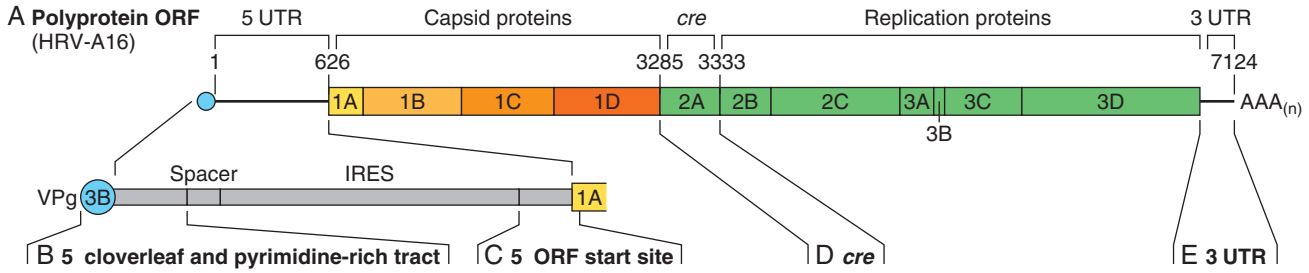

FIG. 175.2 (A) The genome of a human rhinovirus encodes a single polyprotein open reading form *(ORF)*. Important RNA structural motifs include a (B) 5′ cloverleaf, (C) ORF stat-site stem, (D) a *cre* element, and (E) 3′ stem motif. *(Modified from Gern J, Palmenberg AC. Rhinoviruses. In: Knipe DM, Howley PM, eds.* Fields Virology. *Philadelphia: Lippincott, Williams & Wilkins; 2013:533.)*

80% of upper respiratory illnesses during peak seasons.[29] Even during the low periods in the summer months, rhinoviruses may still be responsible for up to 50% of upper respiratory illnesses.[23,26]

The reasons for the seasonality of rhinovirus infections are not fully understood. Environmental high humidity improves rhinovirus survival, and high indoor humidity has been associated with an increased incidence of rhinovirus infections.[30] An increase in transmission has also been attributed to periods of time when schools are in session.[30,31] In tropical climates, rhinovirus infections appear to occur with little relationship to climatic factors.

PATHOGENESIS

The pathogenesis of rhinovirus infections has been studied in experimental infection of volunteers and in naturally occurring infection in patients with various rhinovirus-associated illnesses. Initial infection occurs in the nasal mucus or the eye. Experimental studies have shown that extremely low quantities of rhinovirus (1 median tissue culture infective dose) can induce infection when administered via the conjunctiva or nasal mucosa.[32]

The primary site of infection is the epithelium of the upper respiratory tract via receptors described earlier. Inoculation of rhinoviruses into the nasal cavity is followed by spread to the posterior nasopharynx.[33–36] Infection of the upper respiratory tract includes involvement of the paranasal sinuses, and rhinovirus can be detected in sinus secretions by polymerase chain reaction (PCR).[37] Sinus abnormalities are frequently noted on computed tomography or magnetic resonance imaging in both experimentally induced and naturally occurring colds.[38] Rhinoviruses grow preferentially at temperatures of 33°C to 35°C, rather than at higher temperatures of 37°C or greater. Studies indicate that rhinovirus replication at higher temperatures results in more expression of interferon types 1 and 3 and of interferon-stimulated genes, and this may account for the preferential growth at lower temperatures.[39] These observations led to the early concept that rhinoviruses may not be able to infect lower airways that have higher core temperatures. More recent studies indicate that medium and lower airways have core temperatures lower than 37°C and that they support rhinovirus replication efficiently.[9] After experimental infection of the upper airway, rhinoviruses are found in the lower airway by bronchoscopy or bronchoalveolar lavage in 50% of specimens.[40,41] It has also been noted that RV-C species grow well at 37°C in human sinus epithelial cells in contrast to RV-A and RV-B species.[42] Thus it is clear that rhinoviruses can infect the lower respiratory tract, but how frequently this occurs remains undetermined.[43]

Once symptoms of a cold are already present, biopsy specimens of the nasal mucosa and nasopharynx surprisingly show only small foci of infection, along with large areas of apparently uninfected cells.[36,44] Examination of specimens of nasal epithelium during rhinovirus infection by light or electron microscopy do not show consistent histopathology.[45]

Rhinovirus infection involves primarily epithelial ciliated cells, although nonciliated cells are also infected. It has been suggested that most of the rhinovirus-infected cells are likely shed into nasal secretions.[46]

