107] Eosinophilic nuclear inclusions and intracytoplasmic picornavirus particles can be seen microscopically within cells surrounding dermal vessels.[108]

The vesicular lesions of HFMD disease superficially resemble those caused by herpes simplex or varicella-zoster virus (VZV). Patients with HFMD invariably have lesions of the oral mucosa. In contrast, oral lesions are less common in patients with chickenpox; moreover, these patients generally appear more ill, and their cutaneous lesions are more extensive and centrally distributed, generally with sparing of the palms and soles. Patients with primary herpetic gingivostomatitis also usually appear more ill and have a higher fever and cervical lymphadenopathy; lesions are usually confined to the oral cavity and do not involve the extremities. The enanthem of herpangina also resembles HFMD, but it occurs in the posterior oropharynx and typically involves the fauces and soft palate.

An atypical form of HFMD, caused by a novel CV-A6 strain, has been observed since 2008 in multiple locations globally and is characterized by a wider distribution of skin lesions that enlarge and vesiculate, especially in areas of eczematous skin.[104,105,109,110] Generalized varicella form–like eruptions have also been reported in association with CV-A6 infection.[111] The acute disease is associated with a higher rate of hospitalization than typical HFMD, but it has an invariably benign outcome, with the exception of onychomadesis (loss of fingernails), which occurs in many cases 1 to 2 months after the infection. Generalized vesicular eruptions occurring in crops of lesions are also reported in association with CV-A9[112] and E-11 infection, and one case with disseminated lesions has been described in an infant with preexisting atopic eczema; the condition was given the sobriquet "eczema coxsackium," by analogy with eczema herpeticum and eczema vaccinatum.[113,114] Unlike HSV and VZV infection, the vesicles do not evolve to form pustules and scabs. Vesicular eruptions caused by E-11 have occurred in immunocompromised adult patients.[114] An acute eruption resembling dermatomal zoster, in which E-6 was isolated from the bullous lesions, has been reported.[115]

Petechial Exanthems and Other Cutaneous Manifestations

Petechial and purpuric rashes have been described with E-9[29,116] and CV-A9[117] infections. When these rashes have a hemorrhagic component, the illness is easily confused with meningococcal disease, especially if aseptic meningitis occurs simultaneously. On occasion, cutaneous eruptions of CV-A9 disease have an urticarial nature.[112] One child was reported to have papular acrodermatitis (Gianotti-Crosti syndrome) in association with CV-A16 infection.[118]

ACUTE RESPIRATORY DISEASE (EV-D68)

EVs account for most viruses recovered from children with summertime upper respiratory tract infections, including undifferentiated febrile illnesses ("summer grippe") with sore throat and on occasion cough or coryza.[119,120] EV upper respiratory tract illnesses are generally clinically indistinguishable from disease caused by rhinoviruses, *Mycoplasma pneumoniae* and other respiratory tract agents, unless accompanied by aseptic meningitis, exanthem, or other clinical features suggesting EV infection.

Many EV serotypes are associated with upper respiratory tract disease. Among the best-characterized EV respiratory viruses are CV-A21 and CV-A24, which produce illness resembling the common cold, except for a higher incidence of fever.[121,122] Outbreaks of CV-A21 illness are reported in military populations. Although epidemics in civilians have not been recognized, sporadic infections presumably account for the observed high antibody prevalence rates in the general population.[121] Unlike most EVs, CV-A21 is more readily recovered from throat swabs than from feces. In volunteers receiving small-particle aerosols of the virus, illness has included not only coryza and sore throat but also tracheobronchitis and pneumonia.[123] EV-D68 emerged in 2008–10 as a cause of clusters of upper and lower respiratory tract disease in Europe, Asia, and the United States.[124–129] EV-D68 has been isolated primarily from respiratory specimens during the autumn months. Cases have been reported primarily in children but also occur in adults.[130] Symptoms include no to low-grade fever, cough, wheezing, shortness of breath, dyspnea, tachypnea, retractions, and hypoxia.[130] Clinical syndromes associated with infection have included bronchiolitis, bronchitis, encephalitis, and pneumonia. In some, disease has been severe enough to warrant admission to the intensive care unit.[131] Deaths have been reported.[130] In 2014 the United States experienced a nationwide outbreak of EV-D68. From mid-August 2014 to January 2015, the Centers for Disease Control and Prevention (CDC) or state public health laboratories reported 1153 cases with respiratory illnesses caused by EV-D68 in 49 states and the District of Columbia.[132] Almost all of the confirmed cases were in children, many of whom had asthma or a history of wheezing. There were likely many thousands of mild EV-D68 infections in individuals who did not seek medical attention and who were not tested for EV-D68. Of the 2600 specimens sent to the CDC for EV testing, approximately 36% tested positive for EV-D68, and 33% were positive for another EV or for rhinoviruses.[130] Serious respiratory disease has also been reported in EV-D68 infection in adults,[130] including infections in patients with hematalogic malignancies.[133] The clinical syndromes seen in the 2014 outbreak of EV-D68 were primarily respiratory illnesses, typical of those seen in earlier outbreaks.

The CDC used a "real-time" reverse-transcriptase PCR that identifies all strains of EV-D68 that circulated in 2014. Genomic sequence of 2014 strains of EV-D68 showed that they are related to strains that circulated previously in the United States, Europe, and Asia.[132] The majority of strains in the 2014 outbreak were classified as clade B, but interclade variations were also seen, which led to identification of strains that comprised a new clade D among EV-D68 strains.[134]

E serotypes 4, 8, 9, 11, 20, 22, and 25 are also common causes of respiratory disease.[121] E-11 produces sore throat, coryza, and cough and has also been associated with croup. The spectrum of CV-B respiratory disease includes coryza, laryngotracheobronchitis, bronchiolitis, and pneumonia.[81,135] Pneumonia, which may be interstitial or a patchy bronchopneumonia, has occurred in children[136] and rarely in adults.[137] Severe lower respiratory tract EV infections are uncommon, although some EVs, notably E-6, -9, -11, and -33 and EV-A71, have been isolated after death from infants and young children with severe pneumonia.[5,53,138–140]

Herpangina

Herpangina (herpes: vesicular eruption; angina: quinsy, or inflammation of the throat) is a well-characterized vesicular enanthem of the fauces and soft palate that is accompanied by fever, sore throat, and pain on swallowing (Fig. 172.2). Despite the name, it has no relationship to HSV. Summer outbreaks of herpangina typically affect children age 3 to 10 years and, less commonly, adolescents and young adults. CV-A1 to -A10, -A16, and -A22 have been the most common viruses recovered from herpangina patients. Other serotypes include CV-B1 to CV-B5; CV-A6; E-3, -6, -9, -16, -17, -25, and -30; and EV-A71.[51,54,141]

Clinical Manifestations

Herpangina begins suddenly with fever, vomiting, myalgia, and headache. Sore throat and pain on swallowing are prominent symptoms that precede appearance of the enanthem by several hours to a day. Examination of the throat reveals erythema and mild exudate of the tonsils and the characteristic enanthem, which begins as punctate macules and evolves over a 24-hour period to 2- to 4-mm erythematous papules that vesiculate and then ulcerate centrally. The moderately painful lesions, which are usually small in number, are located on the soft palate, uvula, and less commonly, on the tonsils; the posterior pharyngeal wall; or the buccal mucosa. The fever subsides in 2 to 4 days, but the ulcers may persist

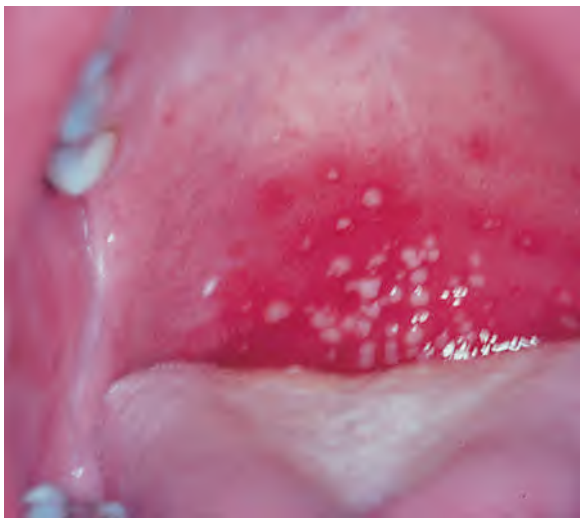

FIG. 172.2 Herpangina on the soft palate in a teenager with severe throat pain. *(From Cohen J, Powderly WG:* Infectious Diseases. *2nd ed. St Louis: Mosby; 2004.)*

for up to a week. Patients with herpangina do not appear very ill and require only symptomatic treatment for sore throat.

A variant of the herpangina, termed acute lymphonodular pharyngitis, has been described in association with CV-A10 infection.[142] Lesions occur in the same distribution as herpangina but consist of tiny nodules of packed lymphocytes that eventually recede without undergoing vesiculation or ulceration.

Differential Diagnosis

Herpangina is most often confused with bacterial tonsillitis or other viral causes of pharyngitis, herpetic gingivostomatitis, HFMD, and aphthous stomatitis. HSV gingivostomatitis occurs in the anterior oral cavity, especially on the inner aspects of the lips, the buccal mucosa, and the tongue. Gingivitis, prominent systemic toxicity, and cervical lymphadenitis are additional features of primary herpes simplex infection that are not seen in herpangina. In HFMD lesions also occur on the extremities in most cases. Aphthous stomatitis is characterized by recurrent large ulcerative lesions of the lips, tongue, and buccal mucosa among older children, adolescents, and adults.

MYOSITIS

Pleurodynia

Pleurodynia (Bornholm disease, "devil's grippe") is an acute EV infection of skeletal muscle, characterized by fever and sharp, spasmodic pain in the chest or upper part of the abdomen, which has been usually observed during infrequent outbreaks. Pleurodynia was first described in 1872 in Norway by Daae and by Homann as an outbreak of "acute muscular rheumatism spread by contagion." Subsequent reports from Scandinavia included a paper by Ejnar Sylvest, a Danish general practitioner, in 1933, in which he described his experience with the disease on the island of Bornholm in the Baltic Sea. This monograph received worldwide attention after it was translated into English in 1934,[143] and little has been added to Sylvest's descriptions of the disease and its epidemiology, pathogenesis, and complications. The etiologic role of CV-B, the most important cause of epidemic pleurodynia, was established in 1949.[144,145] Other agents rarely implicated in pleurodynia include E-1, -6, -9, -16, and -19 and CV-A4, -A6, -A9, and -A10.[146,147]

Epidemiology

Published reports of major epidemics have come primarily from Europe and North America. These epidemics have been reported at infrequent intervals, often 10 to 20 years, and attack rates have been higher in sparsely populated areas than in cities. Persons with pleurodynia are somewhat older than those with most other diseases caused by CVs and Es. Multiple family members may be attacked almost simultaneously or in rapid succession, separated by several days.

Pathogenesis

Although pleurodynia probably results from direct viral invasion of thoracic and abdominal muscles after viremia, direct virologic evidence supporting this hypothesis is lacking. Tenderness mimicking spontaneously occurring pain can be elicited by pressure on affected muscles in most cases; in addition, palpable, often visible muscle swelling is a subtle finding in some cases.[143] A pleural friction rub has been rare or absent in most epidemics, although this sign has occasionally been noted in 7% or greater of patients.[148,149]

Clinical Manifestations

Pleurodynia begins abruptly with an onset of spasmodic pain, typically over the lower part of the rib cage or the upper abdominal region. Fever, sore throat, and headache may occur, but cough and coryza are notably absent. Aseptic meningitis and orchitis occur in a small number of patients with pleurodynia, in general less than 10%.[143,150,151] Pericarditis and pneumonia are rare.[148]

The pain, which varies in intensity, is variously described as lancinating, stabbing, constricting, or viselike. Patients asked to localize the pain are likely to indicate a broad area with the palm of the hand rather than a specific point with the finger. The most common location is the vicinity of the costal margin on one or both sides or occasionally the subxiphoid region. About half the patients have pain primarily in muscles of the thorax, especially the intercostals, the trapezius, and occasionally the erector spinae or pectoralis major. In the remainder pain is primarily in the upper part of the abdomen, especially the hypochondrium (internal and external obliques and transversus abdominis) or the epigastrium (rectus abdominis). Periumbilical pain and pain in the lower abdominal quadrants are also seen, especially in children, in whom abdominal localization of pain is the rule.[122,149] A few patients experience pain in neither the chest nor the abdomen but instead in the neck or limbs[150]; in these cases the diagnosis can be made only by association with other typical cases in the family. Whatever the localization of the pain, it is usual for an individual patient to experience this pain in only one or two areas of the body.

Although the location and severity vary, it is the spasmodic and paroxysmal character of the pain that is its hallmark. If the pain is mild and the patient ambulatory, the patient stoops forward or leans to the side to splint the chest. With more severe pain, the patient lies still in bed and appears acutely ill and apprehensive. Motion produces pain, and patients resist being turned in bed. Chest pain limits deep inspiration, and respirations are shallow and rapid. Auscultation of the chest reveals no abnormalities. Pain can be elicited by pressure on the involved muscles in most patients. Swelling is seen or felt only occasionally and by careful, sequential observations.

Most patients are ill for 4 to 6 days. Children have milder disease than adults, who are often confined to bed. The first paroxysm is the most severe, and subsequent paroxysms are shorter and accompanied by less fever. Although dull aching of involved muscles usually persists between bouts of sharp pain, the patient may look and feel entirely healthy between paroxysms. About one-fourth of patients experience multiple recurrences, often after they have been free of pain for a day or more and have felt well enough to return to work or school.[143,150] In about half of these persons recurrence of pain is at the same site; in the remainder a new site is attacked. Late relapses occur in some patients after they have been free of symptoms for a month or longer.[150]

Diagnosis

The severity, location, and other characteristics of the pain are so protean that pleurodynia is readily confused with other illnesses. Pain in the chest may mimic pneumonia, pulmonary infarction, myocardial ischemia, and the preeruptive phase of zoster. Abdominal pain in epidemic pleurodynia may resemble that in acute abdomen of a variety of causes. Normal auscultatory examination of the chest, together with the characteristic spasmodic and relapsing character of the pain, is helpful in excluding pneumonia. A negative chest radiographic film is also helpful, although pleural effusions may rarely be present.

Management and Prognosis

Analgesics and the application of heat to affected muscles are useful in relieving pain in most cases; in some, opiate analgesics are required for adequate pain control. Despite the distressing tendency of the disease to relapse, all patients eventually recover completely. Debility out of all proportion to the apparent severity of the illness is occasionally observed for several months during convalescence.[143,151]

Other Skeletal Myositis

EVs have been implicated as a cause of acute myositis, not confined to the torso in some patients,[152–159] although the diagnosis has rarely been proved virologically. E-11 has been recovered from clinically involved skeletal muscle of a 3-month-old infant with a fatal systemic infection.[152] In other cases CV-A9, CV-B2 and CV-B6, and E-9 have been etiologically linked to myositis on the basis of serology, recovery of virus from the throat or feces, or demonstration of viral antigen in muscle by immunofluorescence. Both generalized polymyositis and focal myositis have been noted, the latter sometimes localized to the thighs. Clinical myositis is manifested by fever, chills, weakness, hypotonia, tenderness, and edema of the involved muscle groups. Myoglobinemia, myoglobinuria, and an elevated creatine phosphokinase level are often found. Most reported patients have recovered rapidly.

A dermatomyositis-like illness occurs in B-cell–deficient immunocompromised patients with persistent EV infections (see later).

MYOPERICARDITIS

Because EVs rarely, if ever, infect the pericardium alone without involving the subepicardial myocardium, the term *myopericarditis* best describes the disease caused by these viruses when they affect the heart (see Chapter 84).[160] In the clinical setting, however, signs of either myocarditis or pericarditis often predominate. In older children and adults the severity of myopericarditis varies from asymptomatic cardiac involvement to severe disease with intractable heart failure and death. Myocarditis that occurs with generalized EV infection in the newborn is discussed separately in the section on neonatal infections.

An epidemic of CV-B5 myopericarditis occurred in Finland in autumn 1965, when 18 patients were admitted to a single hospital.[161] Epidemic myopericarditis appears to be exceptional, however, and most reported cases beyond the neonatal period have been sporadic, probably because involvement of the heart is a relatively uncommon manifestation of illness even during substantial EV epidemics.

Etiology and Pathogenesis

EVs account for at least one-third of all cases of acute viral myopericarditis.[143,162,163] However, the strength of the evidence linking a given EV serotype with myopericarditis varies considerably. Proof of causation exists for all CV-B serotypes; CV-A4 and CV-A16; and E-6, -7, -9, -13, and -22 by demonstration of infectious virus, viral genome, or viral antigen in the myocardium or pericardial fluid.[164–168] The evidence is less substantive for CV-A1, -A2, -A5, -A8, and -A9 and E-1 to -4, -7, -8, -11, -14, -19, -25, and -30.[163,165–167,169–178] These serotypes have been recovered from noncardiac sources during an episode of acute myopericarditis, some with a significant increase in neutralizing antibody titer to the isolated virus.

Many other viruses and bacteria have been associated with myopericarditis, although adenovirus,[179,180] influenza A virus,[162,181] parvovirus B19,[162,182] mumps virus,[183] cytomegalovirus,[162] HSV,[162] respiratory syncytial virus,[162] Epstein-Barr virus,[162] and vaccinia virus[184] are the principal non-EV agents that have been detected directly in pericardial fluid or myocardial tissue. The weight of clinical evidence suggests that *M. pneumoniae*, VZV, and measles virus also cause myopericarditis.

CV-B and other EVs reach the heart during the viremia that follows replication in the gastrointestinal (GI) or respiratory tract (see Chapter 170). Experimental studies in a murine model demonstrate that virus replication of myocytes results in scattered myocyte necrosis, followed by focal infiltration of polymorphonuclear leukocytes, lymphocytes, plasma cells, and macrophages.[185] The chronic inflammatory response that may persist for weeks to months has been a subject of widespread interest. Some investigators have demonstrated the presence of EV RNA in biopsy samples of cardiac tissue for months after the acute episode, whereas others have reported negative results from similar experiments. Other investigators consider the late-phase inflammatory response to be due to virus-induced, cytotoxic T-lymphocyte destruction of myocytes,[186] whereas still others have postulated the development of a myocardial neoantigen or cross-reactivity between viral and myocardial cell antigens.[187] Healing is accompanied by a variable degree of interstitial fibrosis and evidence of myocyte loss.

Clinical Manifestations

EV myocarditis occurs at all ages but has a special predilection for physically active adolescents and young adults. The incidence in males is at least twice that in females.[163] In two-thirds of cases an upper respiratory tract illness precedes the onset of cardiac manifestations by 7 to 14 days.[163] Common initial symptoms include dyspnea, chest pain, fever, and malaise.[161,163,188–190] Pain in the precordial area is usually dull, but it may resemble angina pectoris or be sharp, pleuritic, and exacerbated by recumbency when pericarditis is present. A pericardial friction rub, often transient, has been observed in 35% to 80% of cases. Enlargement of the cardiac silhouette on chest radiograph films, present in about 50%, may be due to either pericardial effusion or cardiac dilation. A gallop rhythm and other signs of frank congestive heart failure are observed in roughly 20%.[189,190]

Electrocardiographic abnormalities, including ST-segment elevation or nonspecific ST-segment and T-wave abnormalities, are invariably present. More severe myocardial disease leads to the development of Q waves, ventricular tachyarrhythmias, and all degrees of heart block. Echocardiography may confirm the presence of acute ventricular dilation or a diminished cardiac ejection fraction. Serum levels of myocardial enzymes are frequently elevated. Other clinical manifestations of systemic EV disease sometimes occur with myopericarditis and include aseptic meningitis, pleurodynia, hepatitis, and orchitis. Cardiac MRI has been shown to be useful in the diagnosis of myocarditis.[191]

Acute myocardial infarction associated with chest pain, arrhythmias, and congestive heart failure may be difficult to distinguish from myopericarditis. Patients suspected of having acute myocardial infarction sometimes have evidence of concurrent CV-B infection,[192–194] and focal myocarditis has been proved in at least one case of acute CV-B5 infection.[195] Some patients presenting with suspected myocardial infarction who have normal coronary angiographic studies have been shown to have myocarditis by radiolabeled antimyosin antibody cardiac scanning.[196]

Diagnostic Virology

Although CVs have been isolated on numerous occasions from pericardial fluid or heart muscle at autopsy or by open biopsy procedures,[197] in practice, these specimens are rarely available. Cardiac tissue infrequently yields a viral isolate when cultured. The likelihood of a positive PCR result may depend on the number of potential viral pathogens included in the assay.[160,182,198,199] In the absence of identification of virus in cardiac tissue, the diagnosis often rests on circumstantial evidence provided by recovery of the agent from the oropharynx or feces or on serologic evidence of recent infection by a CV-B.

Management

Supportive treatment consists of bed rest, pain relief, and medical management of arrhythmias and heart failure.[200] Although one study reported improved cardiac function and a trend toward increased survival for children with acute myocarditis who received intravenous immune globulin (IVIG), compared with historical controls,[201] randomized trials of immunosuppressive therapy, including IVIG, prednisone, azathioprine, and other drugs, have failed to show any consistent treatment effect.[202–204] In cases of fulminant myocarditis with severe heart failure refractory to medical management, extracorporeal membrane oxygenation or use of a ventricular assist device may be beneficial in recovery.[205,206]

Course and Prognosis

Persistent electrocardiographic abnormalities (10%–20%), cardiomegaly (5%–10%), and chronic congestive heart failure are indications of permanent myocardial injury that occur overall in about one-third of adult patients identified with acute myopericarditis; these abnormalities

may ultimately lead to a diagnosis of dilated cardiomyopathy.[163,189,190] Chronic constrictive pericarditis has occurred after intervals of 5 weeks to 1 year.[207–09]

The prognosis for children with acute myocarditis is better than for adults. Less than 15% of children die during the acute illness from intractable heart failure or uncontrolled arrhythmias, and fewer than 10% develop persistent or recurrent compromise from dilated cardiomyopathy requiring cardiac transplantation.[210] The risk for developing long-term cardiac sequelae may be higher in children with less severe acute myocarditis.[211]

Dilated Cardiomyopathy

Chronic dilated cardiomyopathy, which is second only to ischemic heart disease as a cause of chronic congestive heart failure, is the final result of multiple infectious and noninfectious cardiac insults,[212] including up to one-third of cases of acute myopericarditis and, in some instances, unrecognized past EV infection.[163,189,190] Persistence of EV in cardiac tissues has been proposed as a mechanism for the development of chronic dilated cardiomyopathy.[213] Some investigators have detected positive- or negative-strand EV RNA or EV protein in cardiac tissue months to years after the onset of dilated cardiomyopathy.[203] However, others using similar methods have not.[214–216]

COXSACKIEVIRUS AND ECHOVIRUS DISEASE IN THE NEWBORN

The human neonate is uniquely susceptible to EV disease. Although many EV serotypes cause the same self-limited clinical syndromes in neonates as in older persons (e.g., aseptic meningitis, exanthems), some serotypes are capable of producing fulminant, frequently fatal disease in the newborn infant. CV-B1 to CV-B5 and E-11 are most frequently associated with overwhelming systemic neonatal infections. Rare cases of serious neonatal disease are reported with CV-A3, CV-A9, and CV-A16.[217–219]

Epidemiology

Although most neonatal EV infections are directly transmitted from the mother, some infections are acquired by a nosocomial route. The first description of CV-B disease in newborn infants followed outbreaks occurring in nurseries in South Africa, Zimbabwe, and the Netherlands.[220] Numerous nursery outbreaks of neonatal E infection have been recorded, with the severity of neonatal disease varying according to the viral serotype.[221,222] Introduction of infection into the nursery has been traced to an infected parent or to hospital personnel. Infant-to-infant spread within nurseries probably occurs by the hands of personnel engaged in mouth care, gavage feeding, and other activities requiring close direct contact.[223]

Because most neonatal EV infections are sporadic rather than nosocomial, the incidence and severity of neonatal EV infection generally reflect the occurrence of EV disease in the community. Although many cases occur sporadically during the enterovirus season, clusters of vertically transmitted neonatal infection sometimes occur during community outbreaks with a single EV serotype.[224,225] During the 20-year period between 1983 and 2003, CV-B1 to CV-B4, E-11, and E-25 were reported to the CDC significantly more commonly among neonates than among persons older than 1 month.[226] In 2007 and 2008 a markedly increased number of cases of serious neonatal disease caused by CV-B1 were reported to the CDC, reflecting both widespread circulation of this virus nationally and a higher attack rate in infants, compared with other circulating enteroviruses.[227]

Pathophysiology

Most newborns with life-threatening EV disease are infected by vertical transmission from the infected mother in the perinatal period.[221,222] About 60% to 70% of women who give birth to infected infants have a febrile illness during the last week of pregnancy.[13,221] Experimental evidence indicates that the fetus is relatively protected by the placenta during maternal infection,[222,228] but the newborn has a high risk for infection,[141,229] perhaps as a result of exposure to either virus-positive cervical secretions[230,231] or viremic maternal blood.[232] Although most vertically transmitted EV infections are probably acquired during delivery, some infants are infected before delivery, as evidenced by the recovery of virus from cord blood[230] and the development of disease within the first 2 days of life.[221,232]

In the infected neonate EV spread systemically through the bloodstream. Tropism for and replication within specific organs of the neonatal host depend on both virus and host factors. Experimental evidence suggests that some neonatal tissues are innately more susceptible to infection with some EV than the corresponding tissues from an adult host.[233] In addition, the neonatal immune system is insufficient to control the replication and spread of virulent EV. Both premature and term human infants respond adequately to EV infection with humoral neutralizing antibody.[234] However, macrophage function, which does not mature sufficiently until several weeks of age in the human neonate, is necessary to limit initial EV replication.[235,236]

The outcome of neonatal infection is also strongly influenced by the presence or absence of passively acquired maternal antibody specific for the infecting EV serotype.[228,229,237] Thus the timing of maternal infection in relation to the development of maternal immunoglobulin G (IgG) antibody and delivery of the infant may be the most critical factor in determining the outcome of neonatal EV infection.

Clinical Manifestations

Symptoms develop in most neonates with generalized EV disease between 3 and 10 days of life.[231,232] A small number have signs of illness in the delivery room or within the first 1 to 2 days of life[221,232]; conversely, the onset of fatal infection has been documented in infants as old as 3 months.[152] Male infants and premature infants are overrepresented among infants with serious illness. Early symptoms are generally mild and nonspecific and include listlessness, anorexia, and transient respiratory distress. Fever may or may not be present. About one-third of patients have a biphasic illness with a period of 1 to 7 days of apparent well-being interspersed between the initial symptoms and the appearance of more serious manifestations.

Generalized EV disease in the newborn most often occurs in one of two characteristic clinical syndromes: either myocarditis or fulminant hepatitis. Neonatal myocarditis, which is often accompanied by encephalitis (encephalomyocarditis syndrome) and sometimes by hepatitis,[238] is characteristically a manifestation of CV-B infection[220,226,239] and, less commonly, E-11 infection.[177,240] Fulminant hepatitis is characterized by hypotension, profuse bleeding, jaundice, and multiple organ failure (hemorrhage-hepatitis syndrome).[226] E-11 is responsible for a large proportion of cases, but well-documented cases of severe hepatitis in neonates have resulted from E-4, -6, -7, -9, -12, -14, -19 to -21, -31, and -33.[221,232–235,237–247]

Myocarditis

Signs of neonatal myocarditis include rapid onset of heart failure; respiratory distress; tachycardia, often exceeding 200 beats/min; cardiomegaly; and electrocardiographic evidence of myocardial injury and arrhythmias. Cyanosis and circulatory collapse develop rapidly in severely affected infants. Fatal cases are often accompanied by disseminated viral infection involving other organs (in order of frequency), such as the CNS, liver, pancreas, and adrenal glands. Most affected neonates are lethargic, and seizures, a bulging fontanelle, and CSF pleocytosis indicate the presence of meningoencephalitis. Enlargement of the liver is more often due to congestive heart failure than to viral hepatitis.

The overall mortality rate for neonatal myocarditis is 30% to 50%, and death usually occurs within a week of onset. Myocardial function rapidly improves in surviving infants after defervescence, generally by 1 week, although in a few infants, convalescence is prolonged for several weeks. Some surviving infants develop dilated cardiomyopathy with ventricular aneurysm formation.[238] Pathologic data are limited to information obtained at postmortem examination. Infants dying of myocarditis have enlarged, dilated hearts, extensive myonecrosis, and a variable degree of cardiac inflammation.

Hepatitis

The initial symptoms of severe neonatal hepatitis are lethargy, poor feeding, and increasing jaundice that progress in many infants, within 1 to 2 days, to jaundice, ecchymoses, bleeding from puncture sites, and

metabolic acidosis. Hepatic transaminases may rise rapidly to extremely high levels, and markedly prolonged prothrombin times and partial thromboplastin times confirm profound hepatic failure.

More than half of infants with severe neonatal E hepatitis die within days after the onset of symptoms despite therapy with blood products and intensive supportive care. Some ultimately fatal cases survive for 2 to 3 weeks with supportive care.[224] Postmortem findings include massive hepatic necrosis and extensive hemorrhage into the cerebral ventricles, pericardial sac, renal medullae, and interstitial spaces of many solid organs.[248] Inflammation is commonly limited to the liver and adrenal glands, with sparing of the heart, brain, meninges, and other organs. The long-term prognosis for surviving infants is not well known, although hepatic fibrosis and chronic hepatic insufficiency develop early in life in some.

Pneumonia

Several cases of EV pneumonia occurring in the first few days of life have been reported, all of them fatal and caused by E-6,[138] E-9,[139] and E-11,[140] and CV-A3.

Diagnosis and Differential Diagnosis

The diagnosis of neonatal CV and E infection depends on detection of viral RNA by PCR or isolation of virus in cell culture. Because virus is usually present in the infected neonate in high titer, isolation from oropharyngeal secretions, feces, and urine is relatively rapid; virus may also be recovered from blood, CSF, ascitic fluid, and multiple tissues obtained at biopsy or autopsy.

Neonatal myocarditis is sometimes mistaken for congenital heart disease because, in both conditions, murmurs and evidence of congestive heart failure may be present. However, fever and electrocardiographic evidence of acute myocardial injury are absent in patients with congenital heart disease. The early features of myocarditis and severe hepatitis resemble those of bacterial sepsis. Because of liver and CNS involvement in either syndrome, visceral dissemination with perinatally acquired HSV in the absence of cutaneous lesions may be suspected.

Management

Management of neonatal EV disease is supportive. Infants in congestive heart failure require judicious fluid management and administration of inotropic agents and diuretics. The profuse bleeding that results from hepatic failure necessitates frequent replacement therapy with packed red blood cells, platelets, and fresh-frozen plasma. Vitamin K should be administered intravenously in pharmacologic doses. Large doses of IVIG, which have been reported to improve outcome in at least one case,[249] may be justified, given the extremely poor prognosis, although there is not reliable evidence of efficacy. A phase II clinical trial using the experimental antipicornavirus drug pleconaril demonstrated shorter times to culture and PCR negativity along with greater survival among pleconaril recipients.[250]

CHRONIC MENINGOENCEPHALITIS IN AGAMMAGLOBULINEMIC AND OTHER IMMUNOCOMPROMISED PATIENTS

The EVs have been responsible for persistent, severe, sometimes fatal infections of the CNS in patients with hereditary (X-linked agammaglobulinemia, common variable immunodeficiency, severe combined immunodeficiency syndrome, and hyper-IgM syndrome)[251–254] or acquired defects in B-lymphocyte function (recipients of bone marrow transplants and of rituximab and obinutuzumab).[255–258] Most cases have been caused by Es; single cases caused by CV-A4, CV-A11, and CV-A15 and by CV-B2 and CV-B3 have been reported.[251,259]

Clinical Manifestations

Nervous system manifestations may be totally absent, or mild nuchal rigidity, headache, lethargy, papilledema, seizure disorders, motor weakness, tremors, and ataxia may be present. These neurologic abnormalities may fluctuate in severity, disappear, or steadily progress. The CSF exhibits lymphocytic pleocytosis and a higher protein concentration than is usually seen in cases of acute EV aseptic meningitis. An EV can be repeatedly recovered from the CSF over a period of months to years, usually in high titer. In some cases virus is isolated only intermittently from the CSF or detected only by PCR. For unknown reasons it is usually more difficult to isolate virus from the feces than from the CSF. Persistent skeletal muscle involvement causes a dermatomyositis-like syndrome in more than half of these patients, and some also have chronic hepatitis.

EVs have been recovered from many other sites in these patients, including the brain, lung, liver, spleen, kidney, myocardium, pericardial fluid, skeletal muscle, and bone marrow.[251] Some patients have been infected with more than one EV serotype, either concurrently[260] or sequentially.[251,259] The pathogenesis of the chronic muscle and soft tissue inflammation is not fully understood, but isolation of E from muscle in one case suggests a role for direct virus infection.[261]

In many patients, possibly most, the disease ends fatally. Autopsy findings have included chronic meningitis and encephalitis, with lymphocytic perivascular cuffing, focal loss of neurons, and gliosis of both gray and white matter. However, widespread destruction of motor neurons, such as that seen in poliomyelitis, has not been observed.

Prophylaxis and Therapy

Prophylactic use of IVIG serum globulin reduces the risk for acquiring chronic EV infection by these patients. However, IVIG has been less effective in the treatment of chronic EV meningitis, even when using IVIG lots with relatively high concentrations of specific antibody. Some patients have experienced clinical improvement when IVIG has been injected directly into the ventricles,[251] but relapse of infection may occur even after long-term intraventricular IVIG therapy. The experimental antiviral drug pleconaril has been used in this setting, but the reported experience with it is uncontrolled and limited to a small number of patients.[254,262]

Infections in Other Immunocompromised Patients

Hematopoietic cell allograft recipients have profoundly suppressed immunologic responses during the immediate posttransplantation period, including suppression of the ability to mount a humoral immune response. In some recipients EV infections have developed in the posttransplantation period that were disseminated, prolonged, and contributed to fatal outcomes.[255,262–265] One outbreak of CV-A1 diarrheal illness was observed in a bone marrow transplantation unit in which virus-induced diarrhea was difficult to distinguish from graft-versus-host enteritis.[266] Some patients receiving rituximab (anti-CD20) monoclonal antibody, which profoundly suppresses B-lymphocyte function, have developed paralysis, myocarditis, and other complications of CNS infection.[256,257,267]

ACUTE HEMORRHAGIC CONJUNCTIVITIS

Acute hemorrhagic conjunctivitis (AHC) is a contagious ocular infection characterized by pain, swelling of the eyelids, and subconjunctival hemorrhage that generally resolves spontaneously within a week. Epidemic or pandemic disease has now occurred in most parts of the world, resulting from EV-D70, which has been responsible for tens of millions of cases of AHC, and CV-A24 has caused a similar but geographically more restricted outbreak of disease that afflicted hundreds of thousands of persons. Some epidemics of conjunctivitis in the Far East have involved both viruses sequentially or concurrently. Although the relative contribution of these two agents has not always been defined, it is clear that EV-D70 has accounted for greater total morbidity.

Epidemiology

AHC appeared to emerge as a new disease in 1969 with explosive, pandemic spread from simultaneous foci in Ghana and Indonesia.[268] The initial epidemic caused by EV-D70 spread along the coast of West Africa and ultimately involved many countries on the African continent by 1973, as well as England, the former Soviet Union, Holland, France, and Yugoslavia.[269,270] CV-A24 was identified as the etiology of more than 60,000 cases of AHC in Singapore in 1970.[271–273]

Subsequently, both viruses circulated in Southeast Asia and the Indian subcontinent, causing large seasonal outbreaks.[274–277] Although the geographic distribution of AHC is wide, large-scale epidemics have occurred predominantly in crowded coastal areas of tropical countries during the hot, rainy season.[278] A 2012 outbreak of CV-A24 AHC on the French island of Mayotte, in the Indian Ocean, affected greater than 6% of the population and spread to neighboring islands of the Union of Comoros.[279,280]

Outbreaks in economically developed countries and temperate climates have been much more limited. AHC in the West has been mostly confined to seasonal outbreaks in Central America and the Caribbean. The disease did not appear in the United States until September 1981, when EV-D70 conjunctivitis was first reported in Key West, Florida. Within weeks, about 2500 cases occurred, largely among disadvantaged persons living in Miami.[281] With the exception of a few imported cases, AHC activity has not since been noted in the United States.[282] CV-A24 AHC cases first appeared in the Western Hemisphere in Trinidad, Jamaica, St. Croix, Panama, and Mexico in 1986.[283] Two outbreaks in Cuba in 2008 and 2009 resulted in more than 72,000 cases.[284]

Patterns of Transmission

AHC is highly contagious and spreads rapidly. Unlike most other EV infections, AHC is transmitted primarily from fingers or fomites directly to the eye rather than by respiratory secretions or fecal contamination. Both EV-D70 and CV-A24 can be regularly recovered from the conjunctiva early in the illness but only infrequently recovered from respiratory secretions or feces. Both serotypes appear to be naturally occurring, temperature-sensitive viruses whose optimal replication is at 33° to 35°C and reflects their adaptation to the temperature of the conjunctiva.[285,286] The observed rapid serial transmission at about 24-hour intervals is consistent with direct spread of virus from hand to eye. During a 1980 EV-D70 outbreak in Singapore, the secondary attack rate within affected households was 73%.[287] Contagion is favored by crowding and unsanitary living conditions.[280] AHC occurs substantially more often among the poor than among others living in the same country.[288,289] Reuse of water for bathing and sharing of towels are implicated as factors contributing to the spread of infection. Limited outbreaks of AHC in Europe have been primarily nosocomial, particularly in ophthalmology clinics, where infection appears to have been spread directly by physicians' fingers or by instruments.

Postepidemic antibody prevalence rates of nearly 50% have been observed in Ghana and Indonesia but only in 6% of affected populations of Japan. These findings are consistent with less intense spread of AHC in economically developed regions. Antibody prevalence rates are highest in children younger than 10 years, whereas attack rates for clinical disease are greatest in young adults, which indicates that many infections in children must be inapparent or mild.[290,291]

Clinical Manifestations

The clinical manifestations of AHC begin abruptly and peak within 24 hours. Burning, foreign body sensation, ocular pain, photophobia, eyelid swelling, and watery discharge begin in one eye and rapidly progress to the other eye.[281] Constitutional symptoms, such as fever, malaise, and headache, are observed in 20% of cases. The most distinctive sign is subconjunctival hemorrhage, which is present in 70% to 90% of patients with AHC caused by EV-D70,[291] but it is less frequent in cases caused by CV-A24.[272,273,275] The hemorrhages may be pinpoint or occupy the entire bulbar conjunctiva and are precipitated by everting the upper lid or by rubbing the eyes. Conjunctival edema is said to be more common in elderly people; hemorrhage is more profuse in young patients.[291] Small follicles appear on the tarsal conjunctiva after 3 to 5 days in 90% of patients. In most cases corneal erosion or a fine punctate epithelial keratitis can be demonstrated by slit-lamp examination after staining with fluorescein. The ocular discharge is serous or seromucoid and contains abundant neutrophils in the first 24 hours. Preauricular lymph nodes are often enlarged and tender by the second day of illness. Recovery is usually noticeable by the second or third day and is complete in most cases in 10 days, although discoloration from the hemorrhages sometimes persists for many days.

Complications

In severe cases of AHC, keratitis occasionally persists for several weeks but almost never leads to permanent scarring. Uveitis has not been reported. Conjunctivitis may be complicated by secondary bacterial infection.

More than 200 cases of acute motor paralysis have been reported as a complication of EV-D70 AHC in India, Thailand, Taiwan, and Senegal.[276,292–295] The paresis is clinically indistinguishable from poliomyelitis except for its temporal association with AHC, which it generally follows by 2 to 5 weeks. Neuroparalytic disease has been reproduced clinically and pathologically in monkeys by inoculation of EV-D70 into the spinal cord.[268,296]

Differential Diagnosis

Small outbreaks or sporadic cases may be mistaken for adenovirus infection causing epidemic keratoconjunctivitis (see Chapter 142), which has a longer incubation period (5–7 days), peaks several days after onset, and persists for up to 2 or 3 weeks, compared with AHC.

Laboratory Diagnosis

EV-D70 and CV-A24 can be recovered from conjunctival swabs or scrapings of patients with AHC during the first 3 days of illness.[271,297] Isolation rates exceeding 90% from conjunctival scrapings have been reported for CV-A24, but recovery rates for EV-D70 have been somewhat lower.[288] NAAT and sequencing of the VP1 coding region can readily identify the causative agent.[280,283] EV-D70 has not been recovered from the CSF in persons with paralysis, but high titers of specific neutralizing antibody to EV-D70 have been demonstrated in the CSF in virtually all affected patients.[295]

Treatment and Prevention

Treatment of conjunctivitis is symptomatic. Antimicrobial agents are not indicated. Contagion can be prevented by careful hand washing, use of separate towels, and sterilization of ophthalmologic instruments.

ILLNESSES IN WHICH THE ETIOLOGIC ROLE OF ENTEROVIRUSES IS MINOR OR POORLY DEFINED

Gastrointestinal Diseases

Acute hepatitis occurring beyond the neonatal period is described in association with CV-B and echovirus infections.[236,298–300] Most cases have been mild and self-limited. Prospective studies indicated that 2% to 20% of patients with acute pancreatitis have concurrent EV infection.[301,302] CV-B1 to CV-B5 and E-6, E-11, E-22, and E-30 are all reported to cause acute pancreatitis.[301–303]

CVs and echoviruses, possibly as a result of their replication in the small bowel, are frequently cited as causes of nonbacterial diarrhea or gastroenteritis. However, conflicting results have been obtained in several studies that compared rates of EV isolation from children with acute diarrheal illness with matched healthy control subjects.[304–307] Overall, the data favor a variable, generally small excess of EV infections in subjects with diarrhea. Evidence is somewhat stronger that certain Es, particularly E-11, E-14, and E-18, have occasionally been responsible for epidemic diarrhea in young infants.[304,306,308] Most of these studies were performed before the discovery of toxigenic *Escherichia coli*, rotaviruses, enteric adenoviruses, and caliciviruses, now established as major causes of diarrheal illness. In light of this knowledge, additional epidemiologic investigations encompassing all these agents are required before the contribution of EVs to diarrheal disease can be accurately assessed. Nonetheless, their role is probably minor.

Similarly, hemolytic-uremic syndrome (HUS) has been temporally associated with CV-A4, CV-B2, and CV-B4,[309,310] and CV-B5 has been reported associated with acute renal failure.[311] The well-established link between enterohemorrhagic *E. coli* infection and HUS renders the relationship between EV infection and acute renal disease questionable at best.

Other Diseases

Orchitis has been observed in adolescent boys during infection with CV-A9; CV-B2, CV-B4, and CV-B5; and E-6,[312–315] including CV-B5

isolated from a testicular biopsy specimen in one case.[312] Splenomegaly and a heterophile-negative mononucleosis-like syndrome have also been reported.[304] Es have been associated with acute arthritis, including E-11, which was recovered from synovial fluid in one case.[316,317] In separate case reports, E-25[318,319] and an untyped EV resembling a CV-A[319] have been recovered from the GI tracts of children with acute infectious lymphocytosis; however, further evidence of an etiologic association is lacking. Multiple reports of EV-induced lymphocytic hemophagocytosis occurring in infants, children, and adults have been published.[320–322]

Diabetes Mellitus

An accumulating body of epidemiologic, clinical, and experimental evidence suggests an intriguing link between the enteroviruses, particularly the CV-Bs, and type 1 diabetes mellitus (T1DM). However, the role of the EVs in the pathogenesis of T1DM remains controversial. Knowledge of the potential interactions of enteroviruses with beta cells in the islets of Langerhans, as well as the host's innate and adaptive arms of the immune system, is complex and evolving.[323] The reader is referred to several excellent reviews for more detailed analyses.[323–325]

The hygiene hypothesis of TIDM[323] contends that in areas where EV infections are frequent, infants develop early infection and immunity to them or are born with maternal antibodies to the common EV serotypes that partially protect the infant from severe infection. In areas of low prevalence, such as industrialized nations where sanitary conditions are better, EV infections may occur at older ages. Thus EV infection in infancy or early childhood may protect individuals from the risk of developing T1DM.

The observation that new-onset T1DM cases occur in seasonal patterns,[326] and sometimes in clusters or small outbreaks, has been cited as evidence for the role of viral disease in the pathophysiology of T1DM. The peak occurrence of new T1DM cases is late in the calendar year, 1 to 2 months later than peak EV activity. However, the occurrence of EV infection and T1DM during the same season could be independent, and several studies found no increase in new onset of T1DM after outbreaks of CV-B disease.[324,325,327,328] Evidence for direct infection of beta cells by EVs of patients with T1DM exists. Murine studies have documented that EVs cause specific destruction of beta cells in the islets of Langerhans.[329] Detection of EV RNA in serum or viral VP1 in the islets at the onset or recent onset of T1DM[330,331]; postmortem isolation of CV-B4[332,333] and CV-B5[334] from the pancreatic tissue of children dying of ketoacidosis, as their initial manifestation of T1DM; and the demonstration of CV-B IgM antibody in the serum of children with recent-onset T1DM all support the direct-infection hypothesis. However, inconsistency regarding this finding has been noted across different studies.[335–337]

Direct infection may result in viral-induced cytolytic death of islet cells or set the stage for an autoimmune response by the host or both.[323,338] EV infection of islet cells might activate the innate immune system, leading to inflammation. Human islet cells infected with CV-B5 results in the expression of Toll-like receptor 3, retinoic acid–inducible protein 1, and interferon (IFN)-induced helicase 1 pattern-recognition receptors that can detect EVs. Replication of the EVs results in the generation of double-stranded RNA that can bind to pattern-recognition receptors, leading to production of IFN-α and IFN-β, apoptosis, expression of major class I histocompatibility complex antigens, and production of cytokines. These innate immune responses serve to control the EV infection but can also be harmful to beta-cell function.

Evidence for "bystander activation" of preexisting autoreactive T cells exists in a transgenic mouse model overexpressing a T-cell receptor specific for an autoantigen in islet cells infected with CV-B4.[339] The resulting inflammation leads to the development of diabetes mellitus. Some investigations suggest that molecular mimicry between an EV protein (2C) and islet autoantigens or other cellular proteins may result in autoimmune destruction of pancreatic islet cell tissue.[340]

ENTEROVIRUS A71 INFECTIONS

EV-A71 is closely related to CV-A16, and both viruses cause skeletal myositis in suckling mice and myelitis with paralysis in cynomolgus monkeys.[341,342] Genomic analysis of strains from around the world has revealed the existence of three lineages, genogroups A to C, with several genotypes within the B and C genogroups.[343] The B lineage predominates in Singapore and Malaysia, whereas C lineage viruses predominate in east Asia.[55]

EV-A71 was first isolated in California in 1969 from young children with encephalitis and aseptic meningitis[344,345] and has since been found to cause infection globally, including large outbreaks of HFMD, which have been associated with aseptic meningitis and serious CNS complications in young children.[51,55,56,345–348] The largest recorded outbreak of EV-A71 HFMD occurred in Taiwan in 1998, where approximately 1.5 million individuals were infected.[53] In that epidemic 405 children were hospitalized because of neurologic complications, 78 of whom died. Infants and young children who develop a rhombencephalitis have a high mortality related to rapid cardiovascular collapse and pulmonary edema.[51–53]

EV-A71 is also unique among the nonpolio EVs as a cause of epidemic AFP, in which localized outbreaks have involved small numbers of patients over several years,[344,345] and regional epidemics have involved hundreds to thousands of persons within a single season.[51,346] Other, less common manifestations attributed to EV-A71 infection include generalized maculopapular rash,[345] interstitial pneumonia,[54] and myocarditis.[53] EV-A71 can be isolated from vesicle fluid, feces, oropharyngeal secretions, urine, and CSF. Primary isolation is most successful in African green monkey kidney cell culture, although a cytopathic effect may take 5 to 8 days to develop. Isolation rates are highest from vesicle swabs and lowest from the CSF.[347] Detection using NAAT facilitates and speeds the identification of the virus.[349] However, NAAT detection of EV-A71 from the CSF is also low.[350]

Treatment of EV-A71 infection is symptomatic and supportive. The large-scale epidemics occurring in the Far East have led to the development of an EV-A71 vaccine that has recently undergone a successful phase III trial, with greater than or equal to 90% efficacy against EV-A71–associated HFMD.[351–353] It remains to be determined if the vaccine will confer cross-protection against other C genotypes or strains from the A and B genogroups.

Key References

The complete reference list is available online at Expert Consult.

2. Berlin LE, Rorabaugh ML, Heldrich F, et al. Aseptic meningitis in infants less than two years of age: diagnosis and etiology. *J Infect Dis*. 1993;168:888.
3. Hasbun R, Rosenthal N, Balada-Llasat JM, et al. Epidemiology of meningitis and encephalitis in the United States, 2011-2014. *Clin Infect Dis*. 2017;65:359.
6. Rorabaugh ML, Berlin LE, Heldrich F, et al. Aseptic meningitis among infants less than two years of age: acute illness and neurologic complications. *Pediatrics*. 1993;92:206.
8. Rotbart H, Brennan PJ, Fife KH, et al. Enterovirus meningitis in adults. *Clin Infect Dis*. 1998;27:896.
13. Lake AM, Lauer BA, Clark JC, et al. Enterovirus infections in neonates. *J Pediatr*. 1976;89:787.
17. Feigin RD, Shackelford PG. Value of repeat lumbar puncture in the differential diagnosis of meningitis. *N Engl J Med*. 1973;289:571.
23. Rotbart HA. Diagnosis of enteroviral meningitis with the polymerase chain reaction. *J Pediatr*. 1990;117:85.
24. Stellrecht KA, Lamson DA, Romero JR, et al. Enteroviruses and parechoviruses. In: Murray PR, Baron EJ, Jorgensen JH, eds. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: American Society for Microbiology Press; 2011:1388.
35. King RL, Lorch SA, Cohen DM, et al. Routine cerebrospinal fluid enterovirus polymerase chain reaction testing reduces hospitalization and antibiotic use for infants 90 days of age or younger. *Pediatrics*. 2007;120:489.
36. Lyons TW, McAdam AJ, Cohn KA, et al. Impact of in-hospital enteroviral polymerase chain reaction testing on the clinical management of children with meningitis. *J Hosp Med*. 2012;7:517.
43. Fowlkes AL, Honarmand S, Glaser C, et al. Enterovirus-associated encephalitis in the California encephalitis project, 1998-2005. *J Infect Dis*. 2008;198:1685.
51. Ho M, Chen ER, Hsu KH, et al. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. *N Engl J Med*. 1999;341:929.
52. Huang C-C, Liu C-C, Chang Y-C, et al. Neurologic complications in children with enterovirus 71 infection. *N Engl J Med*. 1999;341:936.
55. Solomon T, Lewthwaite P, Perera D, et al. Virology, epidemiology, pathogenesis, and control of enterovirus 71. *Lancet Infect Dis*. 2010;10:778.
60. Huang MC, Wang SM, Hsu YW, et al. Long-term cognitive and motor deficits after enterovirus 71 brainstem encephalitis in children. *Pediatrics*. 2006;118:e1785.
63. Melnick JL. Enteroviruses. In: Evans A, ed. *Viral Infections of Human Epidemiology and Control*. 3rd ed. New York: Plenum; 1991:191.
71. Messacar K, Schreiner TL, Maloney JA, et al. A cluster of acute flaccid paralysis and cranial nerve dysfunction temporally associated with an outbreak of enterovirus D68 in children in Colorado, USA. *Lancet*. 2015;385:1662.