Inflammatory Responses

Although histopathologic changes in the nasal mucosa are not prominent in rhinovirus infection, even when symptoms are clearly present, a variety of inflammatory and cellular immune responses can be seen. Polymorphonuclear leukocytes are present in both nasal mucosa and secretions.[47,48] A modest increase in peripheral polymorphonuclear leukocytes is seen during the first 2 to 3 days after experimental virus infection, which is not observed in volunteers who did not become ill.[49] Modest increases in T-cell lymphocyte counts in the nasal mucosa and nasal secretions also occur during rhinovirus infection, but data are conflicting on the effect of rhinovirus infection on peripheral lymphocyte counts.[50,51]

Rhinovirus infections stimulate a variety of innate immune responses including proinflammatory cytokines and mediators of inflammation. The signaling pathways that induce these effects are not fully determined, but Toll-like receptor 3, phosphatidylinositol 3-kinase, virus-induced oxidant stress, and mitogen protein kinases all have been implicated.[17,52,53] Ceramide-enriched cell membrane platforms may also play a role in stimulation of the signaling pathways.[17,54,55] Chemokines and cytokines that are present in increased concentrations in nasal secretions include interleukin-1β, interleukin-6, interleukin-8, and interferon-γ-induced protein 10.[17] Concentrations of interleukin-6, interleukin-8, and interferon-γ-induced protein 10 in nasal secretions are increased during colds, correlate with symptom severity, and decrease as symptoms abate.

Kinins, including bradykinin and lysyl-bradykinin are found in nasal mucosa during colds, and their concentrations correlate with the severity and time course of illness.[48,56] However, administration of a bradykinin antagonist did not reduce cold symptoms in one study[57]; reduction of the concentration of kinins by administration of steroids also did not reduce symptoms.[58] Histamine is present in nasal mucosa, and although antihistamines are commonly used for symptomatic treatment of colds, a trial of a second-generation antihistamine did not show an effect on rhinovirus-induced colds.[59]

Nasal Fluid Production

Parasympathetic innervation controls secretory function of nasal seromucous glands. Most of the nasal fluid produced by colds is derived from these glands, along with fluid that emerges by transudation.[60] Intranasal administration of drugs with anticholinergic activity such as atropine or ipratropium and oral administration of first-generation antihistamines reduce nasal fluid by one-third in experimentally induced colds.

The inflammatory processes that result in increased passage of serum into the nasal mucosa and nasal secretions are particularly prominent early in the course of illness and are major components of nasal obstruction. Increased secretion from nasal glands becomes more prominent later during the course of a cold.[16,17]

TRANSMISSION

Person-to-person transmission of rhinovirus infection occurs by direct contact or by aerosol. Direct contact appears to be the more efficient route of transmission. Rhinovirus survives for several hours on the skin of infected volunteers and can be recovered from 65% of fingers of infected subjects after finger-to-nose contact.[61,62] Self-inoculation into the eye or nasal mucosa can result via rhinovirus-contaminated fingers. Larger particle aerosols such as those produced by sneezing or coughing transmit infection in experimental studies.[63,64] Small particle

aerosols appear to have a lesser role in transmission, although rhinovirus RNA has been detected in such aerosols.[17] In experimentally induced infection, rhinovirus transmission was most efficient when there were large concentrations of virus in the nose (>1000 median tissue culture infective dose), when virus was present on the hands and nasal mucosa, and when symptoms of a cold were most severe.[65] These findings occurred most commonly during the second or third day after virus inoculation.[17]

The efficiency of transmission of rhinovirus infection is increased by time and closeness of contact between infected and susceptible individuals. Children not only have the highest rates of infection but also have the highest rates of transmission to other children and to adults.[66,67] In experimental studies, the highest transmission rates occurred among married couples, under crowded living conditions, and in association with severity of illness.[68–70]

In studies of naturally occurring rhinovirus infection, the importance of the direct contact route of transmission is less clear cut. Rhinovirus can be isolated from approximately 40% of individuals with colds and from 6% to 15% of objects found in their environment.[17,61,62] Individuals routinely make finger-to-nose or finger-to-eye contact that can self-inoculate infection. Application of 2% aqueous iodine to fingers of mothers of children with colds appeared to prevent infection in the mothers in one study.[61] However, a study of the use of a virucidal hand disinfectant in young adults did not reduce acquisition of rhinovirus infections.[71]

The role of fomites in transmission of rhinovirus infection remains uncertain. Although objects in the environment can be readily contaminated by contact with hands that contain virus, attempts to demonstrate experimental transmission from such fomites have not succeeded.[72]

CLINICAL MANIFESTATIONS OF COMMON COLD

The most frequent illness associated with rhinovirus infection is the common cold, an upper respiratory syndrome (Fig. 175.3). After experimental infection, there is a 1- to 2-day incubation period followed by the onset of throat discomfort, usually described as "scratchy" or sore. Rhinorrhea and nasal obstruction develop shortly thereafter, along with various combinations of hoarseness, sneezing, headache, malaise, and feverishness. Cough occurs in approximately 30% of cases, usually begins after the appearance of nasal signs and symptoms, and may persist after those subside.[17] Illness is similar in adults and children except that fever occurs in approximately one-third of children but is usually absent in adults.[73]