72. Sejvar JJ, Lopez AS, Cortese MM, et al. Acute flaccid myelitis in the United States, August-December 2014: results of nationwide surveillance. *Clin Infect Dis*. 2016;63:737.
73. Messacar K, Asturias EJ, Hixon AM, et al. Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. *Lancet Infect Dis*. 2018;18:e239–e247.
74. Messacar K, Schreiner TL, Van Haren K, et al. Acute flaccid myelitis: a clinical review of US cases 2012-2015. *Ann Neurol*. 2016;80:326.
76. Hixon AM, Clarke P, Tyler KL. Evaluating treatment efficacy in a mouse model of enterovirus D68-associated paralytic myelitis. *J Infect Dis*. 2017;216:1245–1253.
77. Dietz V, Andrus J, Olivé JM, et al. Epidemiology and clinical characteristics of acute flaccid paralysis associated with non-polio enterovirus isolation: the experience in the Americas. *Bull World Health Organ*. 1995;73:597.
78. Saeed M, Zaidi SZ, Naeem A, et al. Epidemiology and clinical findings associated with enteroviral acute flaccid paralysis in Pakistan. *BMC Infect Dis*. 2007;7:6.
90. Ramsay M, Reacher M, O'Flynn C, et al. Causes of morbilliform rash in a highly immunised English population. *Arch Dis Child*. 2002;87:202.
94. Hall CB, Cherry JD, Hatch MH, et al. The return of Boston exanthem: echovirus 16 infections in 1974. *Am J Dis Child*. 1977;131:323.
95. Neva FA. A second outbreak of Boston exanthem disease in Pittsburgh during 1954. *N Engl J Med*. 1956;254:838.
99. Lindenbaum JE, Van Dyck PC, Allen RG. Hand, foot and mouth disease associated with coxsackievirus group B. *Scand J Infect Dis*. 1975;7:161.
102. Blomqvist S, Klemola P, Kaijalainen S, et al. Co-circulation of coxsackieviruses A6 and A10 in hand, foot and mouth disease outbreak in Finland. *J Clin Virol*. 2010;48:49–54.
104. Bracho MA, González-Candelas F, Valero A, et al. Enterovirus co-infections and onychomadesis after hand, foot, and mouth disease, Spain, 2008. *Emerg Infect Dis*. 2011;17:2223.
105. Centers for Disease Control and Prevention. Notes from the field: severe hand, foot, and mouth disease associated with coxsackievirus A6—Alabama, Connecticut, California, and Nevada, November 2011-February 2012. *MMWR Morb Mortal Wkly Rep*. 2012;61:213–214.
126. Jacobson LM, Redd JT, Schneider E, et al. Outbreak of lower respiratory tract illness associated with human enterovirus 68 among American Indian children. *Pediatr Infect Dis J*. 2012;31:309.
129. Centers for Disease Control and Prevention. Enterovirus D68. https://www.cdc.gov/non-polio-enterovirus/about/ev-d68.html. Accessed June 8, 2018.
130. Prill MM, Dahl RM, Midgley CM, et al. Severe respiratory illness associated with rhinovirus during the enterovirus D68 outbreak in the United States, August 2014-November 2014. *Clin Infect Dis*. 2018;66:1528.
131. Schuster JE1, Selvarangan R, Hassan F, et al. Clinical course of enterovirus D68 in hospitalized children. *Pediatr Infect Dis J*. 2017;36:290.
143. Sylvest E. *Epidemic Myalgia: Bornholm Disease*. London: Oxford University Press; 1934.
145. Weller TH, Enders JF, Buckingham M, et al. Etiology of epidemic pleurodynia: study of two viruses isolated from typical outbreak. *J Immunol*. 1950;65:337.
149. Disney ME, Howard EM, Wood BSB. Bornholm disease in children. *Br Med J*. 1953;1:1351.
162. Bowles NE, Ni J, Kearney DL, et al. Detection of viruses in myocardial tissues by polymerase chain reaction: evidence of adenovirus as a common cause of myocarditis in children and adults. *J Am Coll Cardiol*. 2003;42:466.
205. Pages ON, Aubert S, Combes A, et al. Paracorporeal pulsatile biventricular assist device versus extracorporal membrane oxygenation-extracorporal life support in adult fulminant myocarditis. *J Thorac Cardiovasc Surg*. 2009;137:194–197.
206. Madden K, Thiagarajan RR, Rycus PT, et al. Survival of neonates with enteroviral myocarditis requiring extracorporeal membrane oxygenation. *Pediatr Crit Care Med*. 2011;12:314.
211. McCarthy RE III, Boehmer JP, Hruban RH, et al. Long-term outcome of fulminant myocarditis as compared with acute (nonfulminant) myocarditis. *N Engl J Med*. 2000;342:690.
221. Modlin JF. Perinatal echovirus infection: insights from a literature of 61 cases of serious infection and 16 outbreaks in nurseries. *Rev Infect Dis*. 1986;8:918.
224. Modlin JF. Fatal echovirus 11 disease in premature neonates. *Pediatrics*. 1980;66:775.
226. Khetsuriani N, Lamonte A, Oberste M, et al. Neonatal enterovirus infections reported to the national enterovirus surveillance system in the United States, 1983-2003. *Pediatr Infect Dis J*. 2006;25:889.
232. Kaplan MH, Klein SW, McPhee J, et al. Group B coxsackievirus infections in infants younger than three months of age: a serious childhood illness. *Rev Infect Dis*. 1983;5:1019.
250. Abzug MJ, Michaels MG, Wald E, et al. A randomized, double-blind, placebo-controlled trial of pleconaril for the treatment of neonates with enterovirus sepsis. *J Pediatric Infect Dis Soc*. 2016;5:53.
251. McKinney RE, Katz SL, Wilfert CM. Chronic enteroviral meningoencephalitis in agammaglobulinemic patients. *Rev Infect Dis*. 1987;9:334.
268. Kono R. Apollo 11 disease or acute hemorrhagic conjunctivitis: a pandemic of a new enterovirus infection of the eyes. *Am J Epidemiol*. 1975;101:383.
270. Mirkovic RR, Kono R, Yin-Murphy M, et al. Enterovirus type 70: the etiologic agent of pandemic acute hemorrhagic conjunctivitis. *Bull World Health Organ*. 1973;49:341.
284. Fonseca MC, Sarmiento L, Resik S, et al. Isolation of Coxsackievirus A24 variant from patients with hemorrhagic conjunctivitis in Cuba, 2008-2009. *J Clin Virol*. 2012;53:77.
350. Pérez-Vélez CM, Anderson MS, Robinson CC, et al. Outbreak of neurologic enterovirus type 71 disease: a diagnostic challenge. *Clin Infect Dis*. 2007;45:950.
351. Zhu FC, Meng FY, Li JX, et al. Efficacy, safety, and immunology of an inactivated alum-adjuvant enterovirus 71 vaccine in children in China: a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2013;381:2024.
352. Zhu F, Xu W, Xia J, et al. Efficacy, safety, and immunogenicity of an enterovirus 71 vaccine in China. *N Engl J Med*. 2014;370:818.
353. Li R, Liu L, Mo Z, et al. An inactivated enterovirus 71 vaccine in healthy children. *N Engl J Med*. 2014;370:829.
355. Magoffin RL, Lennette EH. Nonpolioviruses and paralytic disease. *Calif Med*. 1962;97:1.

References

1. Kogon A, Spigland I, Frothingham TE, et al. The virus watch program: a continuing surveillance of viral infections in metropolitan New York families. VII. Observations on viral excretion, seroimmunity, intrafamilial spread and illness association in coxsackie and echovirus infections. *Am J Epidemiol*. 1969;89:51.
2. Berlin LE, Rorabaugh ML, Heldrich F, et al. Aseptic meningitis in infants less than two years of age: diagnosis and etiology. *J Infect Dis*. 1993;168:888.
3. Hasbun R, Rosenthal N, Balada-Llasat JM, et al. Epidemiology of meningitis and encephalitis in the United States, 2011-2014. *Clin Infect Dis*. 2017;65:359.
4. Mullins JA, Khetsuriani N, Nix WA, et al. Emergence of echovirus type 13 as a prominent enterovirus. *Clin Infect Dis*. 2004;38:70.
5. Huang QS, Carr JM, Nix WA, et al. An echovirus type 33 outbreak in New Zealand. *Clin Infect Dis*. 2003;37:650.
6. Rorabaugh ML, Berlin LE, Heldrich F, et al. Aseptic meningitis among infants less than two years of age: acute illness and neurologic complications. *Pediatrics*. 1993;92:206.
7. Dagan R, Jenista J, Menegus MA. Association of clinical presentation, laboratory findings, and virus serotypes with the presence of meningitis in hospitalized infants with enterovirus infection. *J Pediatr*. 1988;113:975.
8. Rotbart H, Brennan PJ, Fife KH, et al. Enterovirus meningitis in adults. *Clin Infect Dis*. 1998;27:896.
9. Chemtob S, Reece ER, Mills EL. Syndrome of inappropriate secretion of antidiuretic hormone in enteroviral meningitis. *Am J Dis Child*. 1985;139:292.
10. Bernit E, de Lamballerie X, Zandotti C, et al. Prospective investigation of a large outbreak of meningitis due to echovirus 30 during summer 2000 in Marseilles, France. *Medicine (Baltimore)*. 2004;83:245.
11. Desmond RA, Accortt NA, Talley L, et al. Enteroviral meningitis: natural history and outcome of pleconaril therapy. *Antimicrob Agents Chemother*. 2006;50:2409.
12. Haynes RE, Cramblett HG, Kronfol HJ. Echovirus 9 meningoencephalitis in infants and children. *JAMA*. 1969;208:1657.
13. Lake AM, Lauer BA, Clark JC, et al. Enterovirus infections in neonates. *J Pediatr*. 1976;89:787.
14. Seiden JA, Zorc JJ, Hodinka RL, et al. Lack of cerebrospinal fluid pleocytosis in young infants with enterovirus infections of the central nervous system. *Pediatr Emerg Care*. 2010;26:77.
15. Yun KW, Choi EH, Cheon DS, et al. Enteroviral meningitis without pleocytosis in children. *Arch Dis Child*. 2012;97:874.
16. Amir J, Harel L, Frydman M, et al. Shift in cerebrospinal polymorphonuclear cell percentage in the early stage of aseptic meningitis. *J Pediatr*. 1991;119:938.
17. Feigin RD, Shackelford PG. Value of repeat lumbar puncture in the differential diagnosis of meningitis. *N Engl J Med*. 1973;289:571.
18. Shah SS, Hodinka RL, Turnquist JL, et al. Cerebrospinal fluid mononuclear cell predominance is not related to symptom duration in children with enteroviral meningitis. *J Pediatr*. 2006;148:118.
19. Avner E, Satz J, Plotkin SA. Hypoglycorrhachia in young infants with viral meningitis [letter]. *J Pediatr*. 1975;87:883.
20. Singer JI, Mauer PR, Riley JP, et al. Management of central nervous system infections during an epidemic of enteroviral aseptic meningitis. *J Pediatr*. 1980;96:559.
21. Sumaya CV, Corman LI. Enteroviral meningitis in early infancy: significance in community outbreaks. *Pediatr Infect Dis*. 1982;3:151.
22. Malcom BS, Eiden JJ, Hendley JO. Echovirus type 9 meningitis simulating tuberculous meningitis. *Pediatrics*. 1980;65:725.
23. Rotbart HA. Diagnosis of enteroviral meningitis with the polymerase chain reaction. *J Pediatr*. 1990;117:85.
24. Stellrecht KA, Lamson DA, Romero JR. Enteroviruses and parechoviruses. In: Versalovic J, Pfaller MA, Carroll KC, et al, eds. *Manual of Clinical Microbiology*. 11th ed. Washington, DC: American Society for Microbiology Press; 2015:1536.
25. Lennette EH, Magoffin R, Knouf EG. Viral central nervous system disease: an etiologic study conducted at the los Angeles County General Hospital. *JAMA*. 1962;179:687.
26. Lepow ML, Carver DH, Wright HT, et al. A clinical, epidemiologic and laboratory investigation of aseptic meningitis during the four-year period, 1955-1958. I. Observations concerning etiology and epidemiology. *N Engl J Med*. 1962;266:1181.
27. Marier R, Rodriguez W, Chloupek RJ, et al. Coxsackievirus B5 infection and aseptic meningitis in neonates and children. *Am J Dis Child*. 1975;129:321.
28. Torphy DE, Ray GC, Thompson RS, et al. An epidemic of aseptic meningitis due to echovirus type 30: epidemiologic features and clinical and laboratory findings. *Am J Public Health*. 1970;60:1447.
29. Sabin AB, Krumbiegel ER, Wigand R. ECHO type 9 virus disease. *Am J Dis Child*. 1958;96:197.
30. Rotbart HA, Ahmed A, Hickey S, et al. Diagnosis of enterovirus infection by PCR of multiple specimen types. *Pediatr Infect Dis J*. 1997;16:409.
31. Kupila L, Vuorinen T, Vainionpaa R, et al. Diagnosis of enteroviral meningitis by use of polymerase chain reaction of cerebrospinal fluid, stool, and serum specimens. *Clin Infect Dis*. 2005;40:982.
32. Converse GM, Gwaltney JMJ, Strasburg DA, et al. Alteration of cerebrospinal fluid findings by partial treatment of bacterial meningitis. *J Pediatr*. 1973;83:220.
33. Dalton HP, Allison MJ. Modification of laboratory results by partial treatment of bacterial meningitis. *Am J Clin Pathol*. 1968;49:410.
34. Nigrovic LE, Malley R, Macias CG, et al. Effect of antibiotic pretreatment on cerebrospinal fluid profiles of children with bacterial meningitis. *Pediatrics*. 2008;122:726.
35. King RL, Lorch SA, Cohen DM, et al. Routine cerebrospinal fluid enterovirus polymerase chain reaction testing reduces hospitalization and antibiotic use for infants 90 days of age or younger. *Pediatrics*. 2007;120:489.
36. Lyons TW, McAdam AJ, Cohn KA, et al. Impact of in-hospital enteroviral polymerase chain reaction testing on the clinical management of children with meningitis. *J Hosp Med*. 2012;7:517.
37. Abzug MJ, Cloud G, Bradley J, et al. Double blind placebo-controlled trial of pleconaril in infants with enterovirus meningitis. *Pediatr Infect Dis J*. 2003;22:335.
38. Lepow ML, Coyne N, Thompson LB, et al. A clinical, epidemiologic and laboratory investigation of aseptic meningitis during the four-year period, 1955-1958. II. The clinical disease and its sequelae. *N Engl J Med*. 1962;266:1188.
39. Farmer K, MacArthur BA, Clay MM. A follow-up study of 15 cases of neonatal meningoencephalitis due to Coxsackie virus B5. *J Pediatr*. 1975;87:568.
40. Sells CJ, Carpenter RL, Ray CG. Sequelae of central-nervous-system enterovirus infections. *N Engl J Med*. 1975;293:1.
41. Bergman I, Painter MJ, Wald ER, et al. Outcome in children with enteroviral meningitis during the first year of life. *J Pediatr*. 1987;110:705.
42. Rorabaugh ML, Berlin LE, Rosenberg L, et al. Absence of neurodevelopmental sequelae from aseptic meningitis. Baltimore: Society for Pediatric Research; 1992.
43. Fowlkes AL, Honarmand S, Glaser C, et al. Enterovirus-associated encephalitis in the California encephalitis project, 1998-2005. *J Infect Dis*. 2008;198:1685.
44. Child N, Croxson MC, Rahnama F, et al. A retrospective review of acute encephalitis in adults in Auckland over a five-year period (2005-2009). *J Clin Neurosci*. 2012;19:1483.
45. de Ory F, Avellon A, Echevarria JE, et al. Viral infections of the central nervous system in Spain: a prospective study. *J Med Virol*. 2013;85:554.
46. Meyer HM, Johnson RT, Crawford IP, et al. Central nervous system syndromes of viral etiology. A study of 713 cases. *Am J Med*. 1960;29:334.
47. Peters ACB, Vielvoye GJ, Versteeg J, et al. Echo 25 focal encephalitis and subacute hemichorea. *Neurology*. 1979;29:676.
48. Roden VJ, Cantor HE, O'Connor DM, et al. Acute hemiplegia of childhood associated with Coxsackie A9 viral infection. *J Pediatr*. 1975;86:56.
49. Liow K, Spanaki MV, Boyer RS, et al. Bilateral hippocampal encephalitis caused by enteroviral infection. *Pediatr Neurol*. 1999;21:836.
50. Modlin JF, Dagan R, Berlin LE, et al. Focal encephalitis with enterovirus infections. *Pediatrics*. 1991;88:841.
51. Ho M, Chen ER, Hsu KH, et al. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. *N Engl J Med*. 1999;341:929.
52. Huang C-C, Liu C-C, Chang Y-C, et al. Neurologic complications in children with enterovirus 71 infection. *N Engl J Med*. 1999;341:936.
53. Chan KP, Goh KT, Chong CY, et al. Epidemic hand, foot and mouth disease caused by enterovirus 71, Singapore. *Emerg Infect Dis*. 2003;9:78.
54. Kreuter JD, Barnes A, McCarthy JE, et al. A fatal central nervous system enterovirus 68 infection. *Arch Pathol Lab Med*. 2011;135:793.
55. Solomon T, Lewthwaite P, Perera D, et al. Virology, epidemiology, pathogenesis, and control of enterovirus 71. *Lancet Infect Dis*. 2010;10:778.
56. Ma H, He F, Wan J, et al. Glucocorticoid and pyrazolone treatment of acute fever is a risk factor for critical and life-threatening human enterovirus 71 infection during an outbreak in China, 2008. *Pediatr Infect Dis J*. 2010;29:524.
57. Chalhub E, Devivo D, Siegel BA, et al. Coxsackie A9 focal encephalitis associated with acute infantile hemiplegia and porencephaly. *Neurology*. 1977;27:574.
58. Klapper PE, Bailey AS, Longson M, et al. Meningoencephalitis caused by coxsackievirus group B type 2: diagnosis confirmed by measuring intrathecal antibody. *J Infect*. 1984;8:227.
59. Price RA, Garcia JH, Rightsel WA. Choriomeningitis and myocarditis in an adolescent with isolation of coxsackie B5 virus. *Am J Clin Pathol*. 1970;53:825.
60. Huang MC, Wang SM, Hsu YW, et al. Long-term cognitive and motor deficits after enterovirus 71 brainstem encephalitis in children. *Pediatrics*. 2006;118:e1785.
61. Grimwood K, Huang QS, Sadleir LG, et al. Acute flaccid paralysis from echovirus type 33 infection. *J Clin Microbiol*. 2003;41:2230.
62. Chumakov M, Voroshilova M, Shindarov L, et al. Enterovirus 71 isolated from cases of epidemic poliomyelitis-like disease in Bulgaria. *Arch Virol*. 1979;60:329.
63. Melnick JL. Enteroviruses. In: Evans A, ed. *Viral Infections of Human Epidemiology and Control*. 3rd ed. New York: Plenum; 1991:191.
64. Grist NR. Type A7 Coxsackie (type 4 poliomyelitis) virus infection in Scotland. *J Hyg (Lond)*. 1962;60:323.
65. Rao CD, Yergolkar P, Shankarappa KS. Antigenic diversity of enteroviruses associated with nonpolio acute flaccid paralysis, India, 2007-2009. *Emerg Infect Dis*. 2012;18:1833.
66. Angez M, Shaukat S, Alam MM. Genetic relationships and epidemiological links between wild type 1 poliovirus isolates in Pakistan and Afghanistan. *Virol J*. 2012;9:51.
67. Gilbert GL, Dickson KE, Waters MJ, et al. Outbreak of enterovirus 71 infection in Victoria, Australia, with a high incidence of neurologic involvement. *Pediatr Infect Dis J*. 1988;7:484–488.
68. Shindarov LM, Chumakov MP, Voroshilova MK, et al. Epidemiological, clinical, and pathophysiological characteristics of epidemic poliomyelitis-like disease caused by enterovirus 71. *J Hyg Epidemiol Microbiol Immunol*. 1979;23:284.
69. Voroshilova MK, Chumakov MP. Poliomyelitis-like properties of AB-IV-Coxsackie A7 group of viruses. *Prog Med Virol*. 1959;2:106.
70. Grist NR. Type A and coxsackie (type 4 poliomyelitis) virus infection in Scotland. *J Hyg (Lond)*. 1962;60:323.
71. Messacar K, Schreiner TL, Maloney JA, et al. A cluster of acute flaccid paralysis and cranial nerve dysfunction temporally associated with an outbreak of enterovirus D68 in children in Colorado, USA. *Lancet*. 2015;385:1662.
72. Sejvar JJ, Lopez AS, Cortese MM, et al. Acute flaccid myelitis in the United States, August-December 2014: results of nationwide surveillance. *Clin Infect Dis*. 2016;63:737.
73. Messacar K, Asturias EJ, Hixon AM, et al. Enterovirus D68 and acute flaccid myelitis-evaluating the evidence for causality. *Lancet Infect Dis*. 2018;18:e239–e247.
74. Messacar K, Schreiner TL, Van Haren K, et al. Acute flaccid myelitis: a clinical review of US cases 2012-2015. *Ann Neurol*. 2016;80:326.
75. Ulferts R, van der Linden L, Thibaut HJ, et al. Selective serotonin reuptake inhibitor fluoxetine inhibits replication of human enteroviruses B and D by targeting viral protein 2C. *Antimicrob Agents Chemother*. 2013;57:1952.
76. Hixon AM, Clarke P, Tyler KL. Evaluating treatment efficacy in a mouse model of enterovirus D68-associated paralytic myelitis. *J Infect Dis*. 2017;216:1245.
77. Dietz V, Andrus J, Olivé JM, et al. Epidemiology and clinical characteristics of acute flaccid paralysis associated with non-polio enterovirus isolation: the experience in the Americas. *Bull World Health Organ*. 1995;73:597.
78. Saeed M, Zaidi SZ, Naeem A, et al. Epidemiology and clinical findings associated with enteroviral acute flaccid paralysis in Pakistan. *BMC Infect Dis*. 2007;7:6.
79. Hertenstein JR, Sarnat HB, O'Connor DM. Acute unilateral oculomotor palsy associated with echo 9 viral infection. *J Pediatr*. 1976;89:79.
80. Steigman AJ, Lipton MM. Fatal bulbospinal paralytic poliomyelitis due to ECHO 11 virus. *JAMA*. 1960;174:178.
81. Dery P, Marks MI, Shapera R. Clinical manifestations of coxsackievirus infections in children. *Am J Dis Child*. 1974;128:464.
82. Geer J. Coxsackie virus infections in Southern Africa. *Yale J Biol Med*. 1961;34:289.
83. Barak Y, Schwartz JF. Acute transverse myelitis associated with ECHO type 5 infection. *Am J Dis Child*. 1988;142:128.

84. Majima E, Harima R, Okuni H, et al. Case of transverse myelitis with Coxsackie B 5 virus isolated from the spinal fluid. *Nihon Shonika Gakkai Zasshi*. 1969;73:1593.
85. Kaul A, Cohen ME, Broffman G, et al. Reye-like syndrome associated with Coxsackie B2 virus infection. *J Pediatr*. 1979;94:67.
86. Alvira MM, Mendoza M. Reye's syndrome: a viral myopathy? *N Engl J Med*. 1975;292:1297.
87. Brunberg JA, Bell WE. Reye syndrome. *Arch Neurol*. 1974;30:304.
88. Kuban KC, Ephros MA, Freeman RL, et al. Syndrome of opsoclonus-myoclonus caused by Coxsackie B3 infection. *Ann Neurol*. 1983;13:69.
89. Akiyama K, Imazeki R, Yoshii F, et al. An adult case of hand, foot, and mouth disease caused by enterovirus 71 accompanied by opsoclonus myoclonica. *Tokai J Exp Clin Med*. 2008;33:143.
90. Ramsay M, Reacher M, O'Flynn C, et al. Causes of morbilliform rash in a highly immunised English population. *Arch Dis Child*. 2002;87:202.
91. Bell EJ, Ross CAC, Grist NR. Echo 9 infection in pregnant women with suspected rubella. *J Clin Pathol*. 1975;28:267.
92. Lerner AM, Klein JO, Levin HS, et al. Infections due to Coxsackie virus group A, type 9, in Boston, 1959, with special reference to exanthems and pneumonia. *N Engl J Med*. 1960;263:1265.
93. Annunziato D. Koplik spots and echo 9 virus. *N Y State J Med*. 1987;87:667.
94. Hall CB, Cherry JD, Hatch MH, et al. The return of Boston exanthem: echovirus 16 infections in 1974. *Am J Dis Child*. 1977;131:323.
95. Neva FA. A second outbreak of Boston exanthem disease in Pittsburgh during 1954. *N Engl J Med*. 1956;254:838.
96. Cherry JD, Lerner AM, Klein JO, et al. Coxsackie B5 infections with exanthems. *Pediatrics*. 1963;31:455.
97. Moritsugu Y, Sawada K, Hinohara M, et al. An outbreak of type 25 echovirus infection with exanthem in an infant home near Tokyo. *Am J Epidemiol*. 1968;87:599.
98. Hughes RO, Roberts C. Hand, foot, mouth disease associated with Coxsackie A9 virus. *Lancet*. 1972;2:751.
99. Lindenbaum JE, Van Dyck PC, Allen RG. Hand, foot and mouth disease associated with coxsackievirus group B. *Scand J Infect Dis*. 1975;7:161.
100. Robinson CR, Doane FW, Rhodes AJ. Report of an outbreak of febrile illness with pharyngeal lesions and exanthem. *Can Med Assoc J*. 1958;79:615.
101. Wang S-M, Liu C-C, Tseng H-W, et al. Clinical spectrum of enterovirus 71 infection in children in southern Taiwan, with an emphasis on neurological complications. *Clin Infect Dis*. 1999;29:184.
102. Blomqvist S, Klemola P, Kaijalainen S, et al. Co-circulation of coxsackieviruses A6 and A10 in hand, foot and mouth disease outbreak in Finland. *J Clin Virol*. 2010;48:49–54.
103. Wei SH, Huang YP, Liu MC, et al. An outbreak of coxsackievirus A6 hand, foot, and mouth disease associated with onychomadesis in Taiwan, 2010. *BMC Infect Dis*. 2011;11:346.
104. Bracho MA, González-Candelas F, Valero A, et al. Enterovirus co-infections and onychomadesis after hand, foot, and mouth disease, Spain, 2008. *Emerg Infect Dis*. 2011;17:2223.
105. Centers for Disease Control and Prevention. Notes from the field: severe hand, foot, and mouth disease associated with coxsackievirus A6—Alabama, Connecticut, California, and Nevada, November 2011-February 2012. *MMWR Morb Mortal Wkly Rep*. 2012;61:213–214.
106. Adler JL, Mostow SR, Mellin H, et al. Epidemiologic investigation of hand, foot, and mouth disease: infection caused by coxsackievirus A16 in baltimore, June through September 1968. *Am J Dis Child*. 1970;120:309.
107. Miller GD. Hand-foot-and-mouth disease. *JAMA*. 1968;203:827.
108. Kimura A, Abe M, Nakao T. Light and electron microscopic study of skin lesions in patients with hand, foot, and mouth disease. *Tohoku J Exp Med*. 1977;122:237.
109. Fujimoto T, Iizuka S, Enomoto M, et al. Hand, foot, and mouth disease caused by coxsackievirus A6, Japan, 2011. *Emerg Infect Dis*. 2012;18:337–339.
110. Flett K, Youngster I, Huang J, et al. Hand, foot, and mouth disease caused by coxsackievirus A6. *Emerg Infect Dis*. 2012;18:1702.
111. Buttery VW, Kenyon C, Grunewald S, et al. Atypical presentations of hand, foot, and mouth disease caused by coxsackievirus A6—Minnesota, 2014. *MMWR Morb Mortal Wkly Rep*. 2015;64:805.
112. Cherry JD, Lerner AM, Klein JO, et al. Coxsackie A9 infections with exanthems with particular reference to urticaria. *Pediatrics*. 1963;31:819.
113. Nahmias AJ, Froeschle JE, Feorino PM, et al. Generalized eruption in a child with eczema due to coxsackievirus A16. *Arch Dermatol*. 1968;97:147.
114. Deseda-Tous J, Byatt PH, Cherry JD. Vesicular lesions in adults due to echovirus 11 infections. *Arch Dermatol*. 1977;113:1705.
115. Meade RH, Chang TW. Zosterlike eruption due to echovirus 6. *Am J Dis Child*. 1979;133:283.
116. Frothingham TE. ECHO virus type 9 associated with three cases simulating meningococcemia. *N Engl J Med*. 1958;259:484.
117. Cherry JD, Jahn CL. Virologic studies of exanthems. *J Pediatr*. 1966;68:204.
118. James WD, Odom RB, Hatch MH. Gianotti-Crosti-like eruption associated with coxsackievirus A-16 infection. *J Am Acad Dermatol*. 1982;6:862.
119. Kepfer P, Hable DA, Smith TF. Viral isolation rates during summer from children with acute upper respiratory tract disease and healthy children. *Am J Clin Pathol*. 1974;61:1.
120. Rotbart HA, McCracken GH, Whitley RJ, et al. The clinical significance of enteroviruses in serious summer febrile illnesses of children. *Pediatr Infect Dis J*. 1999;18:869.
121. Jackson GG, Muidoon RL. Viruses causing common respiratory infections in man. II. Enteroviruses and paramyxoviruses. *J Infect Dis*. 1973;128:387.
122. Johnson KM, Bloom HH, Forsyth B, et al. The role of enteroviruses in respiratory disease. *Am Rev Respir Dis*. 1963;88:240.
123. Couch RB, Cate TR, Gerone PJ, et al. Production of illness with a small particle aerosol of Coxsackie A21. *J Clin Invest*. 1965;44:535.
124. Meijer A, van der Sanden S, Snijders BE, et al. Emergence and epidemic occurrence of enterovirus 68 respiratory infections in The Netherlands in 2010. *Virology*. 2012;42:49.
125. Centers for Disease Control and Prevention. Clusters of acute respiratory illness associated with human enterovirus 68—Asia, Europe, and United States, 2008-2010. *MMWR Morb Mortal Wkly Rep*. 2011;60:1301.
126. Jacobson LM, Redd JT, Schneider E, et al. Outbreak of lower respiratory tract illness associated with human enterovirus 68 among American Indian children. *Pediatr Infect Dis J*. 2012;31:309.
127. Renois F, Bouin A, Andreoletti L. Enterovirus 68 in pediatric patients hospitalized for acute airway diseases. *J Clin Microbiol*. 2013;51:640.
128. Kreuter JD, Barnes A, McCarthy JE, et al. A fatal central nervous system enterovirus 68 infection. *Arch Pathol Lab Med*. 2011;135:793.
129. Centers for Disease Control and Prevention. Enterovirus D68. https://www.cdc.gov/non-polio-enterovirus/about/ev-d68.html. Accessed June 8, 2018.
130. Prill MM, Dahl RM, Midgley CM, et al. Severe respiratory illness associated with rhinovirus during the enterovirus D68 outbreak in the United States, August 2014-November 2014. *Clin Infect Dis*. 2018;66:1528.
131. Schuster JE1, Selvarangan R, Hassan F, et al. Clinical course of enterovirus D68 in hospitalized children. *Pediatr Infect Dis J*. 2017;36:290.
132. Du J, Zheng B, Zheng W, et al. Analysis of enterovirus 68 strains from the 2014 North American outbreak reveals a new clade, indicating viral evolution. *PLoS ONE*. 2015;10:e0144208.
133. Waghmare A, Pergam SA, Jerome KR, et al. Clinical disease due to enterovirus D68 in adult hematologic malignancy patients and hematopoietic cell transplant recipients. *Blood*. 2015;125:1724–1729.
134. Du J, Zheng B, Zheng W, et al. Analysis of enterovirus 68 strains from the 2014 North American outbreak reveals a new clade, indicating viral evolution. *PLoS ONE*. 2015;10:e0144208.
135. Eckert HL, Portnoy B, Salvatore MA, et al. Group B Coxsackie virus infection in infants with acute lower respiratory disease. *Pediatrics*. 1967;39:526.
136. Flewett TH. Histological study of two cases of Coxsackie B virus pneumonia in children. *J Clin Pathol*. 1965;18:743.
137. Jahn CL, Felton OL, Cherry JD. Coxsackie B1 pneumonia in an adult. *JAMA*. 1964;189:236.
138. Boyd MT, Jordan SW, Davis LE. Fatal pneumonitis from congenital echovirus type 6 infection. *Pediatr Infect Dis J*. 1987;6:1138.
139. Cheeseman SH, Hirsch MS, Keller EW, et al. Fatal neonatal pneumonia caused by Echovirus type 9. *Am J Dis Child*. 1977;131:1169.
140. Toce SS, Keenan WJ. Congenital echovirus 11 pneumonia in association with pulmonary hypertension. *Pediatr Infect Dis J*. 1988;7:360.
141. Cherry JL, Soriano F, Jahn CL. Search for perinatal enterovirus infection. *Am J Dis Child*. 1968;116:245.
142. Steigman AJ, Lipton MM, Braspennickx H. Acute lymphonodular pharyngitis: a newly described condition due to Coxsackie A virus. *J Pediatr*. 1962;61:331.
143. Sylvest E. *Epidemic Myalgia: Bornholm Disease*. London: Oxford University Press; 1934.
144. Curnen EC, Shaw EW, Melnick JL. Disease resembling nonparalytic poliomyelitis associated with virus pathogenic for infant mice. *JAMA*. 1949;141:894.
145. Weller TH, Enders JF, Buckingham M, et al. Etiology of epidemic pleurodynia: study of two viruses isolated from typical outbreak. *J Immunol*. 1950;65:337.
146. Bell EJ, Grist NR. ECHO viruses, carditis, and acute pleurodynia. *Am Heart J*. 1971;82:133.
147. Madhaven HN, Bedninath S, Chanraseker S. A case of pleurodynia associated with Coxsackie virus type A9. *J Assoc Physicians India*. 1977;25:491.
148. Bain HW, McLean DM, Walker SJ. Epidemic pleurodynia (Bornholm disease) due to Coxsackie B-5 virus: the interrelationship of pleurodynia, benign pericarditis, and aseptic meningitis. *Pediatrics*. 1961;27:889.
149. Disney ME, Howard EM, Wood BSB. Bornholm disease in children. *Br Med J*. 1953;1:1351.
150. Warin JF, Davies JBM, Sanders FK, et al. Oxford epidemic of Bornholm disease, 1951. *Br Med J*. 1953;1:1345.
151. Gordon RB, Lennette EH, Sandrock RS. The varied clinical manifestations of Coxsackie viral infections. *Arch Intern Med*. 1959;103:63.
152. Halfon N, Spector SA. Fatal echovirus type 11 infections. *Am J Dis Child*. 1981;135:1017.
153. Fukuyama Y, Ando T, Yokota J. Acute fulminant myoglobinuric polymyositis with picornavirus-like crystals. *J Neurol Neurosurg Psychiatry*. 1977;40:775.
154. Gyorkey F, Cabral GA, Gorkey PK, et al. Coxsackievirus aggregates in muscle cells of polymyositis patient. *Intervirology*. 1978;10:69.
155. Schiraldi O, Iandolo E. Polymyositis accompanying Coxsackie virus B2 infection. *Infection*. 1978;6:32.
156. Josselson J, Pula T, Sadler JH. Acute rhabdomyolysis associated with echovirus 9 infection. *Arch Intern Med*. 1980;140:1671.
157. Jehn UW, Fink MW. Myositis, myoglobinemia, and myoglobinuria associated with enterovirus echo 9 infection. *Arch Neurol*. 1980;33:457.
158. Bowles NE, Dubowitz V, Sewry CA, et al. Dermatomyositis, polymyositis, and Coxsackie-B-virus infection. *Lancet*. 1987;1:1004.
159. De Renck J, De Coster W, Inderadjaja N. Acute viral polymyositis with predominant diaphragm involvement. *J Neurol Sci*. 1977;33:453.
160. Smith WG. Adult heart disease due to the Coxsackievirus group B. *Br Heart J*. 1966;28:204.
161. Helin M, Savola J, Lapinleimu K. Cardiac manifestations during a Coxsackie B5 epidemic. *Br Med J*. 1968;3:97.
162. Bowles NE, Ni J, Kearney DL, et al. Detection of viruses in myocardial tissues by polymerase chain reaction: evidence of adenovirus as a common cause of myocarditis in children and adults. *J Am Coll Cardiol*. 2003;42:466.
163. Sainani GS, Krompotic E, Slodki SJ. Adult heart disease due to the Coxsackie virus B infection. *Medicine (Baltimore)*. 1968;47:133.
164. Guarner J, Bhatnagar J, Shieh WJ, et al. Histopathologic, immunohistochemical, and polymerase chain reaction assays in the study of cases with fatal sporadic myocarditis. *Hum Pathol*. 2007;38:1412.
165. Russell SJM, Bell EJ. Echoviruses and carditis. *Lancet*. 1970;1:784.
166. Grist NR, Bell EJ. Coxsackieviruses and the heart. *Am Heart J*. 1969;77:295.
167. Lerner AM, Wilson FM. Virus myocardiopathy. *Prog Med Virol*. 1973;15:63.
168. Woodruff JF. Viral myocarditis. A review. *Am J Pathol*. 1980;101:427.
169. Grist NR, Bell EJ. Coxsackie virus and heart diseases. *Br Med J*. 1968;3:556.
170. Grist NR, Bell EJ, Assad F. Enteroviruses in human disease. *Prog Med Virol*. 1978;24:114.
171. Meehan WF, Bertrand CA. Ventricular tachycardia associated with echovirus infection. *JAMA*. 1970;212:1701.
172. Schleissner LA, Fiala M, Imagawa DT, et al. Application of systolic time intervals to acute cardiomyopathy with echovirus 2. *Chest*. 1976;69:563.
173. Kanra G, Dogruel N, Tinaztepe K, et al. Myocarditis caused by echovirus 11 virus. *Turk J Pediatr*. 1978;20:24.
174. Lewes D, Rainford DJ, Lane WF. Symptomless myocarditis and myalgia in viral and *Mycoplasma pneumoniae* infections. *Br Heart J*. 1974;36:924.
175. Van Loon GR, Masson AM. Viral pericarditis. *Can Med Assoc J*. 1968;99:163.
176. Bell EJ, Grist NR. Echoviruses, carditis, and acute pleurodynia. *Lancet*. 1970;1:326.
177. Berkovich S, Rodriguez-Torres R, Lin J-S. Virologic studies in children with acute myocarditis. *Am J Dis Child*. 1968;115:207.

178. Johnson RT, Portnoy B, Rogers NG, et al. Acute benign pericarditis: virologic study of 34 patients. *Arch Intern Med*. 1961;108:823.
179. Martin AB, Webber S, Fricker FJ, et al. Acute myocarditis. Rapid diagnosis by PCR in children. *Circulation*. 1994;90:330.
180. Lozinski GM, Davis GG, Krous HF, et al. Adenovirus myocarditis: retrospective diagnosis by gene amplification from formalin-fixed, paraffin-embedded tissues. *Hum Pathol*. 1994;25:831.
181. Hildebrandt HM, Massab HF, Willis PW. Influenza virus pericarditis. *Am J Dis Child*. 1962;104:579.
182. Mahrholdt H, Wagner A, Deluigi CC, et al. Presentation, patterns of myocardial damage, and clinical course of viral myocarditis. *Circulation*. 2006;114:1581.
183. Centers for Disease Control and Prevention. Fatal mumps myocarditis in England. *MMWR Morb Mortal Wkly Rep*. 1980;27:425.
184. Caldera R, Sarrut S, Mallet R, et al. Existetil des complications cardiaques de la vaccine? *Sem Hop Paris*. 1961;37:1281.
185. Woodruff JF, Woodruff JJ. Involvement of T lymphocytes in the pathogenesis of coxsackievirus B3 heart disease. *J Immunol*. 1974;113:1726.
186. Rose NR, Wolfgram LJ, Herskowitz A, et al. Postinfectious autoimmunity: two distinct phases of coxsackievirus B3-induced myocarditis. *Ann N Y Acad Sci*. 1986;475:146.
187. Rose NR. Autoimmunity in coxsackievirus infection. *Curr Top Microbiol Immunol*. 2008;323:293–314.
188. Sainani GS, Dekate MP, Rao CP. Heart disease caused by coxsackievirus B infection. *Br Heart J*. 1975;37:819.
189. Smith WG. Coxsackie B myopericarditis in adults. *Am Heart J*. 1970;80:34.
190. Koontz CH, Ray CG. The role of Coxsackie group B virus infections in sporadic myopericarditis. *Am Heart J*. 1971;82:750.
191. Friedrich MG, Sechtem U, Schulz-Menger J, et al. Cardiovascular magnetic resonance in myocarditis: a JACC White Paper. *J Am Coll Cardiol*. 2009;53:1475.
192. Woods JD, Nimmo MJ, MacKay-Scollay EM. Acute transmural myocardial infarction associated with acute Coxsackie virus B infection. *Am Heart J*. 1975;89:283.
193. Griffiths PD, Hannington G, Booth JC. Coxsackie B virus infection and myocardial infarction. *Lancet*. 1980;1:1387.
194. Chrysohoou C, Tsiamis E, Brili S, et al. Acute myocarditis from coxsackie infection, mimicking subendocardial ischaemia. *Hellenic J Cardiol*. 2009;50:147–150.
195. DeSa'Neto A, Bullington JD, Bullington RH, et al. Coxsackie B5 heart disease. Demonstration of inferolateral wall myocardial necrosis. *Am J Med*. 1980;68:295.
196. Narula J, Khaw BA, Dec GW Jr, et al. Brief report: recognition of acute myocarditis masquerading as acute myocardial infarction. *N Engl J Med*. 1993;328:100.
197. Sutton GC, Harding HB, Truehart RP, et al. Coxsackie B4 myocarditis in an adult: successful isolation of virus from ventricular myocardium. *Aerospace Med*. 1967;38:66.
198. Jin O, Sole MJ, Butany JW, et al. Detection of enterovirus RNA in myocardial biopsies from patients with myocarditis and cardiomyopathy using gene amplification by polymerase chain reaction. *Circulation*. 1990;82:8.
199. Weiss LM, Movahed LA, Billingham ME, et al. Detection of coxsackievirus B3 RNA in myocardial tissues by polymerase chain reaction. *Am J Pathol*. 1991;138:497.
200. Feldman AM, McNamara D. Myocarditis. *N Engl J Med*. 2000;343:1388.
201. Drucker NA, Colan SD, Lewis AB, et al. γ-Globulin treatment of acute myocarditis in the pediatric population. *Circulation*. 1994;89:252.
202. Mason JW, O'Connell JB, Herskowitz A, et al. A clinical trial of immunosuppressive therapy for myocarditis. *N Engl J Med*. 1995;333:269.
203. Garg A, Shaiu J, Guyatt G. The ineffectiveness of immunosuppressive therapy in lymphocytic myocarditis: an overview. *Ann Intern Med*. 1998;129:317.
204. Frustaci A, Chimenti C, Calabrese F, et al. Immunosuppressive therapy for active lymphocytic myocarditis: virological and immunologic profile of responders versus nonresponders. *Circulation*. 2003;107:857.
205. Pages ON, Aubert S, Combes A, et al. Paracorporeal pulsatile biventricular assist device versus extracorporal membrane oxygenation-extracorporal life support in adult fulminant myocarditis. *J Thorac Cardiovasc Surg*. 2009;137:194–197.
206. Madden K, Thiagarajan RR, Rycus PT, et al. Survival of neonates with enteroviral myocarditis requiring extracorporeal membrane oxygenation. *Pediatr Crit Care Med*. 2011;12:314.
207. Gibbons JE, Goldbloom RB, Dobell ARC. Rapidly developing pericardial constriction in childhood following acute nonspecific pericarditis. *Am J Cardiol*. 1965;15:863.
208. Howard EJ, Maier HC. Constrictive pericarditis following acute Coxsackie viral pericarditis. *Am Heart J*. 1968;75:247.
209. Matthews JD, Cameron SJ, George M. Constrictive pericarditis following Coxsackie virus infection. *Thorax*. 1970;25:624.
210. Lee KJ, McCrindle BW, Bohn DJ, et al. Clinical outcomes of acute myocarditis in childhood. *Heart*. 1999;82:226.
211. McCarthy RE III, Boehmer JP, Hruban RH, et al. Long-term outcome of fulminant myocarditis as compared with acute (nonfulminant) myocarditis. *N Engl J Med*. 2000;342:690.
212. Codd MB, Sugrue DD, Gersh BJ, et al. Epidemiology of idiopathic dilated and hypertrophic cardiomyopathy. A population-based study in Olmsted County, Minnesota, 1975-1984. *Circulation*. 1989;80:564.
213. Chapman NM, Kim KS. Persistent coxsackievirus infection: enterovirus persistence in chronic myocarditis and dilated cardiomyopathy. *Curr Top Microbiol Immunol*. 2008;323:275.
214. Giacca M, Severini GM, Mestroni L, et al. Low frequency of detection by nested polymerase chain reaction of enterovirus ribonucleic acid in endomyocardial tissue of patients with idiopathic dilated cardiomyopathy. *J Am Coll Cardiol*. 1994;24:1033.
215. de Leeuw N, Melchers WJG, Balk AH, et al. No evidence for persistent enterovirus infection in patients with end-stage idiopathic dilated cardiomyopathy. *J Infect Dis*. 1998;178:256.
216. Mahon NG, Zal B, Arno G, et al. Absence of viral nucleic acids in early and late dilated cardiomyopathy. *Heart*. 2001;86:687.
217. Baker DA, Phillips CA. Maternal and neonatal infection with coxsackievirus. *Obstet Gynecol*. 1980;55:12.
218. Talsma M, Vegting M, Hess J. Generalized Coxsackie A9 infection in a neonate presenting with pericarditis. *Br Heart J*. 1984;52:683.
219. Wright HT, Landing BH, Lennette EH, et al. Fatal infection in an infant associated with Coxsackie virus group A, type 16. *N Engl J Med*. 1963;268:1041.
220. Gear JHS, Measroch V. Coxsackievirus infection of the newborn. *Prog Med Virol*. 1973;15:42.
221. Modlin JF. Perinatal echovirus infection: insights from a literature of 61 cases of serious infection and 16 outbreaks in nurseries. *Rev Infect Dis*. 1986;8:918.
222. Modlin JF, Kinney JS. Perinatal enterovirus infections. In: Aronoff SC, Hughes WT, Kohl S, et al, eds. *Advances in Pediatric Infectious Diseases*. Vol. 2. Chicago: Year Book Medical Publishers; 1986:57.
223. Kinney JS, McCray E, Kaplan JE, et al. Risk factors associated with echovirus 11 infection in a newborn nursery. *Pediatr Infect Dis J*. 1986;5:192.
224. Modlin JF. Fatal echovirus 11 disease in premature neonates. *Pediatrics*. 1980;66:775.
225. Piraino FF, Sedmak G, Raab K. Echovirus 11 infections of newborns with mortality during the 1979 enterovirus season in Milwaukee, Wis. *Public Health Rep*. 1982;97:346.
226. Khetsuriani N, Lamonte A, Oberste M, et al. Neonatal enterovirus infections reported to the national enterovirus surveillance system in the United States, 1983-2003. *Pediatr Infect Dis J*. 2006;25:889.
227. Wikswo ME, Khetsuriani N, Fowlkes AL, et al. Increased activity of Coxsackievirus B1 strains associated with severe disease among young infants in the United States, 2007-2008. *Clin Infect Dis*. 2009;49:e44.
228. Modlin JF, Bowman M. Perinatal transmission of Coxsackie B3 virus in a murine model. *J Infect Dis*. 1987;156:21.
229. Modlin JF, Polk BF, Horton P, et al. Perinatal echovirus 11 infection: risk of transmission during a community outbreak. *N Engl J Med*. 1981;305:368.
230. Jones MJU, Kolb M, Votava HJ, et al. Intrauterine echovirus type 11 infection. *Mayo Clin Proc*. 1980;55:509.
231. Reyes MP, Ostrea EM, Roskamp J, et al. Disseminated neonatal echovirus 11 disease following antenatal maternal infection with a virus-positive cervix and virus negative gastrointestinal tract. *J Med Virol*. 1983;12:155.
232. Kaplan MH, Klein SW, McPhee J, et al. Group B coxsackievirus infections in infants younger than three months of age: a serious childhood illness. *Rev Infect Dis*. 1983;5:1019.
233. Kunin CM. Virus-tissue union and the pathogenesis of enterovirus infections. *J Immunol*. 1962;88:556.
234. Eichenwald HF, Kotsevalov O. Immunologic responses of premature and full-term infants to infection with certain viruses. *Pediatrics*. 1960;25:829.
235. Rager-Zisman B, Allison AC. The role of antibody and host cells in the resistance of mice against infection by Coxsackie B-3 virus. *J Gen Virol*. 1973;19:329.
236. Lansky LL, Krugman S, Huq G. Anicteric Coxsackie B hepatitis. *J Pediatr*. 1979;94:64.
237. Berry PJ, Nagington J. Fatal infection with echovirus 11. *Arch Dis Child*. 1982;57:22.
238. Kibrick S, Benirschke K. Severe generalized disease (encephalohepatomyocarditis) occurring in the newborn period and due to infection with Coxsackie virus, group B: evidence of intrauterine infection with this agent. *Pediatrics*. 1958;22:857.
239. Freund MW, Kleinveld G, Krediet TG, et al. Prognosis for neonates with enterovirus myocarditis. *Arch Dis Child Fetal Neonatal Ed*. 2010;95:F206.
240. Drew JH. Echo 11 virus outbreak in a nursery associated with myocarditis. *Aust Paediatr J*. 1973;9:90.
241. Yoshioka I, Horstmann DM. Viremia in infection due to echo virus type 9. *N Engl J Med*. 1961;262:224.
242. Woodruff J. Lack of correlation between neutralizing antibody production and suppression of Coxsackie B-3 replication in target organs: evidence for involvement of mononuclear inflammatory cells in host defense. *J Immunol*. 1979;123:31.
243. Georgieff MK, Johnson DE, Thompson TR, et al. Fulminant hepatic necrosis in an infant with perinatally acquired echovirus 21 infection. *Pediatr Infect Dis J*. 1987;6:71.
244. Spector SA, Straube RC. Protean manifestations of perinatal enterovirus infection. *West J Med*. 1983;138:847.
245. Speer ME, Yawn DH. Fatal hepatoadrenal necrosis in the neonate associated with echovirus types 11 and 12 presenting as a surgical emergency. *J Pediatr Surg*. 1984;19:591.
246. Wreghitt TG, Gandy GM, King A, et al. Fatal neonatal echo 7 virus infection. *Lancet*. 1984;2:465.
247. Chambon M, Delage C, Bailly J-L, et al. Fatal hepatic necrosis in a neonate with echovirus 20 infection: use of the polymerase chain reaction to detect enterovirus in liver tissue. *Clin Infect Dis*. 1997;24:523.
248. Mostoufizadeh G, Lack EE, Gang DL, et al. Postmortem manifestations of echovirus 11 sepsis in five newborn infants. *Hum Pathol*. 1983;14:819.
249. Johnston JM, Overall JC. Intravenous immunoglobulin in disseminated neonatal echovirus 11 infection. *Pediatr Infect Dis J*. 1989;8:254.
250. Abzug MJ, Michaels MG, Wald E, et al. A randomized, double-blind, placebo-controlled trial of pleconaril for the treatment of neonates with enterovirus sepsis. *J Pediatric Infect Dis Soc*. 2016;5:53.
251. McKinney RE, Katz SL, Wilfert CM. Chronic enteroviral meningoencephalitis in agammaglobulinemic patients. *Rev Infect Dis*. 1987;9:334.
252. Rudge P, Webster AD, Revesz T, et al. Encephalomyelitis in primary hypogammaglobulinaemia. *Brain*. 1996;119:1–15.
253. Cunningham CK, Bonville CA, Ochs HD, et al. Enteroviral meningoencephalitis as a complication of X-linked hyper IgM syndrome. *J Pediatr*. 1999;134:584–588.
254. Nowak-Wegrzyn A, Phipatanakul W, Winkelstein JA, et al. Successful treatment of enterovirus infection with the use of pleconaril in 2 infants with severe combined immunodeficiency. *Clin Infect Dis*. 2001;32:E13.
255. Fischmeister G, Wiesbauer P, Holzmann HM, et al. Enteroviral meningoencephalitis in immunocompromised children after matched unrelated donor-bone marrow transplantation. *Pediatr Hematol Oncol*. 2000;17:393.
256. Padate BP, Keidan J. Enteroviral meningoencephalitis in a patient with non-Hodgkin's lymphoma treated previously with rituximab. *Clin Lab Haematol*. 2006;28:69.
257. Quartier P, Tournilhac O, Archimbaud C, et al. Enteroviral meningoencephalitis after anti-CD20 (rituximab) treatment. *Clin Infect Dis*. 2003;26:e47.
258. Dendle C, Gilbertson M, Korman TM, et al. Disseminated enteroviral infection associated with obinutuzumab. *Emerg Infect Dis*. 2015;21:1661.
259. O'Neil KM, Pallansch MA, Winkelstein JA, et al. Chronic group A coxsackievirus infection in agammaglobulinemia: demonstration of genomic variation of serotypically identical isolates persistently excreted from the same patient. *J Infect Dis*. 1988;157:183.
260. Webster ADB. Echovirus disease in hypogammaglobulinaemic patients. *Clin Rheum Dis*. 1984;10:189.
261. Mease PJ, Ochs HD, Wedgewood RJ. Successful treatment of echovirus meningoencephalitis and myositis-fasciitis with intravenous immune globulin therapy in a patient with X-linked hypogammaglobulinemia. *N Engl J Med*. 1981;304:1278.
262. Rotbart HA, Webster ADB. Treatment of potentially life-threatening enterovirus infections with pleconaril. *Clin Infect Dis*. 2001;32:228.
263. Biggs DD, Toorkey BC, Carrigan DR, et al. Disseminated echovirus infection complicating bone marrow transplantation. *Am J Med*. 1990;88:421.