The duration and severity of rhinovirus-induced colds are quite variable in severity and duration. Naturally occurring illness has been reported to last 1 to 33 days, with a median duration of 7 days, and approximately one-fourth last for 2 weeks.[17,74] Peak signs and symptoms of illness generally occur 2 to 4 days after onset, and illness usually subsides or ends by 1 week. Some minor symptoms may linger longer and likely account for the prolonged illness reported in some studies.[9,68,69]

Computed tomography performed by Gwaltney and colleagues[38] showed that paranasal involvement, consisting of mucosal thickening, swelling, or fluid accumulation, occurred in one or more sinuses in 87% of subjects with clinically typical colds. Rhinovirus RNA has also

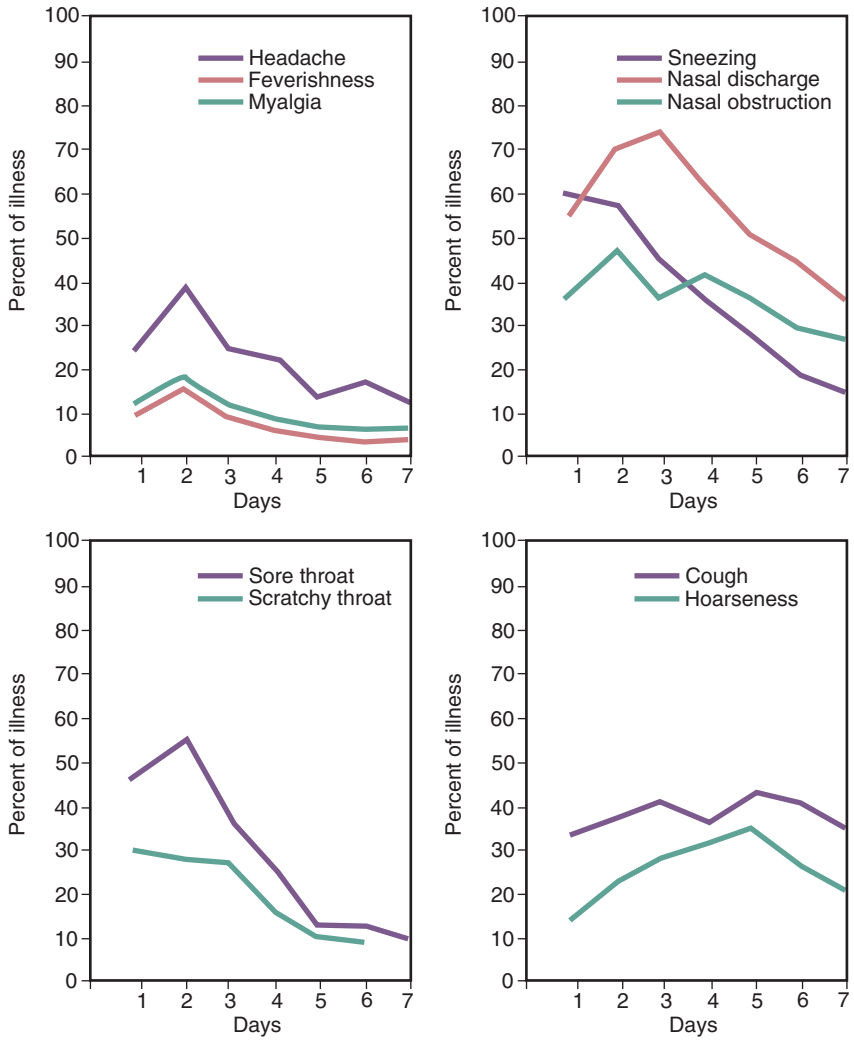

FIG. 175.3 **Rhinovirus cold symptoms (139 adults with natural infection).**

been found in brushings from sinus cavities,[75] and rhinoviruses have been isolated from sinus aspirates. On this basis, it has been suggested that colds might be appropriately characterized as a viral rhinosinusitis.[46] This common rhinovirus involvement of sinuses resolves without specific therapy,[75,76] in contrast to the complication of bacterial sinusitis described next.

COMPLICATIONS

Acute Bacterial Sinusitis

The frequency of acute bacterial sinusitis as a complication of a cold is difficult to ascertain, in part because of the frequent involvement of sinuses in uncomplicated colds. Various studies have reported that 0.5% to 8% of colds have been complicated by bacterial sinusitis.[77] The precipitating factors for development of acute bacterial sinusitis in association with colds have not been fully elucidated. Infection may result from retrograde propulsion of bacteria into sinuses by nose blowing, by occlusion of drainage of paranasal ostia, and by obstruction of the ethmoid and infundibulum.[17] Facial swelling, pressure, pain, and tenderness accompanied by fever should increase the suspicion that bacterial sinusitis may be present. The persistence of a respiratory illness beyond the typical 2-week duration of a cold should also increase that suspicion. (Also see Chapter 62.)