264. Aquino VM, Farah RA, Lee ME, et al. Disseminated Coxsackie A9 infection complicating bone marrow transplantation. *Pediatr Infect Dis J*. 1996;15:1053.
265. Galama JMD, de Leeuw N, Wittebol S, et al. Prolonged enteroviral infection in a patient who developed pericarditis and heart failure after bone marrow transplantation. *Clin Infect Dis*. 1996;22:1004.
266. Townsend TR, Bolyard EA, Yolken RH, et al. Outbreak of Coxsackie A1 gastroenteritis: a complication of bone-marrow transplantation. *Lancet*. 1982;1:820.
267. Sellier-Leclerc AL, Belli E, Guérin V, et al. Fulminant viral myocarditis after rituximab therapy in pediatric nephrotic syndrome. *Pediatr Nephrol*. 2013;28:1875.
268. Kono R. Apollo 11 disease or acute hemorrhagic conjunctivitis: a pandemic of a new enterovirus infection of the eyes. *Am J Epidemiol*. 1975;101:383.
269. Kono R, Sasagawa A, Ishii K, et al. Pandemic of new type of conjunctivitis. *Lancet*. 1972;2:1191.
270. Mirkovic RR, Kono R, Yin-Murphy M, et al. Enterovirus type 70: the etiologic agent of pandemic acute hemorrhagic conjunctivitis. *Bull World Health Organ*. 1973;49:341.
271. Mirkovic RR, Schmidt NJ, Yin-Murphy M, et al. Enterovirus etiology of the 1970 Singapore epidemic of acute conjunctivitis. *Intervirology*. 1974;4:119.
272. Yin-Murphy M, Lim KH, Yo YM. A coxsackievirus type A24 epidemic of acute conjunctivitis. *Southeast Asian J Trop Med Public Health*. 1976;7:1.
273. Yin-Murphy M, Lim KH. Picornavirus epidemic conjunctivitis in Singapore. *Lancet*. 1972;2:857.
274. Higgins PG, Scott RJ, Davies PM, et al. A comparative study of viruses associated with acute hemorrhagic conjunctivitis. *J Clin Pathol*. 1974;27:292.
275. Christopher S, Theogaraj S, Godbole S, et al. An epidemic of acute hemorrhagic conjunctivitis due to coxsackievirus A24. *J Infect Dis*. 1982;146:16.
276. Kono R, Miyamura K, Tajiri E, et al. Neurologic complications associated with acute hemorrhagic conjunctivitis virus infection and its serologic confirmation. *J Infect Dis*. 1974;129:590.
277. Ray I, Roy IS, Sarkhar JK, et al. Laboratory investigations of an epidemic of conjunctivitis in Calcutta: a preliminary report. *Bull Calcutta Sch Trop Med*. 1972;20:1.
278. Hierholzer JC, Hilliard KA, Esposito JJ. Serosurvey for "acute hemorrhagic conjunctivitis" virus (enterovirus 70) antibodies in the southeastern United States, with review of the literature and some epidemiologic implications. *Am J Epidemiol*. 1975;102:533.
279. Aubry C, Gautret P, Nougairede A, et al. 2012 outbreak of acute haemorrhagic conjunctivitis in Indian ocean islands: identification of Coxsackievirus A24 in a returned traveller. *Euro Surveill*. 2012;17:1.
280. Lernout T, Maillard O, Boireaux S, et al. A large outbreak of conjunctivitis on Mayotte Island, France, February to May 2012. *Euro Surveill*. 2012;17:1.
281. Sklar VE, Patriarca PA, Onorato IM, et al. Clinical findings and results of treatment in an outbreak of acute hemorrhagic conjunctivitis in southern Florida. *Am J Ophthalmol*. 1983;95:45.
282. Kuritsky JN, Weaver JH, Bernard KW, et al. An outbreak of acute hemorrhagic conjunctivitis in central Minnesota. *Am J Ophthalmol*. 1983;96:449.
283. Centers for Disease Control. Acute hemorrhagic conjunctivitis caused by coxsackievirus A24—caribbean. *MMWR Morb Mortal Wkly Rep*. 1987;36:245.
284. Fonseca MC, Sarmiento L, Resik S, et al. Isolation of Coxsackievirus A24 variant from patients with hemorrhagic conjunctivitis in Cuba, 2008-2009. *J Clin Virol*. 2012;53:77.
285. Miyamura K, Tanimura M, Takeda N, et al. Evolution of enterovirus 70 in nature: all isolates were recently derived from a common ancestor. *Arch Virol*. 1986; 89:1.
286. Stanton GJ, Langford MP, Baron S. Effect of interferon, elevated temperature, and cell type on replication of acute hemorrhagic conjunctivitis viruses. *Infect Immun*. 1977;18:370.
287. Goh KT, Doraisingham S, Yin-Murphy M. An epidemic of acute conjunctivitis caused by enterovirus-70 in Singapore in 1980. *Southeast Asian J Trop Med Public Health*. 1981;12:473.
288. Arnow PM, Hierholzer JC, Higbee J, et al. Acute hemorrhagic conjunctivitis: a mixed virus outbreak among Vietnamese refugees on Guam. *Am J Epidemiol*. 1977;105:69.
289. Onorato IM, Morens DM, Schonberger LB, et al. Acute hemorrhagic conjunctivitis caused by enterovirus type 70: an epidemic in American Samoa. *Am J Trop Med Hyg*. 1985;34:984.
290. Kono R, Sasagawa A, Miyamura K, et al. Serologic characterization and seroepidemiologic studies on acute hemorrhagic conjunctivitis (AHC) virus. *Am J Epidemiol*. 1975;101:444.
291. Kono R, Uchida Y. Acute hemorrhagic conjunctivitis. *Ophthalmol Dig*. 1977;39:14.
292. Kono R, Miyamura K, Tajiri E, et al. Virological and serological studies of neurological complications of acute hemorrhagic conjunctivitis in Thailand. *J Infect Dis*. 1977;135:706.
293. Hung TS, Sung SM, Liang HC, et al. Radiculomyelitis following acute hemorrhagic conjunctivitis. *Brain*. 1976;99:771.
294. Katiyar BC, Misra S, Singh RB, et al. Adult polio-like syndrome following enterovirus 70 conjunctivitis (natural history of the disease). *Acta Neurol Scand*. 1983;67:263.
295. Wadia NH, Katrak SM, Misra VP, et al. Polio-like motor paralysis associated with acute hemorrhagic conjunctivitis in an outbreak in 1981 in Bombay. *J Infect Dis*. 1983;147:660.
296. Kono R, Uchida N, Sasagawa A, et al. Neurovirulence of acute hemorrhagic conjunctivitis virus in monkeys. *Lancet*. 1973;1:61.
297. Yin-Murphy M. Simple tests for the diagnosis of picornavirus epidemic conjunctivitis (acute haemorrhagic conjunctivitis). *Bull World Health Organ*. 1976;54:675.
298. Leggiadro RJ, Chwatsky DN, Zucker SW. Echovirus 3 infection associated with anicteric hepatitis. *Am J Dis Child*. 1982;136:744.
299. Morris JA, Elisberg BL, Pond WL, et al. Hepatitis associated with Coxsackie virus group A, type 4. *N Engl J Med*. 1962;267:1230.
300. Sun NC, Smith VM. Hepatitis associated with myocarditis: unusual manifestation of infection with Coxsackie virus group B, type 3. *N Engl J Med*. 1966;274:190.
301. Arnesjo B, Eden T, Ihse I, et al. Enterovirus infections in acute pancreatitis: a possible etiologic connection. *Scand J Gastroenterol*. 1976;11:645.
302. Imrle CW, Ferguson JC, Sommerville RG. Coxsackie and mumps virus infection in a prospective study of acute pancreatitis. *Gut*. 1977;18:53.
303. Ursing B. Acute pancreatitis in Coxsackie B infection. *Br Med J*. 1973;3:524.
304. Kibrick S. Current status of Coxsackie and ECHO viruses in human disease. *Prog Med Virol*. 1964;6:27.
305. Ramos-Alvarez M, Olarte J. Diarrheal diseases of children. *Am J Dis Child*. 1964;107:218.
306. Steinhoff MC. Viruses and diarrhea: a review. *Am J Dis Child*. 1978;132:302.
307. Yow DM, Melnick JL, Blattner JR, et al. Enteroviruses in infantile diarrhea. *Am J Hyg*. 1963;77:283.
308. Patel JR, Daniel J, Mathan VI. An epidemic of acute diarrhoea in rural southern India associated with echovirus type 11 infection. *J Hyg (Lond)*. 1985;95:483.
309. Glasgow LA, Balduzzi P. Isolation of Coxsackie virus group A, type 4, from a patient with hemolytic uremic syndrome. *N Engl J Med*. 1965;273:754.
310. Ray CG, Tucker VL, Harris DJ, et al. Enteroviruses associated with the hemolytic-uremic syndrome. *Pediatrics*. 1970;46:378.
311. Aronson MD, Phillips CA. Coxsackievirus B5 infections in acute oliguric renal failure. *J Infect Dis*. 1975;132:303.
312. Craighead JE, Mahoney EM, Carver DH, et al. Orchitis due to Coxsackie virus group B, type 5. *N Engl J Med*. 1962;267:498.
313. Welliver RC, Cherry JD. Aseptic meningitis and orchitis associated with echovirus 6 infection. *J Pediatr*. 1978;92:239.
314. Ager EA, Felsenstein WC, Alexander ER, et al. An epidemic of illness due to Coxsackie virus group B, type 2. *JAMA*. 1964;187:251.
315. Willems WR, Hornig C, Bauer H, et al. A case of Coxsackie A9 virus infection with orchitis. *J Med Virol*. 1978;3:137.
316. Blotzer JW, Myers AR. Echovirus associated polyarthritis: report of a case with synovial fluid and synovial histologic characterization. *Arthritis Rheum*. 1978;21:978.
317. Kujala G, Newman JH. Isolation of echovirus type 11 from synovial fluid in acute monocytic arthritis. *Arthritis Rheum*. 1985;28:98.
318. Van der Sar A. Acute infectious lymphocytosis with echovirus type 25. *West Indian Med J*. 1979;28:185.
319. Norwitz MS, Moore GT. Acute infectious lymphocytosis: an etiologic study of an outbreak. *N Engl J Med*. 1968;279:399.
320. Guerin C, Pozzetto B, Berthoux F. Hemophagocytic syndrome associated with coxsackie virus A 9 infection in a non-immunosuppressed adult. *Intensive Care Med*. 1989;15:547–548.
321. Letsas KP, Filippatos GS, Delimpasi S, et al. Enterovirus-induced fulminant myocarditis and hemophagocytic syndrome. *J Infect*. 2007;54:e75.
322. Lindamood KE, Fleck P, Narla A, et al. Neonatal enteroviral sepsis/meningoencephalitis and hemophagocytic lymphohistiocytosis: diagnostic challenges. *Am J Perinatol*. 2011;28:337.
323. Hober D, Sauter P. Pathogenesis of type 1 diabetes mellitus: interplay between enterovirus and host. *Nat Rev Endocrinol*. 2010;6:279.
324. Yeung WC, Rawlinson WD, Craig ME. Enterovirus infection and type 1 diabetes mellitus: systematic review and meta-analysis of observational molecular studies. *BMJ*. 2011;342:d35.
325. Stene LC, Rewers M. Immunology in the clinic review series; focus on type 1 diabetes and viruses: the enterovirus link to type 1 diabetes: critical review of human studies. *Clin Exp Immunol*. 2012;168: 12.
326. Gleason RE, Kahn CB, Funk IB, et al. Seasonal incidence of insulin-dependent diabetes in Massachusetts, 1964-1973. *Int J Epidemiol*. 1982;11:39.
327. Hierholzer JC, Farris WA. Follow-up of children infected in a coxsackievirus B3 and B4 outbreak: no evidence of diabetes mellitus. *J Infect Dis*. 1974;129:741.
328. Dippe SE, Bennett PH, Miller M, et al. Lack of causal association between Coxsackie B4 virus infection and diabetes. *Lancet*. 1975;1:1314.
329. Hartig PC, Madge GE, Webb SR. Diversity within a human isolate of Coxsackie B4: relationship to viral-induced diabetes mellitus. *J Med Virol*. 1983; 11:23.
330. Andreoletti L, Hober D, Hober-Vandenberghe C, et al. Detection of Coxsackie B virus RNA sequences in whole blood samples from adult patients at the onset of type I diabetes mellitus. *J Med Virol*. 1997;52:121.
331. Richardson SJ, Willcox A, Bone AJ, et al. The prevalence of enteroviral capsid protein VP1 immunostaining in pancreatic islets in human type 1 diabetes. *Diabetologia*. 2009;5:1143.
332. Yoon JW, Austin M, Onodera T, et al. Virus-induced diabetes mellitus: isolation of a virus from the pancreas of a child with diabetic ketoacidosis. *N Engl J Med*. 1979;300:1173.
333. Dotta F, Censini S, van Halteren AG, et al. Coxsackie B4 virus infection of beta cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. *Proc Natl Acad Sci USA*. 2007;104:5115.
334. Gladish R, Hofmann W, Waldherr R. Myocarditis and insulitis in coxsackievirus infection. *Z Kardiol*. 1976;65:835.
335. Helfand RF, Gary HE Jr, Freeman CY, et al; for the Pittsburgh Diabetes Research Group. Serologic evidence of an association between enteroviruses and onset of type 1 diabetes mellitus. *J Infect Dis*. 1995;172:1206.
336. D'Alessio DJ. A case-control study of group B coxsackievirus immunoglobulin M antibody prevalence and HLA-DR antigens in newly diagnosed cases of insulin-dependent diabetes mellitus. *Am J Epidemiol*. 1992;135:1331.
337. Tuvemo T, Dahlquist G, Frisk G, et al. The Swedish Childhood Diabetes Study. III. IgM against Coxsackie B viruses in newly diagnosed type 1 (insulin-dependent) diabetic children: no evidence of increased antibody frequency. *Diabetologia*. 1989;32:745.
338. Hanafusa T, Imagawa A. Insulitis in human type 1 diabetes. *Ann N Y Acad Sci*. 2008;1150:297.
339. Horwitz MS, et al. Diabetes induced by coxsackievirus: initiation by bystander damage and not molecular mimicry. *Nat Med*. 1998;4:781.
340. Vreugdenhil GR, Geluk A, Ottenhoff TH, et al. Molecular mimicry in diabetes mellitus: the homologous domain in coxsackie B virus protein 2c and islet autoantigen GAD65 is highly conserved in the coxsackie B-like enteroviruses and binds to the diabetes associated HLA-DR3 molecule. *Diabetologia*. 1998;41:40.
341. Hagiwara A, Yoneyama T, Takami S, et al. Genetic and phenotypic characteristics of enterovirus 71 isolates from patients with encephalitis and with hand, foot, and mouth disease. *Arch Virol*. 1984;79:273.
342. Hashimoto I, Hagiwara A, Kodama H. Neurovirulence in cynomolgus monkeys of enterovirus 71 isolated from a patient with hand, foot, and mouth disease. *Arch Virol*. 1978;56:257.
343. Brown BA, Oberste MS, Alexander JP Jr, et al. Molecular epidemiology and evolution of enterovirus 71 strains isolated from 1970 to 1998. *J Virol*. 1999;73:9969.
344. Schmidt NJ, Lennette EH, Ho HH. An apparently new enterovirus isolated from patients with disease of the central nervous system. *J Infect Dis*. 1974;129:304.
345. Kennett ML, Birch CJ, Lewis FA, et al. Enterovirus type 71 infection in Melbourne. *Bull World Health Organ*. 1974;51:609.
346. Nagy G, Takatsy S, Kukan E, et al. Virological diagnosis of enterovirus type 71 infections: experiences gained during an epidemic of acute CNS diseases in Hungary in 1978. *Arch Virol*. 1982;71:217.
347. Chumakov MP, Voroshilova MK, Shindarov L, et al. Enterovirus 71 isolated from cases of poliomyelitis-like disease in Bulgaria. *Arch Virol*. 1979;60:329.

348. Alexander JP, Baden L, Pallansch MA, et al. Enterovirus 71 infections and neurologic disease—United States, 1977-1991. *J Infect Dis*. 1994;169:905.
349. Thao NT, Ngoc NT, Tú PV, et al. Development of a multiplex polymerase chain reaction assay for simultaneous identification of human enterovirus 71 and coxsackievirus A16. *J Virol Methods*. 2010;170:134.
350. Pérez-Vélez CM, Anderson MS, Robinson CC, et al. Outbreak of neurologic enterovirus type 71 disease: a diagnostic challenge. *Clin Infect Dis*. 2007;45:950.
351. Zhu FC, Meng FY, Li JX, et al. Efficacy, safety, and immunology of an inactivated alum-adjuvant enterovirus 71 vaccine in children in China: a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2013;381:2024.
352. Zhu F, Xu W, Xia J, et al. Efficacy, safety, and immunogenicity of an enterovirus 71 vaccine in China. *N Engl J Med*. 2014;370:818.
353. Li R, Liu L, Mo Z, et al. An inactivated enterovirus 71 vaccine in healthy children. *N Engl J Med*. 2014;370:829.

173 Parechoviruses

José R. Romero and Vini Vijayan

SHORT VIEW SUMMARY

Definition
- The human parechoviruses (HPeVs) are members of a relatively newly created genus designated *Parechovirus* in the Picornaviridae family. Four species (*Parechovirus* A through D) comprise the genus. Only serotypes within species A and B infect humans; A is the most important, with 19 serotypes currently identified.
- HPeVs are responsible for multiple clinical syndromes involving many organ systems, including undifferentiated febrile illness, sepsis-like syndrome, meningitis, encephalitis, paralysis, and respiratory illnesses.

Epidemiology
- HPeVs are found worldwide.
- The majority of HPeV infections occur during the summer and autumn months in infants and young children.

Microbiology
- Morphologically they are small (28 nm in diameter), nonenveloped, icosahedral-shaped viruses that possess a single-stranded, positive-sense RNA genome.

Diagnosis
- HPeVs can be isolated, or their genomic RNA can be detected from oral secretions, feces, cerebrospinal fluid, and blood.
- Nucleic acid amplification has been quicker and more sensitive than cell culture for their detection.
- Type identification is accomplished by sequencing of the major capsid protein, VP1.

Therapy
- Therapy is supportive. No specific antiviral therapy is available.

Prevention
- Contagion can be prevented by hand washing.

HISTORICAL BACKGROUND

The initial two serotypes of the genus *Parechovirus* were isolated in 1956 from children with diarrhea.[1,2] On the basis of the scheme used at that time for the classification of the enteroviruses, they were classified as echovirus (E) serotypes 22 (Harris strain) and 23 (Williamson strain).[2–4] After their classification as enteroviruses, it became evident that they differed from other serotypes within the genus. Their growth in cell culture produced a cytopathic effect that differed from that of the enteroviruses.[5] E22 and E23 produce incomplete cytopathic effects of the cell monolayer and a distinctive nuclear cytopathology consisting of thickening of the nuclear membrane and the disappearance of the nucleolus, and of nuclear chromatin.[2,5] As molecular methods for the evaluation of viral replication became available, additional discordances were identified. Salient among these were the inability of E22 and E23 to cleave the eukaryotic translation initiation factor 4G (eIF4G) and thereby shut off host cell protein synthesis—a notable characteristic of the enteroviruses—and the efficient translation of their genome in rabbit reticulocyte lysates, something not observed in the enteroviruses.[6,7] Furthermore, the cleavage of the nascent capsid proteins of E22 resulted in three rather than four mature capsid proteins, as with the enteroviruses.[8] With the advent of tools for direct and computational analysis of the viral genome, it became evident that E22 and E23 were clearly dissimilar to other members of the genus. Nuclease digestion of the RNA genome of E22 revealed that it possessed a greater mean relative resistance to digestion than that of poliovirus type 1, suggesting that it contained significantly more secondary structure (i.e., double-stranded or base-paired regions).[9] Failure to hybridize subgenomic probes to the E22 genome, known to be reactive with other serotypes of the *Enterovirus* genus, or amplify subgenomic segments using pan-enterovirus reactive primers, added further support that it was not a true enterovirus.[6,10,11] Ultimately, the sequencing and phylogenetic analysis of the complete genomes of E22 and E23 confirmed that they were indeed genetically distinct from the enteroviruses and led to their assignation to a new genus: *Parechovirus,* members of the Picornaviridae family.[12–14] The genus is currently comprised of 4 (A—D). Only members of species A and B infect humans.

Human parechoviruses (HPeVs) 1 and 2 (formerly E22 and E23, respectively) were soon joined by 17 newly identified members of the species bringing the total number of types to 19.[15,16] Potentially new types continue to be identified.[17]

VIROLOGY

Only the major differences between HPeVs and enteroviruses are discussed here. Several reviews of HPeVs have been published in recent years.[18,19] Readers are directed to those sources, and to Chapter 170 in this text, for a more detailed discussion of the virology of HPeVs and enteroviruses.

HPeVs are small (28 nm in diameter), nonenveloped viruses possessing a single-stranded, positive (messenger)-sense RNA genome. The external appearance of the virion is much smoother than that of the enteroviruses owing to shorter surface loops on the capsid protein, VP1, resulting in a less pronounced plateau at the fivefold axis of symmetry and a shallower canyon (Fig. 173.1).[20] The HPeV genome is approximately 7.35 kb in length and organized in a manner comparable with that of enteroviruses.[12–14,16,21–27] The open reading frame (ORF) codes for a polyprotein of approximately 2180 amino acids, depending on HPeV type and strain, that is shorter than that of enteroviruses.[16,17]

Computational support has been generated for the existence of linear regions of RNA and higher-order RNA structures within the HPeV 5′ and 3′ nontranslated regions (NTRs) analogous to those of enteroviruses (see Chapter 172).[14,25,27] Analysis of the HPeV internal ribosome entry site (IRES), a region essential for efficient translation of the picornavirus genome, reveals it to be more closely related to that of the *Cardiovirus* and *Aphthovirus* genera.[27] Conserved regions of nucleotide identity exist among the 16 types within the HPeV 5′ NTR and are used in nucleic acid amplification tests (NAATs) for their detection.[28,29]

Translation of the ORF yields three capsid (VP0, VP3, and VP1) and seven nonstructural proteins essential for replication and viral assembly. The structures and functions of all of the HPeV proteins have not been extensively studied. Many have functions similar to cognate enterovirus proteins, but differences are emerging. Unlike the enteroviruses, the HPeV genome codes for three, rather than four, capsid proteins as a result of failure to cleave the precursor protein, VP0, of capsid proteins

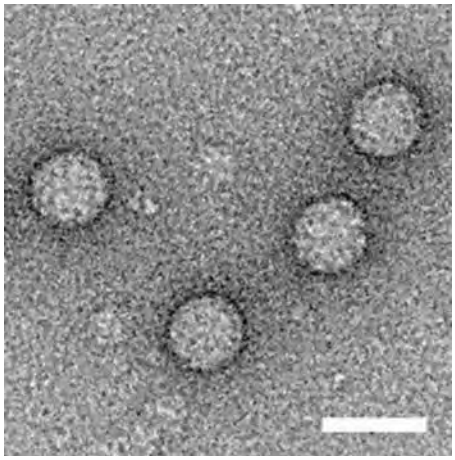

FIG. 173.1 Electron micrograph of parechoviruses (bar = 50 nm). *(From Stanway G, Williams C, Hyypia T. Parechovirus. In: Tidona C, Darai G, eds.* The Springer Index of Viruses. *2nd ed. New York: Springer; 2011.)*

VP2 and VP4.[8] The result is a capsid composed of VP0, VP3, and VP1.[12,14,23–26] The VP0 coding sequence contains a stem-loop structure that may function as a *cis*-acting replication element (CRE) critical for replicaton.[25] The C-terminal region of the VP1 protein of several HPeVs contains an arginine-glycine-aspartic acid (RGD) motif functional in binding to host cell integrins.[a] Evidence points to the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_6$ as receptors for some HPeVs.[20,30] The cell receptors used by HPeVs lacking the RGD motif are currently unknown. The VP3 of HPeV varies from that of the enteroviruses as the result of the N-terminal extension of 28-34 amino-acid residues.[24]

Among the nonstructural proteins, 2A protein differs functionally from that of the enteroviruses in that it lacks proteolytic activity and is incapable of mediating the cleavage between the 1D and 2A proteins.[31] The HPeVs fail to shut off host cell protein synthesis, a function mediated in enteroviruses by 2A cleavage of eIF4G.[6,7,8] The 2A protein also binds to RNA and may play a role in RNA replication.[32] The HPeV 2C protein also appears to differ from its cognate enterovirus protein. Like the enterovirus 2C, it localizes to endoplasmic reticulum membrane–associated replication complexes, supporting a role in viral replication. However, it is also associated with cellular structures not directly involved in replication.[33,34] 2C also has AMP (adenosine monophosphate) kinase activity, a property whose significance is unknown.

EPIDEMIOLOGY

Our understanding of the epidemiology of the HPeV is lacking for multiple reasons: continued discovery of new types[17]; inability of all HPeV types to be identified through cell culture[35]; and an NAA that is pan-amplifying for all HPeVs.[28]

HPeVs have been identified worldwide. HPeV infections occur throughout the year; however, they exhibit seasonal epidemiology. Worldwide, the peak incidence for HPeV infection is during the summer and autumn months.[24,36–50]

In the case of HPeV3, a biennial pattern has been reported,[44,51,52,53] but several reports specifically have not supported this finding.[26,37,46,54,55] Cocirculation of multiple types within a community or geographic region is common.[b] The HPeVs have been associated with community[45] and nosocomial outbreaks in pediatric and neonatal units.[36,58,59,60,61]

The contribution of the HPeV to the disease burden, and the relative proportion due to each of the types, is yet unclear. With use of traditional cell culture methods, the HPeVs accounted for approximately 2% of all "enteroviruses" isolated by clinical laboratories. HPeV1 is the most frequently identified member of the genus, followed by HPeV3. In the United States, HPeV1 (E22) accounted for 1.8% and HPeV2 (E23) for 0.1% of all typed enterovirus isolates reported to the Centers for Disease Control and Prevention (CDC) over a 39-year period (1970–2008) and ranked among the top 15 isolates isolated for 74% all years.[62,63] Reports from two clinical laboratories in Japan found that HPeV accounted for 2.2% to 2.8% of all enteroviruses isolated over a period of 15 and 9 years, respectively.[23,26] Typing revealed that HPeV1 and HPeV3 accounted for 85% of the isolates, whereas types 6 and 4 accounted for the remainder in decreasing order of frequency. A Canadian report found that over a 20-year period, of 28 HPeV isolates that were identified, types 1, 2, and 3 accounted for 71.4%, 10.7%, and 17.9%, respectively, of the total number.[24]

However, with use of molecular techniques for the detection of these viruses, it appears that HPeV may play a more significant role in disease burden than previously was thought. Surveillance by the CDC (2009–2013) found that HPeV3 accounted for 12.3% of 1819 reports with a known enterovirus or HPeV type.[64] However, most HPeV3 detections originated from a single hospital that routinely tested for HPeVs. A report from Northern Italy documented that HPeVs were detected in 5.2% of 812 samples collected from children younger than 5 years over a 4-year period. For comparison, enteroviruses were identified in 16.1% of samples. HPeV1 accounted for 66.7% of the HPeVs identified, followed by HPeV3 (20%) and HPeV6 (13.3%).[50]

HPeV infections occur early in life. Seroprevalence data and longitudinal studies of the shedding of HPeV in stools of healthy infants suggest that approximately 70% to 80% of infants have evidence of at least one HPeV infection by 2 years of age, increasing to approximately 90% by 5 years in some studies.[39,54,65–68,69] In the United States, approximately 70% of cases of HPeV1 and HPeV2 infection reported to the CDC occurred in infants younger than 1 year.[62] Children younger than 5 years accounted for 95.6% and 88.2% of all HPeV1 and HPeV2 isolates, respectively.[62] The seroprevalence for some HPeV types approaches 100% in adults.[69] However, in some geographic areas, certain HPeV types fail to circulate or do so at low levels.[41] A limited serologic survey of the prevalence of HPeV3 among adults in the Milwaukee, Wisconsin, area failed to detect any seropositive individuals[70]

CLINICAL MANIFESTATIONS

Transmission of HPeV occurs primarily by the fecal-oral and, less frequently, the respiratory routes. Fecal-oral transmission is favored by high viral titers in stool.[42] After infection, HPeVs are shed from the gastrointestinal and upper respiratory tracts.[37,42,71] The duration of shedding is still not well defined but may range from less than 2 weeks to as long as 5 months in stool and 1 to 3 weeks from the upper respiratory tract.[37,42,71] In one report, after symptomatic infection, shedding of HPeV lasted for 2 to 24 weeks (median, 58 days) and most of the shedding occurred while infants were asymptomatic.[72] Reports of HPeV infections occurring in the first 2 days after birth may support the possibility of in utero transmission.[44,73] Identification of genetically identical HPeV3 in infected patients and in asymptomatic children in their families have suggested that family members may be a source of infection in neonates and young infants.[74]

The finding of HPeV in the stool of asymptomatic infants, along with the high seroprevalence among young infants and children in the general population, provides evidence that the majority of HPeV infections are subclinical.[37,42,54] Given the high prevalence of HPeV infections, it is likely that the reported cases of severe HPeV infections represent infrequent events.

UNDIFFERENTIATED FEBRILE ILLNESS/SEPSIS SYNDROME

HPeVs have been isolated from the stool, nasopharyngeal swabs, urine, and blood of neonates and infants with an undifferentiated febrile illness (UFI).[40,54,71,75–82] The overwhelming majority of reported cases have occurred in male infants younger than 2 months. Seven percent of infants younger than 36 months evaluated for fever without a source in the emergency department of a children's hospital had a HPeV infection.[83] In some cases, cardiovascular and respiratory dysfunction is severe enough to warrant a clinical diagnosis of sepsis. Although multiple HPeV types have been associated with UFI, cases of sepsis have been most frequently associated with infection due to HPeV3.[40,76,80–82] HPeV4 infection may also be associated with serious, sepsis-like disease.[81,84,85]

In addition to fever, irritability, and poor feeding, tachycardia and tachypnea are typically present.[c] A rash may be present on the trunk

[a]References 8, 12, 14, 20, 23, 25.
[b]References 38, 39, 43, 47, 48, 53, 54, 56, 57.

and extremities, including the palms and soles, which has been described as macular, maculopapular, or erythematous.[76,79,80,86–88] Mild respiratory (rhinorrhea, cough) and gastrointestinal symptoms (diarrhea, abdominal distention) may be present. Some may present with or develop a sepsis-like syndrome with severe tachycardia, retractions, apnea, oxygen desaturation, poor capillary filling, mottling, or shock.[80–83] In these patients, hepatitis may be present.[80,83]

Admission to the intensive care unit may be necessary for monitoring and cardiovascular or respiratory support and may be more common for infants younger than 1 to 2 months.[80–83] In a review of three recent series involving very young infants, totaling over 200 cases, only a single death was reported.[80,81,82] A recent study raised concerns regarding normal neurodevelopment among infants hospitalized with HPeV sepsis-like presentation.[89] With use of a standard questionnaire, follow-up was performed 1 year after hospitalization in 42 infants who had been hospitalized with HPeV sepsis-like syndrome, 12% of whom had encephalitis. The investigators found that half of the infants may have had some abnormal neurodevelopment, including possible significant abnormalities in one-fifth of the infants, most prominently in the gross motor and problem solving domains. These findings suggest that careful follow-up of young infants hospitalized with HPeV disease may be warranted.

CENTRAL NERVOUS SYSTEM INFECTIONS

HPeVs have been conclusively documented to cause various central nervous system (CNS) syndromes, including acute flaccid paralysis (AFP),[23,26,90,91] encephalitis,[44,48,75,80–82,92] and meningitis.[d]

Meningitis

HPeVs have been detected from the cerebrospinal fluid (CSF) or blood of individuals with meningitis.[51] Retrospective analysis of archival CSF specimens negative for enterovirus and a prospective report detected HPeV in approximately 1% to 8.8% of individuals undergoing evaluation for possible infection.[e] The annual prevalence varies widely and ranges from 0% to 17% (mean, 5.3%). With the exception of one report, the overwhelming cause of HPeV meningitis is HPeV3.[75] More than 80% of cases occur in infants younger than 3 months. Males account for the majority of cases.

Clinical findings consist of fever and irritability in nearly all cases.[f] Poor feeding and decreased activity are frequent manifestations. An exanthem may be present and has been described as maculopapular or erythematous, affecting primarily the extremities and involving the palms and soles as noted earlier.[48,80,88] Gastrointestinal signs, such as emesis, diarrhea, and distention, and respiratory symptoms such as rhinorrhea, cough, tachypnea, apnea, and wheezing, may also be present. Conspicuously absent are clinical findings indicative of increased intracranial pressure (bulging fontanel) or meningeal irritation (nuchal stiffness, Kernig, or Brudzinski signs).

Minimal or no abnormalities are found at CSF evaluation in the overwhelming majority of patients.[g] Pleocytosis is absent in the overwhelming majority of cases and, if present, is generally minimal (<25 cells/mm^3). Hypoglycorrhachia and increased CSF protein are uncommon.[46,48,94]

The majority of infants with HPeV meningitis are hospitalized as a result of the need to exclude bacterial infection.[44,46,48,94] The median duration of hospitalization varies from 3 to 7 days.[g] Death is extremely rare.[g]

Encephalitis

HPeV encephalitis occurs almost exclusively in neonates and very young infants.[h] Although HPeV3 is the dominant cause, cases due to HPeV1 have been reported.[92,96] Seizures, which may be intractable, constitute the predominant presenting clinical finding, followed by fever and irritability. An exanthem is seen in the majority of patients. Apnea and hypotension, requiring inotropic support, may occur. Additional findings include stupor, hypertonia, decreased appetite, and diarrhea.

The CSF is normal in nearly all cases. Cerebral magnetic resonance imaging (MRI) or ultrasonography reveals abnormalities in nearly all cases.[75,86,92,95,97] White matter changes consisting of high signal intensity and punctate lesions are seen at MRI in a majority of cases. These changes occur even in children without seizures.[75,86] Ultrasound usually reveals severe periventricular echogenicity. Electroencephalography findings are abnormal and document epileptic discharges and background slowing.[95]

Fatalities rarely occur.[76,91,97] Limited information on long-term neurologic outcome is available; however, in more than half of reported cases, patients were neurologically normal at the time of the clinical report. A recent report documented neurodevelopment sequelae in the majority of infants with confirmed HPeV encephalitis. At discharge, one-third were noted to have sequelae. However, at the 12-month follow-up, 62.5% were assessed to have sequelae: cerebral palsy, visual impairment, and concern regarding gross motor development.[95] Other neurologic sequelae include epilepsy, learning disability, and mild distal hypotonia.[43,86] Repetitive seizures and a discontinuous background on electroencephalogram were found to correlate with poor neurodevelopmental outcome.[86,95] Striking similarities between the clinical features and neurologic sequelae caused by HPeV3 encephalitis and hypoxic ischemic encephalopathy of the newborn have been noted.[98]

Acute Flaccid Paralysis

HPeV types 1, 3, 5, 6, 7, and 12 have been identified from stools and CSF in patients with AFP.[i] With the exception of a single report of an outbreak of HPeV1-associated AFP,[87] all cases have been sporadic occurrences. The outbreak occurred in Jamaica and involved five individuals—one adult and four children.[90] Individuals with AFP are older than those with other HPeV-related CNS diseases, ranging in age from 1 month to 27 years.[23,90,91,96,99] Detailed clinical data are limited.[23,90,96] The onset is acute and in the majority of cases involves the lower extremities. Loss of deep tendon reflexes occurs in the involved extremities. Respiratory failure due to involvement of the muscles of respiration may occur. Fever and diarrhea may be present at the onset. Other findings include paresthesias, cranial nerve involvement, and signs of meningeal irritation. CSF examination generally reveals increased protein concentration and absence of pleocytosis. Although occasional cases of transient paralysis have been reported,[23] the majority of reports have indicated residual weakness or palsy of the involved extremity.

GASTROINTESTINAL DISEASE

Hemorrhage-Hepatitis Syndrome

Neonates and very young infants may develop a syndrome characterized by hepatitis, thrombocytopenia, and coagulopathy reminiscent of hemorrhage-hepatitis syndrome observed with enteroviruses (see Chapter 172) after HPeV infection.[43,75,87,100] All infants have been younger than 2 months, and HPeV3 has been isolated in all cases. Infants present with fever, fussiness, and poor feeding. As the illness progresses, ecchymosis and bleeding from puncture sites occur. Marked elevation of aspartate and alanine aminotransferases levels, prolongation of prothrombin, and partial thromboplastin times attest to hepatitis and liver failure. Thrombocytopenia is universally present. Deaths have occurred, and liver transplantation as a result of severe liver necrosis has been necessary.[43,100]

Necrotizing Enterocolitis

Infection with HPeV1 and HPeV3 has been associated with necrotizing enterocolitis in neonates and very young infants.[36,100] Clinical findings include feeding intolerance, abdominal distention, and bloody stools. Nonspecific symptoms such as apnea, rhinitis, tachypnea, and lethargy may also occur. Radiograph findings include dilated loops of bowel and pneumatosis intestinalis.

Acute Gastroenteritis

Multiple studies that vary in stringency, controls, number of specimens, and clinical venues have examined the role of HPeV in the etiology of

[c]References 40, 43, 71, 75, 76, 79.
[d]References 44, 46, 48, 51, 73, 75, 81–82, 87, 93.
[e]References 44, 46, 48, 51, 73, 87, 93.
[f]References 46, 48, 73, 80–82, 87, 93.
[g]References 44, 46, 48, 80, 81, 82, 94.
[h]References 43, 44, 48, 58, 75, 80, 81, 82, 86, 95, 96, 97.
[i]References 23, 26, 90, 91, 96, 99.

acute gastroenteritis (AGE) in children[j] and adults.[42] Further befuddling the analysis of the role of HPeV in AGE is that gastrointestinal signs and symptoms accompany almost every syndrome caused by these agents.

An analysis of reports of stool specimens shown to be negative for known viral cases of AGE, and screened for HPeV with NAATs, indicates that prevalence of HPeV among hospitalized children ranges from 0% to 2.7%.[42,47,102] No difference in prevalence was observed among children from developing versus industrialized nations.

The prevalence of HPeV in cases of AGE has been examined in the outpatient setting in different countries by using large numbers of stool samples negative for other viral causes of AGE. A report from Germany found that only 1.8% of all samples contained HPeV. No difference was found in HPeV-AGE patients compared with a control non-AGE group.[42] All positive specimens were found in children younger than 6 years. The prevalence in children younger than 2 years was 11.6%. Among nonhospitalized Japanese children with AGE, 8.3% had detectable HPeV.[57] Lastly, a report from the CDC found HPeV present in only 4.4% of 782 stool specimens submitted from children younger than 5 years with AGE.[103] These researchers concluded that there was no causal role for HPeV in AGE. On the basis of the available information, HPeV appears to have at best a small role as a cause of AGE.

Respiratory Disease

Respiratory symptoms, either alone or in combination with other HPeV syndromes, are common.[k] Several recent large studies cast doubt on the role of HPeV as a significant cause of respiratory disease. In a report from Norway, 161 asymptomatic children attending daycare were studied, and 9% were found to be positive for HPeV.[104] A study conducted in Northern Italy found that only 0.4% (14) of 3525 patients with respiratory syndromes had HPeV isolated. Of those, all but 4 had a coinfection with other respiratory viruses.[78] In a report from Scotland, the prevalence of HPeV was 1.2% among individuals with respiratory tract illness.[42] HPeV accounted for 2.9% of cases of those in whom any respiratory virus was identified. Of significance, coinfection with one or more respiratory viral pathogens was found in 76% of HPeV-positive children. All PeVs were isolated from children 0.5 to 5 years of age, and 89% were younger than 3 years. The observed incidence of HPeV infections in children younger than 5 years was within the expected frequency of the general population. Comparison of the clinical profile of those in whom HPeV was detected coincided more closely with those with negative samples.[41] Lastly, a report from a Midwest children's hospital in the United States found a 3% prevalence rate for HPeV infection among 637 children for whom respiratory specimens were submitted.[105] All children were younger than 3 years, and 79% were younger than 3 months. Only nonspecific respiratory symptoms were present. Taken together, these findings fail to support a significant etiologic role for HPeV in respiratory disease.

[j]References 38, 42, 47, 49, 54, 56, 57.
[k]References 42, 44, 47, 57, 59, 76, 103.

MISCELLANEOUS CLINICAL ASSOCIATIONS

HPeVs have been isolated from patients with a wide array of syndromes, including sudden infant death,[70] Reye syndrome,[25,26,106] Guillain-Barré syndrome,[90,107] myocarditis,[43,108,109] myositis and epidemic myalgia,[26,110,111] hemolytic uremic syndrome,[112] hand-foot-mouth disease,[26,54] herpangina,[52] otitis media,[113] hemophagocytic lymphohistiocytosis,[114,115] and hyperferritinemia.[116,117] In the majority of reports, HPeVs were detected from sites where prolonged shedding is known to occur and not from the involved organ system or tissue. Some reports fail to indicate whether other infectious agents known to be associated with the syndrome were excluded. Further adding to the complexity of ascribing a HPeV etiology to an illness is the codetection of a second agent known to be linked to the disease in question.[41,54,57]

DIAGNOSIS

Samples for detection of HPeV include throat swabs, stool specimens, CSF, or blood. The HPeVs produce an enterovirus-like cytopathic effect on appropriate cell lines.[35,118] However, optimal recovery of the HPeV requires the use of multiple cell lines because not all types replicate equally on the same primary cells or cell lines. Many of these are not routinely found in diagnostic laboratories.

Reverse-transcriptase polymerase chain reaction (RT-PCR) is the methodology of choice for detection of HPeV because of its sensitivity and ability to detect all known HPeV types in a clinically meaningful time frame.[28] Speciation of HPeV is accomplished by amplification and sequencing of the VP1 coding region of the viral genome.[28,29]

THERAPY AND PREVENTION

No specific antiviral therapy has been described for HPeVs, although it is an area of active research.[119,120,121] Intravenous immunoglobulin has been used to treat serious HPeV infections, but its efficacy is not established.[122] Monoclonal antibodies with neutralizing activity against HPeV have been reported and may offer an approach to specific therapy.[123] Because their major mode of transmission is the fecal-oral route, hand hygiene prevents their spread. For hospitalized patients with HPeV-related syndromes, infection control measures similar to those for the enteroviruses, consisting of use of standard precautions, are sufficient.[124] No vaccine is currently available for the prevention of HPeV-associated disease.

Key References

The complete reference list is available online at Expert Consult.

2. Wigand R, Sabin AB. Properties of ECHO types 22, 23 and 24 viruses. *Arch Gesamte Virusforsch*. 1961;11: 224–247.
6. Coller BA, Chapman NM, Beck MA, et al. Echovirus 22 is an atypical enterovirus. *J Virol*. 1990;64: 2692–2701.
7. Coller BG, Tracy SM, Etchison D. Cap-binding complex protein p220 is not cleaved during echovirus 22 replication in HeLa cells. *J Virol*. 1991;65:3903–3905.
11. Rotbart HA. Enzymatic RNA amplification of the enteroviruses. *J Clin Microbiol*. 1990;28:438–442.
12. Hyypia T, Horsnell C, Maaronen M, et al. A distinct picornavirus group identified by sequence analysis. *Proc Natl Acad Sci USA*. 1992;89:8847–8851.
16. Picornaviridae Study Group. Human Parechoviruses. http://www.picornastudygroup.com/types/parechovirus/hpev.htm. Accessed May 1, 2018.
28. Nix WA, Maher K, Johansson ES, et al. Detection of all known parechoviruses by real-time PCR. *J Clin Microbiol*. 2008;46:2519–2524.
29. Nix WA, Maher K, Pallansch MA, et al. Parechovirus typing in clinical specimens by nested or semi-nested PCR coupled with sequencing. *J Clin Virol*. 2010;48:202–207.
35. Westerhuis BM, Jonker SC, Mattao S, et al. Growth characteristics of human parechovirus 1 to 6 on different cell lines and cross-neutralization of human parechovirus antibodies: a comparison of the cytopathic effect and real time PCR. *Virol J*. 2013;10:146.
36. Birenbaum E, Handsher R, et al. Echovirus type 22 outbreak associated with gastro-intestinal disease in a neonatal intensive care unit. *Am J Perinatol*. 1997;14:469–473.
37. Tapia G, Cinek O, Witsø E, et al. Longitudinal observation of parechovirus in stool samples from Norwegian infants. *J Med Virol*. 2008;80:1835–1842.
40. Benschop KS, Schinkel J, Minnaar RP, et al. Human parechovirus infections in Dutch children and the association between serotype and disease severity. *Clin Infect Dis*. 2006;42:204–210.
41. Harvala H, Robertson I, McWilliam Leitch EC, et al. Epidemiology and clinical associations of human parechovirus respiratory infections. *J Clin Microbiol*. 2008;46:3446–3453.
48. Selvarangan R, Nzabi M, Selvaraju SB, et al. Human parechovirus 3 causing sepsis-like illness in children from midwestern United States. *Pediatr Infect Dis*. 2011;30:238–242.
51. Harvala H, Robertson I, Chieochansin T, et al. Specific association of human parechovirus type 3 with sepsis and fever in young infants, as identified by direct typing of cerebrospinal fluid samples. *J Infect Dis*. 2009;199:1753–1760.
52. van der Sanden S, de Bruin E, Vennema H, et al. Prevalence of human parechovirus in the Netherlands in 2000 to 2007. *J Clin Microbiol*. 2008;46:2884–2889.
62. Centers for Disease Control and Prevention. Enterovirus surveillance—United States, 1970-2005. *MMWR Surveill Summ*. 2006;55:1–20.
64. Abedi GR, Watson JT, Pham H, et al. Enterovirus and human parechovirus surveillance—United States, 2009-2013. *MMWR Morb Mortal Wkly Rep*. 2015;64:940–943.
68. Kolehmainen P, Oikarinen S, Koskiniemi M, et al. Human parechoviruses are frequently detected in stool of healthy Finnish children. *J Clin Virol*. 2012;54:156–161.
72. Wildenbeest JG, Benschop KS, Bouma-de Jongh S, et al. Prolonged shedding of human parechovirus in feces of young children after symptomatic infection. *Pediatr Infect Dis J*. 2016;35:580–583.
80. Khatami A, McMullan BJ, Webber M, et al. Sepsis-like disease in infants due to human parechovirus type 3 during an outbreak in Australia. *Clin Infect Dis*. 2015;60:228–236.
81. Midgley CM, Jackson MA, Selvarangan R, et al. Severe parechovirus 3 infections in young infants—Kansas and Missouri, 2014. *J Pediatric Infect Dis Soc*. 2017.
82. Vergnano S, Kadambari S, Whalley K, et al. Characteristics and outcomes of human parechovirus infection in infants (2008-2012). *Eur J Pediatr*. 2015;174:919–924.
83. Colvin JM, Muenzer JT, Jaffe DM, et al. Detection of viruses in young children with fever without an apparent source. *Pediatrics*. 2012;130:e1455–e1462.
86. Verboon-Maciolek MA, Groenendaal F, Hahn CD, et al. Human parechovirus causes encephalitis with white

matter injury in neonates. *Ann Neurol*. 2008;64:266–273.
88. Shoji K, Komuro H, Miyata I, et al. Dermatologic manifestations of human parechovirus type 3 infection in neonates and infants. *Pediatr Infect Dis J*. 2013;32:233–236.
89. Britton PN, Khandaker G, Khatami A, et al. High prevalence of developmental concern amongst infants at 12 months following hospitalised parechovirus infection. *J Paediatr Child Health*. 2018;54:289–295.
94. Sharp J, Harrison CJ, Puckett K, et al. Characteristics of young infants in whom human parechovirus, enterovirus or neither were detected in cerebrospinal fluid during sepsis evaluations. *Pediatr Infect Dis J*. 2013;32:213–216.
95. Britton PN, Dale RC, Nissen MD, et al. Parechovirus encephalitis and neurodevelopmental outcomes. *Pediatrics*. 2016;137:e20152848.
98. Gupta S, Fernandez D, Siddiqui A, et al. Extensive white matter abnormalities associated with neonatal parechovirus (HPeV) infection. *Eur J Paediatr Neurol*. 2010;14:531–534.
103. Chhabra P, Payne DC, Szilagyi PG, et al. Etiology of viral gastroenteritis in children <5 years of age in the United States, 2008-2009. *J Infect Dis*. 2013;208:790–800.
104. Moe N, Pedersen B, Nordbø SA, et al. Respiratory virus detection and clinical diagnosis in children attending day care. *PLoS ONE*. 2016;11:e0159196.
105. Sharp J, Bell J, Harrison CJ, et al. Human parechovirus in respiratory specimens from children in Kansas City, Missouri. *J Clin Microbiol*. 2012;50:4111–4113.
124. Healthcare Infection Control Practices Advisory Committee. Centers for Disease Control and Prevention: 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. www.cdc.gov/hicpac/2007IP/2007ip_appendA.html. Accessed March 17, 2014.