Acute Bacterial Otitis Media

Rhinovirus infections frequently result in abnormalities and dysfunction in the eustachian tube and middle ear.[78,79] Increased middle ear pressures occur in three-fourths of patients with rhinovirus colds, sometimes in association with middle ear effusions.[17] Rhinovirus has been found alone or in combination with bacteria in middle ear fluids of 24% of patients with otitis media.[80] It has been estimated that otitis media is associated with 30% of colds in children and with 2% of colds in adults.[77,81] (Also see Chapter 61.)

Asthma

Rhinovirus infections are major causes of wheezing syndromes and of exacerbations of asthma.[82,83] They are associated with 60% to 70% of asthma exacerbations in school-aged children and have been implicated in precipitation of asthma attacks in older children and adults. The basis for this association is not well understood. Exacerbation appears to occur most often when rhinovirus infection is superimposed on airway inflammation caused by allergens, as reflected by serum immunoglobulin E (IgE) concentrations or by airway eosinophilia.[17,84–87] Precipitation of asthma episodes by rhinovirus infection in association with allergen-specific IgE appears to be particularly important in children older than 2 years of age. Studies suggest that rhinovirus infection may interact with a genetic risk for development of childhood asthma.[88]

Exacerbation of Chronic Bronchitis

Rhinoviruses have also been implicated in up to 40% of exacerbations of chronic bronchitis, as characterized by fever, increased production of purulent sputum, and deterioration of ventilation.[17] Exacerbation of bronchiectasis, chronic obstructive pulmonary disease, and cystic fibrosis and induction of bronchiolitis have also been described.

Lower Respiratory Tract Infections

The precise role of rhinovirus as pathogens in infections of the lower respiratory tract is also difficult to ascertain. This is due in part to the frequency of rhinovirus infection and shedding in the upper respiratory tract but also to other microbial pathogens that are often isolated concomitantly with rhinovirus from the lower respiratory tract.

Using reverse-transcriptase (RT)–PCR techniques, rhinoviruses have been detected as the sole pathogen in approximately 10% of children hospitalized with bronchiolitis.[89,90] Another study found rhinovirus in 21% of children with bronchiolitis, but it was the only pathogen in 2% of patients.[91,92]

Rhinovirus can be frequently detected in the upper respiratory tract of patients with pneumonia.[93] Using RT-PCR, one study reported that 24% of children with pneumonia had rhinovirus detected in the upper respiratory tract, but more than half of these had evidence of a concomitant bacterial pathogen.[94] Broadly based surveillance studies of community-acquired pneumonia have described detection of multiple viral and bacterial potential pathogens. In specimens from these studies, rhinoviruses have been frequently detected in the United States (9%),[95] Norway (12%),[96] and New Zealand (13%).[97] The rate of detection of rhinoviruses in subjects without pneumonia was significantly less.[95,97] Rhinoviruses were also frequently detected in patients with community-acquired pneumonia who required mechanically assisted ventilation.[98] Based on these and other studies, investigators have suggested that rhinoviruses should be considered as potential etiologic agents of pneumonia.[99,100]

Infections in Immunocompromised Patients

Assessment of the impact of rhinovirus infections in immunocompromised patients is challenging because of the clinical complexity of immunocompromised patients, the frequency of rhinovirus infections throughout the year, and the concomitant detection of other potential pathogens along with rhinoviruses. It is well documented that rhinovirus infection is frequent and can be prolonged in immunocompromised patients. Prolonged shedding is seen, ranging from 30 to 455 days (means of 61 and 92 days), and has been described in patients with hematologic malignancies, hematopoietic stem cell transplants, and other immunosuppressed states.[101–104]

Upper respiratory illnesses were most frequently encountered in immunocompromised patients, but lower respiratory tract illnesses were also seen. Most of the lower respiratory illnesses had concomitant bacterial, fungal, or other viral pathogens noted, but there were instances in which rhinoviruses were the only pathogens detected in the lower respiratory tract.