References

1. Ramos-Alvarez M, Sabin AB. Enteropathogenic viruses and bacteria. Role in summer diarrheal diseases of infancy and early childhood. *JAMA*. 1958;167:147–156.
2. Wigand R, Sabin AB. Properties of ECHO types 22, 23 and 24 viruses. *Arch Gesamte Virusforsch*. 1961;11:224–247.
3. Committee on the ECHO Viruses. Enteric cytopathogenic human orphan (ECHO) viruses. *Science*. 1955;122:1187–1188.
4. Committee on the Enteroviruses. The enteroviruses. *Am J Pub Health*. 1957;47:1556–1566.
5. Shaver DN, Barron AL, Karzon DT. Distinctive cytopathology of ECHO viruses type 22 and 23. *Proc Soc Exp Biol Med*. 1961;106:648–652.
6. Coller BA, Chapman NM, Beck MA, et al. Echovirus 22 is an atypical enterovirus. *J Virol*. 1990;64:2692–2701.
7. Coller BG, Tracy SM, Etchison D. Cap-binding complex protein p220 is not cleaved during echovirus 22 replication in HeLa cells. *J Virol*. 1991;65.
8. Stanway G, Kalkkinen N, Roivainen M, et al. Molecular and biological characteristics of echovirus 22, a representative of a new picornavirus group. *J Virol*. 1994;68:8232–8238.
9. Seal LA, Jamison RM. Evidence for secondary structure within the virion RNA of echovirus 22. *J Virol*. 1984;50:641–644.
10. Rotbart HA, Levin MJ, Villareal L. Use of subgenomic poliovirus DNA hybridization probes to detect the major subgroup of enteroviruses. *J Clin Microbiol*. 1984;20:1105–1108.
11. Rotbart HA. Enzymatic RNA amplification of the enteroviruses. *J Clin Microbiol*. 1990;28:438–442.
12. Hyypia T, Horsnell C, Maaronen M, et al. A distinct picornavirus group identified by sequence analysis. *Proc Natl Acad Sci USA*. 1992;89:8847–8851.
13. Oberste MS, Maher K, Pallansch MA. Complete sequence of echovirus 23 and its relationship to echovirus 22 and other human enteroviruses. *Virus Res*. 1998;5:217–223.
14. Ghazi F, Hughes PJ, Hyypia T, et al. Molecular analysis of human parechovirus type 2 (formerly echovirus 23). *J Gen Virol*. 1998;79:2641–2650.
15. King AMQ, Brown F, Christian P, et al. Picornaviridae. In: King AMQ, Adams MJ, Carstens EB, et al, eds. *Ninth Report of the International Committee on Taxonomy of Viruses*. New York: Academic Press; 2012:855.
16. Picornaviridae Study Group. Human Parechoviruses. http://www.picornastudygroup.com/types/parechovirus/hpev.htm. Accessed May 1, 2018.
17. Böttcher S, Obermeier PE, Diedrich S, et al. Genome sequence of novel human parechovirus type 17. *Genome Announc*. 2017;5.
18. Olijve L, Jennings L, Walls T. Human parechovirus: an increasingly recognized cause of sepsis-like illness in young infants. *Clin Microbiol Rev*. 2018;31:e00047-17.
19. Romero JR, Selvarangan R. The human parechoviruses: an overview. *Adv Pediatr*. 2011;58:65–68.
20. Seitsonen J, Susi P, Heikkilä O, et al. Interaction of alphaVbeta3 and alphaVbeta6 integrins with human parechovirus 1. *J Virol*. 2010;84:8509–8519.
21. Knowles NJ, Hovi T, King AMQ, et al. Overview of taxonomy. In: Ehrenfeld E, Domingo E, Roos RP, eds. *The Picornaviruses*. Washington, DC: American Society for Microbiology Press; 2010:19–32.
22. Jamison RM. Morphology of echovirus 22. *J Virol*. 1969;4:904–906.
23. Ito M, Yamashita T, Tsuzuki H, et al. Isolation and identification of a novel human parechovirus. *J Gen Virol*. 2004;85:391–398.
24. Abed Y, Boivin G. Molecular characterization of a Canadian human parechovirus (HPeV)-3 isolate and its relationship to other HPeVs. *J Med Virol*. 2005;77:566–570.
25. Al-Sunaidi M, Williams CH, Hughes PJ, et al. Analysis of a new human parechovirus allows the definition of parechovirus types and the identification of RNA structural domains. *J Virol*. 2007;81:1013–1021.
26. Watanabe K, Oie M, Higuchi M, et al. Isolation and characterization of novel human parechovirus from clinical samples. *Emerging Infect Dis*. 2007;13:889–895.
27. Nateri AS, Hughes PJ, Stanway G. In vivo and in vitro identification of structural and sequence elements of the human parechovirus 5′ untranslated region required for internal initiation. *J Virol*. 2000;74:6269–6277.
28. Nix WA, Maher K, Johansson ES, et al. Detection of all known parechoviruses by real-time PCR. *J Clin Microbiol*. 2008;46:2519–2524.
29. Nix WA, Maher K, Pallansch MA, et al. Parechovirus typing in clinical specimens by nested or semi-nested PCR coupled with sequencing. *J Clin Virol*. 2010;48:202–207.
30. Merilahti P, Tauriainen S, Susi P. Human parechovirus 1 infection occurs via αVβ1 integrin. *PLoS ONE*. 2016;11:e0154769.
31. Schultheiss T, Emerson SU, Purcell RH, et al. Polyprotein processing in echovirus 22: a first assessment. *Biochem Biophys Res Commun*. 1995;217:1120–1127.
32. Samuilova O, Krogerus C, Pöyry T, et al. Specific interaction between human parechovirus nonstructural 2A protein and viral RNA. *Biol Chem*. 2004;279:37822–37831.
33. Krogerus C, Egger D, Samuilova O, et al. Replication complex of human parechovirus 1. *J Virol*. 2003;77:8512–8523.
34. Krogerus C, Samuilova O, Pöyry T, et al. Intracellular localization and effects of individually expressed human parechovirus 1 non-structural proteins. *J Gen Virol*. 2007;88:831–841.
35. Westerhuis BM, Jonker SC, Mattao S, et al. Growth characteristics of human parechovirus 1 to 6 on different cell lines and cross-neutralization of human parechovirus antibodies: a comparison of the cytopathic effect and real time PCR. *Virol J*. 2013;10:146.
36. Birenbaum E, Handsher R, et al. Echovirus type 22 outbreak associated with gastro-intestinal disease in a neonatal intensive care unit. *Am J Perinatol*. 1997;14:469–473.
37. Tapia G, Cinek O, Witsø E, et al. Longitudinal observation of parechovirus in stool samples from Norwegian infants. *J Med Virol*. 2008;80:1835–1842.
38. Pham NTK, Trinh QD, Khamrin P, et al. Diversity of human parechoviruses isolated from stool samples collected from Thai children with acute gastroenteritis. *J Clin Microbiol*. 2010;48:115–119.
39. Tauriainen S, Martiskainen M, Oikarinen S, et al. Human parechovirus 1 infections in young children—no association with type 1 diabetes. *J Med Virol*. 2007;79:457–462.
40. Benschop KS, Schinkel J, Minnaar RP, et al. Human parechovirus infections in Dutch children and the association between serotype and disease severity. *Clin Infect Dis*. 2006;42:204–210.
41. Harvala H, Robertson I, McWilliam Leitch EC, et al. Epidemiology and clinical associations of human parechovirus respiratory infections. *J Clin Microbiol*. 2008;46:3446–3453.
42. Baumgarte S, de Souza Luna LK, Grywna K, et al. Prevalence, types, and RNA concentrations of human parechoviruses, including a sixth parechovirus type, in stool samples from patients with acute enteritis. *J Clin Microbiol*. 2008;46:242–248.
43. Verboon-Maciolek MA, Krediet TG, Gerards LJ, et al. Severe neonatal parechovirus infection and similarity with enterovirus infection. *Pediatr Infect Dis J*. 2008;27:241–245.
44. Wolthers KC, Benschop KS, Schinkel J, et al. Human parechoviruses as an important viral cause of sepsis like illness and meningitis in young children. *Clin Infect Dis*. 2008;47:358–363.
45. Yamamoto M, Abe K, Kuniyori K, et al. Epidemic of human parechovirus type 3 in Hiroshima City, Japan in 2008. *Jpn J Infect Dis*. 2009;62:244–245.
46. Piñeiro L, Vicente D, Montes M, et al. Human parechovirus in infants with systemic infection. *J Med Virol*. 2010;82:1790–1796.
47. Pham NT, Takanashi S, Tran DN, et al. Human parechovirus infection in children hospitalized with acute gastroenteritis in Sri Lanka. *J Clin Microbiol*. 2011;49:364–366.
48. Selvarangan R, Nzabi M, Selvaraju SB, et al. Human parechovirus 3 causing sepsis-like illness in children from midwestern United States. *Pediatr Infect Dis*. 2011;30:238–242.
49. Chiang GPK, Chen Z, Chan MCW, et al. Clinical features and seasonality of parechovirus infection in an Asian subtropical city, Hong Kong. *PLoS ONE*. 2017;12:e0184533.
50. Bubba L, Martinelli M, Pellegrinelli L, et al. A 4-year study on epidemiologic and molecular characteristics of human parechoviruses and enteroviruses circulating in children younger than 5 years in northern Italy. *Pediatr Infect Dis J*. 2017;36:13–19.
51. Harvala H, Robertson I, Chieochansin T, et al. Specific association of human parechovirus type 3 with sepsis and fever in young infants, as identified by direct typing of cerebrospinal fluid samples. *J Infect Dis*. 2009;199:1753–1760.
52. van der Sanden S, de Bruin E, Vennema H, et al. Prevalence of human parechovirus in the Netherlands in 2000 to 2007. *J Clin Microbiol*. 2008;46:2884–2889.
53. Harvala H, McLeish N, Kondracka J, et al. Comparison of human parechovirus and enterovirus detection frequencies in cerebrospinal fluid samples collected over a 5-year period in Edinburgh: HPeV type 3 identified as the most common picornavirus type. *J Med Virol*. 2011;83:889–896.
54. Ito M, Yamashita T, Tsuzuki H, et al. Detection of human parechoviruses from clinical stool samples in Aichi, Japan. *J Clin Microbiol*. 2010;48:2683–2688.
55. Schuffenecker I, Javouhey E, Gillet Y, et al. Human parechovirus infections, Lyon, France, 2008-10: evidence for severe cases. *J Clin Virol*. 2012;54:337–341.
56. Drexler JF, Grywna K, Stöcker A, et al. Novel human parechovirus from Brazil. *Emerg Infect Dis*. 2009;15:310–313.
57. Pham NTK, Chan-It W, Khamrin P, et al. Detection of human parechovirus in stool samples collected from children with acute gastroenteritis in Japan during 2007-2008. *J Med Virol*. 2011;83:331–336.
58. Ehrnst A, Eriksson M. Echovirus 23 observed as a nosocomial infection in infants. *Scand J Infect Dis*. 1996;28:205–206.
59. Berkovich S, Pangan J. Recoveries of virus from premature infants during outbreaks of respiratory disease: the relation of ECHO virus type 22 to disease of the upper and lower respiratory tract in the premature infant. *Bull N Y Acad Med*. 1968;44:377–387.
60. Barbi Guidotti M, Cappellini D, Pedretti CC, et al. Spread of echovirus 23 in a neonatal pathology unit. *Ann Sclava*. 1982;24:76–84.
61. Strenger V, Diedrich S, Boettcher S, et al. Nosocomial outbreak of parechovirus 3 infection among newborns, Austria, 2014. *Emerg Infect Dis*. 2016;22:1631–1634.
62. Centers for Disease Control and Prevention. Enterovirus surveillance—United States, 1970-2005. *MMWR Surveill Summ*. 2006;55:1–20.
63. Centers for Disease Control and Prevention. Nonpolio enterovirus and human parechovirus surveillance—United States, 2006-2008. *MMWR Morb Mortal Wkly Rep*. 2010;59:1577–1580.
64. Abedi GR, Watson JT, Pham H, et al. Enterovirus and human parechovirus surveillance—United States, 2009-2013. *MMWR Morb Mortal Wkly Rep*. 2015;64:940–943.
65. Joki-Korpela P, Hyypiä T. Diagnosis and epidemiology of echovirus 22 infections. *Clin Infect Dis*. 1998;27:129–136.
66. Takao S, Shimazu Y, Fukuda S, et al. Seroepidemiological study of human parechovirus 1. *Jpn J Infect Dis*. 2001;54:85–87.
67. Abed Y, Wolf D, Dagan R, et al. Development of a serological assay based on a synthetic peptide selected from the VP0 capsid protein for detection of human parechoviruses. *J Clin Microbiol*. 2007;45:2037–2039.
68. Kolehmainen P, Oikarinen S, Koskiniemi M, et al. Human parechoviruses are frequently detected in stool of healthy Finnish children. *J Clin Virol*. 2012;54:156–161.
69. Westerhuis B, Kolehmainen P, Benschop K, et al. Human parechovirus seroprevalence in Finland and the Netherlands. *J Clin Virol*. 2013;58:211–215.
70. Sedmak G, Nix WA, Jentzen J, et al. Infant deaths associated with human parechovirus infection in Wisconsin. *Clin Infect Dis*. 2010;50:357–361.
71. Pajkrt D, Benschop KS, Westerhuis B, et al. Clinical characteristics of human parechoviruses 4-6 infections in young children. *Pediatr Infect Dis J*. 2009;28:1008–1010.
72. Wildenbeest JG, Benschop KS, Bouma-de Jongh S, et al. Prolonged shedding of human parechovirus in feces of young children after symptomatic infection. *Pediatr Infect Dis J*. 2016;35:580–583.
73. Zhong H, Lin Y, Su L, et al. Prevalence of human parechoviruses in central nervous system infections in children: a retrospective study in Shanghai, China. *J Med Virol*. 2013;85:320–326.
74. Aizawa Y, Yamanaka T, Watanabe K, et al. Asymptomatic children might transmit human parechovirus type 3 to neonates and young infants. *J Clin Virol*. 2015;70:105–108.
75. Levorson RE, Jantausch BA, Wiedermann BL, et al. Human parechovirus-3 infection: emerging pathogen in neonatal sepsis. *Pediatr Infect Dis J*. 2009;28:545–547.
76. Boivin G, Abed Y, Boucher FD. Human parechovirus 3 and neonatal infections. *Emerg Infect Dis*. 2005;11:103–107.
77. Benschop KSM, Schinkel J, Luken ME, et al. Fourth human parechovirus serotype. *Emerging Infect Dis*. 2006;12:1572–1575.
78. Piralla A, Furione M, Rovida F, et al. Human parechovirus infections in patients admitted to hospital in Northern Italy, 2008-2010. *J Med Virol*. 2012;84:686–690.
79. Eis-Hübinger AM, Eckerle I, Helmer A, et al. Two cases of sepsis-like illness in infants caused by human parechovirus traced back to elder siblings with mild gastroenteritis and respiratory symptoms. *J Clin Microbiol*. 2013;51:715–718.
80. Khatami A, McMullan BJ, Webber M, et al. Sepsis-like disease in infants due to human parechovirus type 3

during an outbreak in Australia. *Clin Infect Dis*. 2015;60:228–236.
81. Midgley CM, Jackson MA, Selvarangan R, et al. Severe parechovirus 3 infections in young infants—Kansas and Missouri, 2014. *J Pediatric Infect Dis Soc*. 2017.
82. Vergnano S, Kadambari S, Whalley K, et al. Characteristics and outcomes of human parechovirus infection in infants (2008-2012). *Eur J Pediatr*. 2015;174:919–924.
83. Colvin JM, Muenzer JT, Jaffe DM, et al. Detection of viruses in young children with fever without an apparent source. *Pediatrics*. 2012;130:e1455–e1462.
84. Jääskeläinen AJ, Kolehmainen P, Kallio-Kokko H, et al. First two cases of neonatal human parechovirus 4 infection with manifestation of suspected sepsis, Finland. *J Clin Virol*. 2013;58:328–330.
85. Kolehmainen P, Jääskeläinen A, Blomqvist S, et al. Human parechovirus type 3 and 4 associated with severe infections in young children. *Pediatr Infect Dis J*. 2014;33:1109–1113.
86. Verboon-Maciolek MA, Groenendaal F, Hahn CD, et al. Human parechovirus causes encephalitis with white matter injury in neonates. *Ann Neurol*. 2008;64:266–273.
87. Renaud C, Kuypers J, Ficken E, et al. Introduction of a novel parechovirus RT-PCR clinical test in a regional medical center. *J Clin Virol*. 2011;51:50–53.
88. Shoji K, Komuro H, Miyata I, et al. Dermatologic manifestations of human parechovirus type 3 infection in neonates and infants. *Pediatr Infect Dis J*. 2013;32:233–236.
89. Britton PN, Khandaker G, Khatami A, et al. High prevalence of developmental concern amongst infants at 12 months following hospitalised parechovirus infection. *J Paediatr Child Health*. 2018;54:289–295.
90. Figueroa JP, Ashley D, King D, et al. An outbreak of acute flaccid paralysis in Jamaica associated with echovirus type 22. *J Med Virol*. 1989;29:315–319.
91. Alam MM, Khurshid A, Shaukat S, et al. Identification of human parechovirus genotype, HPeV-12, in a paralytic child with diarrhea. *J Clin Virol*. 2012;55:339–342.
92. Koskiniemi M, Paetau R, Linnavuori K. Severe encephalitis associated with disseminated echovirus 22 infection. *Scand J Infect Dis*. 1989;21:463–466.
93. Walters B, Peñaranda S, Nix WA, et al. Detection of human parechovirus (HPeV)-3 in spinal fluid specimens from pediatric patients in the Chicago area. *J Clin Virol*. 2011;52:187–191.
94. Sharp J, Harrison CJ, Puckett K, et al. Characteristics of young infants in whom human parechovirus, enterovirus or neither were detected in cerebrospinal fluid during sepsis evaluations. *Pediatr Infect Dis J*. 2013;32:213–216.
95. Britton PN, Dale RC, Nissen MD, et al. Parechovirus encephalitis and neurodevelopmental outcomes. *Pediatrics*. 2016;137:e20152848.
96. Legay V, Chomel JJ, Fernandez E, et al. Encephalomyelitis due to human parechovirus type 1. *J Clin Virol*. 2002;25:193–195.
97. van Zwol AL, Lequin M, Aarts-Tesselaar C, et al. Fatal neonatal parechovirus encephalitis. *BMJ Case Rep*. 2009;2009.
98. Gupta S, Fernandez D, Siddiqui A, et al. Extensive white matter abnormalities associated with neonatal parechovirus (HPeV) infection. *Eur J Paediatr Neurol*. 2010;14:531–534.
99. Li L, Victoria J, Kapoor A, et al. Genomic characterization of novel human parechovirus type. *Emerg Infect Dis*. 2009;15:288–291.
100. Sainato R, Flanagan R, et al. Severe human parechovirus sepsis beyond the neonatal period. *J Clin Virol*. 2011;51:73–74.
101. Deleted in review.
102. Han TH, Kim CH, Park SH, et al. Detection of human parechoviruses in children with gastroenteritis in South Korea. *Arch Virol*. 2011;156:1471–1475.
103. Chhabra P, Payne DC, Szilagyi PG, et al. Etiology of viral gastroenteritis in children <5 years of age in the United States, 2008-2009. *J Infect Dis*. 2013;208:790–800.
104. Moe N, Pedersen B, Nordbø SA, et al. Respiratory virus detection and clinical diagnosis in children attending day care. *PLoS ONE*. 2016;11:e0159196.
105. Sharp J, Bell J, Harrison CJ, et al. Human parechovirus in respiratory specimens from children in Kansas City, Missouri. *J Clin Microbiol*. 2012;50:4111–4113.
106. Schnurr D, Dondero M, Holland D, et al. Characterization of echovirus 22 variants. *Arch Virol*. 1996;141:1749–1756.
107. Linden S, Vuorinen T, Österback R, et al. Parechovirus infection preceding Guillain-Barré syndrome. *J Neurovirol*. 2012;18:434–436.
108. Wildenbeest JG, Wolthers KC, Straver B, et al. Successful IVIG treatment of human parechovirus-associated dilated cardiomyopathy in an infant. *Pediatrics*. 2013;132:e243–e247.
109. Eisenhut M. Features of myocarditis in infants with human parechovirus infection. *Clin Infect Dis*. 2015;61:139.
110. Mizuta K, Kuroda M, Kurimura M, et al. Epidemic myalgia in adults associated with human parechovirus type 3 infection, Yamagata, Japan, 2008. *Emerg Infect Dis*. 2012;18:1787–1793.
111. Mizuta K, Yamakawa T, Nagasawa H, et al. Epidemic myalgia associated with human parechovirus type 3 infection among adults occurs during an outbreak among children: findings from Yamagata, Japan, in 2011. *J Clin Virol*. 2013;58:188–193.
112. O'Regan S, Robitaille P, Mongeau JG, et al. The hemolytic uremic syndrome associated with ECHO 22 infection. *Clin Pediatr (Phila)*. 1980;19:125–127.
113. Tauriainen S, Oikarinen S, Taimen K, et al. Temporal relationship between human parechovirus 1 infection and otitis media in young children. *J Infect Dis*. 2008;198:35–40.
114. Yuzurihara SS, Ao K, Hara T, et al. Human parechovirus-3 infection in nine neonates and infants presenting symptoms of hemophagocytic lymphohistiocytosis. *J Infect Chemother*. 2013;19:144–148.
115. Aviner S, Sofer D, Shulman LM, et al. Hemophagocytic lymphohistiocytosis associated with parechovirus 3 infection. *J Pediatr Hematol Oncol*. 2014;36:e251–e253.
116. Casas-Alba D, Martínez-Monseny A, Monfort L, et al. Extreme hyperferritinemia in dizygotic twins with human parechovirus-3 infection. *Pediatr Infect Dis J*. 2016;35:1366–1368.
117. Hara S, Kawada J, Kawano Y, et al. Hyperferritinemia in neonatal and infantile human parechovirus-3 infection in comparison with other infectious diseases. *J Infect Chemother*. 2014;20:15–19.
118. Benschop K, Minnaar R, Koen G, et al. Detection of human enterovirus and human parechovirus (HPeV) genotypes from clinical stool samples: polymerase chain reaction and direct molecular typing, culture characteristics, and serotyping. *Diagn Microbiol Infect Dis*. 2010;68:166–173.
119. Wildenbeest JG, Harvala H, Paijkrt D, et al. The need for treatment against human parechoviruses: how, why, when. *Expert Rev Anti Infect Ther*. 2010;8:1417–1429.
120. Esposito S, Rahamat-Langedoen J, Ascolese B, et al. Pediatric parechovirus infections. *J Clin Virol*. 2014;60:84–89.
121. van der Linden L, Wolthers KC, van Kuppeveld FJ. Replication and inhibitors of enteroviruses and parechoviruses. *Viruses*. 2015;7:4529–4562.
122. Wildenbeest JG, Wolthers KC, Straver B, et al. Successful IVIG treatment of human parechovirus-associated dilated cardiomyopathy in an infant. *Pediatrics*. 2013;132:e243–e247.
123. Westerhuis BM, Benschop KS, Koen G, et al. Human memory B cells producing potent cross-neutralizing antibodies against human parechovirus: implications for prevalence, treatment and diagnosis. *J Virol*. 2015;89:7457–7464.
124. Healthcare Infection Control Practices Advisory Committee. Centers for Disease Control and Prevention: 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. www.cdc.gov/hicpac/2007IP/2007ip_appendA.html. Accessed March 17, 2014.

174 Hepatitis A Virus

Francisco Averhoff, Yury Khudyakov, and Claudia Vellozzi

SHORT VIEW SUMMARY

Definition
- Hepatitis A is an acute inflammatory condition of the liver caused by hepatitis A virus (HAV) and characterized by constitutional symptoms including anorexia, fatigue and weight loss, and jaundice.

Microbiology
- HAV is a nonenveloped RNA virus member of the picornavirus family. Three genotypes infect humans (I, II, and III).
- HAV is relatively resistant to heat but can be inactivated at higher temperatures.
- HAV is resistant to organic solvents and detergents and can survive in acidic environments to a pH of 3.
- HAV can be inactivated by hypochlorite (bleach) and quaternary ammonium formulations containing hydrochloric acid (HCl) (found in many toilet cleaners).

Epidemiology
- Transmission is via the fecal-oral route, with peak viral shedding before onset of symptoms.
- Humans are the only important reservoir for HAV.
- Spread is most commonly person to person (including sexual contact) but can occur through foodborne and waterborne transmission, among injection drug users and men who have sex with men, and rarely through use of infected blood products.
- Risk factors in the United States include common-source (foodborne) outbreaks, and close contact (sexual, household or other) with a hepatitis A–infected person in the context of a community outbreak.
- In the United States, the incidence of hepatitis A has decreased dramatically over the past 20 years as a result of implementation of routine vaccination of children. Large community-wide epidemics previously common are now rare. However, high-profile foodborne outbreaks and multistate outbreaks among persons who experience homelessness, persons who use injection and noninjection drugs, and their close direct contacts have emerged; such outbreaks are now more apparent with the overall decreases in background incidence.
- Globally, hepatitis A is a common cause of jaundice and kills an estimated 11,000 persons annually. Disease incidence paradoxically may increase as improvement in living conditions delays infection to older ages, when symptoms are likely to be more severe.

Diagnosis
- Hepatitis A is clinically indistinguishable from other forms of viral hepatitis.
- Laboratory diagnosis is made through detection of immunoglobulin M (IgM) antibodies to HAV in a single, acute-phase serum sample.

Therapy
- Most illnesses are self-limited and can be managed with supportive and symptomatic measures.
- Fulminant cases are rare but need to be managed appropriately and may necessitate transplantation in extremely ill patients.
- Chronic hepatitis A does not occur, although relapse can occur.

Prevention
- In the United States, vaccination with hepatitis A vaccine is recommended for all children at age 12 to 23 months. Globally, few countries recommend hepatitis A vaccination for children. Vaccination of older children may be warranted, and vaccination of international travelers and persons in selected high-risk groups is recommended, including patients with chronic hepatitis B and C (see Table 174.4).
- Administer postexposure prophylaxis with hepatitis A vaccine (preferred) or immune globulin (IG) if exposure occurred within the previous 2 weeks. IG is recommended over vaccination if the patient is younger than 12 months, is immunocompromised, has chronic liver disease, or is a person with a contraindication to vaccine (see Table 174.2).

Hepatitis A is generally an acute, self-limited infection of the liver by an enterically transmitted picornavirus, hepatitis A virus (HAV). Infection may be asymptomatic or result in acute hepatitis. Rarely, fulminant hepatitis can ensue. Although the duration and severity of symptoms vary widely, HAV infections never cause chronic liver disease. The availability of effective vaccines against hepatitis A has markedly affected the epidemiology of hepatitis A in places where the vaccines are widely used, as in the United States (see later).

HISTORY

The earliest accounts of contagious jaundice are from ancient China.[1] Although the signs and symptoms that were described are similar to those currently found in people with hepatitis A, it should be remembered that a number of other infections produce a similar clinical picture. The earliest outbreaks of hepatitis that were almost certainly hepatitis A were documented in Europe in the 17th and 18th centuries, especially during periods of war. From 1855, the disease became known as "catarrhal jaundice" because the pathologists Bamberger and Virchow believed that the disease was caused by blockage of the common bile duct by a plug of inspissated mucus.[2] The first suggestion that the disease was caused by an infectious agent was made by McDonald,[3] who, unable to demonstrate the involvement of enteric bacteria, suggested that the infection might be caused by a virus. Shortly thereafter, Cockayne[4] proposed that the sporadic and epidemic forms of jaundice were manifestations of the same disease. In 1923, Blumer analyzed a large number of epidemics of hepatitis in the United States and identified its predilection for young adults and children and peak incidence in winter and fall.[5] Hepatitis A had an incubation period of between 15 and 49 days and was transmitted by the fecal-oral route.[6–8] Later studies demonstrated that the virus could be detected in feces or blood during the acute infection, that infection could be transmitted experimentally by both the oral and parenteral routes, and that infection was followed by long-term immunity and could be prevented by previous administration of normal human immune globulin (IG).[5,9] In addition, the disease was shown to be associated with a filterable agent resistant to heating at 56°C for 30 minutes and resistant to diethyl ether. In the 1950s and 1960s, Krugman[10] expanded these observations with a series of studies in human volunteers that further defined the incubation period, period of infectivity, and period of viremia, and developed standardized reagents representing hepatitis A. In 1973, Feinstone and colleagues[11] detected

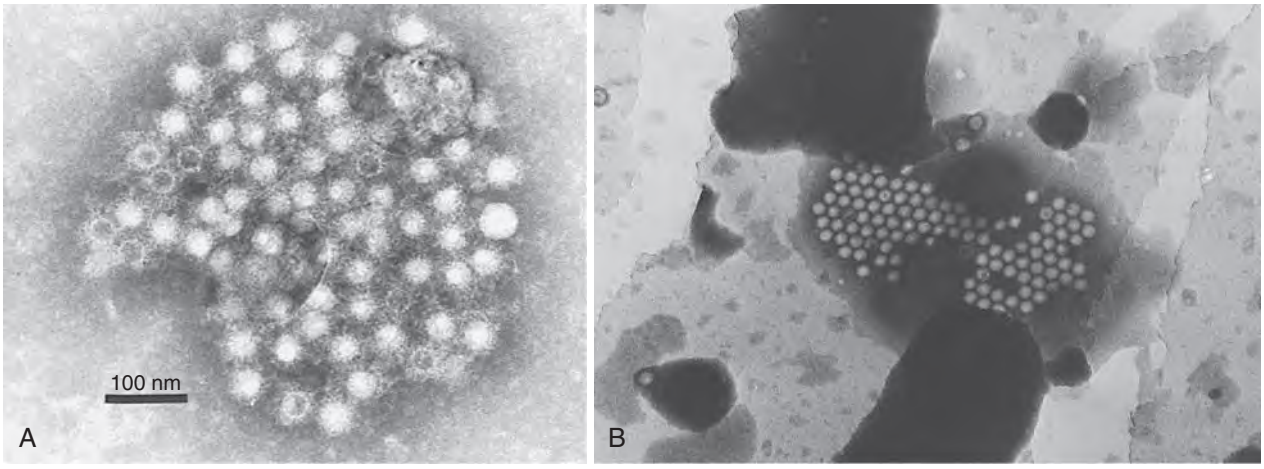

FIG. 174.1 Electron micrographs of hepatitis A virus particles (A) aggregated by antibody, 27 to 28 nm in diameter and highly concentrated, purified hepatitis A virus from human feces (B).

27-nm virus-like particles in the stools of volunteers infected with hepatitis A and demonstrated that they were aggregated by convalescent but not by preinfection serum, thus indicating that the particles represented the etiologic agent of the disease. The identification of HAV, transmission of the disease to marmosets and chimpanzees, propagation of HAV in cell culture, and molecular cloning of the viral genome ushered in a new era of research that culminated almost 2 decades later in the development and licensing of effective vaccines.[12–18]

CLASSIFICATION AND PHYSICOCHEMICAL AND BIOLOGIC PROPERTIES OF HEPATITIS A VIRUS

HAV is a nonenveloped, positive-strand RNA virus member of the Picornaviridae family. This family contains 35 genera such as *Aphthovirus* (foot-and-mouth disease virus), *Enterovirus, Parechovirus,* and others. Although HAV shares general structure and genomic organization with the other picornaviruses, it has limited nucleotide sequence homology and certain distinguishing characteristics that have resulted in its being classified in its own genus, *Hepatovirus.*[19–21] Novel *Hepatovirus* species related to HAV have been found in seals and small mammals.[22,23] Human isolates of HAV are relatively closely related on the basis of partial genomic sequences. However, human HAV strains have been divided into three genotypes—I, II, and III—on the basis of phylogenetic analysis of sequences of the complete VP1 region.[24] The other three genotypes—genotypes IV, V, and VI—are all single simian HAV isolates.[25,26] Phenotypic differences such as disease severity among the genotypes have not been well recognized. Recently, however, some association of fulminant hepatitis with infections with certain genotype IB strains in the United States was suggested.[27] In Korea, patients infected with genotype IIIA were reported to have significantly higher aminotransferase levels, prothrombin time and leukocyte count, and more severe symptoms than patients infected with IA.[28] In addition, mutations in the 2B coding region were shown to be associated with severe and fulminant disease.[29,30] Although antigenic variants exist among all genotypes, there is only one recognized serotype of HAV on the basis of cross-neutralization studies.

Structure

HAV virions recovered from feces of infected individuals are 27- to 28-nm spherical, nonenveloped particles,[11] (Fig. 174.1) with a surface structure suggesting icosahedral symmetry.[31] Good-quality cryoelectron microscopy has confirmed the icosahedral symmetry of the virion with typical picornavirus threefold and fivefold axes and 60 repeated pentamers comprising the capsid (Fig. 174.2). The cryoelectron microscopic views of HAV reveal the plateau surrounding the fivefold axis that is typical for picornaviruses, but not the pit or canyon believed to be the receptor-binding site of enteroviruses and rhinoviruses (see Fig. 174.2). The prominence at the threefold axis of symmetry is distinct from other picornaviruses and is believed to be the major antigenic site at which neutralizing antibodies are targeted. High-resolution x-ray crystallography has confirmed the icosahedral symmetry and the lack of a canyon.[32] It has also revealed that the structure of HAV is notably different from that of typical picornaviruses and bears similarities to insect picorna-like viruses, suggesting that HAV may be a link between modern and primordial picornaviruses.

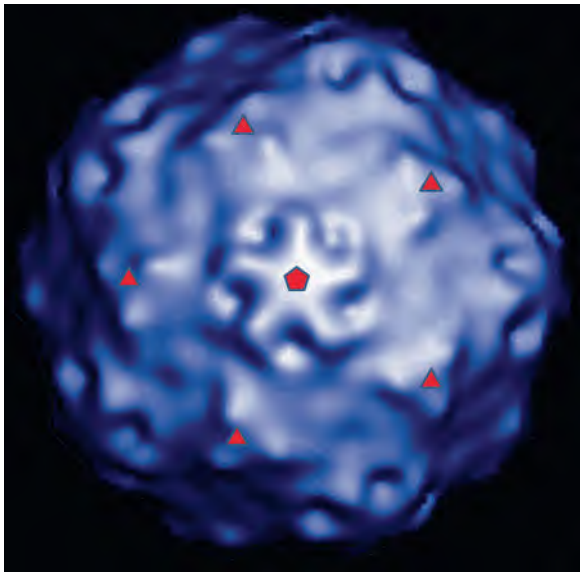

FIG. 174.2 Cryoelectron micrograph showing the surface structure of hepatitis A virus (HAV). The pentagon designates the fivefold axis of symmetry plateau typical of picornaviruses. The triangles designate the prominence of the threefold axis that is unique among picornaviruses and represents the major antigenic site of HAV. *(Courtesy Dr. Holland Cheng, University of California at Davis.)*

Purification of virus from clinical samples or tissue culture yields three distinct populations of particles[33]: (1) mature hepatitis A virions that band at 1.32 to 1.34 g/cm^3 in cesium chloride and sediment at approximately 160 S (similar to enteroviruses and cardioviruses), (2) a lower-density fraction that bands at approximately 1.27 g/cm^3 in cesium chloride, and (3) sediments at 70 to 80 S that may represent empty capsids or particles with incomplete genomes and a high-density fraction (1.4 g/cm^3) that may represent particles with a more open virion structure that allows increased penetration and binding of cesium chloride to

the viral particle. These high-density particles have been shown to contain RNA but tend to be less stable than mature virions.[34,35]

HAV released from cells was shown to be enveloped in host-derived membranes that protect the virion from antibody-mediated neutralization. These quasienveloped virions are fully infectious and circulate in the blood of infected humans. They are secreted nonlytically from infected cells in the form of small extracellular vesicles approximately 50 to 110 nm in diameter. Antibodies to capsid restrict HAV replication after infection with the enveloped virus, suggesting a possible mechanism for postexposure prophylaxis (PEP).[36]

Resistance to Physical and Chemical Agents

HAV is a relatively stable virus under a variety of environmental conditions. HAV is more resistant to heat than other picornaviruses and may not be completely inactivated (depending on the conditions) by exposure to 60°C for 10 to 12 hours.[37–39] Complete inactivation in food requires heating to higher than 85°C for at least 1 minute.[39] Outbreaks of hepatitis A have been reported after ingestion of steamed shellfish, suggesting that the internal temperature achieved by steaming sometimes may be insufficient to destroy the virus.[40] However, HAV can be reliably inactivated by autoclaving (121°C for 30 minutes).[41] Studies have also shown that HAV can be inactivated in shellfish by a nonthermal method using high hydrostatic pressure.[42] The virus is resistant to most organic solvents and detergents and to a pH as low as 3.[41,43] HAV can be inactivated by many common disinfecting chemicals including hypochlorite (bleach) and quaternary ammonium formulations containing 23% hydrochloric acid (HCl) (found in many toilet bowl cleaners).[41] Currently licensed vaccines are inactivated by 1:4000 formalin at room temperature for at least 15 days to exceed complete inactivation by at least threefold. As a result of several outbreaks of hepatitis A in hemophiliacs who received factor VIII concentrates that had been treated by means of a solvent detergent method for inactivation of lipid-enveloped viruses, interest has focused on techniques capable of inactivating nonenveloped viruses without compromising the biologic activity of the product.[44] Various manufacturers use different techniques to inactivate or remove HAV and human parvovirus B19. These include nanofiltration and other purification techniques, sensitive nucleic acid testing methods such as polymerase chain reaction (PCR) on minipools of source plasma, pasteurization at 60°C for 10 hours, and dry heat on lyophilized products.

Genome and Proteins

Although HAV had the characteristics of a picornavirus and indirect tests suggested an RNA genome, molecular cloning and sequence analysis demonstrated that the HAV genome is composed of single-stranded, positive-sense linear RNA of 7478 nucleotides (strain HM175) and a molecular weight of approximately 2.25×10^6 Da, with an overall structure and gene order typical of picornaviruses.[17,19,45]

The 5′ end of the genome does not have a cap structure, but instead, as is typical for picornaviruses, has a small, covalently bound protein termed VPg.[42] The genome itself has a long 5′ untranslated region beginning with UU as found in all picornaviruses. This 5′ untranslated region folds to form a highly ordered secondary structure that is important for both replication and translation. These structured regions include an internal ribosome entry site (IRES) that directs the initiation of translation at either of two AUG codons at nucleotide positions 735–737 and 741–743 (Fig. 174.3).[45] The AUG codon initiates translation of a single long open reading frame of 6681 nucleotides that encodes a polyprotein that is 2227 amino-acid residues in length. The coding region of picornaviruses has been arbitrarily divided into three parts, termed P1, P2, and P3, and the peptides that are ultimately cleaved from the translation products of these regions are referred to as 1A, 1B, 1C, 2A, 2B, 2C, and so forth, in order of translation from the 5′ to the 3′ end of the genome.[46] The HAV genome ends with a 3′ noncoding region of 63 nucleotides that is followed by a virus-encoded poly (A) tail.

A peculiar property of the HAV coding region is a highly deoptimized codon usage. The HAV genome is significantly biased against codons used abundantly by human cells. The accumulation of rare codons was hypothesized to result in the reduced translation rate and modulation of translation kinetics of HAV RNA, thus playing an important role in HAV replication and evolution.[47,48]

The four capsid proteins of mature virus particles are coded by the 5′-end 2373 nucleotides of the long open reading frame (P1 part) and the nonstructural proteins by the remainder (P2 and P3 parts). The four HAV capsid polypeptides named by analogy with other picornaviruses are referred to in descending order of size as virion proteins (VP): VP1 = peptide 1D (molecular weight, 32,800 Da), VP2 = 1B (24,800 Da), VP3 = 1C (27,300 Da), and VP4 = 1A (2500 Da).[49,50] A VP0 protein (1AB) that is the precursor to VP4 and VP2 can also be detected, especially from cell cultures where immature virions (provirions) may accumulate in large amounts.[51] The VP4 molecule is believed to be liberated during the maturation cleavage of VP0, which converts

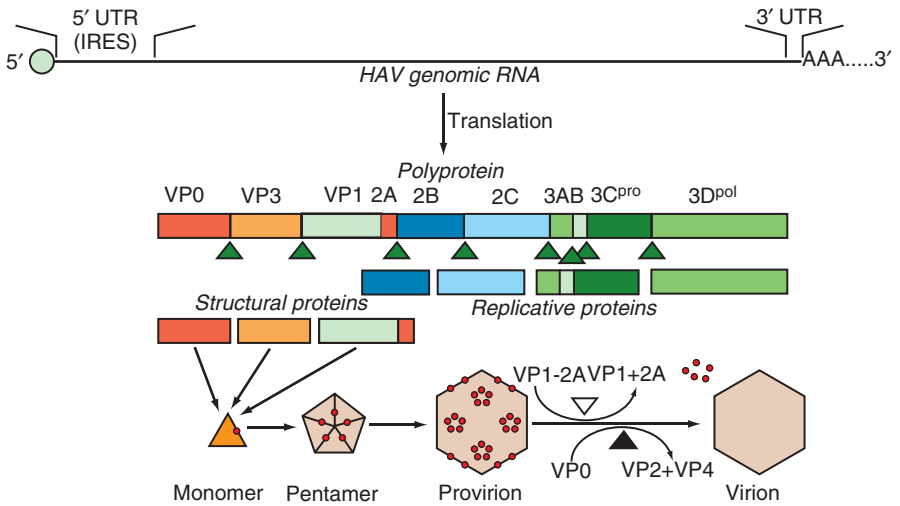

FIG. 174.3 Organization of the RNA genome of hepatitis A virus (HAV), polyprotein cleavage, and viral assembly. The 7.5-kb positive-strand RNA is covalently attached to VPg (5′ end) and has a poly (A) tail. The 5′ untranslated region *(UTR)* of 734 nucleotides functions as an internal ribosome entry site *(IRES)* to initiate translation *(vertical arrow)* of the precursor polyprotein of 2227 amino acids. Regions of the polyprotein are indicated according to standard nomenclature. The single viral protease, $3C^{pro}$, cleaves itself from the polyprotein and subsequently cleaves elsewhere in the polyprotein to yield the structural protein precursors VP0, VP3, and VP1-2A (PX) and replicative proteins 2B, 2C, 3A, 3B, and 3D (RNA-dependent RNA polymerase). VP0, VP3, and VP1-2A probably remain associated as a monomer and then form pentamers that are a stable precursor in capsid formation. Assembly of 12 pentamers together with RNA forms the provirion, after which 2A is susceptible to cleavage by host cell protease(s) *(open arrowhead)*. The final maturation cleavage of VP0 to VP2 and VP4 *(solid arrowhead)* is dependent on the encapsidated viral RNA.

provirions to virions (see later), but VP4 has never been experimentally determined to be within the virion particle and, at just 23 amino acids, is approximately one-third the size of the VP4 proteins of other picornaviruses.

Assembly of HAV particles proceeds through several steps (see Fig. 174.3). Cleavage of the polyprotein by the 3C protease yields three capsid-related proteins—VP0, VP3, and VP1-2A (also known as PX)—which constitute a monomer and subsequently assemble into pentameric subunits. Twelve copies of the pentamer then associate with viral RNA to form provirions or to form empty capsids (procapsids) without viral RNA. The involvement of the VP1-2A precursor in assembly is unique to HAV, and it has been shown that the 2A extension is essential for proper processing and assembly of the pentameric subunit.[52–54] After assembly, 2A is removed from VP1 by cellular proteases,[55,56] and in the final maturation step, VP0 is cleaved to yield VP2 and VP4. The VP0 cleavage is dependent on the presence of viral RNA in the particle, and procapsids therefore fail to cleave VP0, but HAV procapsids are quite stable and seem to have the same antigenic structure as mature virions.

Antigenic Composition and Viral Diversity

Although a variety of genotypes of HAV have been identified through analysis of genome sequences (Fig. 174.4), there seems to be only one serotype.[57–59] This view is supported by the observation that IG prepared in developed countries and monovalent vaccines prepared from strains originating in Australia, Central America, or Europe protect travelers from disease equally well, irrespective of their destination.[60,61]

Neutralization sites for HAV are located primarily on the structural proteins VP1 and VP3, with possibly a minor contribution from VP2.[57] Monoclonal antibodies in competition binding assays and the generation of neutralization escape mutants suggest that the dominant neutralization site is composed of overlapping epitopes on VP1 and VP3 that combine to form a conformational antigenic site at which neutralizing antibodies are targeted.[57,59,62]

Classification of HAV into genotypes and subtypes through use of a 168-nucleotide fragment containing the VP1/P2A junction was originally suggested. Genotypes differ by at least 15% and subtype by 7% to 7.5% from each other in this region.[26] Seven genotypes, I to VII, identified with this approach were recently reclassified into six genotypes, I to VI, using the complete VP1 region. Genotypes I, II, and III are divided into subtypes A and B. The former genotype VII is classified as subtype IIB (2). These three genotypes infect humans, whereas genotypes IV to VI have a simian origin.[26,63,64] Genotypes have a particular geographic distribution.[26,65,66] Genotype I is most prevalent worldwide, with subtype IA being more common than IB, and is the most common in the United States.[26,65] Although IB usually constitutes only a small fraction of genotype I strains circulating in South and North America, Europe, Asia, and Africa,[64] it was reported among 35% to 70% of patients infected with HAV genotype I in Brazil and was identified in an outbreak in Thailand.[67–69] Genotype IB is predominant in the Middle East[70,71] and South Africa.[72] Genotype II isolates originally identified in France in 1979 and Sierra Leone in 1988[26] are not frequently reported. Subtype IIA was suggested to have West African origin.[73] Genotype III can be identified globally,[64] but it is endemic in Southeast and Central Asia.[26] An increase in genotype IIIA infections was reported in Korea and Russia.[28,74] In India, reported hepatitis A outbreaks have all been caused by genotype IIIA infections.[75–77] Genomic heterogeneity of HAV is used to track transmissions during outbreak investigations,[66] with the VP1/P2A region being most frequently applied for genetic identification of HAV strains.[66,78] Although individual strains of HAV have differences at the molecular level that may be useful for epidemiologic studies, a high degree of identity in nucleic acid (up to 90%) and amino-acid sequence (up to 98%) is generally seen between strains.[66,79]

Biology of Hepatitis A Virus in Cell Culture

HAV was first propagated in marmoset liver explant cultures and a cloned line of fetal rhesus monkey kidney cells (FRhK-6) with a strain of virus (CR326) that had been adapted by multiple passages in *Saguinus mystax* and *Saguinus labiatus* marmosets.[14] Many HAV strains have subsequently been isolated from clinical material, although the procedure may take several weeks. Until recently, only epithelial or fibroblast cells of primate origin had been shown to support growth of the virus.[15,16,80] However, in a systematic search for cells that would support HAV replication, growth was detected in cells of guinea pig, dolphin, and porcine origin.[81]

The major characteristics of HAV in cell culture are slow growth and low yields relative to other picornaviruses, which is likely associated with the inefficient IRES,[82–84] inability to induce the cellular protein synthesis shutoff,[85] and highly deoptimized codon use.[47,48] In addition, the virus remains largely cell associated, does not usually produce a cytopathic effect, and readily leads to persistently infected cell lines.[86] With adaptation, more rapid replication and higher yields can be obtained and cytopathic variants have been selected. These cell culture–adapted viruses are useful for virus titrations, neutralization, inactivation kinetics, and virus replication and have made the production of inactivated vaccines practical.[14,16,87–89]

In one study, direct isolation of a wild-type strain of HAV was achieved through use of a modified cell line.[90] Detection of HAV in either patients' or environmental samples is now done primarily using PCR or other nucleic acid–based technology. The kinetics of viral replication and biosynthetic events has been studied in cells infected with cell culture–adapted strains of HAV, and a number of differences from most other picornaviruses have been revealed. After attachment to cells, the uncoating of virus is delayed for more than 8 hours[91,92]; this exceeds the duration of an entire growth cycle for many picornaviruses. The delayed uncoating seems to be related to the protracted maturation cleavage of VP0 to VP2 and VP4 because virions are uncoated more rapidly than provirions.[93] Accumulation of new viral RNA can be detected as early as 12 hours after infection of BS-C-1 cells with a fast-growing, cytopathic variant of strain HM175, but levels of viral replicative intermediates (double-stranded RNAs) remain much lower than in cells infected with other picornaviruses.[91] As outlined previously, translation of the viral polyprotein is directed by the IRES within the 5′ untranslated region, but the IRES of HAV is relatively inefficient, with initiation approximately 1% of that seen for the IRES of encephalomyocarditis virus.[82–84]

The initial proteolytic processing of HAV polyprotein is accomplished by the viral 3C protease, and assembly of viral particles proceeds via monomers and pentamers (see Fig. 174.3).[52] After infection of cell cultures with rapidly replicating and cytopathic variants of HAV, pentamers are first detected at 9 hours after infection and reach peak levels after approximately 18 hours even though the amount of viral RNA (and presumably viral translation) increases beyond this time.[94] These cells continue to produce virus for 2 to 3 days before cell death, whereas most HAV variants progress to a persistent infection with reduced levels of virus production over many weeks and subsequent cell passages.