The overall impact of rhinovirus infection is difficult to ascertain. In one study, the mortality of patients with bronchoalveolar lavage confirmed rhinovirus infection at 1 year was 55% compared with 41% in the total hematopoietic stem cell transplant cohort. Another study reported a similar severity of disease in immunocompromised patients with influenza A/H1N1 infections in 2009 and immunocompromised patients with rhinovirus infections.[105] A recent study reported higher morbidity and mortality in hospitalized elderly patients with rhinovirus infections compared with hospitalized elderly patients with influenza, most of whom had infections with A/H3N2.[106]

DIAGNOSIS

The clinical characteristics of infections caused by rhinoviruses are not sufficiently distinctive either clinically or epidemiologically to make a specific etiologic diagnosis without laboratory techniques. Historically, detection of rhinoviruses has been carried out by isolation of virus in tissue cultures, and much of our understanding of rhinovirus infection has been derived on that basis. Culture has now been largely supplanted by molecular techniques such as RT-PCR, which are considerably more sensitive and efficient

Rhinovirus can be detected in nasal aspirates, swabs, or washings. After experimental infection, virus shedding in the upper airways peaks at 2 to 4 days after inoculation and generally lasts 1 to 2 weeks and occasionally up to 3 weeks.[9,35]

Virus Isolation in Tissue Culture

Detection of rhinoviruses by growth in tissue cultures is carried out using human diploid embryonic lung fibroblasts (WI-38, MRC-5) or HeLa cells. Growth of rhinoviruses is most efficient at 33°C or 34°C and when cultures are incubated in rolling drum conditions. Cultures are maintained for 10 to 14 days after incubation, and cytopathic effect can be readily observed. Tissue cultures may vary in sensitivity for rhinovirus growth, and lots should be monitored for this characteristic. Blind passage in HeLa cells may increase sensitivity for growth. As discussed earlier, rhinovirus species A and B can be grown in tissue culture, but species C does not grow in standard cultures and requires molecular techniques for detection.

Molecular Techniques

RT-PCR has become the standard technique for detection of rhinovirus. Primers for this technique take advantage of the fact that the 5′

untranslated region contains subregions that are highly conserved among all types of rhinoviruses.[107–109] Additional regions such as those on VP2-4 or VP1 can be used for genotyping. The use of multiple different primers increases sensitivity.[110]

Antigen Detection

Serotype-specific antibodies conjugated with immunofluorescence or immunoperoxidase techniques can be used to detect individual serotypes but lack the sensitivity and breadth for use as diagnostic aids.[17]

Neutralizing Antibody Assays

Development of serotype-specific neutralizing antibodies occurs within 7 to 14 days after infection and consists mostly of the IgG1 subclass in serum and of IgA in nasal secretions.[22,111,112] Because the assays are serotype specific, they are diagnostically useful only when the serotype is known and as in experimentally induced infection or in outbreaks in families or in closed environments.

THERAPY

Symptomatic Therapy

A wide variety of symptomatic therapies have been proposed and advocated for common colds regardless of their etiology including colds caused by rhinoviruses. Studies of several of these have shown varying degrees of effectiveness of treatments for nasal congestion, rhinorrhea, and sore throat. These are discussed in detail in Chapter 58. It should be remembered that the common cold, although very frequent, is overwhelmingly a self-limited illness that resolves spontaneously; hence any potential benefit of treatment should be weighed carefully against risks and costs.

Antivirals

Antivirals with in vitro activity against rhinoviruses by a variety of mechanisms have been described. The most intensively studied of these have been agents that bind to the rhinovirus capsid at the hydrophobic pocket formed by VP1.These agents block attachment by rhinovirus to the cell receptor, inhibit ingress of virus into the cell, and interfere with release of viral RNA intracellularly. The most extensively studied of these in clinical trials is pleconaril, which is active against 90% of rhinovirus clinical isolates in vitro (see Chapter 48).[113] Pleconaril administered at 400 mg three times a day for 5 days reduced the duration of naturally occurring uncomplicated colds by approximately 1 day in two studies.[114,115] An intranasal formulation of pleconaril was found to be ineffective in prevention of naturally occurring colds.[116] The US Food and Drug Administration has not approved pleconaril for these indications. The drug was also noted to induce CYP3A enzymes and consequently has the potential for multiple drug interactions.

Vapendavir (BTA798) is another capsid-binding agent that binds to VP1 of RV-A and RV-B. The drug was well tolerated after oral administration, and given 2 days before a rhinovirus challenge, it reduced the frequency of rhinovirus infection and peak virus load.[117] A phase IIb study in patients with asthma failed to show a beneficial effect in naturally occurring rhinovirus infection, although an antiviral effect was noted in subjects who received drug during the first 24 hours of symptoms.

The 3C protease of rhinoviruses is also an attractive target for antiviral inhibition. It cleaves the rhinovirus polyprotein that provides essential structural and enzymatic proteins for rhinovirus replication and is highly conserved among rhinovirus serotypes. Rupintrivir, a 3C protease inhibitor, was studied in clinical trials of experimental rhinovirus infection and was shown to have a modest effect on infection rate and severity of illness.[118]

Interferon

Intranasally administered leukocyte and recombinant interferon have been studied extensively in experimentally induced and naturally occurring colds in both prophylaxis and treatment of rhinovirus infections.[119] Intranasally administered interferon-α was effective in prophylaxis of naturally occurring rhinovirus-induced colds but was ineffective in treatment. Intranasal application of interferons for 1 to 2 weeks was associated with nasal side effects and local toxicity, which halted further development of this approach.

PREVENTION

The economic impact of the common cold in the industrialized world based on added costs and loss of productivity is enormous. In the United States, it has been estimated to exceed $40 billion per year. Because of this, there has been great interest in development of preventive measures, but few have been demonstrated to have any effects.

Virus Inactivation on Skin

As direct contact via hand to nose or hand to eye is a major route of transmission of rhinovirus, it appears reasonable that measures to remove or inactivate virus on hands may prevent infections that occur through self-inoculation. However, the effectiveness of such measures has been difficult to demonstrate. Hand sanitizers that contain 62% ethanol clearly inactivate virus on skin, but use in the setting of naturally occurring infection did not prevent colds.[120–122] Hand treatment with 2% aqueous iodine solution resulted in virus inactivation and did provide prevention against rhinovirus infection. However, the iodine stains on fingers made its use unacceptable.[61]

Acid Inactivation of Virus

The acid lability of rhinoviruses has led to the addition of organic acids to nasal tissues, hand treatments, and sprays in attempts to prevent infection. Studies of nasal tissues that contain citric acid, malic acid, and sodium laurel sulfate showed little effect on the frequency of colds under natural conditions.[123] Organic acids incorporated into hand treatments have also been ineffective in prevention of colds.[71] A study of a nasal spray containing low pH saline found a very modest reduction in virus titer and had no effect on symptoms.[124]

Intercellular Adhesion Molecule 1 Receptor Blockade

Monoclonal antibody directed at the major cellular receptor of rhinovirus, ICAM-1, has been demonstrated to block rhinovirus infection in vitro. Studies of this antibody in prophylaxis of experimental infection did not show an overall consistent benefit on virus shedding or on symptoms of colds.

An additional approach used generation of truncated forms of ICAM-1 through deletion of transmembrane and intracellular domains of the protein, referred to as soluble ICAMs, which inhibited rhinovirus infection in vitro. However, in a clinical trial of experimental infections, soluble ICAMs administered 7 hours before or 12 hours after challenge did not affect the rate of infection, although symptoms were reduced.[125]

VACCINES

The discovery of rhinovirus more than 60 years ago was associated with great interest in the development of vaccines. It was recognized early that the presence of rhinovirus serotype-specific neutralizing antibodies was associated with some protection against infection with homologous, but not with heterologous, serotypes.[126] Early trials with formalin-inactivated rhinoviruses as vaccine candidates, given intramuscularly or intranasally, showed protection against homologous challenge in terms of reduction of symptoms but not in reduction of rates of infection.[127,128] However, the multiplicity of rhinovirus antigenic types has presented a major impediment to the development of vaccines since those early studies. Molecular studies have identified a region of the N-terminus that can raise type cross-reactive neutralizing antibodies, and this may be useful as a vaccine candidate.[129] Use of modern techniques has led to generation of polyvalent rhinovirus vaccines with up to a 50-valent composition, which is broadly immunogenic in nonhuman primates, that are undergoing further development.[130]

ACKNOWLEDGMENT

Sections of this chapter are based in part on previous chapters in *Principles and Practice of Infectious Diseases* authored by Jack Gwaltney and Ron Turner.

Key References

The complete reference list is available online at Expert Consult.