Repeated passage in cell culture has been used to apply mutational pressure to HAV to alter the phenotype. For example, HAV variants that grow more rapidly or are resistant to neutralization by monoclonal antibodies have been selected.[89,95,96] Attenuated strains of HAV have been selected by means of multiple tissue culture passages, and cold adaptation has been achieved by means of passage at reduced temperature.[31,97] Some of the mutations responsible for these altered phenotypes have been identified through molecular cloning and sequencing of the mutant. Mutations within the 5′ untranslated region and mutations within the 2B and 2C coding regions of HAV RNA have been shown to enhance virus replication in vitro.[96,98,99] However, mutations within the VP1-2A and 2C proteins seem to be most important for attenuation of virulence.[96]

Most viruses initiate infection by first binding to a specific cell surface receptor molecule or, in many cases, may require binding to both receptors and coreceptors to facilitate virus entry and uncoating. Identification of a specific receptor for HAV remained elusive for many years. However, Kaplan and colleagues[100] succeeded in the isolation of one specific receptor molecule, havcr-1, first in cells of simian origin and later in human cells.[101] This molecule is a novel mucin-like class I integral membrane glycoprotein of 451 amino acids, known as T-cell immunoglobulin and mucin domain1 (TIM-1), with the amino-terminal, cysteine-rich domain responsible for binding to HAV.[101] Because this

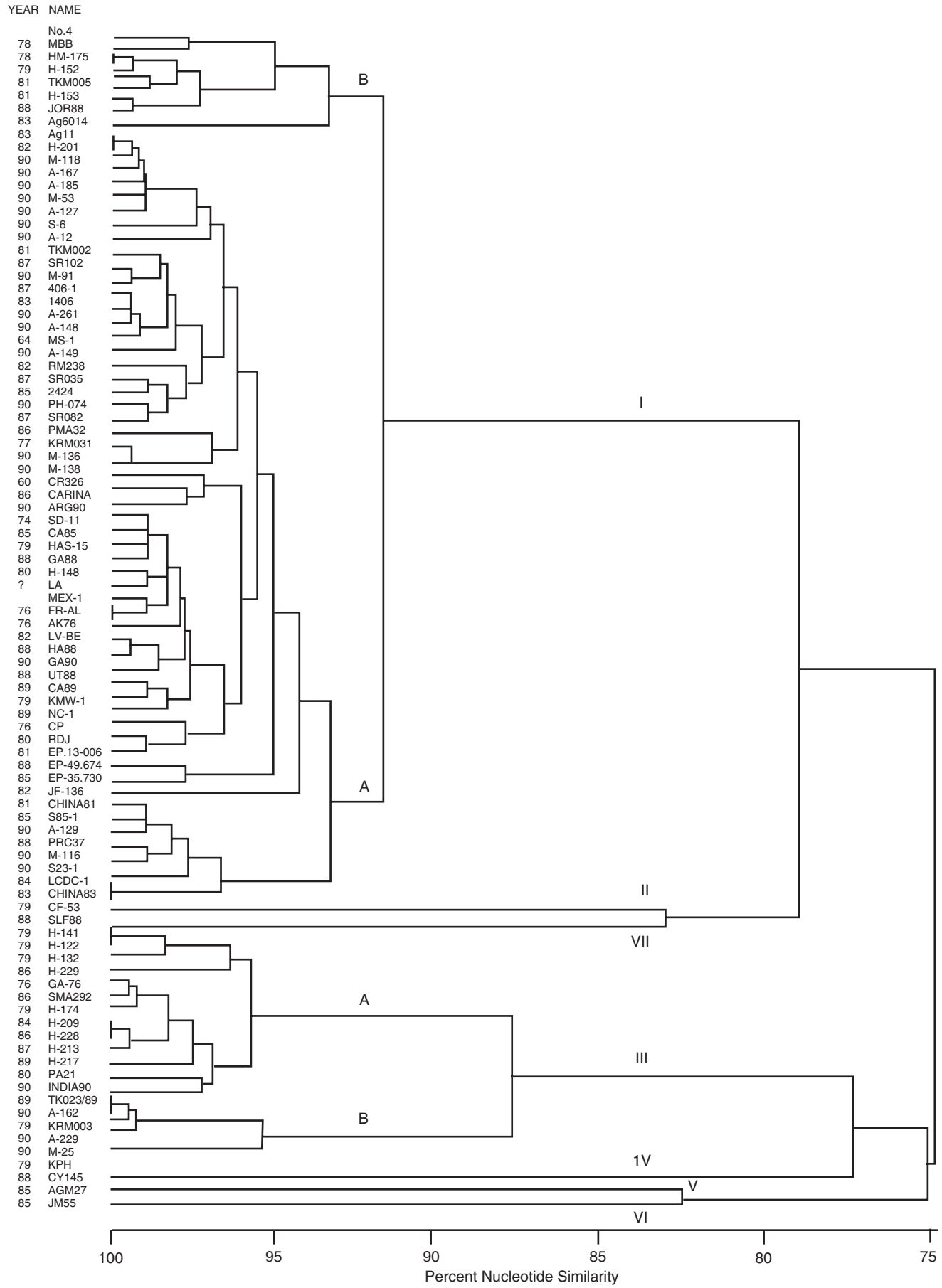

FIG. 174.4 Relationship among hepatitis A virus strains determined by the sequence of the VP1-2A region. Nucleotide sequence alignments of 92 isolates were compared. The genotypes were divided into groups I to VII, and groups I and III were divided into subgroups A and B. *(Courtesy S.M. Lemon, Galveston, TX.)*

molecule is expressed on cells from many tissues that are not susceptible to HAV infection, it is likely that specific coreceptors contribute to the organ tropism of HAV. However, a soluble form of havcr-1 has some ability to neutralize particles of HAV directly, which is consistent with roles in both attachment and uncoating of virus.[102]

It remains possible that HAV may use other pathways for cell entry in addition to havcr-1. Studies have demonstrated that the asialoglycoprotein receptor can also mediate infection of cells with HAV when the virus is first complexed with specific immunoglobulin A (IgA), leading to the interesting hypothesis that IgA may play a role as both carrier and targeting molecule during infection and transmission, particularly in relapsing cases of HAV.[103]

Two forms of HAV virions, naked and quasienveloped, appear to use different mechanisms for cell entry. The quasienveloped HAV enters cells via an endocytic pathway. Slow removal of the membrane in endosomal compartment exposes the capsid to neutralizing antibody for 6 to 8 hours but allows for interaction with the cell receptor. The naked HAV uncoats rapidly without endosomal acidifications and is not affected by postendocytic neutralization. Both forms likely bind to the same cell receptor, TIM-1 (HAVCR1).[104] However, recent studies have shown that TIM-1 does not seem to be essential for cell entry of either HAV form.[104]

Host Range

Humans are considered to be the only important reservoir of HAV. However, the existence of extrahuman reservoirs of infection remains possible. In 1961, Hillis[105] described an outbreak of hepatitis A among chimpanzee handlers who apparently contracted the infection from the chimpanzees. Epidemiologic data suggested that the animals had become infected during captivity but before their importation into the United States. Interesting to note, although epidemics of hepatitis were recognized in American primate handlers, the disease was rarely seen in Africa, presumably because most handlers were already immune. Widespread screening of nonhuman primates revealed antibodies to HAV in chimpanzees, gorillas, orangutans, gibbons, macaques, owl monkeys, pigtail monkeys, rhesus monkeys, and several species of South American tamarin monkeys.[106,107] It is unclear whether such primates may serve as reservoirs of infection or as transient hosts after exposure to HAV from human sources. However, both the PA21 and AGM-27 strains of HAV seem to be true simian viruses.[108,109] Interesting to note, the AGM-27 virus produces attenuated disease in chimpanzees and was the subject of some study as a potential live-attenuated vaccine.[110]

Deinhardt[111] pioneered the use of nonhuman primates for hepatitis A studies in both chimpanzees and tamarins (*Saguinus* species). He showed that in chimpanzees, liver function test abnormalities developed on inoculation with known infectious material of human origin. Since that time, the chimpanzee, several species of tamarins, and the owl monkey have all been shown to be susceptible to HAV infection and to develop hepatitis.[111] These animals have been valuable tools in the study of hepatitis A pathogenesis and for the development of vaccines. Recently, it was shown that guinea pigs express a receptor similar to the human HAV receptor and that both guinea pig cells and guinea pigs can be infected with HAV. However, the animals did not have evidence of hepatitis.[81,112]

Molecular mechanisms of the HAV host specificity are not well understood. Recent research using a murine model of HAV infection suggested a role of the intrahepatic immune responses in defining the host range, with the HAV evasion of MAVS-mediated type I interferon responses being essential for restricting the infection to humans and nonhuman primates.[113] Although restricted in its natural infection of host species, adaptation of HAV to growth in murine and guinea pig cells suggests a certain capability to replicate in other hosts.[112,114]

EPIDEMIOLOGY

Modes of Transmission

HAV is primarily transmitted via the fecal-oral route. After replication in the liver, the virus is excreted in bile and is found in highest concentrations in stool. In experimental studies, infectivity of stools was demonstrated from 21 days before to 8 days after onset of jaundice, but the highest concentrations occur during the 2-week period before jaundice

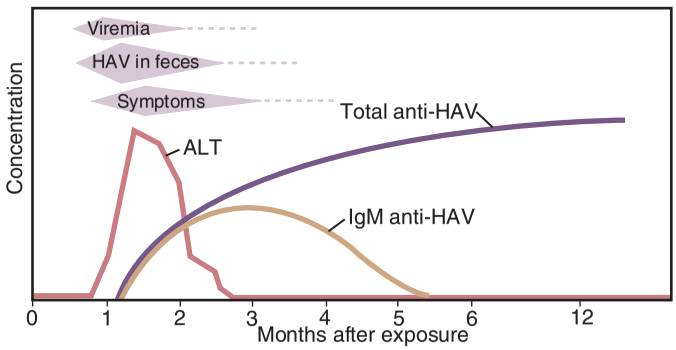

FIG. 174.5 Clinical, virologic, and serologic events associated with hepatitis A virus *(HAV)* infection. *ALT,* Alanine aminotransferase.

develops or liver enzymes increase, followed by a rapid decrease after the appearance of jaundice (Fig. 174.5).[11,115,116] Data from epidemiologic studies also suggest that peak infectivity (risk of transmission) occurs during the 2 weeks before the onset of symptoms.[117] Shedding of HAV in stool may continue for longer periods in infected infants and children than in adults. HAV RNA has been detected in stool of infected newborns for as long as 6 months after infection.[118] Excretion in older children and adults was demonstrated 1 to 3 months after clinical illness.[61,118] Although chronic shedding of HAV does not occur, the virus has been detected in stool during relapsing illness.[119]

During the period of viremia, which begins during the prodrome and extends through the period of increased liver enzymes (see Fig. 174.5), HAV concentrations in serum are several orders of magnitude lower than in stool.[120–122] However, in experiments conducted in nonhuman primates, HAV was several orders of magnitude more infectious when administered via the intravenous compared with the oral route, and animals were successfully infected with low concentrations of HAV administered via the intravenous route.[123] Although HAV may occasionally be detected in saliva in experimentally infected animals and may be present in human saliva,[124,125] transmission by saliva has not been demonstrated.

The infectious dose of HAV in humans is unknown. Enzyme immunoassays and PCR may detect defective (noninfectious) and infectious viral particles. Thus, the detection of HAV antigen in the stool with enzyme immunoassays or HAV RNA in the serum, stool, or saliva with PCR does not mean that an infected person is necessarily infectious, and it is likely that the period of infectivity is shorter than the period during which HAV RNA is detectable. For practical purposes, both children and adults with hepatitis A can be assumed to be noninfectious 1 week after jaundice appears.

Person to Person

Person-to-person transmission via the fecal-oral route remains the primary means of HAV transmission throughout most of the world.[126,127] Transmission can occur among close contacts, particularly in households and extended family settings.[128] In the United States prior to the introduction of hepatitis A vaccine, young children had the highest rates of infection, and children often served as a source of HAV infection for others because HAV infection is often asymptomatic and standards of hygiene are generally lower in young children compared with adults, as in daycare facilities.[128–130] Since the introduction of vaccine, person-to-person transmission in the United States is now more commonly seen among special populations including people experiencing homelessness, people who use injection and noninjection drugs, and men who have sex with men (MSM).[131,131a]

Similar patterns are emerging in Western Europe.[132–134]

Foodborne and Waterborne

HAV can remain infectious in the environment for long periods of time,[135] allowing for common source outbreaks and sporadic cases to occur from exposure to fecally contaminated food or water. Many uncooked foods have been recognized as the source of outbreaks. Cooked

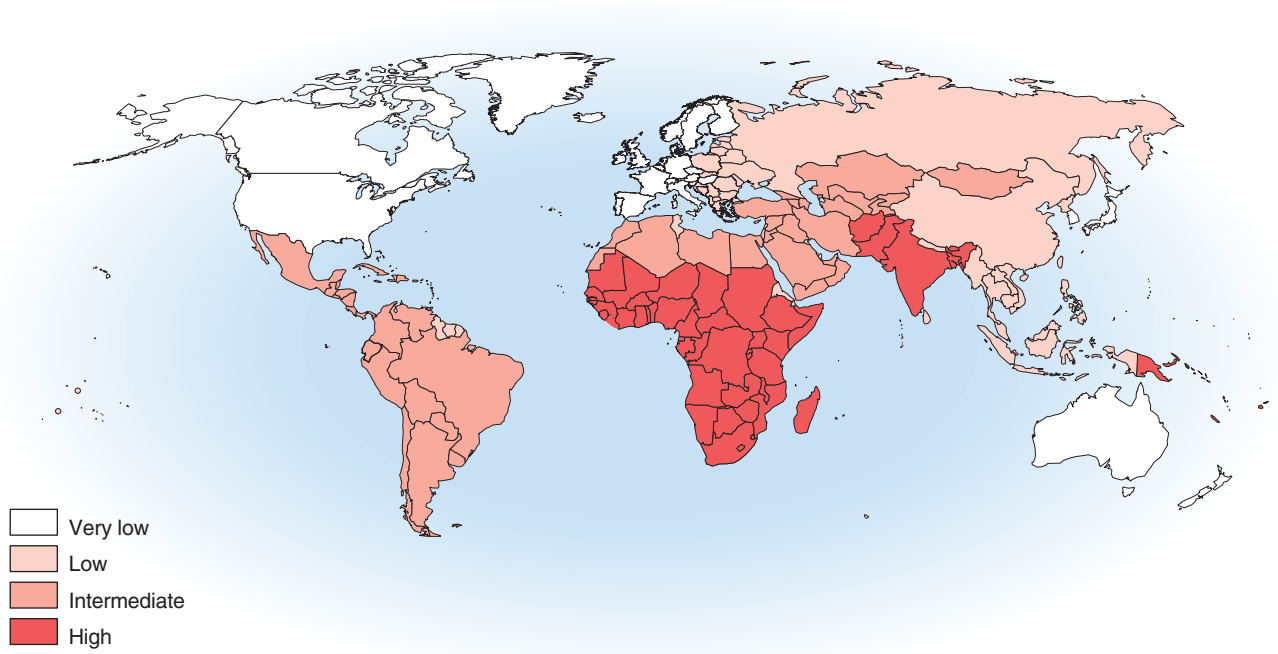

FIG. 174.6 World map indicating patterns of endemicity of hepatitis A virus infection, 2005. The patterns of high, transitional, intermediate, low, and very low endemicity are shown. *(Modified from Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005.* Vaccine. *2010;28:6653–6657.)*

foods also can transmit HAV if the cooking is inadequate to kill the virus or if the food is contaminated after cooking, as commonly occurs in outbreaks associated with infected food handlers.[136,138,139] Contaminated shellfish were responsible for a large outbreak in Shanghai, China, in 1988.[140,141] Although such outbreaks continue to be reported,[142–144,145,146] shellfish-associated HAV outbreaks in the United States and other developed countries have become increasingly uncommon. In Italy, detection of HAV in samples of shellfish has decreased, believed to be the result of hepatitis A vaccination programs in that country.[147] Several large outbreaks traced to produce contaminated before distribution have been reported in recent years from the United States, Europe, and Australia.[148–150,151–157] Waterborne outbreaks of hepatitis A are also uncommon in developed countries, and are difficult to differentiate from other forms of transmission in the absence of well-designed studies in less developed countries. Molecular techniques now allow for identification of common source foodborne hepatitis A outbreaks around the globe.[153–155,158]

Bloodborne

Transfusion-related hepatitis A is rare because HAV does not result in chronic infection, and, in the developed world, blood donors have been screened for many years for elevated aminotransferase levels, further decreasing the risk of transmission during the viremic prejaundice phase of infection. However, transmission through transfusion of blood or blood derivatives collected from donors during the viremic phase of their infection has been reported, including outbreaks in Europe and the United States among patients who received factor VIII and IX concentrates prepared with solvent-detergent treatment to inactivate lipid-containing viruses.[44,122,159,160–162] HAV is resistant to solvent-detergent treatment, and contamination presumably occurred from plasma donors with hepatitis A who donated during the incubation period. The degree to which transfusion-related transmission occurs in countries where screening for blood and blood products may be limited is unknown.

Vertical

Two published case reports describe intrauterine transmission of HAV during the first trimester, resulting in fetal meconium peritonitis.[163,164] After delivery, both infants were found to have a perforated ileum. The risk of transmission from pregnant women who develop hepatitis A in the third trimester of pregnancy to newborns appears to be low.[165] However, newborns who acquire infection in this manner are usually asymptomatic, and an outbreak among hospital staff related to exposure to such an infant has been reported.[166]

Worldwide Disease Patterns

Globally, tens of millions of hepatitis A infections occur annually,[167–169] resulting in an estimated 11,000 deaths annually from hepatitis A.[170] Although hepatitis A occurs worldwide, major geographic differences exist in endemicity and resulting varying epidemiologic patterns (Fig. 174.6).[167–169] The degree of endemicity is closely related to hygienic and sanitary conditions and other indicators of the level of development. In less developed areas, especially when there is limited access to clean water and inadequate disposal of human feces, HAV infects most people early in life, when infection is rarely clinically apparent When high standards of hygiene and sanitation apply, the majority of adults remain susceptible. Distinct patterns of HAV infection can be described, each characterized by particular age-specific anti-HAV prevalence (Fig. 174.7) and hepatitis A incidence; these patterns are a result of prevailing environmental (hygienic and sanitary) and socioeconomic conditions.[126,167–169]

In areas of high endemicity, represented by the least developed countries (i.e., parts of Africa and South Asia), poor hygienic and sanitary conditions allow HAV to spread readily (see Figs. 174.6 and 174.7). Infection is nearly universal in early childhood, when asymptomatic infection predominates, and most of the population is infected before reaching adolescence, as demonstrated by the age-specific prevalence of anti-HAV (see Fig. 174.7).[167,171,172] Because most adults are immune, reported disease rates in this population are low and few outbreaks occur. However, susceptible adolescents and adults in these areas are at high risk for hepatitis A. Recent surveillance data have demonstrated increasing numbers of cases among adults and frequent outbreaks, and small seroprevalence studies have identified greater susceptibility of some children into adolescence, in some highly endemic countries.[173–176] Differences in susceptibility to hepatitis A in low- and middle-income countries can be defined by socioeconomic status, wherein more affluent children living under more hygienic conditions are more likely to escape HAV infection as infants and young children, leaving them susceptible to HAV infection as adolescents and adults.[177] In past years, some ethnic

or geographically defined groups within highly developed countries also experienced high endemicity, including Native Americans, aboriginal populations in Australia, and Roma populations in Slovakia.[178–180]

In areas of intermediate endemicity, HAV is not transmitted as readily because of better sanitary and living conditions, and the predominant age at infection is older than in areas of high endemicity (see Figs. 174.6 and 174.7).[168,181] Paradoxically, the overall incidence and average age in reported cases are often higher than in highly endemic areas because high levels of virus circulate in a population that includes many susceptible older children, adolescents, and young adults who are more likely to develop symptoms with HAV infection.[182] Large common source food- and water-associated outbreaks can occur because of the relatively high rate of virus transmission and large number of susceptible persons, especially among those of higher socioeconomic level. The largest such outbreak was reported from Shanghai in 1988, with more than 300,000 cases associated with consumption of clams harvested from water contaminated with human sewage.[140] Nevertheless, person-to-person transmission in community-wide epidemics continues to account for much of the disease in these countries.

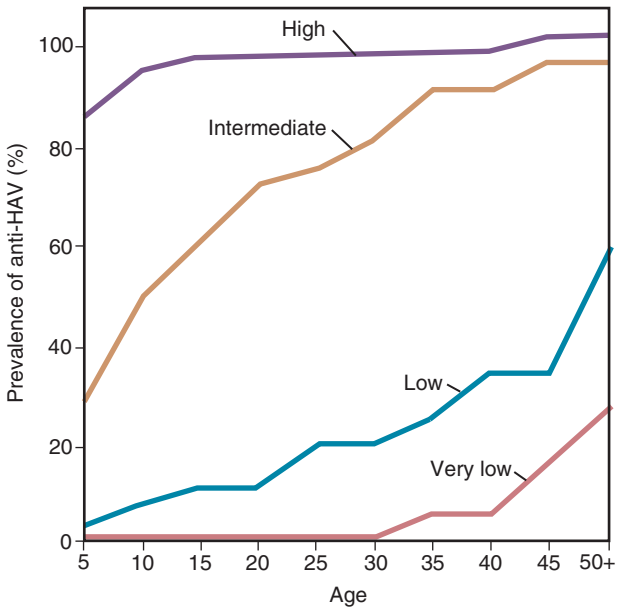

FIG. 174.7 Patterns of hepatitis A virus *(HAV)* infection worldwide. Age-specific prevalence of anti-HAV in areas of high, intermediate, low, and very low endemicity is shown.

Shifts in age-specific prevalence patterns that reflect a transition from high to intermediate endemicity are occurring in many parts of the world (see Fig. 174.6).[183–188] A feature of this transitional pattern is striking variations in hepatitis A epidemiology among countries and within countries and cities, with some areas displaying a pattern typical of high endemicity and others of intermediate endemicity.[126,183,189–194,195–200] Considerable hepatitis A–related morbidity and associated costs occur with this transition, even in developing countries.[201,202] For example, hepatitis A was the cause of fulminant hepatitis in two-thirds of children presenting to two hospitals in Argentina during a 15-year period, and, in one of these hospitals in which liver transplantations were performed, one-third of liver transplantations among children were performed for fulminant hepatitis A.[201]

In high-income, developed countries, including the United States, Canada, and much of western Europe, the endemicity of HAV infection is low or very low (see Fig. 174.6). Few children are infected, the incidence of disease is generally low, and disease is usually sporadic. Community-wide outbreaks are becoming increasingly rare; in the United States this may be because of routine hepatitis A vaccination of children.[126,162,179,203–207] Population-based seroprevalence surveys show a gradual increase in the prevalence of anti-HAV with increasing age, primarily reflecting declining incidence, changing endemicity, and resultant lower childhood infection rates over time. In countries where hepatitis A vaccine has been introduced, such as the United States, anti-HAV prevalence is increasing among children as a result of vaccination.[208] In low-and very-low-endemicity countries, most cases occur in defined risk groups such as travelers returning from endemic areas, MSM, and users of injection drugs.[162,207,209–214] The United States has recently experienced outbreaks among people experiencing homelessness and people who use injection and noninjection drugs.[131a] There is documented risk of hepatitis A among children and other contacts of family members who are residing in low endemic countries and travel to their high or intermediate endemic country of origin.[215–218] An emerging risk for hepatitis A transmission in low endemic countries stems from asylum seekers or other persons displaced from their home country owing to conflict.[219–221]

Epidemiology in the United States

Although large national outbreaks were recognized during the 1950s, data collection for hepatitis A in the United States started in 1966. Surveillance data demonstrates that periodic outbreaks, approximately every decade, occurred through the 1990s, before hepatitis A vaccine was widely available (Fig. 174.8).[222,223] The highest rates and the majority of cases occurred in the western and southwestern states.[224–226] With the advent of routine vaccination of children, large community-wide outbreaks and cyclic peaks have ceased. The geographic variations that characterized hepatitis A incidence in the past have also disappeared.[225]

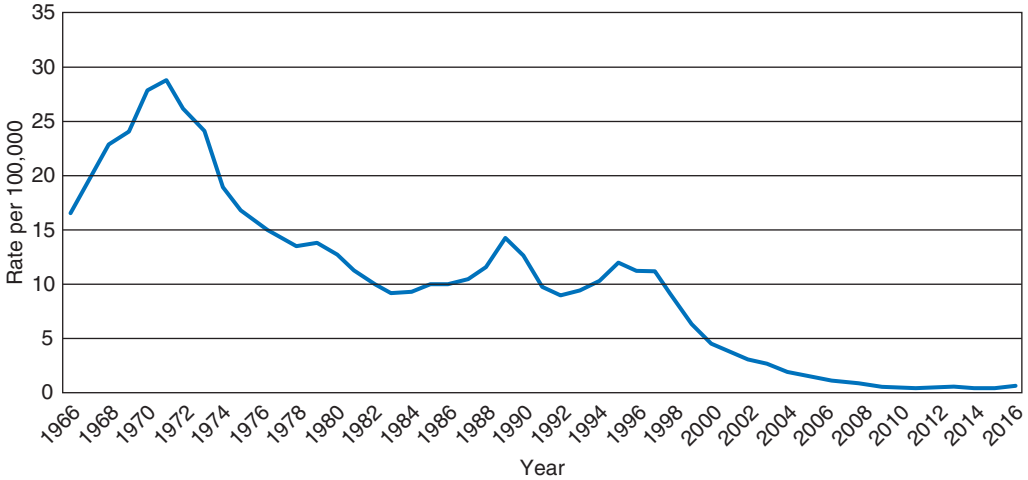

FIG. 174.8 Hepatitis A incidence, United States, 1966–2016. *(From Centers for Disease Control and Prevention, National Notifiable Diseases Surveillance System, Atlanta, GA.)*

In 1995, when hepatitis A vaccine was licensed in the United States, more than 31,000 cases of hepatitis A were reported (incidence, 12 cases per 100,000 persons), making hepatitis A one of the most frequently reported vaccine-preventable diseases.[227] In 1996–99 the Advisory Committee on Immunization Practices (ACIP) recommended targeted vaccination for children at 2 years of age in states with high hepatitis A rates.[224,228] Incidence rates declined sharply after implementation of these recommendations (see Fig. 174.8).[223,229] In 2006, universal vaccination was recommended in all states for all US infants starting at 1 year of age.[226] The number of cases reported annually in the United States dropped steadily from the late 1990s through 2010, plateauing at between 1500 and 2000 cases reported per year (i.e., <1 case per 100,000 population) through 2016.[223] Since 2012, high-profile multistate outbreaks of hepatitis A and increased reporting associated with heightened awareness around the outbreaks may have accounted for the plateauing of the number of reports.[155] Nevertheless, it is clear that hepatitis A rates have declined since vaccine introduction from 11.7 cases per 100,000 population in 1996 to less than 1 per 100,000 population in 2016, a greater than 10-fold decline.[229a]

Young children typically have asymptomatic or unrecognized infections, and have historically, before vaccine introduction, played an important role in the epidemiology of hepatitis A in the United States, serving as a major reservoir for HAV transmission.[230] Before vaccine introduction in the United States, the highest hepatitis A rates were reported among children 5 to 14 years old, with approximately one-third of cases occurring among children younger than 15 years;[224] the majority of infections were among asymptomatic children <5 years of age.[130] Hepatitis A rates among children have declined more sharply than among adults since vaccine introduction and, through 2016, rates among both adults and children have been around 1 case per 100,000, or less, with children <10 years of age having the lowest rates of any age group (Fig. 174.9).[223] Historically, rates of hepatitis A among Hispanics and Native Americans/Alaska Natives have been higher than those of other racial or ethnic populations, but currently these differences have largely disappeared.[223] Mortality associated with hepatitis A in the United States is uncommon; 60 to 80 cases were reported annually during 2012 to 2016, with highest rates among persons aged 55 years and older.[223]

In the United States, before the introduction of vaccine when community outbreaks and outbreaks in child care centers were common, studies of outbreaks wherein serologic testing of household contacts was performed found that 25% to 40% of contacts <6 years of age had serologic evidence of acute HAV infection,[128,231] resulting in some immunity among children as they entered adolescence and adulthood.[232,233] A nationally representative serosurvey conducted during 2007–2010 found that among persons ages 6 to 19, the prevalence of anti-HAV was 37.6%, compared with an anti-HAV prevalence of 13.1% conducted during 1988–94, before the availability of hepatitis A vaccine, reflecting increasing immunity among children due to vaccination. However, results from serial national serosurveys among the entire population conducted for the periods 1988–94 and 2003–06 indicate an overall decline in the age-adjusted seroprevalence of anti-HAV in the US population from 32.5% in 1988–94 to 26.7% in 2003–06, reflecting the decreased incidence in the country over time.[208] Substantial declines in HAV incidence, likely fueled in part by the childhood vaccination program, have resulted in an overall decrease in seroprevalence rates among US adults and therefore an increase in susceptibility to HAV among older adults.[208,234] A 2013 multistate outbreak of hepatitis A occurred largely in adults, with few cases among vaccinated and unvaccinated children.[155] In summary, the epidemiology of hepatitis A in the United States has fundamentally changed as endemic transmission, outbreaks in child care centers, and large community outbreaks have been virtually eliminated, in part through improvements in hygiene, but largely through the childhood vaccination program. We are likely to continue to see a greater proportion of cases among adults due to international travel, outbreaks associated with MSM, outbreaks associated with imported food contaminated with HAV, and transmission among marginalized populations including people experiencing homelessness and people who use illicit drugs.

Potential Sources of Infection

Risk factor and exposure data for hepatitis A cases are often lacking in the United States. Before 2010, on the basis of the data collected from enhanced disease surveillance, the most commonly reported exposure to HAV in the United States was international travel (accounting for 46% of cases), followed by household or sexual contact with individuals with hepatitis A.[235] Although data are limited by underreporting, approximately three-quarters of hepatitis A cases reported through routine surveillance in the United States do not have a recognized source of infection.[223] Surveillance data from 2016 identified food (and waterborne) outbreaks as the most common source of infection (75.8%) among case reports with an identified risk factor or exposure.[223] Cases associated with child care centers or preschools, which previously accounted for a

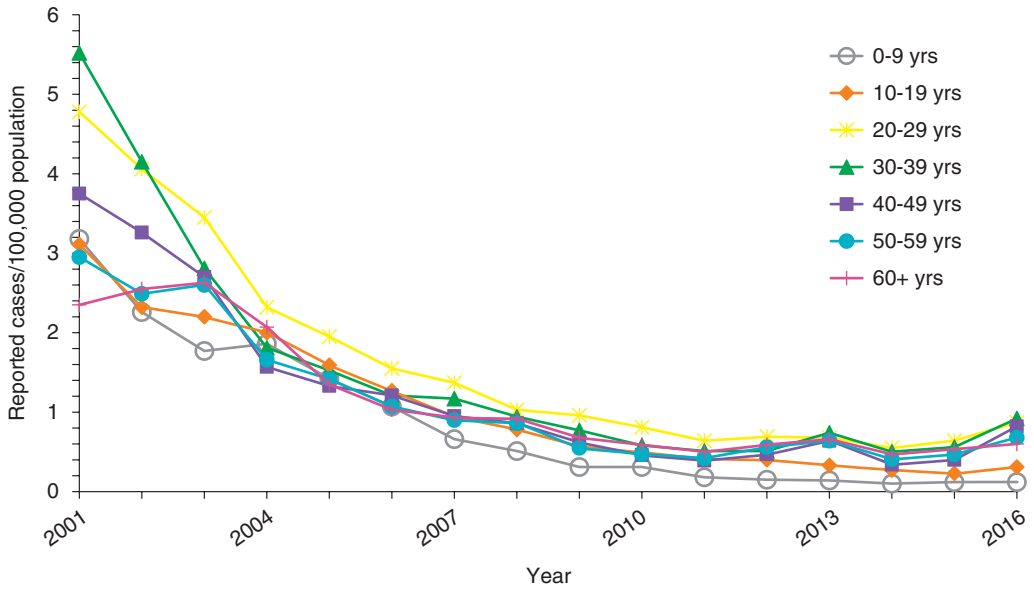

FIG. 174.9 Incidence of hepatitis A by age group—United States, 2001–2016. Rates of reported hepatitis A reached a low point in 2014 for all age groups except 10 to 19 years; for that group the low point occurred in 2015. Rates increased for all age groups from 2015 and 2016, except for those aged 0 to 9, whose rates remained stable. When the 2016 hepatitis A rates of all age groups are compared, persons aged 20 to 29 years and 30 to 39 years had the highest rate (0.9 cases per 100,000 population) and persons aged 0 to 9 years had the lowest rate (0.1 cases per 100,000 population). *(From Centers for Disease Control and Prevention, National Notifiable Diseases Surveillance System [NNDSS].)*

substantial proportion of reported exposures, were responsible for only 8.6% of all reported exposures in 2016.[223] Other reported exposures include international travel (6.5%), household or sexual contact (3.8%), illicit drug use (3.6%), and MSM (1.5%).[223] Increasingly in the United States, outbreaks among people experiencing homelessness are being recognized[131a]; however, data regarding homelessness as an exposure are not currently collected with routine surveillance.[223]

Specific Groups and Settings of Infection

International Travel

Hepatitis A is a risk for unvaccinated persons from developed countries who travel to regions with high, transitional, or intermediate endemicity (see Fig. 174.6).[236–241] A study of Swiss travelers that was published in 2006 estimated the risk to be 6 to 30 cases per 100,000 months of stay in developing countries among persons who did not receive IG or vaccine before departure.[242] A more recent study from the Netherlands found rates of 3.5 to 7.5 per 100,000 Dutch travelers.[240] The risk may be higher among travelers staying in areas with poor hygienic conditions[243] and varies according to the region and the length of stay. Declines in the number of hepatitis A cases attributed to improved hygiene and infrastructure in endemic countries have been reported for travelers from some European countries.[240,244,245] In the United States, international travel is a rarely reported risk factor.[223] In the United States and Europe, the risk of hepatitis A for travelers, especially children, traveling to endemic countries to visit relatives and friends poses a particular risk.[218] Compared with persons traveling for work or recreation, these persons generally have trips of longer duration and, by staying in family settings rather than in hotels, may have greater exposure to HAV circulating in the community. These persons also are less likely to seek or to adhere to pretravel advice.[215,246–252] Travelers who acquire hepatitis A infection during their trip may also transmit the virus to others on their return.[253] Vaccination of international travelers, including those visiting friends and family in endemic regions, remains an important consideration for pretravel preparations.[254]

International Adoption and Migrant and Refugee Populations

International adoptees with asymptomatic hepatitis A may transmit the virus to family members and close contacts. Most adoptee-associated cases of hepatitis A are among unvaccinated nontraveling contacts of the adoptee exposed during the first 60 days after arrival in the United States; secondary and tertiary transmission has been described.[255–258] Serologic results from 270 adoptees who received testing within 4 months of arrival in the United States showed 1% with evidence of acute hepatitis A; rates varied by country of origin. All acutely infected adoptees were asymptomatic.[258] A retrospective review from Minnesota, United States, from 2007 to 2009 identified 21 hepatitis A cases of among international adoptees within ≤60 months of age.[259] Another study found the prevalence of HAV infection to be 4.6% among international adoptees based on screening of adoptees from 2006 to 2010; the study noted the substantial interruption of care and social support associated with secondary caretaker infection.[260] Screening of international adoptees from endemic areas was suggested as a preventive measure; however, there is a need for a better understanding of the impact and public health importance of international adoptees introducing hepatitis A into the community.

In recent years, there has been an increase in persons displaced by conflict, resulting in refugees and migrants and particularly affecting Europe and the Mediterranean. Increased transmission of hepatitis A has reemerged as a public health problem associated with conflict and war. Conflict can lead to increases in refugees, migrants, and internally displaced people (IDPs). In recent years, conflict has directly resulted in increases in cases and outbreaks of hepatitis A being recognized in Sri Lanka among IDPs in the wake of a war[261] and among refugees and asylum seekers in Greece, Turkey, and Germany.[219–221]

Users of Injection and Noninjection Drugs and People Experiencing Homelessness

Outbreaks of hepatitis A have been reported among people who use injection and noninjection drugs in North America, Australia, and Europe.[131a,262–272] In the United States, these outbreaks have involved users of injection and noninjection drugs.[127,131a,263,267,273] Cross-sectional serologic surveys have demonstrated that injection drug users have a higher prevalence of anti-HAV than the general US population,[274–279] although a small study from Switzerland found a decrease in the seroprevalence in this population compared with previous years.[280] A randomized intervention trial of 469 injection (70%) and noninjection (30%) drug users in methadone maintenance found 56% were anti-HAV positive.[281] Transmission among injection drug users probably occurs predominantly through fecal-oral routes, but percutaneous transmission may occur.[267] People experiencing homelessness in the United States and Europe may be at increased risk of hepatitis A infection, may have a higher seroprevalence of anti-HAV, and may be associated with outbreaks.[131a,282] The risk of hepatitis A among people experiencing homelessness may be related to lack of access to water and sanitation, sexual and illicit drug use practices, years experiencing homelessness, and country of origin.[269,282–284] It is anticipated that the childhood vaccination program that began in the United States in the late 1990s will offer protection to future generations of people experiencing homelessness who were vaccinated as children.

Men Who Have Sex With Men

Hepatitis A outbreaks among MSM have been reported in urban areas in the United States, Canada, Europe, and Australia and may occur in the context of an outbreak in the larger community or a mass gathering such as a Pride Festival.[a] Seroprevalence surveys have not consistently demonstrated an elevated prevalence of anti-HAV among MSM compared with a general population of similar age.[274,294] Some studies conducted during outbreaks and seroprevalence surveys have identified specific sex practices associated with illness, whereas others have not demonstrated such associations.[275,286,289] A large cross-sectional prevalence study among MSM conducted from 1994 to 2000 in the United States found a similar prevalence of anti-HAV to the general population of similar age.[295] The study also found that factors associated with HAV infection among MSM in nonoutbreak settings are likely similar to those among non-MSM.

Transfusion and Health Care Settings

Transfusion-related hepatitis A is rare.[296] The risk of infection in patients with hemophilia is unknown, but results of one serologic survey of persons with hemophilia before the licensure of hepatitis A vaccine suggested they might have been at increased risk.[297] Outbreaks have been reported in Europe and the United States among patients who received factor VIII and factor IX concentrates.[160,298] However, no HAV infections attributed to blood products were identified in an analysis of serosurveillance data collected from 140 hemophilia treatment centers in the United States between 1998 and 2002, suggesting that improved viral inactivation procedures, donor screening, and increased hepatitis A vaccination coverage among clotting factor recipients has reduced the risk of HAV transmission.[299]

Outbreaks of hepatitis A have been reported in neonatal intensive care units after transmission to hospital staff from a neonate with asymptomatic HAV infection acquired from a blood transfusion.[118,300–302] Nosocomial transmission of hepatitis A from adult patients to health care workers also is rare because most patients with hepatitis A are hospitalized after the onset of jaundice, when infectivity is low, but it has been reported in association with fecal incontinence of patie nts.[115,303,304] Health care workers were not found to have an increased prevalence of anti-HAV compared with control populations in serologic surveys conducted in the United States before vaccine introduction.[305]

Foodborne and Waterborne Hepatitis A

Foodborne hepatitis A outbreaks are of increasing concern globally, particularly in Europe and the United States. Historically, in the United States foodborne outbreaks were most commonly recognized in association with contamination of food during preparation by a food handler with HAV infection.[136–138,306–309] Food contaminated before retail distribution, such as lettuce or fruit contaminated at the growing or processing

[a]References **127**, 131, 132, 134, 212, 272, 285–293.

stage, can be the source of hepatitis A outbreaks, some of which can be large or spread over a wide geographic area.[148–151,310–313] Since 2000, common source food exposures, such as the 2003 outbreak associated with contaminated green onions[150,308] and more recently contaminated berries imported from countries with endemic transmission of hepatitis A, have been increasingly recognized in hepatitis A low and very low endemic regions.[b] According to the Rapid Alert System for Food and Feed database in Europe, 35 notifications of HAV in food were reported between 1999 and 2013 in eight EU countries from multiple food sources.

Mixed frozen berries were implicated in a hepatitis A genotype IA outbreak in Italy in 2013.[314,316–318] An outbreak likely associated with berry mix buttermilk cake imported frozen from Germany[319] occurred in 2013–14. Other recent outbreaks of HAV genotype IB illness associated with food from the Middle East were also reported. A Nordic outbreak (Denmark, Finland, Sweden, and Norway) occurred in 2012–13 from contaminated strawberries, suspected to have originated from Egypt and Morocco.[320] Frozen pomegranate arils from Egypt were implicated in an outbreak in Canada in 2012, and imported pomegranate arils from Turkey were associated with a multistate outbreak in the United States in 2013.[155] Semidried tomatoes from Turkey were associated with cases in Europe and Australia in 2009–11.[153,154,321] Foodborne outbreaks can be resource intensive to investigate and control, and it may be challenging to identify the point source or source country of the contaminated produce.[156,157]

In low and very low endemic countries, outbreaks related to contaminated shellfish[78] have become less common but are still occasionally identified.[143–147] Waterborne hepatitis A outbreaks are rare and generally are related to sewage contamination or inadequate treatment of water.[322–324]

Although results of some serologic surveys conducted among sewage workers in Europe indicated a possible elevated risk of HAV infection, findings have not been consistent.[325–327] In published reports of three serologic surveys conducted among US sewage workers and appropriate comparison populations, no substantial or consistent increase in prevalence of anti-HAV was found among sewage workers.[328–330] No work-related instances of HAV transmission have been reported among sewage workers in the United States.

Institutions Including Daycare for Children and Facilities for Persons With Developmental Disabilities

Historically, HAV infection was commonly reported from daycare centers and endemic in institutions for developmentally disabled people in the United States. With the adoption of universal hepatitis A vaccination, transmission in daycare settings is now rarely reported.[223] For persons with disabilities, with deinstitutionalization and the shift to smaller facilities, some with improved conditions and increased availability of vaccine, the incidence and prevalence of infection have decreased and outbreaks rarely are reported in the United States.[226,227,331] However, unvaccinated disabled adults cared for in group homes are at risk because they typically live in close quarters and often are incontinent and nonverbal.[332] During 2013, an outbreak in Michigan occurred in which eight unvaccinated residents of five group homes for adults with disabilities were diagnosed with hepatitis A, and one died.[332] Unvaccinated staff working in group homes may also be at risk during such outbreaks.

PATHOGENESIS

Although HAV shares many virologic characteristics with enteroviruses, it has several differentiating features that influence the pathogenesis and clinical expression of the disease. HAV is resistant to heat, solvents, and acid and grows slowly in living cells, where it has been shown to be relatively noncytolytic and to have little effect on the rate of host protein synthesis.

Incubation Period

Determination of the incubation period of hepatitis A is imprecise because the early signs and symptoms are often vague and nonspecific. Jaundice may not be noticed by the patient, so a change in urine color to very dark urine, which is almost always recognized by the patient, is the most common reason for seeking medical attention for patients with hepatitis A. The range of incubation is between 15 and 50 days, with a mean of approximately 28 days (see Fig. 174.5). Although HAV can be transmitted orally or parenterally, the incubation period is independent of the route of inoculation.[333] Experiments in primates and observations in humans suggest that the incubation period is dependent on the infectious dose.[334]

Site of Viral Replication

HAV is generally transmitted by the fecal-oral route. Because the virus is acid resistant, it passes through the stomach, replicates lower in the intestine,[335–337] and is then transported to the liver, which is the major site of replication.[335,338,339] Evidence of replication in the oropharynx has been obtained in chimpanzees, and HAV has been identified in human saliva.[124,125] HAV, like many other picornaviruses, is highly organ specific with little evidence of significant replication outside the liver. Virus is shed from infected liver cells into the hepatic sinusoids and canaliculi, passes into the intestine, and is excreted in feces. In humans and nonhuman primates, HAV has been detected in the liver, bile, and feces.[340,341] The first indirect evidence that virus may replicate in the gut was the detection of co-proantibodies in the feces,[342,343] followed by the demonstration of hepatitis A antigen in duodenal lining cells.[335] Nonetheless, the major pathology is restricted to the liver.

Pathology

HAV is generally not cytopathic in cell culture, and histopathologic findings in experimental animals and humans do not show widespread hepatocyte necrosis, although the vast majority of hepatocytes at the peak of viral replication appear to be infected by immunohistochemical staining. Therefore, immune mechanisms have been invoked to explain the pathogenesis of the disease.[344] It has been postulated that liver cell damage occurs through a cell-mediated immune response, whereas circulating antibodies are probably more important in limiting the spread of virus to uninfected liver cells and other organs. This hypothesis is consistent with observations in animal models and humans. For example, intravenous inoculation of marmosets with a large dose of HAV resulted in mildly abnormal liver function test results and detectable hepatitis A antigen in hepatocytes within 1 week. Enzyme levels stabilized or even decreased until the third week after inoculation, when a second, higher peak was observed coincident with the appearance of serum antibodies.[345] One explanation is that the early mild hepatitis was due to a direct viral effect, but the second, more severe episode was due to an immune response. The presence of large quantities of virus in hepatocytes before the onset of hepatitis also argues against a major direct cytopathic effect of HAV.[336] It has been suggested that virally elicited T cells target infected liver cells and induce immunopathology. In human studies, Vallbracht and colleagues[344,346] found that lymphocytes from convalescing patients produced cytotoxic effects against autologous epidermal cell lines infected with HAV and that $CD8^+$ T-cell clones demonstrated cytotoxic activity against autologous fibroblasts infected with hepatitis A. These findings are consistent with the hypothesis that $CD8^+$ T lymphocytes mediate liver cell damage. Furthermore, natural killer cells have been demonstrated to be capable of lysing HAV-infected tissue culture cells.[347]

Although liver damage occurs at the time that circulating antibodies become detectable, it has not been proved that the pathology is antibody dependent.[347] Circulating immune complexes containing HAV and specific immunoglobulin M (IgM) antibodies have been found during infection. However, IG and complement deposits were not found at the sites of liver cell damage, and resolution of disease occurred even when antibody levels were increasing and hepatitis A antigen could still be detected in the liver.[348,349,350]

MANIFESTATIONS

Hepatitis A is an acute infection of the liver and is indistinguishable from other forms of acute viral hepatitis on clinical grounds, although the diagnosis may be suspected in a patient with typical symptoms during an outbreak. Although the clinical expression of infection varies widely, the disease is usually self-limited, sometimes subclinical, but

[b]References 154, **155**, 156, 157, 314, 315.

typically is symptomatic with jaundice. The most important determinant of the likelihood of clinical expression is the age at which infection occurs.[351] The vast majority of infections in children younger than 5 years are silent, and the proportion of symptomatic infections increases with age. The proportion of icteric cases (compared with anicteric cases) varies by age at time of infection and ranges from <10% among children younger than 5 years to over 70% among adolescents and adults.[232,352,353] In an epidemic in Greenland during the 1970s, the frequency of clinically recognizable hepatitis increased from 1% in children younger than 1 year to 24% in 15-year-olds.[354,355] Similar low rates of clinical symptoms have been noted in children involved in outbreaks in daycare centers in the United States[356]; however, adults infected in these outbreaks usually became jaundiced.

Signs and Symptoms

Patients with hepatitis A often describe a mild illness, the prodrome (see Fig. 174.5), that appears 1 to 7 days before the onset of dark urine (bilirubinuria) and jaundice, although longer periods have been recorded.[236,351] These symptoms may not be severe enough to cause the patient to seek medical attention or to stay home from work. In the early stages, flulike symptoms are common; fever (as high as 40°C) may be accompanied by chills, mild headache, malaise, and fatigue. Loss of appetite, nausea, vomiting, and weight loss are common. Occasionally, children may experience atypical symptoms such as diarrhea, cough, coryza, and arthralgia.

A common early sign of disease and the one that causes most patients to seek medical attention is dark urine (bilirubinuria), which is usually followed within a few days by pale or clay-colored feces (lacking bilirubin pigment), jaundice (yellow discoloration of the skin and mucous membranes), and scleral icterus (yellow discoloration of the sclera). The return of color to the stool occurs 2 or 3 weeks after the onset of illness and is an indication of resolution of disease. Pruritus, a sign of cholestasis, occurs in less than 50% of patients but may be severe enough to require antipruritics or other medications.

On physical examination, hepatomegaly is common and usually associated with tenderness to palpation. Palpable splenomegaly may be found in 5% to 15% of patients. Other extrahepatic manifestations such as skin rashes and arthralgias may be seen in up to 15% of patients, and immune complex–associated disorders and vasculitis occur rarely.

The duration of illness varies, but by the third week most patients feel better, have lost their hepatomegaly, and have normal or nearly normal levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In many patients, the appearance of jaundice is associated with rapid resolution of symptoms. In a study of 59 patients in the United States, approximately two-thirds recovered within 2 months, 85% within 3 months, and nearly all by 6 months.[357]

The clinical course and histologic findings do not differ in pregnancy.[358,359] Intrauterine transmission of HAV during the first trimester that resulted in fetal meconium peritonitis has been described in two case reports.[163,164] At delivery, both infants had a perforated ileum. No evidence has suggested that more severe infection or subsequent loss of immunity occurs in the presence of human immunodeficiency virus infection.[360] Although not demonstrated in all published studies, on balance, it seems that HAV infection in persons with chronic liver disease is more severe and more likely to result in fulminant hepatitis A.[361–363]

Complications

The clinical course of hepatitis A is usually benign, and long-term sequelae in recovered patients have not been observed. However, complications can occur, including cholestasis and acalculous cholecystitis, hemolysis, prolonged and relapsing disease, fulminant hepatitis, autoimmune hepatitis, pancreatitis, and autoimmune extrahepatic disease. During a large 1995 community-wide hepatitis A outbreak in Memphis, Tennessee, a total of 14% of hospitalized patients experienced serious complications. The most common complications were acalculous cholecystitis and hemolysis. Consistent with other series, complications were more common among older adults (25% of patients 40 years of age or older) but did occur among younger adults and children (11% of younger patients).[363,364,365,366] Hepatitis A may be a common cause of acute hepatic failure and hospitalization of children with jaundice in endemic countries.[367,368] Although unusual in low endemic countries, fulminant hepatic failure requiring transplantation and death from hepatitis A can occur.