14. Bochkov YA, Watters K, Ashraf S, et al. Cadherin-related family member 3, a childhood asthma susceptibility gene product, mediates rhinovirus C binding and replication. *Proc Natl Acad Sci USA*. 2015;112:5485–5490.
17. Turner RB. Rhinovirus. In: Bennett JE, Dolin R, Blaser MJ, eds. *Principles and Practice of Infectious Diseases*. Philadelphia, PA: Elsevier; 2015:2113–2121.
39. Foxman EF, Storer JA, Fitzgerald ME, et al. Temperature-dependent innate defense against the common cold virus limits viral replication at warm temperature in mouse airway cells. *Proc Natl Acad Sci USA*. 2015;112:827–832.
95. Jain S, Self WH, Wunderink RG, et al. Community-acquired pneumonia requiring hospitalization among U.S. adults. *N Engl J Med*. 2015;373:415–427.
96. Holter JC, Müller F, Bjørang O, et al. Etiology of community-acquired pneumonia and diagnostic yields of microbiological methods: a 3-year prospective study in Norway. *BMC Infect Dis*. 2015;15:64.
101. Piralla A, Zecca M, Comoli P, et al. Persistent rhinovirus infection in pediatric hematopoietic stem cell transplant recipients with impaired cellular immunity. *J Clin Virol*. 2015;67:38–42.
102. Engelmann I, Dewilde A, Lazrek M, et al. In vivo persistence of human rhinoviruses in immunosuppressed patients. *PLoS ONE*. 2017;12:e0170774.
104. Jacobs SE, Lamson DM, Soave R, et al. Clinical and molecular epidemiology of human rhinovirus infections in patients with hematologic malignancy. *J Clin Virol*. 2015;71:51–58.
106. Hung IF, Zhang AJ, To KK, et al. Unexpectedly higher morbidity and mortality of hospitalized elderly patients associated with rhinovirus compared with influenza virus respiratory tract infection. *Int J Mol Sci*. 2017;18:pii: E259.
116. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2006 Nov 6. Identifier NCT00394914, Effects of Pleconaril Nasal Spray on Common Cold Symptoms and Asthma Exacerbations Following Rhinovirus Exposure (Study P04295); 2013 Oct 31 [cited 2015 Jul 16]. https://clinicaltrials.gov/ct2/show/NCT00394914. Accessed March 21, 2018.
126. Glanville N, Johnston SL. Challenges in developing a cross-serotype rhinovirus vaccine. *Curr Opin Virol*. 2015;11:83–88.
130. Lee S, Nguyen MT, Currier MG, et al. A polyvalent inactivated rhinovirus vaccine is broadly immunogenic in rhesus macaques. *Nat Commun*. 2016;7:12838.