Cholestatic hepatitis, characterized by fever, pruritus, and prolonged jaundice, has been reported as an occasional complication. Cholestasis developed in 4 of 59 (7%) patients in a hospital-based study.[357] In a detailed description of 6 patients, peak serum bilirubin levels of 12 to 29 mg/dL were recorded and jaundice lasted for 12 to 18 weeks. In each case, peak ALT levels were less than 500 IU/L.[369] Liver biopsy specimens revealed centrilobular cholestasis and portal inflammation.

Relapsing disease has been reported as an occasional complication in both adults and children.[119,357,370] It has been reported that a relapse occurs after a typical initial course in 3% to 20% of cases. Typically, symptoms decrease but may not completely resolve during the recovery phase, and the relapse disease is usually milder than the first. In one clinical series,[357] the mean ALT level was 3500 mIU/mL and the mean bilirubin level was 4.9 mg/dL during the first peak and 1554 mIU/mL and 2.5 mg/dL, respectively, during the second. Viral excretion during the relapse has been detected. Although the pathogenesis of relapses has not been elucidated, these cases resolve without sequelae.[370,371] Extrahepatic manifestations of hepatitis A rarely include cardiac involvement, although patients with acute hepatitis may have bradycardia and electrocardiograms may show prolongation of the PR interval and some mild T-wave depression. These changes resolve rapidly during convalescence.[372] Less common extrahepatic complications include postviral encephalitis, Guillain-Barré syndrome, cholecystitis, acute pancreatitis, acute renal failure secondary to interstitial nephritis, aplastic or hemolytic anemia, agranulocytosis, thrombocytopenic purpura, and pancytopenia. Several cases of arthritis, vasculitis, and cryoglobulinemia have also been reported.[349,373,374] Some patients may become depressed, and occasionally the depression may be severe enough to require treatment, but it is usually mild and self-limited.

The most serious complication of hepatitis A is fulminant hepatic failure, defined by the appearance of severe acute liver disease with hepatic encephalopathy in a previously healthy person.[375,376] Signs may include excitability, irritability, insomnia, confusion, and severe vomiting. Laboratory and clinical evidence of deteriorating liver function, especially prolonged prothrombin times or abnormal international normalized ratio (INR), correlates with the histologic picture of almost complete destruction of the hepatic parenchyma, with only a reticulin framework and portal tracts remaining. Occasionally, small groups of surviving hepatocytes can be seen close to the portal tracts, which may represent foci of regeneration. Surprisingly, little indication of a vigorous inflammatory response has been noted. Fulminant hepatitis A is now rarely seen in the United States. However, hepatitis A can be more severe in older patients and in patients with other comorbidities or hepatitis B or C, resulting in a greater proportion of patients requiring hospital care,[376,377] although overall hospitalization rates for hepatitis A have been decreasing in the United States.[378] Spontaneous survival from fulminant hepatitis A occurs more commonly than from fulminant hepatitis of other causes.[376]

Recent hepatitis A outbreaks in the United States among people who use illicit substances and people experiencing homelessness have resulted in a large proportion of patients with acute hepatitis A requiring hospitalization (71%), and a high proportion of deaths (3%).[131a] This is likely associated with high rates of comorbid conditions, including chronic hepatitis B and hepatitis C, in this population. Another reason for this may include a demographic shift among hepatitis A outbreaks of the past, with more recent outbreaks having a greater proportion of affected individuals who are older and/or have more comorbid conditions.[377]

THERAPY AND GENERAL MANAGEMENT

There is no specific therapy available for hepatitis A, and management is supportive. In the rare event of fulminant hepatitis, identification of patients requiring liver transplantation is difficult because up to 60% of patients, especially children, with fulminant hepatic failure caused

by hepatitis A survive.[376,379] Transplantation is used for the management of carefully selected patients who have a poor prognosis with medical management alone. The survival rate is reported to be 80%, although reinfection has been reported.[380,381]

For most patients with hepatitis A, admission to the hospital is not indicated, provided that patients have access to supportive care. If hospitalized, fecally incontinent patients, patients with diarrhea, and small children should be given a separate room and toilet, and contact precautions should be implemented. There is no objective evidence that bed rest or restriction of physical activity affects the outcome of the disease. There also are few data indicating that dietary restrictions, including prohibition of even modest amounts of alcohol, affect outcome. Nevertheless, abstaining from alcohol is usually recommended because alcohol has been linked with relapse of jaundice.[382]

LABORATORY DIAGNOSIS

Hepatitis A is not clinically distinguishable from other forms of viral hepatitis, although the diagnosis may be suspected in a patient with typical symptoms during an outbreak. Liver enzyme tests (see Fig. 174.5), especially serum levels of ALT and AST, are sensitive measures of parenchymal liver damage but are not specific for hepatitis A. In one case series[357] the peak mean ALT level was 1952 mIU/mL and the mean peak AST level was 1442 mIU/mL, with the highest ALT level being 9711 mIU/mL, although values greater than 20,000 mIU/mL have been observed. The ALT levels returned to normal by a mean of 7.4 weeks (range, 1–29 weeks). Although elevated ALT levels are detected in patients with severe hepatitis, high levels are not necessarily correlated with an adverse outcome. Alkaline phosphatase levels are usually only mildly elevated, and persisting elevated levels suggest hepatitis-associated cholestasis.[369] Peak bilirubin levels can exceed 30 mg/dL and tend to be higher with increasing age.[357] The presence of mild lymphocytosis and occasional atypical mononuclear cells is a common laboratory finding among patients with acute hepatitis A.[357,383]

The diagnosis of acute hepatitis A is most commonly confirmed by detection of specific IgM in a single acute-phase serum sample.[384] The hepatitis A–specific IgM antibody is usually present at the initial evaluation and may be detectable at the time of the first increase in ALT (see Fig. 174.5). However, serologic assays can be initially negative in 10% of patients who are tested soon after the onset of symptoms; testing should be repeated for persons testing negative for IgM anti-HAV if hepatitis A is clinically suspected.[385,386] IgM anti-HAV can be detected in nearly 100% of patients with acute hepatitis A at least 2 days after their peak ALT and remains positive in most for 3 to 6 months; it is rarely detected more than 2 years after initial exposure. False-positive test results occur particularly among persons who have no other evidence of recent infection, suggesting a low positive predictive value when testing is performed in asymptomatic persons with no known recent HAV exposures.[387] Assays for total antibody to the virus are of little diagnostic value because immunoglobulin G (IgG) persists for many years and may be related to a past infection or vaccination. Antibodies to naturally acquired HAV infection are primarily directed against the virion and do not react well with the individual peptides that make up the virion capsid. Low levels of antibodies to nonstructural proteins are found in the serum of convalescing patients, but attempts to use this phenomenon to distinguish the antibody response to natural infection from the response to vaccination have not yielded consistent results.[388,389]

HAV or viral antigen can be detected in the stools of patients 1 to 2 weeks before symptoms develop, but such detection has little place in routine clinical diagnosis because the tests are not widely available and shedding may have ceased before the patient seeks medical attention.[390,391] Nucleic acid–based diagnostic techniques, primarily PCR or other nucleic acid amplification assays, have been used in research laboratories when a highly sensitive test for the presence of HAV is required. PCR has been useful in the study of environmental samples.[392,393] In response to several outbreaks of hepatitis A associated with pooled plasma products, screening by nucleic acid testing of plasma pools intended for manufacture into various plasma components has been instituted by most plasma fractionators for process testing. Although these nucleic acid tests are now commercially available for testing plasma, they are not recommended for use as diagnostics for patients with acute hepatitis. The performance of these assays in the diagnostic setting has not been evaluated. The use of PCR for identifying HAV in stool samples also has not been validated as a diagnostic assay. Many stools have inhibitors of PCR, which could result in false-negative results. The use of these types of assays for diagnosis is currently not recommended outside the research setting.

Liver biopsy is rarely indicated to establish a diagnosis in acute hepatitis because this procedure is associated with a small, but measurable, risk and the histopathologic findings are not usually diagnostic. In one study done in Japan, where biopsy for acute hepatitis was routine, 86 patients with serologically established acute hepatitis A were evaluated for quantitative and qualitative light microscopic features, together with biopsy samples from 78 patients with acute hepatitis B and from 76 patients with acute non-A, non-B hepatitis. Hepatitis A was characterized by more pronounced portal inflammation than was non-A, non-B hepatitis, but less conspicuous parenchymal changes such as focal necrosis, Kupffer cell proliferation, acidophil bodies, and ballooning. Nonspecific reactive hepatitis with slightly increased serum transaminase levels was often seen during recovery from hepatitis A and needs to be distinguished from the longer-lasting cases of acute hepatitis B and C.[394,395] Hepatitis A antigen and HAV particles can be detected in the cytoplasm of infected cells with immunostaining techniques or thin-section electron microscopy.[340,396]

IMMUNITY

The high prevalence of antibody in older individuals in countries that now have a low incidence of hepatitis A suggests that anti-HAV IgG usually persists for life. After a natural infection, second attacks of hepatitis A have not been documented in the field and have not been induced experimentally. In two sets of experiments involving a total of 19 volunteers, reinoculation with HAV 6 to 9 months after the initial illness failed to induce disease.[5,6]

Past experience with IG has shown that it provides protection against hepatitis A, suggesting that serum antibody alone is sufficient to prevent infection. It has been difficult to judge the effect of mucosal immunity because IgA antibodies in saliva or feces are not detected or are present at very low levels.[397] The antibody response to HAV infection is generally brisk and high titered. Both IgG and IgM can usually be detected at the time of the first expression of clinical illness (see Fig. 174.5). Neutralizing antibody as measured with in vitro tissue culture assays can also be detected early in disease. Because HAV is not usually cytopathic, a radioimmunofocus reduction test that is equivalent to a plaque reduction assay was devised.[398] With this assay, both IgM and IgG have been shown to possess neutralizing activity. It has also been demonstrated that patients convalescing from hepatitis A may have high titers of in vitro neutralizing antibody. Serum dilutions of 1:100,000 to 1:500,000 or more are not uncommon.

The role of T lymphocytes in protection from HAV infection has not been fully elucidated. Undoubtedly, T-cell responses do occur and $CD8^+$ cytotoxic T lymphocytes and possibly natural killer cells are important in pathogenesis.[399–401]

PREVENTION

Infection Prevention and Control

Hygiene and sanitation are an effective methods to control hepatitis A and other enteric infections. Good hygienic practices with particular emphasis on hand washing and restriction of activities of workers who are ill are of primary importance in the food preparation industry. Travelers to hepatitis A–endemic countries should be advised to eat only properly cooked food, avoid uncooked vegetables and shellfish, and consume only treated or purified water and ice. Nosocomial infections have been reported but are not common, and transmission is usually from a patient who is not suspected of having hepatitis A.[402,403] Hence, hospitalized patients with confirmed or suspected hepatitis A need routine precautions. Private rooms, gowns, and masks are not necessary unless the patient is incontinent, in which case contact precautions should be instituted. Gloves should be worn when handling any material potentially contaminated with feces. Frequent hand washing, whether gloves are worn or not, should be emphasized.

Passive Immunization

Before the licensing of hepatitis A vaccines, the mainstay of hepatitis A immunoprophylaxis was passive immunization with pooled serum IG, which has been referred to as gamma globulin or immune serum globulin. IG has proved useful for the prevention of hepatitis A in travelers, Peace Corps volunteers, and military personnel and for PEP. Although IG has proven effective for prevention of hepatitis A in individuals, it has not been successful in altering the epidemiology of hepatitis A in communities; this can be attributed to the transient nature of the protection and the high population coverage rates necessary to alter the epidemiology and induce herd immunity.

IG is manufactured by means of cold ethanol precipitation from large pools of plasma collected from tens of thousands of donors.[404] At present, the individual plasma units used in these pools are screened for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus by the appropriate serologic and other assays. Minipools of plasma are tested with nucleic acid testing for hepatitis C and human immunodeficiency virus, and the product itself undergoes at least one specific viral inactivation step in its manufacturing process. In the United States, the prevalence of antibody to HAV in the population has been decreasing, raising concern that antibody levels against HAV in IG preparations might drop below effective levels. Although no standard for anti-HAV levels exists in IG preparations in the United States, reduced anti-HAV antibody titers have been documented in pooled plasma in the United States and Europe.[405] Studies have not demonstrated reduced efficacy in the prevention of hepatitis A at differing doses of IG.[226,406] However, in July 2017, the prescribing information for GamaSTAN S/D, the only IG product approved by the US Food and Drug Administration for HAV prophylaxis, was updated owing to concerns around decreasing HAV IgG antibody levels. Changes were made in 2017 to the dosing instructions, increasing the dose, for both preexposure and postexposure HAV prophylaxis (Table 174.1).[407] Eventually the manufacture of IG from selected antibody-positive donors may need to be considered to develop a hyperimmune globulin for hepatitis A prevention, analogous to other agent-specific hyperimmune globulins.[408]

IG is effective for preexposure prophylaxis (PrEP) and PEP of hepatitis A.[409] The efficacy of IG was first demonstrated in an outbreak at a summer camp in 1944 and has been confirmed many times since.[410,411] Several studies have demonstrated the effectiveness of IG in preexposure settings, such as among travelers, military personnel,[412] and Peace Corps workers.[413] The rate of HAV infections in Peace Corps volunteers dropped from 1.6 to 2.1 cases per 100 per year before mandatory administration of IG every 4 months, to 0.1 to 0.3 case per 100 per year after the institution of a mandatory program.[413] When administered before exposure or within 2 weeks after exposure, IG is more than 85% effective in preventing hepatitis A.[410,414,415] Whether IG completely prevents infection or leads to asymptomatic infection and the development of persistent anti-HAV (passive-active immunity) is probably related to the amount of time that has elapsed between exposure and IG administration.[410,416]

The use of IG for PrEP and PEP has become limited with the licensure of inactivated hepatitis A vaccines, but it remains indicated for some rare situations both for PEP (Table 174.2).[407,417,417a] A randomized trial comparing hepatitis A vaccine versus IG for PEP found them to have equivalent efficacy among persons ≤40 years of age.[418] After exposure, IG should be used to protect children younger than 12 months, immunocompromised persons, persons with chronic liver disease, and persons for whom the vaccine is contraindicated.[417] Previously the preference was also to use IG for persons older than 40 years; however, although limited effectiveness data were available, recommendations in the United States changed in 2018 to provide hepatitis A vaccination for all individuals aged ≥12 months and to consider additional use of IG for persons aged ≥40 years based on clinical assessment (Table 174.4).[417a] These changes were driven by the increase in the IG dosing instructions (due to concerns of decreased IG potency) and by the decreased availability of IG during outbreaks.[417a] Similarly, for PrEP such as is needed for travel to endemic areas, recommendations have changed to provide hepatitis A vaccine to children 6 to 11 months of age who also require measles, mumps, and rubella (MMR) vaccine because IG cannot be given with MMR vaccine, which is also recommended for PrEP for this age group prior to travel to countries with endemic measles (see Table 174.3). Children should then receive the recommended two-dose series of HAV beginning at age 12 months or older, and their recommended MMR vaccine series as indicated.[417a] IG should be used instead of vaccine for persons who have a contraindication to the vaccine.

The current recommended dose of IG is a single IM injection of 0.1 mL/kg for postexposure and preexposure travel of up to 1 month; 0.2 mL/kg should be given before exposure for travel up to 2 months and repeated every 2 months for extended travel (see Table 174.1).[407] Hepatitis A vaccine, if not contraindicated, is a better choice for PrEP and PEP (see "Active Immunization" later).

Intramuscular preparations of IG should never be given intravenously, and the intravenous preparations of IG are not intended for hepatitis A prevention and are formulated at a lower globulin concentration.

IG does not interfere with the immune response to oral poliovirus or yellow fever vaccine or, in general, to inactivated vaccines. However, IG can interfere with the immune response to some live-attenuated vaccines (e.g., MMR vaccine; varicella vaccine). Administration of the

TABLE 174.1 Indications and Updated Dosage Recommendations for GamaSTAN S/D Human Immune Globulin for Preexposure and Postexposure Prophylaxis Against Hepatitis A Infection

INDICATION	DOSAGE RECOMMENDATION
Preexposure prophylaxis	
Up to 1 month of travel	0.1 mL/kg
Up to 2 months of travel	0.2 mL/kg
2 months of travel or longer	0.2 mL/kg (repeat every 2 months)
Postexposure prophylaxis	0.1 mL/kg

Modified from Nelson NP. Updated Dosing Instructions for Immune Globulin (Human) GamaSTAN S/D for Hepatitis AVirus Prophylaxis. MMWR Morb Mortal Wkly Rep. *2017;66:959–960.*

TABLE 174.2 Recommendations for Postexposure Prophylaxis, by Age Group and Risk Category

INDICATION/ AGE GROUP	RISK CATEGORY/ HEALTH STATUS	HAV	IG
Postexposure Prophylaxis			
<12 mo	Healthy	No	0.1 mL/kg[a]
12 mo–40 yr	Healthy	1 dose[b]	None
>40 yr	Healthy	1 dose[b]	0.1 mL/kg[c]
≥12 mo	Immunocompromised or chronic liver disease	1 dose[b]	0.1 mL/kg[d]
≥12 mo	Persons who elect not to receive vaccine or for whom vaccine is contraindicated[e]	No	0.1 mL/kg

HAV, Hepatitis A vaccine; *IG,* immune globulin.
[a]Measles, mumps, and rubella vaccine should not be administered for at least 3 months after receipt of IG.
[b]A second dose is not required for postexposure prophylaxis; however, for long-term immunity, the HAV series should be completed with a second dose at least 6 months after the first dose.
[c]The provider's risk assessment should determine the need for IG administration. If the provider's risk assessment determines that both vaccine and IG are warranted, HAV and IG should be administered simultaneously at different anatomic sites.
[d]Vaccine and IG should be administered simultaneously at different anatomic sites.
[e]Life-threatening allergic reaction to a previous dose of HAV, or allergy to any vaccine component.
Data from Nelson NP, Link-Gelles R, Hofmeister MG, et al. Update: Recommendations of the Advisory Committee on Immunization Practices for Use of Hepatitis A Vaccine for Postexposure Prophylaxis and for Preexposure Prophylaxis for International Travel. MMWR Morb Mortal Wkly Rep. *2018;67:1216–1220.*

TABLE 174.3 Recommendations for Preexposure Protection, by Age Group and Risk Category

INDICATION/ AGE GROUP	RISK CATEGORY/ HEALTH STATUS	HAV	IG
<6 mo	Healthy	No	0.1–0.2 mL/kg[b]
6–11 mo	Healthy	1 dose[c]	None
12 mo–40 yr	Healthy	1 dose[d]	None
>40 yr	Healthy	1 dose[d]	0.1–0.2 mL/kg[b,e]
All ages	Immunocompromised or chronic liver disease	1 dose[d]	0.1–0.2 mL/kg[b,e]
>6 mo	Persons who elect not to receive vaccine or for whom vaccine is contraindicated[f]	No	0.1–0.2 mL/kg[b]

HAV, Hepatitis A vaccine; *IG,* immune globulin.
[a]IG should be considered before travel for persons with special risk factors for either HAV infection or increased risk for complications in the event of exposure to HAV.
[b]0.1 mL/kg for travel up to 1 month; 0.2 mL/kg for travel up to 2 months, 0.2 mL/kg every 2 months for travel of ≥2 months' duration.
[c]This dose should not be counted toward the routine 2-dose series, which should be initiated at age 12 months.
[d]For persons not previously vaccinated with HAV, administer dose as soon as travel is considered, and complete series according to routine schedule.
[e]May be administered, based on providers' risk assessment.
[f]Life-threatening allergic reaction to a previous dose of HAV, or allergy to any vaccine component.
Data from Nelson NP, Link-Gelles R, Hofmeister MG, et al. Update: Recommendations of the Advisory Committee on Immunization Practices for Use of Hepatitis A Vaccine for Postexposure Prophylaxis and for Preexposure Prophylaxis for International Travel. MMWR Morb Mortal Wkly Rep. *2018;67:1216–1220.*

TABLE 174.4 ACIP Recommendations for Routine Preexposure Use of Hepatitis A Vaccine

GROUP	COMMENTS
All children at age 12–23 mo	2 doses separated by 6–18 months, between the first and second birthdays; a series begun before the second birthday should be completed even if the child turns 2 before the second dose is administered.
Children aged 2–18 years	Catch-up vaccination; anyone 2 years of age or older may receive hepatitis A vaccine if desired; minimum interval between doses is 6 months
International travelers or workers	Travelers to or workers in high- or intermediate-endemicity countries (see Fig. 174.6)
Persons who anticipate close contact with an international adoptee	Includes unvaccinated household or other close contacts (e.g., regular babysitting) 12 mo of age or older of international adoptees from a country of high or intermediate hepatitis A endemicity during the first 60 days after arrival of the adoptee in the United States; first dose of vaccine should be administered at least 2 weeks before the arrival of the adoptee
Men who have sex with men	Includes adolescents
People who use injection and noninjection drugs	Includes adolescents
Persons with chronic liver disease	Increased risk of fulminant hepatitis A with HAV infection
Persons receiving clotting factor concentrates	
Persons who work with HAV in research settings or work with nonhuman primates	
Anyone who wants to obtain immunity	

ACIP, Advisory Committee on Immunization Practices; *HAV,* hepatitis A virus.
Data from Centers for Disease Control and Prevention (CDC). Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. *2006;55(RR-7):1–23; ACIP CDC. Update: Prevention of hepatitis A after exposure to hepatitis A virus and in international travelers: updated recommendations of the Advisory Committee on Immunization Practices (ACIP).* MMWR Morb Mortal Wkly Rep. *2007;56:1080–1084; CDC. Updated recommendations from the Advisory Committee on Immunization Practices (ACIP) for use of hepatitis A vaccine in close contacts of newly arriving international adoptees.* MMWR Morb Mortal Wkly Rep. *2009;58:1006–1007; ACIP, Fiore AE, Wasley A, Bell BP. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP).* MMWR Recomm Rep. *2006;55(RR-7):1–24.*

MMR vaccine and of varicella-containing vaccines should be delayed for at least 3 months after administration of IG, and IG should not be given within 2 weeks after the administration of the MMR or varicella-containing vaccines, unless the benefits of IG administration are greater than the benefits of vaccination.[419]

Serious adverse events from IG are rare. Because anaphylaxis has been reported after repeated administration to persons with IgA deficiency, these persons should not receive IG.[420] Pregnancy or lactation is not a contraindication to IG administration. For infants and pregnant women, a preparation that does not include thimerosal is preferable; in the United States, the only available preparation, GamaSTAN S/D, does not contain thimerosal.

Active Immunization

Active immunization with hepatitis A vaccines has developed along classic lines similar to the path followed for polio vaccines. As with poliovirus, the initial breakthrough came with the in vitro cultivation of HAV in cell lines suitable for vaccine production.[14] Formalin-inactivated, cell culture–produced, whole-virus vaccines have now been approved in much of the world, and a live-attenuated vaccine is available in China.

Two inactivated hepatitis A vaccines are approved for use in the United States and widely throughout the world. Other inactivated hepatitis A vaccines are available in Europe and other parts of the world.[421,422] Both US-licensed vaccines are produced from highly cell culture–adapted virus strains that have also been shown to be highly attenuated in humans, which gives them an extra measure of safety.[423] The US-licensed vaccines—Havrix (GlaxoSmithKline Biologicals, Philadelphia, PA) and VAQTA (Merck & Co., Whitehouse Station, NJ)—are both grown in MRC-5 cells, purified, inactivated by formalin, and formulated with alum as an adjuvant. Both vaccines are licensed in a two-dose series, with the second dose given 6 to 18 months after the first, depending on the vaccine (Table 174.5). Inactivated hepatitis A vaccines are also manufactured in Europe and China and are available in many countries of the world. Currently there are two hepatitis A vaccines that have

TABLE 174.5 Recommended Doses and Schedules for Inactivated Hepatitis A Vaccines Licensed in the United States

AGE (yr)	VACCINE	DOSE	VOLUME (mL)	NO. OF DOSES	SCHEDULE (mo)[a]
1–18	Havrix	720 ELU[b]	0.5	2	0, 6–12
	VAQTA	25 U[c]	0.5	2	0, 6–18
≥19	Havrix	1440 ELU	1.0	2	0, 6–12
	VAQTA	50 U	1.0	2	0, 6–18
≥18	Twinrix[d]	720 ELU	1.0	3	0, 1, 6

[a]0 months represents timing of initial dose; subsequent numbers represent months after the initial dose.
[b]Enzyme-linked immunosorbent assay units.
[c]Hepatitis A virus antigen unit.
[d]Hepatitis A (720 ELU) and hepatitis B (recombinant; 20 μg) vaccine. Standard dosing: a series of 3 doses (1 mL each) given on a 0-, 1-, and 6-month schedule. Accelerated dosing: a series of 4 doses (1 mL each) given on days 0, 7,and 21 to 30 followed by a booster dose at month 12.

been prequalified by the World Health Organization (WHO): Havrix and Sinovac (Biotech).[424] In the United States, a combination hepatitis A and hepatitis B vaccine, Twinrix (GlaxoSmithKline), is also available for persons aged 18 and older.

Clinical trials have indicated that inactivated hepatitis A vaccines are safe, highly immunogenic, and efficacious.[60,61,421,425] In one US study, 1037 healthy seronegative children 2 to 16 years of age in a community experiencing yearly outbreaks of hepatitis A received either a single dose of formalin-inactivated vaccine (n = 519) or placebo (n = 518). No cases of hepatitis occurred in the vaccinated group, except for a few that appeared within 3 weeks of vaccination. These cases represented patients who were already incubating the infection at the time of vaccination. In the period from 21 days to 103 days, 34 cases of hepatitis A were observed, all in the placebo group, indicating a 100% vaccine protective efficacy during that period of observation.[425,426] In a large field trial of an inactivated vaccine involving more than 40,000 children in Thailand, the vaccine was found to be at least 80% effective compared with placebo and was without serious adverse reactions.[61]

Although the absolute level of antibody required to protect against infection has not been rigorously established, it is accepted on the basis of comparisons with protective antibody levels associated with passive immunization with IG that antibody concentrations of 10 to 20 mIU/mL (depending on the assay used) are protective.[60,427,428] The licensed inactivated hepatitis A vaccines have all been shown to be highly and rapidly immunogenic. They induce seroconversions to protective levels of antibody in as little as 2 weeks after the initial dose.[429,430] The level of antibody after vaccination varies with the dose and schedule of the vaccine. However, after a single dose of vaccine, antibody titers are higher than titers produced by known protective levels of IG but are generally lower than titers measured after natural infection.[431–433] The quality of the antibody response after vaccination has also been studied by comparing antibodies detected with radioimmunoassay, radioimmunoprecipitation, and in vitro neutralization in sera from persons passively immunized with IG and in persons immunized by vaccine. With the antibody normalized between the two groups by radioimmunoassay, the IG recipients had higher neutralization titers but negligible radioimmunoprecipitation titers compared with the group that was vaccinated.[433] However, it has also been shown that IG prepared from the serum of vaccinees could protect a chimpanzee from HAV challenge when the titer of antibody achieved by passive immunization in the chimpanzee was similar to that found in humans receiving IG prophylaxis.[434] Nevertheless, there are no direct correlations between in vitro neutralization assays and seroprotection. Regardless of the results of antibody measurements after vaccination, clinical trials have demonstrated that the vaccine is highly effective within 1 month of the first dose.[418]

Certain factors may reduce the response to the vaccine. For instance, only 50% to 75% of HIV-positive vaccinees develop protective levels of antibody, and those who respond have lower antibody titers than vaccinees without HIV infection.[435–438] However, response improves with higher $CD4^+$ counts and suppression of HIV.[437,438] The final antibody concentrations achieved in patients with chronic liver disease are also lower than in normal subjects, but the seroprotection rates were approximately the same; vaccination before development of cirrhosis is advisable.[439] The common recommended schedule of a single dose followed by a booster dose 6 to 18 months later produces high levels of antibody; the high levels of antibody produced by vaccination are well in excess of the levels achieved after passive immunization with IG, which are known to be effective in preventing hepatitis A. After the completed series, it is estimated that protective levels of antibody will persist for at least 20 to 30 years.[440,441,442,443] Because the incubation period for hepatitis A averages 28 to 30 days, and the anamnestic responses observed after the second dose are rapid and robust, it has been suggested that vaccinees who have seroconverted will be protected even if their antibody levels have fallen below protective levels.[60] Long-term follow-up studies are being performed to confirm the duration of protection.

A freeze-dried live-attenuated vaccine based on the H2 strain, a derivative of the cell culture–adapted, attenuated HM175 strain, has been used in China.[31,423,444–447] The vaccine is administered as a single subcutaneous dose. Antibody has been detected for at least 8 years after vaccination, and postlicensure community-based studies have demonstrated protection against hepatitis A in vaccinated populations.[441,448]

RECOMMENDATIONS FOR PREVENTION

Postexposure Prophylaxis

For decades, IG has been recommended for PEP to prevent infection after known exposure to HAV. However, with the demonstration of postexposure efficacy from hepatitis A vaccine,[418] and the relative public health advantages of vaccine compared with IG including the induction of active immunity and longer protection, greater ease of administration, and greater acceptability and availability, in the United States hepatitis A vaccines are now recommended in most circumstances for PEP (see Table 174.2).[417,417a] Hepatitis A vaccines should be administered for PEP for most persons aged ≥12 months; however, in addition to hepatitis A vaccine, IG may be administered to persons aged >40 years depending on the providers' risk assessment.[417a]

When using hepatitis A vaccine alone for PEP, single-antigen vaccine should be used; no data exist regarding the performance of the combination hepatitis A and hepatitis B vaccine for prophylaxis after exposure to HAV, and the concentration of HAV antigen in the currently available combination vaccine is half that included in the single-antigen vaccine available from the same manufacturer. IG may still be used for PEP in certain cases at a dose of 0.01 mL/kg, such as children younger than 12 months, immunocompromised persons, persons who have had chronic liver disease diagnosed, and persons for whom vaccine is contraindicated (see Table 174.2).

PEP, with vaccine, is recommended to prevent hepatitis A after exposure in certain settings.[226,417] Household and sexual contacts of patients with hepatitis A should receive appropriate PEP as soon as possible. Casual contacts such as school classmates or coworkers who have not had close physical contact usually do not require PEP. PEP may be indicated to control hepatitis A outbreaks in homeless shelters, substance abuse treatment centers, or correctional facilities when a resident, client, or inmate, respectively, or an employee is diagnosed with hepatitis A.[131a] Outbreaks in other settings (e.g., hospitals, facilities for developmentally disabled persons) are rare but can occur. When a food handler is identified with hepatitis A, PEP with vaccine or IG should be administered to other food handlers at the food establishment and can be considered for patrons if certain other conditions exist.[136,138,226,417] Once cases that are associated with a food service establishment are identified, it is generally too late to administer PEP to patrons because the 2-week postexposure period during which IG or vaccine is known to be effective will have passed.

Preexposure Prophylaxis

Hepatitis A vaccine is preferred over IG for PrEP. Hepatitis A vaccine is indicated for preexposure protection of susceptible persons 12 months of age or older, persons at increased risk of hepatitis A, and for any person wishing to obtain immunity. In addition, hepatitis A vaccine should also be administered to children 6 to 11 months of age traveling outside the United States when protection against hepatitis A is recommended (see Table 174.3) (MMWR, ACIP 2018, pending publication).[255,406] IG should be used for children younger than 6 months or other persons who cannot or choose not to receive hepatitis A vaccine but who require protection against hepatitis A when protection against hepatitis A is recommended (see Table 174.3).

Prevaccination serologic testing may be considered to reduce costs by eliminating vaccination of persons with previous immunity, such as older adolescents and adults in certain population groups with a high prevalence of infection (e.g., persons born in areas of high hepatitis A endemicity). However, prevaccination testing should take into account the cost of testing, vaccine cost, and the likelihood that the person will return for vaccination.[449] Vaccination of immune individuals is not harmful, and therefore, for most situations, vaccinating individuals without prevaccination testing is preferred. Postvaccination testing is not indicated because of the high rate of vaccine response and because some commercially available assays may not detect lower anti-HAV concentrations generated by immunization.

Disease-Control Strategies

Routine Vaccination of Children

Hepatitis A vaccine was licensed and became available in the United States in 1995. It was soon recognized that a strategy of widespread routine vaccination of children had the potential to achieve a sustained reduction in the overall incidence of hepatitis A in the community.[450] The initial recommendations, published in 1996, called for routine vaccination of children living in communities with the highest hepatitis A rates (e.g., Native American and Alaska Native communities).[228] Although effective in reducing disease rates in communities covered by these recommendations, implementation of these recommendations had little impact on overall disease incidence nationwide because only a small proportion of nationally reported cases occurred among persons in such communities. Recommendations for use of hepatitis A vaccine were updated and incrementally expanded in 1999, 2006, and 2009.[224,226,228,255,451] In 1999, the recommendation for routine vaccination of children was extended to include those living in states, counties, and communities with consistently elevated hepatitis A rates.[224] Hepatitis A incidence decreased sharply in the states included in the 1999 recommendations for routine vaccination of children, and by 2001, rates were similar across all regions of the country, and the majority of cases were occurring in states that historically had low rates and where hepatitis A vaccination of children had not been widely implemented.[225] In 2006, after hepatitis A vaccines were licensed for children beginning at age 12 months (vs. 24 months previously), the recommendations for routine vaccination were expanded to include all children 12 to 23 months of age nationwide (see Table 174.4).[226] This strategy has resulted in a historic low of reported hepatitis A cases and rates among all age groups in the United States (see Figs. 174.8 and 174.9).[223] Similar decreases in hepatitis A have been documented throughout the world in countries implementing vaccination strategies that target children.[144,452–457] By 2017, 18 countries had introduced hepatitis A vaccine into their routine childhood vaccination programs, including some countries that have a single-dose schedule.[458] In 2012, the WHO Strategic Advisory Group of Experts (SAGE) on Immunization reviewed evidence of efficacy after a single dose of inactivated hepatitis A vaccine in a randomized controlled trial in Nicaragua and from national use of a single-dose schedule of hepatitis A vaccine in Argentina. The SAGE concluded that national programs may consider a single-dose schedule, although additional evidence of long-term immunogenicity is needed, and for certain high-risk and immunocompromised individuals a two-dose schedule is recommended.[459,460]

In the United States, it is recommended that children who did not receive two-dose hepatitis A vaccine by 2 years of age should be offered catch-up vaccination through age 18 years (see Table 174.4).[226] The first dose does not need to be repeated if the first dose was given.[385]

Vaccination of Persons at Increased Risk of Hepatitis A Infection or Severe Consequences

In addition to routine vaccination of children, hepatitis A vaccine is also indicated for persons in the following groups that have an increased risk of HAV infection or of severe consequences if infected (see Table 174.4).[226]

Men Who Have Sex With Men

Adolescent and adult MSM should be vaccinated, regardless of reported level of sexual activity.[226] Prevaccination serologic testing is not necessary for vaccination of adolescents and young adults, but it could be considered for older adults (see Table 174.4).[226]

Users of Illicit Drugs

Vaccination is recommended for users of injection and noninjection illegal drugs (see Table 174.4).[226] Prevaccination testing could be considered for adults; the need might depend on the particular characteristics of the population of drug users, including the type and duration of drug use.[272,274,275]

International Travelers

Susceptible persons ≥6 months of age who travel to or work in countries where hepatitis A is endemic should be vaccinated to prevent infection and illness. Although IG is still an option, hepatitis A vaccination is preferred for most travelers (see Tables 174.3 and 174.4). The first dose should be administered as soon as travel is considered. For most healthy persons, one dose of single-antigen hepatitis A vaccine given any time before departure can provide adequate protection. For travel that will begin within 2 weeks to countries with high or intermediate endemicity of hepatitis A, older adults, immunocompromised persons, and persons with chronic liver disease or other chronic medical conditions should receive the initial dose of the vaccine and may also be simultaneously administered IG (0.01–0.02 mL/kg), depending on providers risk assessment, at a different anatomic injection site (see Tables 174.1 and 174.3). Travelers who elect not to receive hepatitis A vaccine, are aged <6 months, or are allergic to a component of hepatitis A vaccine should receive IG at the appropriate dose (see Table 174.1).

Completion of the vaccine series according to the licensed schedule is recommended for long-term protection. Prevaccination serologic testing could be considered for older travelers or younger travelers who were born in a country in which hepatitis A is endemic.

Close Contacts of Newly Arriving International Adoptees

Susceptible household members and other close personal contacts (e.g., regular babysitters) who anticipate close personal contact during the first 60 days after arrival of adopted children from countries with high or intermediate levels of hepatitis should receive hepatitis A vaccination (see Table 174.4).[255] The first dose should be administered as soon as adoption is planned, ideally 2 or more weeks before the arrival of the adoptee. Parents traveling to high or intermediate endemic countries (see Fig. 174.6) to bring adoptees home should be vaccinated as per the recommendations for international travelers.[417]

Recipients of Blood or Plasma-Derived Products

The risk of hepatitis A from a blood transfusion or from plasma derivatives is extremely low, but both have been reported.[44,122] Individuals who receive these products regularly should receive hepatitis A vaccine (see Table 174.4).

Persons With Chronic Liver Disease

Although individuals with chronic liver disease are not at increased risk of acquiring hepatitis A, acute hepatitis A in such patients can have serious or fatal consequences.[461] Therefore, it is recommended that all such persons, no matter the cause of their liver disease, be vaccinated against hepatitis A (see Table 174.4). Vaccination should be performed early after the diagnosis of chronic liver disease in order to obtain optimal response to the vaccine.

Other Groups and Settings

Persons who work with HAV in research settings or have exposure to nonhuman primates should be vaccinated (see Table 174.4).[226]

Hepatitis A Vaccination During Outbreaks

Large community-wide outbreaks have become rare in the United States since the implementation of routine childhood immunization, and ongoing vaccination of children should reduce the occurrence of these outbreaks further. The United States and Europe have experienced large outbreaks associated with contaminated shellfish, produce, and frozen berries.[146,155–157,319] Beginning in 2017, there have been multiple outbreaks of hepatitis A among substance users and people experiencing homelessness in the United States.[131a] Outbreaks can be very disruptive, resource intensive, and logistically challenging to respond to with accelerated vaccination. Furthermore, such efforts may have limited impact on transmission.[226] During outbreaks, persons who work as food handlers, who are not at increased risk of hepatitis A because of their occupation, may also transmit HAV to others when they contract hepatitis A.[138,307] To reduce the frequency of evaluations of food handlers with hepatitis A and the need for PEP of patrons, public health officials in some jurisdictions have instituted measures to promote hepatitis A vaccination of food handlers.[462] Although outbreaks associated with eating establishments continue to garner media attention, often during an ongoing

community outbreak, transmission from infected food handlers generally accounts for a small proportion of incident cases. Therefore, vaccination of food handlers is not likely to affect overall disease incidence and has not been found to be cost-effective.[463]

DIRECTIONS FOR THE FUTURE

In the United States, Western Europe, and many other high-income countries, low levels of endemic transmission and outbreaks among specific population may occur, whereas international travelers to high- and intermediate-endemicity countries remain at risk. In these countries, hepatitis A is relatively well controlled, and as more children are vaccinated, in countries with childhood vaccination programs the population immunity will continue to increase, further reducing the risk of hepatitis A. However, in much of Europe, where routine vaccination of children has mostly not been implemented, population susceptibility continues to grow.[464] Globally, hepatitis A continues to be responsible for significant morbidity and mortality.[170] As of 2017, only 18 countries had introduced hepatitis A vaccine into their routine vaccination program.[458] In many countries previously thought to be highly endemic and transitioning into intermediate hepatitis A endemicity, a sizable proportion of adolescents and adults are increasingly susceptible to hepatitis A, particularly in urban areas with improving water quality and sanitation. Therefore the relative cost-effectiveness of hepatitis A vaccination strategies compared with other major public health priorities deserves greater scrutiny.[465–467] Establishment of surveillance systems and use of surveillance data can shed light on the burden of hepatitis A and provide important inputs for cost-effectiveness analyses.[173,176] Cost-effectiveness analyses of various vaccination strategies have been conducted and are being used for reevaluating hepatitis A vaccine policy in countries with transitional or intermediate endemicity.[460,465,467–472] The global disease burden associated with hepatitis A is likely to continue to increase in the coming years in the absence of vaccination programs.[126,167,169] If vaccine was available at a low cost and vaccination was shown to be cost-effective, more countries in which a significant susceptible adolescent and adult population has developed might find it useful to include hepatitis A in their vaccination programs. One strategy that has been considered as a potential way to reduce the costs is vaccinating with only a single dose. Experiences in Nicaragua, where a single dose was evaluated for efficacy,[473] and Argentina, where a single-dose childhood vaccination strategy was implemented in response to a large nationwide outbreak, were effective in controlling the outbreak.[456,467,474] This led WHO to issue a permissive recommendation allowing for the use of a single-dose vaccination strategy.[460] However, long-term protection remains an important and unanswered question for countries choosing to implement this policy.[460]

International support from the Gavi Alliance for hepatitis A vaccination for eligible countries could catalyze implementation and accelerate control efforts globally.

In the United States, vaccination of successive cohorts of children has resulted in a sustained reduction in disease incidence nationwide, providing the opportunity to eliminate HAV transmission, although recent outbreaks among people experiencing homelessness and people who use injection and noninjection drugs highlight that significant challenges remain.[131a] HAV has been considered a target for eradication, but international bodies have not made this recommendation, primarily because of considerations of cost and feasibility.[475] At present, the disease can best be controlled by improving living conditions in the developing world and prudent application of vaccination strategies.

ACKNOWLEDGMENT

The authors gratefully acknowledge Anna Koscelski, BS, for her numerous contributions to this chapter.

Key References

The complete reference list is available online at Expert Consult.

11. Feinstone SM, Kapikian AZ, Purceli RH. Hepatitis A: detection by immune electron microscopy of a viruslike antigen associated with acute illness. *Science*. 1973.
23. Drexler JF, Corman VM, Lukashev AN, et al. Evolutionary origins of hepatitis A virus in small mammals. *Proc Natl Acad Sci USA*. 2015;112:15190–15195.
24. Costa-Mattioli M, Cristina J, Romero H, et al. Molecular evolution of hepatitis A virus: a new classification based on the complete VP1 protein. *J Virol*. 2002;76:9516–9525.
32. Wang X, Ren J, Gao Q, et al. Hepatitis A virus and the origins of picornaviruses. *Nature*. 2015;517:85–88.
61. Innis BL, Snitbhan R, Kunasol P, et al. Protection against hepatitis A by an inactivated vaccine. *JAMA*. 1994;271:1328–1334.
78. Bialek SR, George PA, Xia GL, et al. Use of molecular epidemiology to confirm a multistate outbreak of hepatitis A caused by consumption of oysters. *Clin Infect Dis*. 2007;44:838–840.
79. Lemon SM, Jansen RW, Brown EA. Genetic, antigenic and biological differences between strains of hepatitis A virus. *Vaccine*. 1992;10(suppl 1):S40–S44.
98. Emerson SU, Huang YK, McRill C, et al. Molecular basis of virulence and growth of hepatitis A virus in cell culture. *Vaccine*. 1992;10(suppl 1):S36–S39.
115. Skinhoj P, Mathiesen LR, Kryger P. Faecal excretion of hepatitis A virus in patients with symptomatic hepatitis A infection. *Ann Intern Med*. 1987;106:221–226.
118. Rosenblum LS, Villarino ME, Nainan OV, et al. 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*. 1991;164:476–482.
123. Purcell RH. Relative infectivity of hepatitis A virus by the oral and intravenous routes in 2 species of nonhuman primates. *J Infect Dis*. 2002;185:1668–1771.
127. Bell BP, Shapiro CN, Alter MJ, et al. The diverse patterns of hepatitis A epidemiology in the United States-implications for vaccination strategies. *J Infect Dis*. 1998;178:1579–1584.
130. Armstrong GL, Bell BP. Hepatitis A virus infections in the United States: model-based estimates and implications for childhood immunization. *Pediatrics*. 2002;109:839–845.
138. Fiore AE. Hepatitis A transmitted by food. *Clin Infect Dis*. 2004;38:705–715.
144. Chironna M, Prato R, Sallustio A, et al. Hepatitis A in puglia (South Italy) after 10 years of universal vaccination: need for strict monitoring and catch-up vaccination. *BMC Infect Dis*. 2012;12:271.
151. Amon JJ, Devasia R, Xia G, et al. Molecular epidemiology of foodborne hepatitis A outbreaks in the United States, 2003. *J Infect Dis*. 2005;192:1323–1330.
155. Collier MG, Khudyakov YE, Selvage D, et al. Outbreak of hepatitis A in the USA associated with frozen pomegranate arils imported from Turkey: an epidemiological case study. *Lancet Infect Dis*. 2014;14:976–981.
160. Soucie JM, Robertson BH, Bell BP, et al. Hepatitis A virus infections associated with clotting factor concentrate in the United States. *Transfusion*. 1998;38:573–579.
165. Tong MJ, Thursby M, Rakela J, et al. Studies on the maternal-infant transmission of the viruses which cause acute hepatitis. *Gastroenterology*. 1981;80(5 Pt 1):999–1004.
167. Aggarwal R, Goel A. Hepatitis A: epidemiology in resource-poor countries. *Curr Opin Infect Dis*. 2015;28:488–496.
168. Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. *Vaccine*. 2010;28:6653–6657.
170. *Global Hepatitis Report 2017*. Geneva: World Health Organization; 2017.
180. Bialek SR, Thoroughman DA, Hu D, et al. Hepatitis A incidence and hepatitis A vaccination among American Indians and Alaska Natives, 1990-2001. *Am J Public Health*. 2004;94:996–1001.
182. Green MS, Block C, Slater PE. Rise in the incidence of viral hepatitis in Israel despite improved socioeconomic conditions. *Rev Infect Dis*. 1989;11:464–469.
195. Das K, Jain A, Gupta S, et al. The changing epidemiological pattern of hepatitis A in an urban population of India: emergence of a trend similar to the European countries. *Eur J Epidemiol*. 2000;16:507–510.
202. Shah U, Habib Z, Kleinman RE. Liver failure attributable to hepatitis A virus infection in a developing country. *Pediatrics*. 2000;105:436–438.
208. Klevens RM, Denniston MM, Jiles-Chapman RB, et al. Decreasing immunity to hepatitis A virus infection among US adults: findings from the National Health and Nutrition Examination Survey (NHANES), 1999-2012. *Vaccine*. 2015;33:6192–6198.
215. Askling HH, Rombo L, Andersson Y, et al. Hepatitis A risk in travelers. *J Travel Med*. 2009;16:233–238.
226. Advisory Committee on Immunization P, Fiore AE, Wasley A, et al. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 2006;55(RR–7):1–23.
235. Klevens RM, Miller JT, Iqbal K, et al. The evolving epidemiology of hepatitis A in the United States: incidence and molecular epidemiology from population-based surveillance, 2005-2007. *Arch Intern Med*. 2010;170:1811–1818.
241. Steffen R, Kane MA, Shapiro CN, et al. Epidemiology and prevention of hepatitis A in travelers. *JAMA*. 1994;272:885–889.
252. Sonder GJ, Bovee LP, Baayen TD, et al. Effectiveness of a hepatitis A vaccination program for migrant children in Amsterdam, The Netherlands (1992-2004). *Vaccine*. 2006;24:4962–4968.
255. Centers for Disease C, Prevention, Advisory Committee on Immunization P. Updated recommendations from the Advisory Committee on Immunization Practices (ACIP) for use of hepatitis A vaccine in close contacts of newly arriving international adoptees. *MMWR Morb Mortal Wkly Rep*. 2009;58:1006–1007.
349. Inman RD, Hodge M, Johnston ME, et al. Arthritis, vasculitis, and cryoglobulinemia associated with relapsing hepatitis A virus infection. *Ann Intern Med*. 1986;105:700–703.
357. Tong MJ, el-Farra NS, Grew MI. Clinical manifestations of hepatitis A: recent experience in a community teaching hospital. *J Infect Dis*. 1995;171(suppl 1):S15–S18.
361. Vento S, Garofano T, Renzini C, et al. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. *N Engl J Med*. 1998;338:286–290.
365. Willner IR, Uhl MD, Howard SC, et al. Serious hepatitis A: an analysis of patients hospitalized during an urban epidemic in the United States. *Ann Intern Med*. 1998;128:111–114.

371. Bornstein JD, Byrd DE, Trotter JF. Relapsing hepatitis A: a case report and review of the literature. *J Clin Gastroenterol*. 1999;28:355–356.
376. Schiodt FV, Davern TJ, Shakil AO, et al. Viral hepatitis-related acute liver failure. *Am J Gastroenterol*. 2003;98:448–453.
377. Ly KN, Klevens RM. Trends in disease and complications of hepatitis A virus infection in the United States, 1999-2011: a new concern for adults. *J Infect Dis*. 2015;212:176–182.
384. Hoofnagle JH, Di Bisceglie AM. Serologic diagnosis of acute and chronic viral hepatitis. *Semin Liver Dis*. 1991;11:73–83.
387. Castrodale L, Fiore A, Schmidt T. Detection of immunoglobulin M antibody to hepatitis A virus in Alaska residents without other evidence of hepatitis. *Clin Infect Dis*. 2005;41:e86–e88.
405. Farcet MR, Planitzer CB, Stein O, et al. Hepatitis A virus antibodies in immunoglobulin preparations. *J Allergy Clin Immunol*. 2010;125:198–202.
409. Liu JP, Nikolova D, Fei Y. Immunoglobulins for preventing hepatitis A. *Cochrane Database Syst Rev*. 2009;(2):CD004181.
417. Advisory Committee on Immunization Practices Centers for Disease C, Prevention. Update: prevention of hepatitis A after exposure to hepatitis A virus and in international travelers. Updated recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep*. 2007;56:1080–1084.
418. Victor JC, Monto AS, Surdina TY, et al. Hepatitis A vaccine versus immune globulin for postexposure prophylaxis. *N Engl J Med*. 2007;357:1685–1694.
442. Ott JJ, Irving G, Wiersma ST. Long-term protective effects of hepatitis A vaccines. A systematic review. *Vaccine*. 2012;31:3–11.
443. Spradling PR, Bulkow LR, Negus SE, et al. Persistence of seropositivity among persons vaccinated for hepatitis A during infancy by maternal antibody status: 15-year follow-up. *Hepatology*. 2016;63:703–711.
448. Zheng H, Chen Y, Wang F, et al. Comparing live attenuated and inactivated hepatitis A vaccines: an immunogenicity study after one single dose. *Vaccine*. 2011;29:9098–9103.
450. Averhoff F, Shapiro CN, Bell BP, et al. Control of hepatitis A through routine vaccination of children. *JAMA*. 2001;286:2968–2973.
460. WHO position paper on hepatitis A vaccines - June 2012. *Wkly Epidemiol Rec*. 2012;87:261–276.

References

1. Zuckerman AJ. The history of viral hepatitis from antiquity to the present. In: Deinhardt F, Deinhardt J, eds. *Viral Hepatitis: Laboratory and Clinical Science*. New York: Marcel Dekker; 1983:3–32.
2. Virchow R. Ueber das vorkommen und den nachweis des hepatogenen, insbesondere des katarrhalischen icterus. *Virchows Arch (Pathol Anat)*. 1865;32:117.
3. McDonald S. Acute yellow atrophy of the liver. *Edinb Med J*. 1907;1:83.
4. Cockayne E. Catarrhal jaundice, sporadic and epidemic, and its relation to acute yellow atrophy of the liver. *Q J Med*. 1912;6:1–29.
5. Havens WP. Immunity in experimentally induced infectious hepatitis. *J Exp Med*. 1946;84:403–406.
6. Neefe JR, Gellis SS, Stokes J Jr. Homologous serum hepatitis and infectious (epidemic) hepatitis; studies in volunteers bearing on immunological and other characteristics of the etiological agents. *Am J Med*. 1946;1:3–22.
7. Havens WP, Ward R, Drill VA. Experimental production of hepatitis by feeding icterogenic materials. *Proc Soc Exp Biol Med*. 1944;57:206–208.
8. MacCallum FO. Transmission of infective hepatitis to human volunteers. *Lancet*. 1944;2:228.
9. Gellis SS, Stokes J Jr, Brother GM, et al. The use of immune globulin (gamma globulin) in infectious (epidemic) hepatitis in the Mediterranean theatre of operations. *JAMA*. 1945;128:1062.
10. Krugman S. Viral hepatitis: overview and historical perspectives. *Yale J Biol Med*. 1976;49:199–203.
11. Feinstone SM, Kapikian AZ, Purceli RH. Hepatitis A: detection by immune electron microscopy of a viruslike antigen associated with acute illness. *Science*. 1973;182:1026–1028.
12. Maynard JE, Lorenz D, Bradley DW, et al. Review of infectivity studies in nonhuman primates with virus-like particles associated with MS-1 hepatitis. *Am J Med Sci*. 1975;270:81–85.
13. Dienstag JL, Feinstone SM, Purcell RH, et al. Experimental infection of chimpanzees with hepatitis A virus. *J Infect Dis*. 1975;132:532–545.
14. Provost PJ, Hilleman MR. Propagation of human hepatitis A virus in cell culture in vitro. *Proc Soc Exp Biol Med*. 1979;160:213–221.
15. Frosner GG, Deinhardt F, Scheid R, et al. Propagation of human hepatitis A virus in a hepatoma cell line. *Infection*. 1979;7:303–305.
16. Daemer RJ, Feinstone SM, Gust ID, et al. Propagation of human hepatitis A virus in African green monkey kidney cell culture: primary isolation and serial passage. *Infect Immun*. 1981;32:388–393.
17. Ticehurst JR, Racaniello VR, Baroudy BM, et al. Molecular cloning and characterization of hepatitis A virus cDNA. *Proc Natl Acad Sci USA*. 1983;80:5885–5889.
18. Andre FE, D'Hondt E, Delem A, et al. Clinical assessment of the safety and efficacy of an inactivated hepatitis A vaccine: rationale and summary of findings. *Vaccine*. 1992;10(suppl 1):S160–S168.
19. Cohen JI, Rosenblum B, Ticehurst JR, et al. Complete nucleotide sequence of an attenuated hepatitis A virus: comparison with wild-type virus. *Proc Natl Acad Sci USA*. 1987;84:2497–2501.
20. Martin A, Lemon SM. Hepatitis A virus: from discovery to vaccines. *Hepatology*. 2006;43(2 suppl 1):S164–S172.
21. Melnick JL. Properties and classification of hepatitis A virus. *Vaccine*. 1992;10(suppl 1):S24–S26.
22. Anthony SJ, St Leger JA, Liang E, et al. Discovery of a novel hepatovirus (phopivirus of seals) related to human hepatitis A virus. *MBio*. 2015;6.
23. Drexler JF, Corman VM, Lukashev AN, et al. Evolutionary origins of hepatitis A virus in small mammals. *Proc Natl Acad Sci USA*. 2015;112:15190–15195.
24. Costa-Mattioli M, Cristina J, Romero H, et al. Molecular evolution of hepatitis A virus: a new classification based on the complete VP1 protein. *J Virol*. 2002;76:9516–9525.
25. Bennett AJ, Sibley SD, Lauck M, et al. Naturally circulating hepatitis A virus in olive baboons, Uganda. *Emerg Infect Dis*. 2016;22:1308–1310.
26. Robertson BH, Jansen RW, Khanna B, et al. Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. *J Gen Virol*. 1992;73(Pt 6):1365–1377.
27. Ajmera V, Xia G, Vaughan G, et al. What factors determine the severity of hepatitis A-related acute liver failure? *J Viral Hepat*. 2011;18:e167–e174.
28. Yun H, Lee HJ, Jang JH, et al. Hepatitis A virus genotype and its correlation with the clinical outcome of acute hepatitis A in Korea: 2006-2008. *J Med Virol*. 2011;83:2073–2081.
29. Fujiwara K, Yokosuka O, Imazeki F, et al. Analysis of the genotype-determining region of hepatitis A viral RNA in relation to disease severities. *Hepatol Res*. 2003;25:124–134.
30. Fujiwara K, Kojima H, Yonemitsu Y, et al. Phylogenetic analysis of hepatitis A virus in sera from patients with hepatitis A of various severities. *Liver Int*. 2009;29:838–845.
31. Karron RA, Daemer R, Ticehurst J, et al. Studies of prototype live hepatitis A virus vaccines in primate models. *J Infect Dis*. 1988;157:338–345.
32. Wang X, Ren J, Gao Q, et al. Hepatitis A virus and the origins of picornaviruses. *Nature*. 2015;517:85–88.
33. Coulepis AG, Locarnini SA, Westaway EG, et al. Biophysical and biochemical characterization of hepatitis A virus. *Intervirology*. 1982;18:107–127.
34. Lemon SM, Jansen RW, Newbold JE. Infectious hepatitis A virus particles produced in cell culture consist of three distinct types with different buoyant densities in CsCl. *J Virol*. 1985;54:78–85.
35. Siegl G, Frosner GG. Characterization and classification of virus particles associated with hepatitis A. II. Type and configuration of nucleic acid. *J Virol*. 1978;26:48–53.
36. Feng Z, Hensley L, McKnight KL, et al. A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. *Nature*. 2013;496:367–371.
37. Murphy P, Nowak T, Lemon SM, et al. Inactivation of hepatitis A virus by heat treatment in aqueous solution. *J Med Virol*. 1993;41:61–64.
38. Nissen E, Konig P, Feinstone SM, et al. Inactivation of hepatitis A and other enteroviruses during heat treatment (pasteurization). *Biologicals*. 1996;24:339–341.
39. Parry JV, Mortimer PP. The heat sensitivity of hepatitis A virus determined by a simple tissue culture method. *J Med Virol*. 1984;14:277–283.
40. Millard J, Appleton H, Parry JV. Studies on heat inactivation of hepatitis A virus with special reference to shellfish. Part 1. Procedures for infection and recovery of virus from laboratory-maintained cockles. *Epidemiol Infect*. 1987;98:397–414.
41. Peterson DA, Hurley TR, Hoff JC, et al. Effect of chlorine treatment on infectivity of hepatitis A virus. *Appl Environ Microbiol*. 1983;45:223–227.
42. Calci KR, Meade GK, Tezloff RC, et al. High-pressure inactivation of hepatitis A virus within oysters. *Appl Environ Microbiol*. 2005;71:339–343.
43. Siegl G, Weitz M, Kronauer G. Stability of hepatitis A virus. *Intervirology*. 1984;22:218–226.
44. Mannucci PM, Gdovin S, Gringeri A, et al. Transmission of hepatitis A to patients with hemophilia by factor VIII concentrates treated with organic solvent and detergent to inactivate viruses. The Italian collaborative group. *Ann Intern Med*. 1994;120:1–7.
45. Cohen JI, Ticehurst JR, Purcell RH, et al. Complete nucleotide sequence of wild-type hepatitis A virus: comparison with different strains of hepatitis A virus and other picornaviruses. *J Virol*. 1987;61:50–59.
46. Rueckert RR, Wimmer E. Systematic nomenclature of picornavirus proteins. *J Virol*. 1984;50:957–959.
47. Pinto RM, Aragones L, Costafreda MI, et al. Codon usage and replicative strategies of hepatitis A virus. *Virus Res*. 2007;127:158–163.
48. D'Andrea L, Pinto RM, Bosch A, et al. A detailed comparative analysis on the overall codon usage patterns in hepatitis A virus. *Virus Res*. 2011;157:19–24.
49. Tratschin JD, Siegl G, Frosner GG, et al. Characterization and classification of virus particles associated with hepatitis A. III. Structural proteins. *J Virol*. 1981;38:151–156.
50. Updike WS, Tesar M, Ehrenfeld E. Detection of hepatitis A virus proteins in infected BS-C-1 cells. *Virology*. 1991;185:411–418.
51. Bishop NE, Anderson DA. RNA-dependent cleavage of VP0 capsid protein in provirions of hepatitis A virus. *Virology*. 1993;197:616–623.
52. Anderson DA, Ross BC. Morphogenesis of hepatitis A virus: isolation and characterization of subviral particles. *J Virol*. 1990;64:5284–5289.
53. Probst C, Jecht M, Gauss-Muller V. Intrinsic signals for the assembly of hepatitis A virus particles. Role of structural proteins VP4 and 2A. *J Biol Chem*. 1999;274:4527–4531.
54. Cohen L, Benichou D, Martin A. Analysis of deletion mutants indicates that the 2A polypeptide of hepatitis A virus participates in virion morphogenesis. *J Virol*. 2002;76:7495–7505.
55. Graff J, Richards OC, Swiderek KM, et al. Hepatitis A virus capsid protein VP1 has a heterogeneous C terminus. *J Virol*. 1999;73:6015–6023.
56. Rachow A, Gauss-Muller V, Probst C. Homogeneous hepatitis A virus particles. Proteolytic release of the assembly signal 2A from procapsids by factor Xa`. *J Biol Chem*. 2003;278:29744–29751.
57. Stapleton JT, Lemon SM. Neutralization escape mutants define a dominant immunogenic neutralization site on hepatitis A virus. *J Virol*. 1987;61:491–498.
58. Lemon SM, Binn LN. Antigenic relatedness of two strains of hepatitis A virus determined by cross-neutralization. *Infect Immun*. 1983;42:418–420.
59. Ping LH, Lemon SM. Antigenic structure of human hepatitis A virus defined by analysis of escape mutants selected against murine monoclonal antibodies. *J Virol*. 1992;66:2208–2216.
60. Nalin DR, Kuter BJ, Brown L, et al. Worldwide experience with the CR326F-derived inactivated hepatitis A virus vaccine in pediatric and adult populations: an overview. *J Hepatol*. 1993;18(suppl 2):S51–S55.
61. Innis BL, Snitbhan R, Kunasol P, et al. Protection against hepatitis A by an inactivated vaccine. *JAMA*. 1994;271:1328–1334.
62. Lemon SM, Chao SF, Jansen RW, et al. Genomic heterogeneity among human and nonhuman strains of hepatitis A virus. *J Virol*. 1987;61:735–742.
63. Robertson BH, Khanna B, Nainan OV, et al. Epidemiologic patterns of wild-type hepatitis A virus determined by genetic variation. *J Infect Dis*. 1991;163:286–292.
64. Cristina J, Costa-Mattioli M. Genetic variability and molecular evolution of hepatitis A virus. *Virus Res*. 2007;127:151–157.
65. Jansen RW, Siegl G, Lemon SM. Molecular epidemiology of human hepatitis A virus defined by an antigen-capture polymerase chain reaction method. *Proc Natl Acad Sci USA*. 1990;87:2867–2871.
66. Nainan OV, Xia G, Vaughan G, et al. Diagnosis of hepatitis A virus infection: a molecular approach. *Clin Microbiol Rev*. 2006;19:63–79.
67. Villar LM, Morais LM, Aloise R, et al. Co-circulation of genotypes IA and IB of hepatitis A virus in Northeast Brazil. *Braz J Med Biol Res*. 2006;39:873–881.
68. de Paula VS, Baptista ML, Lampe E, et al. Characterization of hepatitis A virus isolates from subgenotypes IA and IB in Rio de Janeiro, Brazil. *J Med Virol*. 2002;66:22–27.
69. Ruchusatsawat K, Wongpiyabovorn J, Kawidam C, et al. An outbreak of acute hepatitis caused by genotype IB hepatitis A viruses contaminating the water supply in Thailand. *Intervirology*. 2016;59:197–203.
70. Nejati A, Makvandi M, Samarbafzadeh A, et al. Molecular epidemiology of hepatitis A virus in patients in the ahwaz region of Iran. *J Med Virol*. 2012;84:582–586.
71. Normann A, Badur S, Onel D, et al. Acute hepatitis A virus infection in Turkey. *J Med Virol*. 2008;80:785–790.
72. Taylor MB. Molecular epidemiology of South African strains of hepatitis A virus: 1982-1996. *J Med Virol*. 1997;51:273–279.
73. Desbois D, Couturier E, Mackiewicz V, et al. Epidemiology and genetic characterization of hepatitis A virus genotype IIA. *J Clin Microbiol*. 2010;48:3306–3315.
74. Mukomolov S, Kontio M, Zheleznova N, et al. Increased circulation of hepatitis A virus genotype IIIA over the last decade in St Petersburg, Russia. *J Med Virol*. 2012;84:1528–1534.
75. Arankalle VA, Sarada Devi KL, Lole KS, et al. Molecular characterization of hepatitis A virus from a large outbreak from Kerala, India. *Indian J Med Res*. 2006;123:760–769.
76. Chadha MS, Lole KS, Bora MH, et al. Outbreaks of hepatitis A among children in western India. *Trans R Soc Trop Med Hyg*. 2009;103:911–916.
77. Chobe LP, Arankalle VA. Investigation of a hepatitis A outbreak from Shimla Himachal Pradesh. *Indian J Med Res*. 2009;130:179–184.
78. Bialek SR, George PA, Xia GL, et al. Use of molecular epidemiology to confirm a multistate outbreak of hepatitis A caused by consumption of oysters. *Clin Infect Dis*. 2007;44:838–840.
79. Lemon SM, Jansen RW, Brown EA. Genetic, antigenic and biological differences between strains of hepatitis A virus. *Vaccine*. 1992;10(suppl 1):S40–S44.
80. Siegl G, Frosner GG, Gauss-Muller V, et al. The physicochemical properties of infectious hepatitis A virions. *J Gen Virol*. 1981;57(Pt 2):331–341.
81. Dotzauer A, Feinstone SM, Kaplan G. Susceptibility of nonprimate cell lines to hepatitis A virus infection. *J Virol*. 1994;68:6064–6068.
82. Brown EA, Zajac AJ, Lemon SM. In vitro characterization of an internal ribosomal entry site (IRES) present within the 5' nontranslated region of hepatitis A virus RNA: comparison with the IRES of encephalomyocarditis virus. *J Virol*. 1994;68:1066–1074.
83. Whetter LE, Day SP, Elroy-Stein O, et al. Low efficiency of the 5' nontranslated region of hepatitis A virus RNA in directing cap-independent translation in permissive monkey kidney cells. *J Virol*. 1994;68:5253–5263.

84. Borman AM, Bailly JL, Girard M, et al. Picornavirus internal ribosome entry segments: comparison of translation efficiency and the requirements for optimal internal initiation of translation in vitro. *Nucleic Acids Res*. 1995;23:3656–3663.
85. Ali IK, McKendrick L, Morley SJ, et al. Activity of the hepatitis A virus IRES requires association between the cap-binding translation initiation factor (eIF4E) and eIF4G. *J Virol*. 2001;75:7854–7863.
86. Siegl G. Replication of hepatitis A virus and processing of proteins. *Vaccine*. 1992;10(suppl 1):S32–S35.
87. Cromeans T, Sobsey MD, Fields HA. Development of a plaque assay for a cytopathic, rapidly replicating isolate of hepatitis A virus. *J Med Virol*. 1987;22:45–56.
88. Anderson DA. Cytopathology, plaque assay, and heat inactivation of hepatitis A virus strain HM175. *J Med Virol*. 1987;22:35–44.
89. Cromeans T, Humphrey C, Sobsey M, et al. Use of immunogold preembedding technique to detect hepatitis A viral antigen in infected cells. *Am J Anat*. 1989;185: 314–320.
90. Konduru K, Kaplan GG. Stable growth of wild-type hepatitis A virus in cell culture. *J Virol*. 2006;80:1352–1360.
91. Anderson DA, Ross BC, Locarnini SA. Restricted replication of hepatitis A virus in cell culture: encapsidation of viral RNA depletes the pool of RNA available for replication. *J Virol*. 1988;62:4201–4206.
92. Wheeler CM, Fields HA, Schable CA, et al. Adsorption, purification, and growth characteristics of hepatitis A virus strain HAS-15 propagated in fetal rhesus monkey kidney cells. *J Clin Microbiol*. 1986;23:434–440.
93. Bishop NE, Anderson DA. Uncoating kinetics of hepatitis A virus virions and provirions. *J Virol*. 2000;74:3423–3426.
94. Borovec SV, Anderson DA. Synthesis and assembly of hepatitis A virus-specific proteins in BS-C-1 cells. *J Virol*. 1993;67:3095–3102.
95. Tedeschi V, Purcell RH, Emerson SU. Partial characterization of hepatitis A viruses from three intermediate passage levels of a series resulting in adaptation to growth in cell culture and attenuation of virulence. *J Med Virol*. 1993;39:16–22.
96. Emerson SU, Huang YK, Purcell RH. 2B and 2C mutations are essential but mutations throughout the genome of HAV contribute to adaptation to cell culture. *Virology*. 1993;194:475–480.
97. Provost PJ, Banker FS, Giesa PA, et al. Progress toward a live, attenuated human hepatitis A vaccine. *Proc Soc Exp Biol Med*. 1982;170:8–14.
98. Emerson SU, Huang YK, McRill C, et al. Molecular basis of virulence and growth of hepatitis A virus in cell culture. *Vaccine*. 1992;10(suppl 1):S36–S39.
99. Funkhouser AW, Purcell RH, D'Hondt E, et al. Attenuated hepatitis A virus: genetic determinants of adaptation to growth in MRC-5 cells. *J Virol*. 1994;68: 148–157.
100. Kaplan G, Totsuka A, Thompson P, et al. Identification of a surface glycoprotein on African green monkey kidney cells as a receptor for hepatitis A virus. *EMBO J*. 1996;15: 4282–4296.
101. Feigelstock D, Thompson P, Mattoo P, et al. The human homolog of HAVcr-1 codes for a hepatitis A virus cellular receptor. *J Virol*. 1998;72:6621–6628.
102. Silberstein E, Dveksler G, Kaplan GG. Neutralization of hepatitis A virus (HAV) by an immunoadhesin containing the cysteine-rich region of HAV cellular receptor-1. *J Virol*. 2001;75:717–725.
103. Dotzauer A, Gebhardt U, Bieback K, et al. Hepatitis A virus-specific immunoglobulin a mediates infection of hepatocytes with hepatitis A virus via the asialoglycoprotein receptor. *J Virol*. 2000;74:10950–10957.
104. Das A, Hirai-Yuki A, Gonzalez-Lopez O, et al. TIM1 (HAVCR1) is not essential for cellular entry of either Quasi-enveloped or naked hepatitis A virions. *MBio*. 2017;8.
105. Hillis WD. An outbreak of infectious hepatitis among chimpanzee handlers at a United States air force base. *Am J Hyg*. 1961;73:316–328.
106. Maynard JE, Krushak DH, Bradley DW, et al. Infectivity studies of hepatitis A and B in non-human primates. *Dev Biol Stand*. 1975;30:229–235.
107. Hilleman MR, Provost PJ, Villarejos VM, et al. Infectious hepatitis (hepatitis A) research in nonhuman primates. *Bull Pan Am Health Organ*. 1977;11:140–152.
108. Brown EA, Jansen RW, Lemon SM. Characterization of a simian hepatitis A virus (HAV): antigenic and genetic comparison with human HAV. *J Virol*. 1989;63: 4932–4937.
109. Emerson SU, McRill C, Rosenblum B, et al. Mutations responsible for adaptation of hepatitis A virus to efficient growth in cell culture. *J Virol*. 1991;65:4882–4886.
110. Emerson SU, Tsarev SA, Govindarajan S, et al. A simian strain of hepatitis A virus, AGM-27, functions as an attenuated vaccine for chimpanzees. *J Infect Dis*. 1996;173:592–597.
111. Deinhardt F. Hepatitis in primates. *Adv Virus Res*. 1976;20:113–157.
112. Hornei B, Kammerer R, Moubayed P, et al. Experimental hepatitis A virus infection in guinea pigs. *J Med Virol*. 2001;64:402–409.
113. Hirai-Yuki A, Hensley L, McGivern DR, et al. MAVS-dependent host species range and pathogenicity of human hepatitis A virus. *Science*. 2016;353:1541–1545.
114. Feigelstock DA, Thompson P, Kaplan GG. Growth of hepatitis A virus in a mouse liver cell line. *J Virol*. 2005;79:2950–2955.
115. Skinhoj P, Mathiesen LR, Kryger P. Faecal excretion of hepatitis A virus in patients with symptomatic hepatitis A infection. *Ann Intern Med*. 1987;106:221–226.
116. Tassopoulos NC, Papaevangelou GJ, Ticehurst JR, et al. Fecal excretion of Greek strains of hepatitis A virus in patients with hepatitis A and in experimentally infected chimpanzees. *J Infect Dis*. 1986;154:231–237.
117. Krugman S, Ward R, Giles JP, et al. Infectious hepatitis: detection of virus during the incubation period and in clinically inapparent infection. *N Engl J Med*. 1959;261:729–734.
118. Rosenblum LS, Villarino ME, Nainan OV, et al. 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*. 1991;164:476–482.
119. Sjogren MH, Tanno H, Fay O, et al. Hepatitis A virus in stool during clinical relapse. *Ann Intern Med*. 1987;106:221–226.
120. Bower WA, Nainan OV, Han X, et al. Duration of viremia in hepatitis A virus infection. *J Infect Dis*. 2000;182: 12–17.
121. Krugman S, Ward R, Giles JP. The natural history of infectious hepatitis. *Am J Med*. 1962;32:717–728.
122. Lemon SM. The natural history of hepatitis A: the potential for transmission by transfusion of blood or blood products. *Vox Sang*. 1994;67(suppl 4):19–23, discussion 24–26.
123. Purcell RH. Relative infectivity of hepatitis A virus by the oral and intravenous routes in 2 species of nonhuman primates. *J Infect Dis*. 2002;185:1668–1771.
124. Cohen JI, Feinstone S, Purcell RH. Hepatitis A virus infection in a chimpanzee: duration of viremia and detection of virus in saliva and throat swabs. *J Infect Dis*. 1989;160:887–890.
125. Mahboobi N, Porter SR, Karayiannis P, et al. Oral fluid and hepatitis A, B and C: a literature review. *J Oral Pathol Med*. 2012;41:505–516.
126. Bell BP. Global epidemiology of hepatitis A: implications for control strategies. In: Margolis HS, Alter HJ, Liang TJ, et al, eds. *Viral Hepatitis and Liver Disease*. London: International Medical Press; 2002:359–365.
127. Bell BP, Shapiro CN, Alter MJ, et al. The diverse patterns of hepatitis A epidemiology in the United States-implications for vaccination strategies. *J Infect Dis*. 1998;178:1579–1584.
128. Staes CJ, Schlenker TL, Risk I, et al. Sources of infection among persons with acute hepatitis A and no identified risk factors during a sustained community-wide outbreak. *Pediatrics*. 2000;106:E54.
129. Smith PF, Grabau JC, Werzberger A, et al. The role of young children in a community-wide outbreak of hepatitis A. *Epidemiol Infect*. 1997;118: 243–252.
130. Armstrong GL, Bell BP. Hepatitis A virus infections in the United States: model-based estimates and implications for childhood immunization. *Pediatrics*. 2002;109:839–845.
131. Latash J, Dorsinville M, Del Rosso P, et al. Notes from the field: increase in reported hepatitis A infections among men who have sex with men - New York City, January-August 2017. *MMWR Morb Mortal Wkly Rep*. 2017;66:999–1000.
131a. Foster M, Ramachandran S, Myatt K, et al. Hepatitis A virus outbreaks associated with drug use and homelessness - California, Kentucky, Michigan, and Utah, 2017. *MMWR Morb Mortal Wkly Rep*. 2018;67:1208–1210.
132. Freidl GS, Sonder GJ, Bovee LP, et al. Hepatitis A outbreak among men who have sex with men (MSM) predominantly linked with the EuroPride, the Netherlands, July 2016 to February 2017. *Euro Surveill*. 2017;22.
133. Werber D, Michaelis K, Hausner M, et al. Ongoing outbreaks of hepatitis A among men who have sex with men (MSM), Berlin, November 2016 to January 2017 - linked to other German cities and European countries. *Euro Surveill*. 2017;22.
134. Martinez A, Broner S, Sala MR, et al. Changes in the epidemiology of hepatitis A outbreaks 13 years after the introduction of a mass vaccination program. *Hum Vaccin Immunother*. 2015;11:192–197.
135. McCaustland KA, Bond WW, Bradley DW, et al. Survival of hepatitis A virus in feces after drying and storage for 1 month. *J Clin Microbiol*. 1982;16:957–958.
136. Carl M, Francis DP, Maynard JE. Food-borne hepatitis A: recommendations for control. *J Infect Dis*. 1983;148:1133–1135.
137. Massoudi MS, Bell BP, Paredes V, et al. An outbreak of hepatitis A associated with an infected foodhandler. *Public health reports (Washington, DC : 1974)*. 1999;114:157–164.
138. Fiore AE. Hepatitis A transmitted by food. *Clin Infect Dis*. 2004;38:705–715.
139. Centers for Disease C. Foodborne hepatitis A–Alaska, Florida, North Carolina, Washington. *MMWR Morb Mortal Wkly Rep*. 1990;39:228–232.
140. Halliday ML, Kang LY, Zhou TK, et al. An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. *J Infect Dis*. 1991;164:852–859.
141. Wang JY. [Risk factors analysis of epidemic hepatitis A in a factory in shanghai]. *Zhonghua Yu Fang Yi Xue Za Zhi*. 1990;24:90–92.
142. Outbreak of hepatitis A in Hawaii linked to raw scallops.
143. Bellou M, Kokkinos P, Vantarakis A. Shellfish-borne viral outbreaks: a systematic review. *Food Environ Virol*. 2013;5:13–23.
144. Chironna M, Prato R, Sallustio A, et al. Hepatitis A in puglia (South Italy) after 10 years of universal vaccination: need for strict monitoring and catch-up vaccination. *BMC Infect Dis*. 2012;12:271.
145. Guillois-Becel Y, Couturier E, Le Saux JC, et al. An oyster-associated hepatitis A outbreak in France in 2007. *Euro Surveill*. 2009;14.
146. Boxman IL, Verhoef L, Vennema H, et al. International linkage of two food-borne hepatitis A clusters through traceback of mussels, the Netherlands, 2012. *Euro Surveill*. 2016;21:30113.
147. Prato R, Martinelli D, Tafuri S, et al. Safety of shellfish and epidemiological pattern of enterically transmitted diseases in Italy. *Int J Food Microbiol*. 2013;162:125–128.
148. Hutin YJ, Pool V, Cramer EH, et al. A multistate, foodborne outbreak of hepatitis A. National hepatitis A investigation team. *N Engl J Med*. 1999;340:595–602.
149. Dentinger CM, Bower WA, Nainan OV, et al. An outbreak of hepatitis A associated with green onions. *J Infect Dis*. 2001;183:1273–1276.
150. Wheeler C, Vogt TM, Armstrong GL, et al. An outbreak of hepatitis A associated with green onions. *N Engl J Med*. 2005;353:890–897.
151. Amon JJ, Devasia R, Xia G, et al. Molecular epidemiology of foodborne hepatitis A outbreaks in the United States, 2003. *J Infect Dis*. 2005;192:1323–1330.
152. Chancellor DD, Tyagi S, Bazaco MC, et al. Green onions: potential mechanism for hepatitis A contamination. *J Food Prot*. 2006;69:1468–1472.
153. Petrignani M, Harms M, Verhoef L, et al. Update: a food-borne outbreak of hepatitis A in the Netherlands related to semi-dried tomatoes in oil, January-February 2010. *Euro Surveill*. 2010;15.
154. Donnan EJ, Fielding JE, Gregory JE, et al. A multistate outbreak of hepatitis A associated with semidried tomatoes in Australia, 2009. *Clin Infect Dis*. 2012;54:775–781.
155. Collier MG, Khudyakov YE, Selvage D, et al. Outbreak of hepatitis A in the USA associated with frozen pomegranate arils imported from Turkey: an epidemiological case study. *Lancet Infect Dis*. 2014;14:976–981.
156. Scavia G, Alfonsi V, Taffon S, et al. A large prolonged outbreak of hepatitis A associated with consumption of frozen berries, Italy, 2013-14. *J Med Microbiol*. 2017;66:342–349.
157. Severi E, Verhoef L, Thornton L, et al. Large and prolonged food-borne multistate hepatitis A outbreak in Europe associated with consumption of frozen berries, 2013 to 2014. *Euro Surveill*. 2015;20:21192.
158. Guzman-Herrador BR, Panning M, Stene-Johansen K, et al. Importance of molecular typing in confirmation of the source of a national hepatitis A virus outbreak in Norway and the detection of a related cluster in Germany. *Arch Virol*. 2015;160:2823–2826.
159. Hollinger FB, Khan NC, Oefinger PE, et al. Posttransfusion hepatitis type a. *JAMA*. 1983;250: 2313–2317.
160. Soucie JM, Robertson BH, Bell BP, et al. Hepatitis A virus infections associated with clotting factor concentrate in the United States. *Transfusion*. 1998;38:573–579.
161. Benjamin RJ. Nucleic acid testing: update and applications. *Semin Hematol*. 2001;38(4 suppl 9):11–16.
162. Perevoscikovs J, Lenglet A, Lucenko I, et al. Assessing the risk of a community outbreak of hepatitis A on blood safety in Latvia, 2008. *Euro Surveill*. 2010;15:19640.

163. Leikin E, Lysikiewicz A, Garry D, et al. Intrauterine transmission of hepatitis A virus. *Obstet Gynecol*. 1996;88(4 Pt 2):690–691.
164. McDuffie RS Jr, Bader T. Fetal meconium peritonitis after maternal hepatitis A. *Am J Obstet Gynecol*. 1999;180:1031–1032.
165. Tong MJ, Thursby M, Rakela J, et al. Studies on the maternal-infant transmission of the viruses which cause acute hepatitis. *Gastroenterology*. 1981;80(5 Pt 1):999–1004.
166. Watson JC, Fleming DW, Borella AJ, et al. Vertical transmission of hepatitis A resulting in an outbreak in a neonatal intensive care unit. *J Infect Dis*. 1993;167:567–571.
167. Aggarwal R, Goel A. Hepatitis A: epidemiology in resource-poor countries. *Curr Opin Infect Dis*. 2015;28:488–496.
168. Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. *Vaccine*. 2010;28:6653–6657.
169. Jacobsen KH. Globalization and the changing epidemiology of hepatitis A virus. *Cold Spring Harb Perspect Med*. 2018.
170. *Global Hepatitis Report 2017*. Geneva: World Health Organization; 2017.
171. Coursaget P, Leboulleux D, Gharbi Y, et al. Etiology of acute sporadic hepatitis in adults in Senegal and Tunisia. *Scand J Infect Dis*. 1995;27:9–11.
172. Tsega E, Mengesha B, Hansson BG, et al. Hepatitis A, B, and delta infection in Ethiopia: a serologic survey with demographic data. *Am J Epidemiol*. 1986;123:344–351.
173. Centers for Disease C, Prevention. Establishment of a viral hepatitis surveillance system–Pakistan, 2009-2011. *MMWR Morb Mortal Wkly Rep*. 2011;60:1385–1390.
174. Saha SK, Saha S, Shakur S, et al. Community-based cross-sectional seroprevalence study of hepatitis A in Bangladesh. *World J Gastroenterol*. 2009;15:4932–4937.
175. Talaat M, El-Sayed N, Kandeel A, et al. Sentinel surveillance for patients with acute hepatitis in Egypt, 2001-04. *East Mediterr Health J*. 2010;16:134–140.
176. Kumar T, Shrivastava A, Kumar A, et al. Viral hepatitis surveillance - India, 2011-2013. *MMWR Morb Mortal Wkly Rep*. 2015;64:758–762.
177. Al-Aziz AM, Awad MA. Seroprevalence of hepatitis A virus antibodies among a sample of Egyptian children. *East Mediterr Health J*. 2008;14:1028–1035.
178. Bowden FJ, Currie BJ, Miller NC, et al. Should aboriginals in the "top end" of the Northern Territory be vaccinated against hepatitis A? *Med J Aust*. 1994;161:372–373.
179. Hrivniakova L, Slacikova M, Kolcunova S. Hepatitis A outbreak in a Roma village in eastern Slovakia, August-November 2008. *Euro Surveill*. 2009;14.
180. Bialek SR, Thoroughman DA, Hu D, et al. Hepatitis A incidence and hepatitis A vaccination among American Indians and Alaska Natives, 1990-2001. *Am J Public Health*. 2004;94:996–1001.
181. Cianciara J. Hepatitis A shifting epidemiology in Poland and Eastern Europe. *Vaccine*. 2000;18(suppl 1):S68–S70.
182. Green MS, Block C, Slater PE. Rise in the incidence of viral hepatitis in Israel despite improved socioeconomic conditions. *Rev Infect Dis*. 1989;11:464–469.
183. Safiabadi M, Rezaee-Zavareh MS, Moayed Alavian S. Estimation of hepatitis A virus infection prevalence among Eastern Mediterranean and middle Eastern countries: a systematic review and pooled analysis. *Hepat Mon*. 2017;17:e44695.
184. Melhem NM, Jaffa M, Zaatari M, et al. The changing pattern of hepatitis A in Lebanese adults. *Int J Infect Dis*. 2015;30:87–90.
185. Kury CM, Pinto MA, Silva JP, et al. Hepatitis A seroprevalence in public school children in Campos dos Goytacazes, Rio de Janeiro State, Brazil, prior to the introduction of the hepatitis A universal childhood vaccination. *Cad Saude Publica*. 2016;32:e00175614.
186. Hayajneh WA, Balbeesi A, Faouri S. Hepatitis A virus age-specific sero-prevalence and risk factors among Jordanian children. *J Med Virol*. 2015;87:569–574.
187. Gargouri S, Fki Berrajah L, Ayadi I, et al. Epidemiological and clinical analysis of hepatitis virus a infections during three successive outbreaks in Sfax (Tunisia) between 2007 and 2010. *Med Sante Trop*. 2016;26:159–164.
188. Chen R, et al. "Antibody detection of hepatitis A in children under 13 years old in rural area of jintang county.". *Modern Preventive Medicine*. 2016;43:2942–2944.
189. Lagos R, Potin M, Munoz A, et al. [Serum antibodies against hepatitis A virus among subjects of middle and low socioeconomic levels in urban area of santiago, Chile]. *Rev Med Chil*. 1999;127:429–436.
190. Gdalevich M, Grotto I, Mandel Y, et al. Hepatitis A antibody prevalence among young adults in Israel–the decline continues. *Epidemiol Infect*. 1998;121:477–479.
191. Innis BL, Snitbhan R, Hoke CH, et al. The declining transmission of hepatitis A in Thailand. *J Infect Dis*. 1991;163:989–995.
192. Kunasol P, Cooksley G, Chan VF, et al. Hepatitis A virus: declining seroprevalence in children and adolescents in Southeast Asia. *Southeast Asian J Trop Med Public Health*. 1998;29:255–262.
193. Pinho JR, Sumita LM, Moreira RC, et al. Duality of patterns in hepatitis A epidemiology: a study involving two socioeconomically distinct populations in Campinas, Sao Paulo State, Brazil. *Rev Inst Med Trop Sao Paulo*. 1998;40:105–106.
194. Poovorawan Y, Vimolkej T, Chongsrisawat V, et al. The declining pattern of seroepidemiology of hepatitis A virus infection among adolescents in Bangkok, Thailand. *Southeast Asian J Trop Med Public Health*. 1997;28:154–157.
195. Das K, Jain A, Gupta S, et al. The changing epidemiological pattern of hepatitis A in an urban population of India: emergence of a trend similar to the European countries. *Eur J Epidemiol*. 2000;16:507–510.
196. Tapia-Conyer R, Santos JI, Cavalcanti AM, et al. Hepatitis A in Latin America: a changing epidemiologic pattern. *Am J Trop Med Hyg*. 1999;61:825–829.
197. Tufenkeji H. Hepatitis A shifting epidemiology in the Middle East and Africa. *Vaccine*. 2000;18(suppl 1):S65–S67.
198. Wang LY, Cheng YW, Chou SJ, et al. Secular trend and geographical variation in hepatitis A infection and hepatitis B carrier rate among adolescents in Taiwan: an island-wide survey. *J Med Virol*. 1993;39:1–5.
199. Abdo AA, Sanai FM, Al-Faleh FZ. Epidemiology of viral hepatitis in Saudi Arabia: are we off the hook? *Saudi J Gastroenterol*. 2012;18:349–357.
200. Lazcano-Ponce E, Conde-Gonzalez C, Rojas R, et al. Seroprevalence of hepatitis A virus in a cross-sectional study in Mexico: implications for hepatitis A vaccination. *Hum Vaccin Immunother*. 2013;9:375–381.
201. Ciocca M. Clinical course and consequences of hepatitis A infection. *Vaccine*. 2000;18(suppl 1):S71–S74.
202. Shah U, Habib Z, Kleinman RE. Liver failure attributable to hepatitis A virus infection in a developing country. *Pediatrics*. 2000;105:436–438.
203. Gil A, Gonzalez A, Dal-Re R, et al. Prevalence of antibodies against varicella zoster, herpes simplex (types 1 and 2), hepatitis B and hepatitis A viruses among Spanish adolescents. *J Infect*. 1998;36:53–56.
204. Shapiro CN, Coleman PJ, McQuillan GM, et al. Epidemiology of hepatitis A: seroepidemiology and risk groups in the USA. *Vaccine*. 1992;10(suppl 1):S59–S62.
205. Prodinger WM, Larcher C, Solder BM, et al. Hepatitis A in Western Austria–the epidemiological situation before the introduction of active immunisation. *Infection*. 1994;22:53–55.
206. Termorshuizen F, Dorigo-Zetsma JW, de Melker HE, et al. The prevalence of antibodies to hepatitis A virus and its determinants in The Netherlands: a population-based survey. *Epidemiol Infect*. 2000;124:459–466.
207. Castkova J, Benes C. Increase in hepatitis A cases in the Czech Republic in 2008 - an update. *Euro Surveill*. 2009;14.
208. Klevens RM, Denniston MM, Jiles-Chapman RB, et al. Decreasing immunity to hepatitis A virus infection among US adults: findings from the National Health and Nutrition Examination Survey (NHANES), 1999-2012. *Vaccine*. 2015;33:6192–6198.
209. Bottiger M, Christenson B, Grillner L. Hepatitis A immunity in the Swedish population. A study of the prevalence of markers in the Swedish population. *Scand J Infect Dis*. 1997;29:99–102.
210. Tortajada C, de Olalla PG, Diez E, et al. Hepatitis A among men who have sex with men in Barcelona, 1989-2010: insufficient control and need for new approaches. *BMC Infect Dis*. 2012;12:11.
211. Girardi E, Scognamiglio P, Sciarrone MR, et al. High HIV prevalence in male patients with acute hepatitis A in the Rome metropolitan area, Italy 2002-2008. *J Hepatol*. 2011;54:1102–1106.
212. Sfetcu O, Irvine N, Ngui SL, et al. Hepatitis A outbreak predominantly affecting men who have sex with men in Northern Ireland, October 2008 to July 2009. *Euro Surveill*. 2011;16.
213. Faber MS, Stark K, Behnke SC, et al. Epidemiology of hepatitis A virus infections, Germany, 2007-2008. *Emerg Infect Dis*. 2009;15:1760–1768.
214. Couturier E, Roque-Afonso AM, Letort MJ, et al. Cluster of cases of hepatitis A with a travel history to Egypt, September-November 2008, France. *Euro Surveill*. 2009;14.
215. Askling HH, Rombo L, Andersson Y, et al. Hepatitis A risk in travelers. *J Travel Med*. 2009;16:233–238.
216. Kumbang J, Ejide S, Tedder RS, et al. Outbreak of hepatitis A in an extended family after importation by non-immune travellers. *Epidemiol Infect*. 2012;140:1813–1820.
217. van Genderen PJ, van Thiel PP, Mulder PG, et al. Trends in knowledge, attitudes, and practices of travel risk groups toward prevention of hepatitis A: results from the Dutch schiphol airport survey 2002 to 2009. *J Travel Med*. 2012;19:35–43.
218. Godoy P, Broner S, Manzanares-Laya S, et al. Outbreaks of hepatitis A associated with immigrants travelling to visit friends and relatives. *J Infect*. 2016;72:112–115.
219. Mellou K, Chrisostomou A, Sideroglou T, et al. Hepatitis A among refugees, asylum seekers and migrants living in hosting facilities, Greece, April to December 2016. *Euro Surveill*. 2017;22.
220. Michaelis K, Wenzel JJ, Stark K, et al. Hepatitis A virus infections and outbreaks in asylum seekers arriving to Germany, September 2015 to March 2016. *Emerg Microbes Infect*. 2017;6:e26.
221. Doganay M, Demiraslan H. Refugees of the Syrian Civil War: impact on reemerging infections, health services, and biosecurity in Turkey. *Health Secur*. 2016;14:220–225.
222. Wasley A, Fiore A, Bell BP. Hepatitis A in the era of vaccination. *Epidemiol Rev*. 2006;28:101–111.
223. Centers for Disease C. Surveillance for Viral Hepatitis – United States, 2016; 2018. https://www.cdc.gov/hepatitis/statistics/2016surveillance/index.htm. Accessed May 2018.
224. Centers for Disease C. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 1999;48(RR–12):1–37.
225. Wasley A, Samandari T, Bell BP. Incidence of hepatitis A in the United States in the era of vaccination. *JAMA*. 2005;294:194–201.
226. Advisory Committee on Immunization Practices, Fiore AE, Wasley A, et al. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 2006;55(RR–7):1–23.
227. Daniels D, Grytdal S, Wasley A. Surveillance for acute viral hepatitis. United States 2007. *MMWR Morb Mortal Wkly Rep*. 2009;58:1–27.
228. Centers for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 1996;45(RR–15):1–30.
229. Centers for Disease Control and Prevention. Surveillance for Viral Hepatitis – United States, 2013; 2013.
229a. Surveillance for Viral Hepatitis – United States, 2016. https://www.cdc.gov/hepatitis/statistics/2016surveillance/pdfs/2016HepSurveillanceRpt.pdf. Accessed February 28, 2019.
230. Franco E, Meleleo C, Serino L, et al. Hepatitis A: epidemiology and prevention in developing countries. *World J Hepatol*. 2012;4:68–73.
231. Victor JC, Surdina TY, Suleimenova SZ, et al. Person-to-person transmission of hepatitis A virus in an urban area of intermediate endemicity: implications for vaccination strategies. *Am J Epidemiol*. 2006;163:204–210.
232. Hadler SC, Webster HM, Erben JJ, et al. Hepatitis A in day-care centers. A community-wide assessment. *N Engl J Med*. 1980;302:1222–1227.
233. Venczel LV, Desai MM, Vertz PD, et al. The role of child care in a community-wide outbreak of hepatitis A. *Pediatrics*. 2001;108:E78.
234. Armstrong GL, Billah K, Rein DB, et al. The economics of routine childhood hepatitis A immunization in the United States: the impact of herd immunity. *Pediatrics*. 2007;119:e22–e29.
235. Klevens RM, Miller JT, Iqbal K, et al. The evolving epidemiology of hepatitis A in the United States: incidence and molecular epidemiology from population-based surveillance, 2005-2007. *Arch Intern Med*. 2010;170:1811–1818.
236. Steffen R, Rickenbach M, Wilhelm U, et al. Health problems after travel to developing countries. *J Infect Dis*. 1987;156:84–91.
237. Steffen R. Risk of hepatitis A in travellers. *Vaccine*. 1992;10(suppl 1):S69–S72.
238. Mele A, Sagliocca L, Palumbo F, et al. Travel-associated hepatitis A: effect of place of residence and country visited. *J Public Health Med*. 1991;13:256–259.
239. Braccio S, Irwin A, Riordan A, et al. Acute infectious hepatitis in hospitalised children: a British Paediatric Surveillance Unit study. *Arch Dis Child*. 2017;102:624–628.
240. Sane J, de Sousa R, van Pelt W, et al. Risk of hepatitis A decreased among Dutch travelers to endemic regions in 2003 to 2011. *J Travel Med*. 2015;22:208–211.
241. Steffen R, Kane MA, Shapiro CN, et al. Epidemiology and prevention of hepatitis A in travelers. *JAMA*. 1994;272:885–889.

242. Mutsch M, Spicher VM, Gut C, et al. Hepatitis A virus infections in travelers, 1988-2004. *Clin Infect Dis*. 2006;42:490–497.
243. Lange WR, Frame JD. High incidence of viral hepatitis among American missionaries in Africa. *Am J Trop Med Hyg*. 1990;43:527–533.
244. Baaten GG, Sonder GJ, Van Der Loeff MF, et al. Fecal-orally transmitted diseases among travelers are decreasing due to better hygienic standards at travel destination. *J Travel Med*. 2010;17:322–328.
245. Nielsen US, Larsen CS, Howitz M, et al. Hepatitis A among Danish travellers 1980-2007. *J Infect*. 2009;58:47–52.
246. Weinberg M, Hopkins J, Farrington L, et al. Hepatitis A in Hispanic children who live along the United States-Mexico border: the role of international travel and food-borne exposures. *Pediatrics*. 2004;114:e68–e73.
247. Baggett HC, Graham S, Kozarsky PE, et al. Pretravel health preparation among US residents traveling to India to VFRs: importance of ethnicity in defining VFRs. *J Travel Med*. 2009;16:112–118.
248. Gosselin C, De Serres G, Rouleau I, et al. Comparison of trip characteristics of children and adults with travel-acquired hepatitis A infection. *Pediatr Infect Dis J*. 2006;25:1184–1186.
249. Slinko VG, Jarvinen KA, Beard FH, et al. Notifications of enteric diseases in returning travelers who visit friends and relatives overseas: a call for action. *Commun Dis Intell*. 2008;32:333–334.
250. Adamson R, Reddy V, Jones L, et al. Epidemiology and burden of hepatitis A, malaria, and typhoid in New York City associated with travel: implications for public health policy. *Am J Public Health*. 2010;100:1249–1252.
251. Boggild AK, Castelli F, Gautret P, et al. Vaccine preventable diseases in returned international travelers: results from the GeoSentinel surveillance network. *Vaccine*. 2010;28:7389–7395.
252. Sonder GJ, Bovee LP, Baayen TD, et al. Effectiveness of a hepatitis A vaccination program for migrant children in Amsterdam, The Netherlands (1992-2004). *Vaccine*. 2006;24:4962–4968.
253. Christenson B. Epidemiological aspects of acute viral hepatitis A in Swedish travellers to endemic areas. *Scand J Infect Dis*. 1985;17:5–10.
254. MacDonald E, Steens A, Stene-Johansen K, et al. Increase in hepatitis A in tourists from Denmark, England, Germany, the Netherlands, Norway and Sweden returning from Egypt, November 2012 to March 2013. *Euro Surveill*. 2013;18:20468.
255. Centers for Disease C, Prevention, Advisory Committee on Immunization P. Updated recommendations from the Advisory Committee on Immunization Practices (ACIP) for use of hepatitis A vaccine in close contacts of newly arriving international adoptees. *MMWR Morb Mortal Wkly Rep*. 2009;58:1006–1007.
256. Fischer GE, Teshale EH, Miller C, et al. Hepatitis A among international adoptees and their contacts. *Clin Infect Dis*. 2008;47:812–814.
257. Pelletier AR, Mehta PJ, Burgess DR, et al. An outbreak of hepatitis A among primary and secondary contacts of an international adoptee. *Public Health Reports (Washington, DC : 1974)*. 2010;125:642–646.
258. Abdulla RY, Rice MA, Donauer S, et al. Hepatitis A in internationally adopted children: screening for acute and previous infections. *Pediatrics*. 2010;126:e1039–e1044.
259. Sweet K, Sutherland W, Ehresmann K, et al. Hepatitis A infection in recent international adoptees and their contacts in Minnesota, 2007-2009. *Pediatrics*. 2011;128:e333–e338.
260. Raabe VN, Sautter C, Chesney M, et al. Hepatitis A screening for internationally adopted children from hepatitis A endemic countries. *Clin Pediatr (Phila)*. 2014;53:31–37.
261. Dahanayaka NJ, Kiyohara T, Agampodi SB, et al. Clinical features and transmission pattern of hepatitis A: an experience from a hepatitis A outbreak caused by two cocirculating genotypes in Sri Lanka. *Am J Trop Med Hyg*. 2016;95:908–914.
262. Vong S, Fiore AE, Haight DO, et al. 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*. 2005;23:1021–1028.
263. Prevention CfDCa. Hepatitis Surveillance Report No. 60 Atlanta, GA; 2005.
264. Harkess J, Gildon B, Istre GR. Outbreaks of hepatitis A among illicit drug users, Oklahoma, 1984-87. *Am J Public Health*. 1989;79:463–466.
265. Shaw DD, Whiteman DC, Merritt AD, et al. Hepatitis A outbreaks among illicit drug users and their contacts in Queensland, 1997. *Med J Aust*. 1999;170:584–587.
266. O'Donovan D, Cooke RP, Joce R, et al. An outbreak of hepatitis A amongst injecting drug users. *Epidemiol Infect*. 2001;127:469–473.
267. Hutin YJ, Sabin KM, Hutwagner LC, et al. Multiple modes of hepatitis A virus transmission among methamphetamine users. *Am J Epidemiol*. 2000;152:186–192.
268. Ngui SL, Granerod J, Jewes LA, et al. Outbreaks of hepatitis A in England and Wales associated with two co-circulating hepatitis A virus strains. *J Med Virol*. 2008;80:1181–1188.
269. Tjon GM, Gotz H, Koek AG, et al. An outbreak of hepatitis A among homeless drug users in Rotterdam, The Netherlands. *J Med Virol*. 2005;77:360–366.
270. Spada E, Genovese D, Tosti ME, et al. An outbreak of hepatitis A virus infection with a high case-fatality rate among injecting drug users. *J Hepatol*. 2005;43:958–964.
271. Roy K, Howie H, Sweeney C, et al. Hepatitis A virus and injecting drug misuse in Aberdeen, Scotland: a case-control study. *J Viral Hepat*. 2004;11:277–282.
272. Ochnio JJ, Patrick D, Ho M, et al. Past infection with hepatitis A virus among Vancouver street youth, injection drug users and men who have sex with men: implications for vaccination programs. *CMAJ*. 2001;165:293–297.
273. Hutin YJ, Bell BP, Marshall KL, et al. Identifying target groups for a potential vaccination program during a hepatitis A communitywide outbreak. *Am J Public Health*. 1999;89:918–921.
274. Ivie K, Spruill C, Bell BP. Prevalence of hepatitis A virus infection among illicit drug users, 1993–1994. *Antivir Ther*. 2000;5(suppl 1):A–7.
275. Villano SA, Nelson KE, Vlahov D, et al. Hepatitis A among homosexual men and injection drug users: more evidence for vaccination. *Clin Infect Dis*. 1997;25:726–728.
276. Bart G, Piccolo P, Zhang L, et al. Markers for hepatitis A, B, and C in methadone maintaned patients; an unexpectedly high co-infection with silent hepatitis B. *Addiction*. 2008;103:681–686.
277. Gonzales R, Marinelli-Casey P, Hillhouse M, et al. Hepatitis A and B infection among methamphetamine-dependent users. *J Subst Abuse Treat*. 2008;35:351–352.
278. Reimer J, Lorenzen J, Baetz B, et al. Multiple viral hepatitis in injection drug users and associated risk factors. *J Gastroenterol Hepatol*. 2007;22:80–85.
279. Wells R, Fisher D, Fenaughty A, et al. Hepatitis A prevalence among injection drug users. *Clin Lab Sci*. 2006;19:12–17.
280. Dickson-Spillmann M, Haug S, Uchtenhagen A, et al. Rates of HIV and hepatitis infections in clients entering Heroin-Assisted treatment between 2003 and 2013 and risk factors for hepatitis C infection. *Eur Addict Res*. 2016;22:181–191.
281. Perlman DC, Jordan AE, McKnight C, et al. Viral hepatitis among drug users in methadone maintenance: associated factors, vaccination outcomes, and interventions. *J Addict Dis*. 2014;0.
282. Syed NA, Hearing SD, Shaw IS, et al. Outbreak of hepatitis A in the injecting drug user and homeless populations in Bristol: control by a targeted vaccination programme and possible parenteral transmission. *Eur J Gastroenterol Hepatol*. 2003;15:901–906.
283. Badiaga S, Raoult D, Brouqui P. Preventing and controlling emerging and reemerging transmissible diseases in the homeless. *Emerg Infect Dis*. 2008;14:1353–1359.
284. Hennessey KA, Bangsberg DR, Weinbaum C, et al. Hepatitis A seroprevalence and risk factors among homeless adults in san francisco: should homelessness be included in the risk-based strategy for vaccination? *Public Health Reports (Washington, DC : 1974)*. 2009;124:813–817.
285. Beebeejaun K, Degala S, Balogun K, et al. Outbreak of hepatitis A associated with men who have sex with men (MSM), England, July 2016 to January 2017. *Euro Surveill*. 2017;22.
286. Cotter SM, Sansom S, Long T, et al. Outbreak of hepatitis A among men who have sex with men: implications for hepatitis A vaccination strategies. *J Infect Dis*. 2003;187:1235–1240.
287. Friedman MS, Blake PA, Koehler JE, et al. Factors influencing a communitywide campaign to administer hepatitis A vaccine to men who have sex with men. *Am J Public Health*. 2000;90:1942–1946.
288. Stokes ML, Ferson MJ, Young LC. Outbreak of hepatitis A among homosexual men in Sydney. *Am J Public Health*. 1997;87:2039–2041.
289. Henning KJ, Bell E, Braun J, et al. A community-wide outbreak of hepatitis A: risk factors for infection among homosexual and bisexual men. *Am J Med*. 1995;99:132–136.
290. Centers for Disease C. Hepatitis A among homosexual men–United States, Canada, and Australia. *MMWR Morb Mortal Wkly Rep*. 1992;41:155, 161–164.
291. Mazick A, Howitz M, Rex S, et al. Hepatitis A outbreak among MSM linked to casual sex and gay saunas in Copenhagen, Denmark. *Euro Surveill*. 2005;10:111–114.
292. Bordi L, Rozera G, Scognamiglio P, et al. Monophyletic outbreak of hepatitis A involving HIV-infected men who have sex with men, Rome, Italy 2008-2009. *J Clin Virol*. 2012;54:26–29.
293. Dabrowska MM, Nazzal K, Wiercinska-Drapalo A. Hepatitis A and hepatitis A virus/HIV coinfection in men who have sex with men, Warsaw, Poland, September 2008 to September 2009. *Euro Surveill*. 2011;16.
294. Katz MH, Hsu L, Wong E, et al. Seroprevalence of and risk factors for hepatitis A infection among young homosexual and bisexual men. *J Infect Dis*. 1997;175:1225–1229.
295. Bialek SR, Barry V, Bell BP, et al. Seroprevalence and correlates of hepatitis A among HIV-negative American men who have sex with men. *Sex Health*. 2011;8:343–348.
296. Gowland P, Fontana S, Niederhauser C, et al. Molecular and serologic tracing of a transfusion-transmitted hepatitis A virus. *Transfusion*. 2004;44:1555–1561.
297. Mah MW, Royce RA, Rathouz PJ, et al. Prevalence of hepatitis A antibodies in hemophiliacs: preliminary results from the Southeastern Delta Hepatitis Study. *Vox Sang*. 1994;67(suppl 1):21–22, discussion 23.
298. Mannucci PM, Santagostino E, Di Bona E, et al. The outbreak of hepatitis A in Italian patients with hemophilia: facts and fancies. *Vox Sang*. 1994;67(suppl 1):31–35.
299. Centers for Disease C, Prevention. Blood safety monitoring among persons with bleeding disorders–United States, May 1998-June 2002. *MMWR Morb Mortal Wkly Rep*. 2003;51:1152–1154.
300. Klein BS, Michaels JA, Rytel MW, et al. Nosocomial hepatitis A. A multinursery outbreak in Wisconsin. *JAMA*. 1984;252:2716–2721.
301. Noble RC, Kane MA, Reeves SA, et al. Posttransfusion hepatitis A in a neonatal intensive care unit. *JAMA*. 1984;252:2711–2715.
302. Chodick G, Ashkenazi S, Lerman Y. The risk of hepatitis A infection among healthcare workers: a review of reported outbreaks and sero-epidemiologic studies. *J Hosp Infect*. 2006;62:414–420.
303. Goodman RA. Nosocomial hepatitis A. *Ann Intern Med*. 1985;103:452–454.
304. Papaevangelou GJ, Roumeliotou-Karayannis AJ, Contoyannis PC. The risk of nosocomial hepatitis A and B virus infections from patients under care without isolation precaution. *J Med Virol*. 1981;7:143–148.
305. Gibas A, Blewett DR, Schoenfeld DA, et al. Prevalence and incidence of viral hepatitis in health workers in the prehepatitis B vaccination era. *Am J Epidemiol*. 1992;136:603–610.
306. Centers for Disease C, Prevention. Foodborne transmission of hepatitis A–Massachusetts, 2001. *MMWR Morb Mortal Wkly Rep*. 2003;52:565–567.
307. Dalton CB, Haddix A, Hoffman RE, et al. The cost of a food-borne outbreak of hepatitis A in denver, colo. *Arch Intern Med*. 1996;156:1013–1016.
308. Latham RH, Schable CA. Foodborne hepatitis A at a family reunion use of IgM-specific hepatitis A serologic testing. *Am J Epidemiol*. 1982;115:640–645.
309. Lowry PW, Levine R, Stroup DF, et al. Hepatitis A outbreak on a floating restaurant in Florida, 1986. *Am J Epidemiol*. 1989;129:155–164.
310. Rosenblum LS, Mirkin IR, Allen DT, et al. A multifocal outbreak of hepatitis A traced to commercially distributed lettuce. *Am J Public Health*. 1990;80:1075–1079.
311. Niu MT, Polish LB, Robertson BH, et al. Multistate outbreak of hepatitis A associated with frozen strawberries. *J Infect Dis*. 1992;166:518–524.
312. Reid TM, Robinson HG. Frozen raspberries and hepatitis A. *Epidemiol Infect*. 1987;98:109–112.
313. Kozak GK, MacDonald D, Landry L, et al. Foodborne outbreaks in Canada linked to produce: 2001 through 2009. *J Food Prot*. 2013;76:173–183.
314. Chiapponi C, Pavoni E, Bertasi B, et al. Isolation and genomic sequence of hepatitis A virus from mixed frozen berries in Italy. *Food and environmental virology*. 2014;6:202–206.
315. *EFSA Journal*. 2014;12:3821.
316. Rizzo C, Alfonsi V, Bruni R, et al. Ongoing outbreak of hepatitis A in Italy: preliminary report as of 31 May 2013. *Euro Surveill*. 2013;18.
317. *Euro Surveill*. 2013;18.
318. Authority EFS. Tracing of food items in connection to the multinational hepatitis A virus outbreak. *EFSA Journal*. 2014;12:3821.
319. Guzman-Herrador B, Jensvoll L, Einoder-Moreno M, et al. Ongoing hepatitis A outbreak in Europe 2013 to 2014: imported berry mix cake suspected to be the source of infection in Norway. *Euro Surveill*. 2014;19.
320. Nordic Outbreak Investigation Team C. Joint analysis by the nordic countries of a hepatitis A outbreak, October

2012 to June 2013: frozen strawberries suspected. *Euro Surveill*. 2013;18.

321. Gallot C, Grout L, Roque-Afonso AM, et al. Hepatitis A associated with semidried tomatoes, France, 2010. *Emerg Infect Dis*. 2011;17:566–567.
322. Bloch AB, Stramer SL, Smith JD, et al. Recovery of hepatitis A virus from a water supply responsible for a common source outbreak of hepatitis A. *Am J Public Health*. 1990;80:428–430.
323. De Serres G, Cromeans TL, Levesque B, et al. Molecular confirmation of hepatitis A virus from well water: epidemiology and public health implications. *J Infect Dis*. 1999;179:37–43.
324. Bergeisen GH, Hinds MW, Skaggs JW. A waterborne outbreak of hepatitis A in meade County, Kentucky. *Am J Public Health*. 1985;75:161–164.
325. Lerman Y, Chodik G, Aloni H, et al. Occupations at increased risk of hepatitis A: a 2-year nationwide historical prospective study. *Am J Epidemiol*. 1999;150:312–320.
326. Glas C, Hotz P, Steffen R. Hepatitis A in workers exposed to sewage: a systematic review. *Occup Environ Med*. 2001;58:762–768.
327. Poole CJ, Shakespeare AT. Should sewage workers and carers for people with learning disabilities be vaccinated for hepatitis A? *BMJ*. 1993;306:1102.
328. Trout D, Mueller C, Venczel L, et al. Evaluation of occupational transmission of hepatitis A virus among wastewater workers. *J Occup Environ Med*. 2000;42:83–87.
329. Weldon M, VanEgdom MJ, Hendricks KA, et al. Prevalence of antibody to hepatitis A virus in drinking water workers and wastewater workers in Texas from 1996 to 1997. *J Occup Environ Med*. 2000;42:821–826.
330. Venczel L, Brown S, Frumkin H, et al. Prevalence of hepatitis A virus infection among sewage workers in Georgia. *Am J Ind Med*. 2003;43:172–178.
331. Szmuness W, Purcell RH, Dienstag JL, et al. Antibody to hepatitis A antigen in institutionalized mentally retarded patients. *JAMA*. 1977;237:1702–1705.
332. Bohm SR, Berger KW, Hackert PB, et al. Hepatitis A outbreak among adults with developmental disabilities in group homes–Michigan, 2013. *MMWR Morb Mortal Wkly Rep*. 2015;64:148–152.
333. Krugman S, Giles JP, Hammond J. Infectious hepatitis. Evidence for two distinctive clinical, epidemiological, and immunological types of infection. *JAMA*. 1967;200:365–373.
334. Purcell RH, Feinstone SM, Ticehurst J, et al. Hepatitis A virus. In: Vyas GN, Dienstag JL, Hoofnagle JH, eds. *Viral Hepatitis and Liver Disease*. Orlando, FL: Grune & Stratton; 1984:9–22.
335. Karayiannis P, Jowett T, Enticott M, et al. Hepatitis A virus replication in tamarins and host immune response in relation to pathogenesis of liver cell damage. *J Med Virol*. 1986;18:261–276.
336. Karayiannis. Detection of hepatitis A virus RNA in tissues and feces of experimentally infected tamarins by CDNA-RNA hybridization. In: Zuckerman AJ, ed. *Viral Hepatitis and Liver Disease*. New York: Alan R. Liss; 1988:117–120.
337. Mathiesen LR, Moller AM, Purcell RH, et al. Hepatitis A virus in the liver and intestine of marmosets after oral inoculation. *Infect Immun*. 1980;28:45–48.
338. Mathiesen LR, Feinstone SM, Purcell RH, et al. Detection of hepatitis A antigen by immunofluorescence. *Infect Immun*. 1977;18:524–530.
339. Schulman AN, Dienstag JL, Jackson DR, et al. Hepatitis A antigen particles in liver, bile, and stool of chimpanzees. *J Infect Dis*. 1976;134:80–84.
340. Mathiesen LR, Drucker J, Lorenz D, et al. Localization of hepatitis A antigen in marmoset organs during acute infection with hepatitis A virus. *J Infect Dis*. 1978;138:369–377.
341. Krawczynski KK, Bradley DW, Murphy BL, et al. Pathogenetic aspects of hepatitis A virus infection in enterally inoculated marmosets. *Am J Clin Pathol*. 1981;76:698–706.
342. Locarnini SA, Coulepis AG, Kaldor J, et al. Coproantibodies in hepatitis A: detection by enzyme-linked immunosorbent assay and immune electron microscopy. *J Clin Microbiol*. 1980;11:710–716.
343. Yoshizawa H, Itoh Y, Iwakiri S, et al. Diagnosis of type a hepatitis by fecal IgA antibody against hepatitis A antigen. *Gastroenterology*. 1980;78:114–118.
344. Vallbracht A, Gabriel P, Maier K, et al. Cell-mediated cytotoxicity in hepatitis A virus infection. *Hepatology*. 1986;6:1308–1314.
345. Feinstone SM, Gust ID. Hepatitis A infection: clinical aspects. In: Seeff LB, Lewis JH, eds. *Current Perspectives in Hepatology*. New York: Plenum; 1989:3–14.
346. Vallbracht A, Fleischer B. Immune pathogenesis of hepatitis A. *Arch Virol Suppl*. 1992;4:3–4.
347. Kurane I, Binn LN, Bancroft WH, et al. Human lymphocyte responses to hepatitis A virus-infected cells: interferon production and lysis of infected cells. *J Immunol*. 1985;135:2140–2144.
348. Margolis HS, Nainan OV. Identification of virus components in circulating immune complexes isolated during hepatitis A virus infection. *Hepatology*. 1990;11:31–37.
349. Inman RD, Hodge M, Johnston ME, et al. Arthritis, vasculitis, and cryoglobulinemia associated with relapsing hepatitis A virus infection. *Ann Intern Med*. 1986;105:700–703.
350. Thomas HC, De Villiers D, Potter B, et al. Immune complexes in acute and chronic liver disease. *Clin Exp Immunol*. 1978;31:150–157.
351. Koff RS. Clinical manifestations and diagnosis of hepatitis A virus infection. *Vaccine*. 1992;10(suppl 1):S15–S17.
352. Mathiesen LR. The hepatitis A virus infection. *Liver*. 1981;1:81–109.
353. Lednar WM, Lemon SM, Kirkpatrick JW, et al. Frequency of illness associated with epidemic hepatitis A virus infections in adults. *Am J Epidemiol*. 1985;122:226–233.
354. Skinhoj P. Natural history of viral hepatitis in Greenland. *Am J Med Sci*. 1975;270:305–307.
355. Skinhoj P, Mikkelsen F, Hollinger FB. Hepatitis A in Greenland: importance of specific antibody testing in epidemiologic surveillance. *Am J Epidemiol*. 1977;105:140–147.
356. Hadler SC, McFarland L. Hepatitis in day care centers: epidemiology and prevention. *Rev Infect Dis*. 1986;8:548–557.
357. Tong MJ, el-Farra NS, Grew MI. Clinical manifestations of hepatitis A: recent experience in a community teaching hospital. *J Infect Dis*. 1995;171(suppl 1):S15–S18.
358. Zhang RL, Zeng JS, Zhang HZ. Survey of 34 pregnant women with hepatitis A and their neonates. *Chin Med J*. 1990;103:552–555.
359. Mishra L, Seeff LB. Viral hepatitis, a though e, complicating pregnancy. *Gastroenterol Clin North Am*. 1992;21:873–887.
360. Puoti M, Moioli MC, Travi G, et al. The burden of liver disease in human immunodeficiency virus-infected patients. *Semin Liver Dis*. 2012;32:103–113.
361. Vento S, Garofano T, Renzini C, et al. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. *N Engl J Med*. 1998;338:286–290.
362. Bianco E, Stroffolini T, Spada E, et al. Case fatality rate of acute viral hepatitis in Italy: 1995-2000. An update. *Dig Liver Dis*. 2003;35:404–408.
363. Vogt TM, Wise ME, Bell BP, et al. Declining hepatitis A mortality in the United States during the era of hepatitis A vaccination. *J Infect Dis*. 2008;197:1282–1288.
364. Wasley A, Grytdal S, Gallagher K, et al. Surveillance for acute viral hepatitis–United States, 2006. *MMWR Surveill Summ*. 2008;57:1–24.
365. Willner IR, Uhl MD, Howard SC, et al. Serious hepatitis A: an analysis of patients hospitalized during an urban epidemic in the United States. *Ann Intern Med*. 1998;128:111–114.
366. Hoofnagle JH, Carithers RL, Shapiro C. Fulminant hepatic failure: summary of a workshop. *Hepatology*. 1995;21:240–252.
367. Cervio G, Trentadue J, D'Agostino D, et al. Decline in HAV-associated fulminant hepatic failure and liver transplant in children in Argentina after the introduction of a universal hepatitis A vaccination program. *Hepat Med*. 2011;3:99–106.
368. Bravo LC, Gregorio GV, Shafi F, et al. Etiology, incidence and outcomes of acute hepatic failure in 0-18 year old Filipino children. *Southeast Asian J Trop Med Public Health*. 2012;43:764–772.
369. Gordon SC, Reddy KR, Schiff L, et al. Prolonged intrahepatic cholestasis secondary to acute hepatitis A. *Ann Intern Med*. 1984;101:635–637.
370. Cobden I, James OF. A biphasic illness associated with acute hepatitis A virus infection. *J Hepatol*. 1986;2:19–23.
371. Bornstein JD, Byrd DE, Trotter JF. Relapsing hepatitis A: a case report and review of the literature. *J Clin Gastroenterol*. 1999;28:355–356.
372. Scully LJ, Ryan AE. Urticaria and acute hepatitis A virus infection. *Am J Gastroenterol*. 1993;88:277–278.
373. Kano Y, Kokaji T, Shiohara T. Photo-accentuated eruption and vascular deposits of immunoglobulin a associated with hepatitis A virus infection. *Dermatology*. 2000;200:266–269.
374. Ilan Y, Hillman M, Oren R, et al. Vasculitis and cryoglobulinemia associated with persisting cholestatic hepatitis A virus infection. *Am J Gastroenterol*. 1990;85:586–587.
375. Hanna JN, Warnock TH, Shepherd RW, et al. Fulminant hepatitis A in indigenous children in north Queensland. *Med J Aust*. 2000;172:19–21.
376. Schiodt FV, Davern TJ, Shakil AO, et al. Viral hepatitis-related acute liver failure. *Am J Gastroenterol*. 2003;98:448–453.
377. Ly KN, Klevens RM. Trends in disease and complications of hepatitis A virus infection in the United States, 1999-2011: a new concern for adults. *J Infect Dis*. 2015;212:176–182.
378. Collier MG, Tong X, Xu F. Hepatitis A hospitalizations in the United States, 2002-2011. *Hepatology*. 2015;61:481–485.
379. O'Grady J. Management of acute and fulminant hepatitis A. *Vaccine*. 1992;10(suppl 1):S21–S23.
380. Gane E, Sallie R, Saleh M, et al. Clinical recurrence of hepatitis A following liver transplantation for acute liver failure. *J Med Virol*. 1995;45:35–39.
381. Fagan E, Yousef G, Brahm J, et al. Persistence of hepatitis A virus in fulminant hepatitis and after liver transplantation. *J Med Virol*. 1990;30:131–136.
382. Mijch AM, Gust ID. Clinical, serologic, and epidemiologic aspects of hepatitis A virus infection. *Semin Liver Dis*. 1986;6:42–45.
383. Norkrans G, Nilsson LA, Frosner G, et al. Serum immunoglobulin levels in hepatitis non-A, non-B: a comparison with hepatitis A and B. *Infection*. 1980;8:98–100.
384. Hoofnagle JH, Di Bisceglie AM. Serologic diagnosis of acute and chronic viral hepatitis. *Semin Liver Dis*. 1991;11:73–83.
385. Lee HK, Kim KA, Lee JS, et al. Window period of anti-hepatitis A virus immunoglobulin M antibodies in diagnosing acute hepatitis A. *Eur J Gastroenterol Hepatol*. 2013;25:665–668.
386. Hyun JJ, Seo YS, An H, et al. Optimal time for repeating the IgM anti-hepatitis A virus antibody test in acute hepatitis A patients with a negative initial test. *Korean J Hepatol*. 2012;18:56–62.
387. Castrodale L, Fiore A, Schmidt T. Detection of immunoglobulin M antibody to hepatitis A virus in Alaska residents without other evidence of hepatitis. *Clin Infect Dis*. 2005;41:e86–e88.
388. Robertson BH, Jia XY, Tian H, et al. Antibody response to nonstructural proteins of hepatitis A virus following infection. *J Med Virol*. 1993;40:76–82.
389. Jia XY, Summers DF, Ehrenfeld E. Host antibody response to viral structural and nonstructural proteins after hepatitis A virus infection. *J Infect Dis*. 1992;165:273–280.
390. Hollinger FB, Bradley DW, Maynard JE, et al. Detection of hepatitis A viral antigen by radioimmunoassay. *J Immunol*. 1975;115:1464–1466.
391. Purcell RH, Wong DC, Moritsugu Y, et al. A microtiter solid-phase radioimmunoassay for hepatitis A antigen and antibody. *J Immunol*. 1976;116:349–356.
392. Tsai YL, Sobsey MD, Sangermano LR, et al. 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*. 1993;59:3488–3491.
393. Le Guyader F, Dubois E, Menard D, et al. Detection of hepatitis A virus, rotavirus, and enterovirus in naturally contaminated shellfish and sediment by reverse transcription-seminested PCR. *Appl Environ Microbiol*. 1994;60:3665–3671.
394. Abe H, Beninger PR, Ikejiri N, et al. Light microscopic findings of liver biopsy specimens from patients with hepatitis type a and comparison with type B. *Gastroenterology*. 1982;82(5 Pt 1):938–947.
395. Kobayashi K, Hashimoto E, Ludwig J, et al. Liver biopsy features of acute hepatitis C compared with hepatitis A, B, and non-A, non-B, non-C. *Liver*. 1993;13:69–72.
396. Shimizu YK, Mathiesen LR, Lorenz D, et al. Localization of hepatitis A antigen in liver tissue by peroxidase-conjugated antibody method: light and electron microscopic studies. *J Immunol*. 1978;121:1671–1679.
397. Stapleton JT, Lange DK, LeDuc JW, et al. The role of secretory immunity in hepatitis A virus infection. *J Infect Dis*. 1991;163:7–11.
398. Lemon SM, Binn LN, Marchwicki RH. Radioimmunofocus assay for quantitation of hepatitis A virus in cell cultures. *J Clin Microbiol*. 1983;17:834–839.
399. Baba M, Takegawa M, Kaito M, et al. Propagation of hepatitis A virus in a renal cell line JTC-12.P3 of cynomolgus monkey origin. *Acta Virol*. 1993;37:209–222.
400. Baba M, Fukai K, Hasegawa H, et al. The role of natural killer cells and lymphokine activated killer cells in the pathogenesis of hepatic injury in hepatitis A [corrected]. *J Clin Lab Immunol*. 1992;38:1–14.
401. Pinto MA, Marchevsky RS, Pelajo-Machado M, et al. Inducible nitric oxide synthase (iNOS) expression in liver and splenic T lymphocyte rise are associated with liver histological damage during experimental hepatitis A virus (HAV) infection in *Callithrix jacchus*. *Exp Toxicol Pathol*. 2000;52:3–10.

402. Doebbeling BN, Li N, Wenzel RP. An outbreak of hepatitis A among health care workers: risk factors for transmission. *Am J Public Health*. 1993;83:1679–1684.
403. Petrosillo N, Raffaele B, Martini L, et al. A nosocomial and occupational cluster of hepatitis A virus infection in a pediatric ward. *Infect Control Hosp Epidemiol*. 2002;23:343–345.
404. Cohn EJ, Oncley JL, Strong LE, et al. Chemical, clinical, and immunological studies on the products of human plasma fractionation. I. The characterization of the protein fractions of human plasma. *J Clin Invest*. 1944;23:417–432.
405. Farcet MR, Planitzer CB, Stein O, et al. Hepatitis A virus antibodies in immunoglobulin preparations. *J Allergy Clin Immunol*. 2010;125:198–202.
406. Lerman Y, Shohat T, Ashkenazi S, et al. Efficacy of different doses of immune serum globulin in the prevention of hepatitis A: a three-year prospective study. *Clin Infect Dis*. 1993;17:411–414.
407. Nelson NP. Updated dosing instructions for immune globulin (Human) GamaSTAN S/D for hepatitis A virus prophylaxis. *MMWR Morb Mortal Wkly Rep*. 2017;66:959–960.
408. Smallwood LA, Tabor E, Finlayson JS, et al. Antibodies to hepatitis A virus in immune serum globulin. *Lancet*. 1980;2:482–483.
409. Liu JP, Nikolova D, Fei Y. Immunoglobulins for preventing hepatitis A. *Cochrane Database Syst Rev*. 2009;(2):CD004181.
410. Stokes EJ, Neefe JR. The prevention and attenuation of infectious hepatitis by gamma globulin. *JAMA*. 1945;127:144–145.
411. Stapleton JT. Passive immunization against hepatitis A. *Vaccine*. 1992;10(suppl 1):S45–S47.
412. Weiland O, Niklasson B, Berg R, et al. Clinical and subclinical hepatitis A occurring after immunoglobulin prophylaxis among Swedish UN soldiers in sinai. *Scand J Gastroenterol*. 1981;16:967–972.
413. Pierce PF, Cappello M, Bernard KW. Subclinical infection with hepatitis A in peace corps volunteers following immune globulin prophylaxis. *Am J Trop Med Hyg*. 1990;42:465–469.
414. Kluge T. Gamma globulin in the prevention of viral hepatitis. A study on the effect of medium size doses. *Rev Int Serv Sante Armees*. 1963;36(suppl 59–68).
415. Mosley JW, Reisler DM, Brachott D, et al. Comparison of two lots of immune serum globulin for prophylaxis of infectious hepatitis. *Am J Epidemiol*. 1968;87:539–550.
416. Lemon SM. Type a viral hepatitis. New developments in an old disease. *N Engl J Med*. 1985;313:1059–1067.
417. Advisory Committee on Immunization Practices Centers for Disease C, Prevention. Update: prevention of hepatitis A after exposure to hepatitis A virus and in international travelers. Updated recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep*. 2007;56:1080–1084.
417a. Nelson NP, Link-Gelles R, Hofmeister MG, et al. Update: Recommendations of the Advisory Committee on Immunization Practices for Use of Hepatitis A Vaccine for Postexposure Prophylaxis and for Preexposure Prophylaxis for International Travel. *MMWR Morb Mortal Wkly Rep*. 2018;67:1216–1220.
418. Victor JC, Monto AS, Surdina TY, et al. Hepatitis A vaccine versus immune globulin for postexposure prophylaxis. *N Engl J Med*. 2007;357:1685–1694.
419. National Center for I, Respiratory D. General recommendations on immunization — recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 2011;60:1–64.
420. Ellis EF, Henney CS. Adverse reactions following administration of human gamma globulin. *J Allergy*. 1969;43:45–54.
421. Vidor E, Fritzell B, Plotkin S. Clinical development of a new inactivated hepatitis A vaccine. *Infection*. 1996;24:447–458.
422. Gluck R, Mischler R, Brantschen S, et al. Immunopotentiating reconstituted influenza virus virosome vaccine delivery system for immunization against hepatitis A. *J Clin Invest*. 1992;90:2491–2495.
423. Sjogren MH, Purcell RH, McKee K, et al. Clinical and laboratory observations following oral or intramuscular administration of a live attenuated hepatitis A vaccine candidate. *Vaccine*. 1992;10(suppl 1):S135–S137.
424. WHO Prequalified Vaccines.
425. Werzberger A, Mensch B, Kuter B, et al. A controlled trial of a formalin-inactivated hepatitis A vaccine in healthy children. *N Engl J Med*. 1992;327:453–457.
426. Werzberger A, Kuter B, Shouval D, et al. Anatomy of a trial: a historical view of the monroe inactivated hepatitis A protective efficacy trial. *J Hepatol*. 1993;18(suppl 2):S46–S50.
427. Clemens R, Safary A, Hepburn A, et al. Clinical experience with an inactivated hepatitis A vaccine. *J Infect Dis*. 1995;171(suppl 1):S44–S49.
428. Dagan R, Amir J, Mijalovsky A, et al. Immunization against hepatitis A in the first year of life: priming despite the presence of maternal antibody. *Pediatr Infect Dis J*. 2000;19:1045–1052.
429. Shouval D, Ashur Y, Adler R, et al. Single and booster dose responses to an inactivated hepatitis A virus vaccine: comparison with immune serum globulin prophylaxis. *Vaccine*. 1993;11(suppl 1):S9–S14.
430. Van Damme P, Mathei C, Thoelen S, et al. Single dose inactivated hepatitis A vaccine: rationale and clinical assessment of the safety and immunogenicity. *J Med Virol*. 1994;44:435–441.
431. Fujiyama S, Iino S, Odoh K, et al. Time course of hepatitis A virus antibody titer after active and passive immunization. *Hepatology*. 1992;15:983–988.
432. Fujiyama S, Odoh K, Kuramoto I, et al. Current seroepidemiological status of hepatitis A with a comparison of antibody titers after infection and vaccination. *J Hepatol*. 1994;21:641–645.
433. Lemon SM, Murphy PC, Provost PJ, et al. Immunoprecipitation and virus neutralization assays demonstrate qualitative differences between protective antibody responses to inactivated hepatitis A vaccine and passive immunization with immune globulin. *J Infect Dis*. 1997;176:9–19.
434. Purcell RH, D'Hondt E, Bradbury R, et al. Inactivated hepatitis A vaccine: active and passive immunoprophylaxis in chimpanzees. *Vaccine*. 1992;10(suppl 1):S148–S151.
435. Hess G, Clemens R, Bienzle U, et al. Immunogenicity and safety of an inactivated hepatitis A vaccine in anti-HIV positive and negative homosexual men. *J Med Virol*. 1995;46:40–42.
436. Jimenez HR, Hallit RR, Debari VA, et al. Hepatitis A vaccine response in HIV-infected patients: are TWINRIX and HAVRIX interchangeable? *Vaccine*. 2013;31:1328–1333.
437. Overton ET, Nurutdinova D, Sungkanuparph S, et al. Predictors of immunity after hepatitis A vaccination in HIV-infected persons. *J Viral Hepat*. 2007;14:189–193.
438. Kourkounti S, Mavrianou N, Paparizos VA, et al. Immune response to hepatitis A vaccination in HIV-infected men in Greece. *Int J STD AIDS*. 2012;23:464–467.
439. Leise MD, Talwalkar JA. Immunizations in chronic liver disease: what should be done and what is the evidence. *Curr Gastroenterol Rep*. 2013;15:300.
440. Bell BP. Hepatitis A vaccine. *Semin Pediatr Infect Dis*. 2002;13:165–173.
441. Raczniak GA, Bulkow LR, Bruce MG, et al. Long-term immunogenicity of hepatitis A virus vaccine in Alaska 17 years after initial childhood series. *J Infect Dis*. 2013;207:493–496.
442. Ott JJ, Irving G, Wiersma ST. Long-term protective effects of hepatitis A vaccines. A systematic review. *Vaccine*. 2012;31:3–11.
443. Spradling PR, Bulkow LR, Negus SE, et al. Persistence of seropositivity among persons vaccinated for hepatitis A during infancy by maternal antibody status: 15-year follow-up. *Hepatology*. 2016;63:703–711.
444. Mao JS. Development of live, attenuated hepatitis A vaccine (H2-strain). *Vaccine*. 1990;8:523–524.
445. Midthun K, Ellerbeck E, Gershman K, et al. Safety and immunogenicity of a live attenuated hepatitis A virus vaccine in seronegative volunteers. *J Infect Dis*. 1991;163:735–739.
446. Mao JS, Dong DX, Zhang HY, et al. Primary study of attenuated live hepatitis A vaccine (H2 strain) in humans. *J Infect Dis*. 1989;159:621–624.
447. Cho MW, Ehrenfeld E. Rapid completion of the replication cycle of hepatitis A virus subsequent to reversal of guanidine inhibition. *Virology*. 1991;180:770–780.
448. Zheng H, Chen Y, Wang F, et al. Comparing live attenuated and inactivated hepatitis A vaccines: an immunogenicity study after one single dose. *Vaccine*. 2011;29:9098–9103.
449. Bryan JP, Nelson M. Testing for antibody to hepatitis A to decrease the cost of hepatitis A prophylaxis with immune globulin or hepatitis A vaccines. *Arch Intern Med*. 1994;154:663–668.
450. Averhoff F, Shapiro CN, Bell BP, et al. Control of hepatitis A through routine vaccination of children. *JAMA*. 2001;286:2968–2973.
451. American Academy of Pediatrics. Hepatitis A. In: Pickering LK, ed. *Red Book: Report of the Committee on Infectious Diseases*. 25th ed. Elk Grove Village, IL: The Academy; 2000:280–289.
452. Chodick G, Heymann AD, Ashkenazi S, et al. Long-term trends in hepatitis A incidence following the inclusion of hepatitis A vaccine in the routine nationwide immunization program. *J Viral Hepat*. 2008;15(suppl 2):62–65.
453. Singleton RJ, Hess S, Bulkow LR, et al. Impact of a statewide childhood vaccine program in controlling hepatitis A virus infections in Alaska. *Vaccine*. 2010;28:6298–6304.
454. Torner N, Broner S, Martinez A, et al. Hepatitis A outbreaks: the effect of a mass vaccination programme. *J Viral Hepat*. 2011;18:e1–e4.
455. Fangcheng Z, Xuanyi W, Mingding C, et al. Era of vaccination heralds a decline in incidence of hepatitis A in high-risk groups in China. *Hepat Mon*. 2012;12:100–105.
456. Vacchino MN. Incidence of hepatitis A in Argentina after vaccination. *J Viral Hepat*. 2008;15(suppl 2):47–50.
457. Levine H, Kopel E, Anis E, et al. The impact of a national routine immunisation programme initiated in 1999 on hepatitis A incidence in Israel, 1993 to 2012. *Euro Surveill*. 2015;20:3–10.
458. WHO vaccine-preventable diseases: monitoring system. 2018 global summary; 2018.
459. Sage W. Meeting of the strategic advisory group of experts on immunization, November 2011- conclusions and recommendations. *Weekly Epidiologic Rev*. 2012;87:1–16.
460. WHO position paper on hepatitis A vaccines - June 2012. *Wkly Epidemiol Rec*. 2012;87:261–276.
461. Bell BP. Hepatitis A and hepatitis B vaccination of patients with chronic liver disease. *Acta Gastroenterol Belg*. 2000;63:359–363.
462. Thorburn KM, Bohorques R, Stepak P, et al. Immunization strategies to control a community-wide hepatitis A epidemic. *Epidemiol Infect*. 2001;127:461–467.
463. Meltzer MI, Shapiro CN, Mast EE, et al. The economics of vaccinating restaurant workers against hepatitis A. *Vaccine*. 2001;19:2138–2145.
464. Carrillo-Santisteve P, Tavoschi L, Severi E, et al. Seroprevalence and susceptibility to hepatitis A in the European union and European economic area: a systematic review. *Lancet Infect Dis*. 2017;17:e306–e319.
465. Carlos F, Gomez JA, Anaya P, et al. Health economic assessment of universal immunization of toddlers against Hepatitis A Virus (HAV) in Mexico. *Hum Vaccin Immunother*. 2016;12:52–63.
466. Mellou K, Sideroglou T, Papaevangelou V, et al. Considerations on the current universal vaccination policy against hepatitis A in Greece after recent outbreaks. *PLoS ONE*. 2015;10:e0116939.
467. Vizzotti C, Pippo T, Uruena A, et al. Economic analysis of the single-dose immunization strategy against hepatitis A in Argentina. *Vaccine*. 2015;33(suppl 1):A227–A232.
468. Zhuang GH, Pan XJ, Wang XL. A cost-effectiveness analysis of universal childhood hepatitis A vaccination in China. *Vaccine*. 2008;26:4608–4616.
469. Quezada A, Baron-Papillon F, Coudeville L, et al. Universal vaccination of children against hepatitis A in Chile: a cost-effectiveness study. *Rev Panam Salud Publica*. 2008;23:303–312.
470. Van Effelterre T, De Antonio-Suarez R, Cassidy A, et al. Model-based projections of the population-level impact of hepatitis A vaccination in Mexico. *Hum Vaccin Immunother*. 2012;8:1099–1108.
471. Sartori AM, de Soarez PC, Novaes HM, et al. Cost-effectiveness analysis of universal childhood hepatitis A vaccination in Brazil: regional analyses according to the endemic context. *Vaccine*. 2012;30:7489–7497.
472. Curran D, de Ridder M, Van Effelterre T. The impact of assumptions regarding vaccine-induced immunity on the public health and cost-effectiveness of hepatitis A vaccination: is one dose sufficient? *Hum Vaccin Immunother*. 2016;12:2765–2771.
473. Mayorga Perez O, Herzog C, Zellmeyer M, et al. Efficacy of virosome hepatitis A vaccine in young children in Nicaragua: randomized placebo-controlled trial. *J Infect Dis*. 2003;188:671–677.
474. Mayorga O, Buhler S, Jaeger VK, et al. Single-Dose hepatitis A immunization: 7.5-Year observational pilot study in Nicaraguan children to assess protective effectiveness and humoral immune memory response. *J Infect Dis*. 2016;214:1498–1506.
475. Goodman RA, Foster KL, Trowbridge FL Global disease elimination and eradication as public health strategies. 23-25 February, 1998, 1998; Atlanta, Georgia, USA.

175 Rhinovirus

Raphael Dolin

SHORT VIEW SUMMARY

Definition
- Rhinoviruses are the most frequent causes of the common cold.
- Rhinoviruses cause combinations of sneezing, sore throat, rhinorrhea, nasal congestion, and coughing.

Epidemiology
- Rhinoviruses are among the most common human pathogens.
- Rhinoviruses cause 0.75 infections per year in adults and 1.2 to 6 infections per year in children.
- Infections occur year-round with seasonal peaks in spring and fall in temperate climates.
- Spread occurs by hand-to-nose or hand-to-eye contact and by aerosol.

Microbiology
- Rhinoviruses are single-stranded, nonenveloped RNA viruses in the family Picornaviridae.
- There are three species, RV-A, RV-B, and RV-C, which have at least 156 serotypes and genotypes.

Clinical Manifestations
- The common cold is the most frequent illness.
- Infection exacerbates asthma and chronic pulmonary diseases.
- Infection can be associated with otitis media and sinusitis.
- Lower respiratory tract illness including pneumonia can occur.
- Infection is more severe in immunosuppressed patients.

Diagnosis
- Specific virologic diagnosis is made through detection of virus in nasal secretions (aspirates, swabs, washings) or pulmonary samples.
- Reverse-transcriptase polymerase chain reaction is the most sensitive and efficient laboratory technique for virus detection.

Therapy
- Common colds are self-limited, but symptomatic therapy can be used to provide relief.
- Antiviral therapy is not available.

Prevention
- No established method of prevention exists.
- Vaccines are under active investigation.

Rhinoviruses are among the most frequent causes of viral infections in humans. They are the major cause of the common cold and are significant contributors to other upper respiratory syndromes as well as certain lower respiratory illnesses. The term *common cold* is believed to derive from ancient descriptions of illness in traditional Chinese and Roman sources, in which the illness was believed to be caused by cold temperatures and being chilled.[1] Scientific investigations into the cause of the common cold began in the early 20th century in studies by Kruse,[2] in which filtrates of nasal secretions from individuals with colds were administered to volunteers and subsequently induced colds. These studies, along with similar investigations conducted later by Dochez and colleagues[3] indicated that colds were caused by filterable agents (i.e., presumed to be viruses).

The first isolation of rhinoviruses was achieved by Price[4] and Pelon and coworkers,[5] who inoculated nasopharyngeal washings from individuals with colds into rhesus monkey kidney tissue cultures. Andrewes and colleagues[6] suggested the name "rhinovirus," and a group designation was proposed by Tyrrell and Chanock in 1963.[7] Once in vitro isolation techniques were established, the filtrates used in transmission experiments were demonstrated to contain rhinoviruses. Subsequently the application of sensitive molecular techniques led to characterization of rhinovirus strains that did not grow in tissue culture and to extensive new knowledge of the epidemiology and pathogenesis of rhinovirus infection.

VIROLOGY

Classification

Rhinoviruses are members of the *Enterovirus* genus of the Piconoviridae family and comprise the RV-A, RV-B, and RV-C species. Classification is based on genome organization, capsid properties, and sequence conservation.[8,9] Within species, rhinoviruses are subdivided into numerical genotypes. Rhinoviruses have also historically been characterized by immunologic serotype, as defined by neutralization with antiserum. A total of 101 serotypes, numbered 1A, 1B, and 2 to 100, were identified. Classification into new serotypes ended in 1987 and has been supplanted by genomic characterization, which includes newly discovered strains that do not grow in tissue cultures.[10] The recognized serotypes are divided into two species, RV-A and RV-B (except for RV-A87, which is actually an enterovirus, EVD68). The third species, RV-C, consists of previously unrecognized strains of rhinoviruses detected by genomic sequencing and currently has 55 genotypes.[11]

Receptor specificity for rhinovirus has also been used for group designations. The major receptor group consists of 88 serotypes that use the intercellular adhesion molecule 1 (ICAM-1) to infect cells.[12,13] The minor receptor group consists of 11 serotypes that use the low-density lipoprotein and related proteins as receptors. The major receptor group comprises both RV-A and RV-B species, whereas the minor receptor group comprises RV-A species only. The receptor for the RV-C species is human cadherin-related family member 3.[14] Susceptibility to antiviral compounds has also been used to divide rhinoviruses into two groups (A and B) by Andries and coworkers,[15] who suggested that those groups reflect sequence and possibly pathogenic differences.

Structure

Rhinoviruses are single-stranded RNA viruses of approximately 30 nm in diameter that have a rigid protein shell (capsid) composed of four viral proteins, VP1, VP2, VP3, and VP4. The protein subunits are called protomers; consist of one copy of each of the viral proteins; and are organized into 12 pentamers, each of which contains 5 protomers (Fig. 175.1). In each of the pentamers, VP1 has a prominent symmetrical depression or "canyon," which extends around the fivefold axis of symmetry and contains the binding site for the ICAM-1 receptor. This canyon also provides immunogenic surfaces. Antibodies with neutralizing activities against rhinovirus bind to the receptor site in the canyon. A hydrophobic "pocket" is present at the base of the canyon, which is the site of binding for antiviral drugs such as pleconaril or WIN 52084.[15]

The overall structure of the rhinovirus capsid is similar to that of enteroviruses in general; however, in contrast to enteroviruses, rhinoviruses are unstable at acid pH (<5 or 6) and are completely inactivated

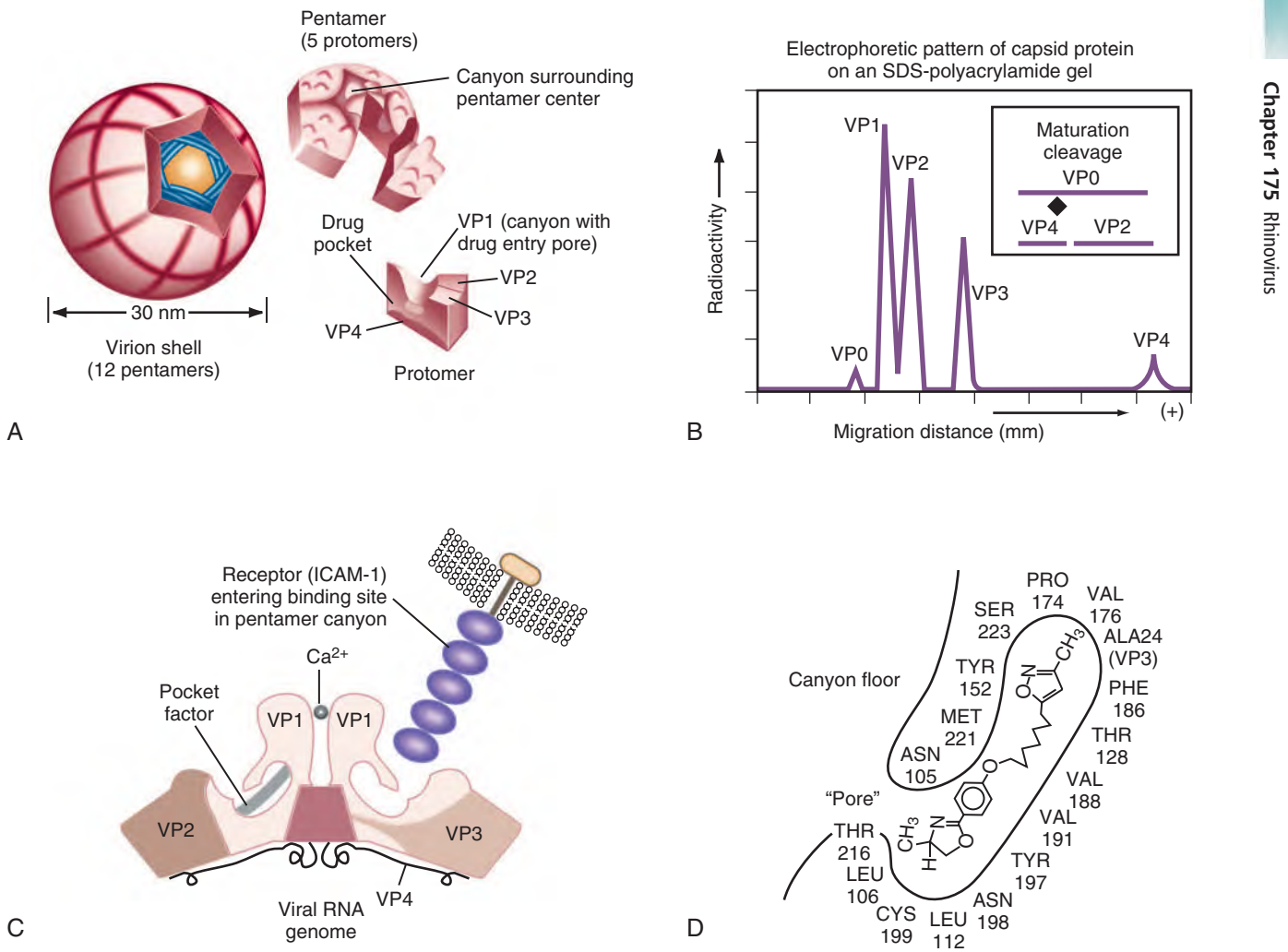

FIG. 175.1 Key features of a human rhinovirus (RV). (A) The virion shell consists of 12 pentamers, one of which has been removed to show the approximate location of the RNA packed tightly into a central cavity. Each pentamer, in turn, consists of five wedge-shaped subunits, called protomers. The canyon *(stippled)* is shown in only 1 of the 12 pentamers. (B) The virion contains four major proteins (VP1, VP2, VP3, and VP4) plus traces of another, VP0, representing residual precursor after the maturation cleavage *(inset)* required for acquisition of infectivity. (C) Transverse section through the center of a pentamer depicting entry of its cellular receptor (intercellular adhesion molecule 1 [ICAM-1]) and the location of the drug-binding pocket just beneath the canyon floor. An ion, located at each pentamer center in RV-1A, RV-14, and RV-16, is tentatively identified as calcium, which is necessary for attachment of some rhinoviruses. (D) Detail showing orientation of a capsid binder (WIN 52084) and identity of amino-acid residues lining the canyon floor and drug-binding pocket in a single protomer. In RV-14, the drug prevents attachment of its receptor, ICAM-1. *SDS,* Sodium dodecyl sulfate.

at pH <3.[16] Rhinoviruses retain stability for hours to days at 24°C to 34°C on environmental surfaces and have viability for years at freezer temperatures of −70°C.[17]

Rhinoviruses lack envelopes and thus are relatively resistant to lipid solvents such as chloroform and ether. Polar organic solvents decrease infectivity of rhinoviruses, perhaps because of partial denaturation of their protein shell or because of inactivation of the hydrophobic pocket. Rhinoviruses are resistant to nonionic detergents but are sensitive to commonly used disinfectants such as chlorine, iodine, hydrogen peroxide, and ozone.[18] Physical treatments such as gentle heating, desiccation, or ultraviolet light also decrease infectivity of rhinoviruses.

Genomic Organization

The rhinovirus genome consists of a single strand of positive sense RNA of approximately 7200 bases, which codes for a single open reading frame for a large polyprotein with almost 2200 amino acids. The RNA genome also contains multiple important RNA motifs.[15] The 5′ end has a cloverleaf structure that is believed to serve as a regulatory element for translation and replication. Distinct secondary and tertiary structures are present that represent internal ribosomal entry sites that enable ribosomes to initiate translation without the necessary "caps" required by most cellular messenger RNAs.[17] A shorter nontranslated region is present at the 3′ end, which is believed to play a role in termination of translation (Fig. 175.2).[19]

EPIDEMIOLOGY

Age

Rhinoviruses are worldwide in distribution. Infections occur most commonly in infants and young children. Infections appear to be less frequent in first the 6 months of life and then have high rates in infancy and early childhood.[9,18,20–22] Among children in the United States, the rate is 1.2 to 6 infections per person-year, and among adults, it is 0.75 infections per person-year.[22–26] Infection rates appear to be higher in young women with children than in men of similar age; however, rates are higher in older men than in older women.[23]

Seasonality

Rhinovirus infection occurs throughout the year, with increased rates in the spring and fall in the temperate climates.[22,23,27,28] The fall peak usually occurs in late August or early September in the Northern Hemisphere, and then the rates are usually lower until the peak in early April and May.[26] Overall, rhinovirus infections may account for up to