References

1. Atzl I, Helms R. A short history of the common cold. In: Eccles R, ed. *Common Cold*. Basal, Switzerland: Birkhauser Verlag; 2009:1–21.
2. Kruse W. The etiology of cough and nasal catarrh. *MMW Munch Med Wochenschr*. 1914;61:1574.
3. Dochez AR, Shibley GS, Mills KC. Studies in the common cold: IV. Experimental transmission of the common cold to anthropoid apes and human beings by means of a filterable agent. *J Exp Med*. 1930;52:701–716.
4. Price WH. The isolation of a new virus associated with respiratory clinical disease in humans. *Proc Natl Acad Sci USA*. 1956;42:892–896.
5. Pelon W, Mogabgab WJ, Phillips IA, et al. A cytopathogenic agent isolated from naval recruits with mild respiratory illnesses. *Proc Soc Exp Biol Med*. 1957;94:262–267.
6. Andrewes CH, Burnet FM, Enders JF, et al. Taxonomy of viruses infecting vertebrates: present knowledge and ignorance. *Virology*. 1961;15:52–55.
7. Tyrrell DAJ, Chanock RM. Rhinoviruses: a description. *Science*. 1963;141:152–153.
8. Palmenberg AC, Rathe JA, Liggett SB. Analysis of the complete genome sequences of human rhinovirus. *J Allergy Clin Immunol*. 2010;125:1190–1199.
9. Gern J, Palmenberg AC. Rhinoviruses. In: Knipe DM, Howley PM, eds. *Fields Virology*. Philadelphia, PA: Lippincott, Williams & Wilkins; 2013:531–549.
10. Palmenberg AC, Spiro D, Kuzmickas R, et al. Sequencing and analyses of all known human rhinovirus genomes reveal structure and evolution. *Science*. 2009;324:55–59.
11. Bochkov YA, Palmenberg AC, Lee WM, et al. Molecular modeling, organ culture and reverse genetics for a newly identified human rhinovirus C. *Nat Med*. 2011;17:627–632.
12. Greve JM, Davis G, Meyer AM, et al. The major human rhinovirus receptor is ICAM-1. *Cell*. 1989;56:839–847.
13. Staunton DE, Merluzzi VJ, Rothlein R, et al. A cell adhesion molecule, ICAM-1 is the major surface receptor for rhinoviruses. *Cell*. 1989;56:849–853.
14. Bochkov YA, Watters K, Ashraf S, et al. Cadherin-related family member 3, a childhood asthma susceptibility gene product, mediates rhinovirus C binding and replication. *Proc Natl Acad Sci USA*. 2015;112:5485–5490.
15. Andries K, Dewindt B, Snoeks J, et al. Two groups of rhinoviruses revealed by a panel of antiviral compounds present sequence divergence and differential pathogenicity. *J Virol*. 1990;64:1117–1123.
16. Gwaltney JM Jr, Colonno RJ, Hamparian VV, et al. Rhinoviruses. In: Schmidt NJ, Emmons RW, eds. *Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections*. 6th ed. Washington, DC: American Public Health Association; 1989:579–614.
17. Turner RB. Rhinovirus. In: Bennett JE, Dolin R, Blaser MJ, eds. *Principles and Practice of Infectious Diseases*. Philadelphia, PA: Elsevier; 2015:2113–2121.
18. Beem M. Rhinovirus infections in nursery school children. *J Pediatr*. 1969;74:818.
19. Palmenberg AC, Spiro D, Kuzmickas R, et al. Sequencing and analyses of all known human rhinovirus genomes reveal structure and evolution. *Science*. 2009;324:55–59.
20. Dick EC, Blumer CR, Evans AS. Epidemiology of infections with rhinovirus types 43 and 55 in a group of university of Wisconsin student families. *Am J Epidemiol*. 1967;86:386–400.
21. Horn ME, Brain E, Gregg I. Respiratory viral infection in childhood. A survey in general practice, Roehampton 1967-1972. *J Hyg (Lond)*. 1975;74:157–168.
22. Fox J, Cooney M, Hall C, et al. Rhinoviruses in Seattle families, 1975-1979. *Am J Epidemiol*. 1985;122:830–846.
23. Gwaltney JM Jr, Hendley JO, Simon G, et al. Rhinovirus infections in an industrial population, I. The occurrence of illness. *N Engl J Med*. 1966;275:1261–1268.
24. Horn ME, Brain E, Gregg I. Respiratory viral infection in childhood. A survey in general practice, Roehampton 1967-1972. *J Hyg (Lond)*. 1975;74:157–168.
25. Blomqvist S, Roivainen M, Puhakka T, et al. Virological and serological analysis of rhinovirus infections during the first two years of life in a cohort of children. *J Med Virol*. 2002;66:263–268.
26. Winther B, Hayden FG, Hendley JO. Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: association with symptomatic illness and effect of season. *J Med Virol*. 2006;78:644–650.
27. Monto AS, Cavallaro JJ. The Tecumseh study of respiratory illness. IV. Prevalence of rhinovirus serotypes, 1966-1969. *Am J Epidemiol*. 1972;96:352–360.
28. Winther B, Hayden FG, Hendley JO. Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: association with symptomatic illness and effect of season. *J Med Virol*. 2006;78:644–650.
29. Makela MJ, Puhakka T, Ruuskanen O, et al. Viruses and bacteria in the etiology of the common cold. *J Clin Microbiol*. 1998;36:539–542.
30. Gwaltney JM Jr. The Jeremiah Metzger lecture. Climatology and the common cold. *Trans Am Clin Climatol Assoc*. 1984;96:159–175.
31. Johnston NW, Johnston SL, Norman GR, et al. The September epidemic of asthma hospitalization: school children as disease vectors. *J Allergy Clin Immunol*. 2006;117:557–562.
32. D'Alessio DJ, Meschievitz CK, Peterson JA, et al. Short-duration exposure and the transmission of rhinoviral colds. *J Infect Dis*. 1984;150:189–194.
33. de Arruda E 3rd, Mifflin TE, Gwaltney JM Jr, et al. Localization of rhinovirus replication in vitro with in situ hybridization. *J Med Virol*. 1991;34:38–44.
34. Bochkov YA, Palmenberg AC, Lee WM, et al. Molecular modeling, organ culture and reverse genetics for a newly identified human rhinovirus C. *Nat Med*. 2011;17:627–632.
35. Mosser AG, Vrtis R, Burchell L, et al. Quantitative and qualitative analysis of rhinovirus infection in bronchial tissues. *Am J Respir Crit Care Med*. 2005;171:645–651.
36. Winther B, Gwaltney JM Jr, Mygind N, et al. Sites of rhinovirus recovery after point inoculation of the upper airway. *JAMA*. 1986;256:1763–1767.
37. Pitkaranta A, Arruda E, Malmberg H, et al. Detection of rhinovirus in sinus brushings of patients with acute community-acquired sinusitis by reverse transcription-PCR. *J Clin Microbiol*. 1997;35:1791–1793.
38. Gwaltney JM Jr, Phillips CD, Miller RD, et al. Computed tomographic study of the common cold. *N Engl J Med*. 1994;330:25–30.
39. Foxman EF, Storer JA, Fitzgerald ME, et al. Temperature-dependent innate defense against the common cold virus limits viral replication at warm temperature in mouse airway cells. *Proc Natl Acad Sci USA*. 2015;112:827–832.
40. Gern JE, Galagan DM, Jarjour NN, et al. Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. *Am J Respir Crit Care Med*. 1997;155:1159–1161.
41. Papadopoulos NG, Bates PJ, Bardin PG, et al. Rhinoviruses infect the lower airways. *J Infect Dis*. 2000;181:1875–1884.
42. Ashraf S, Brockman-Schneider R, Bochkov YA, et al. Biological characteristics and propagation of human rhinovirus-C in differentiated sinus epithelial cells. *Virology*. 2013;436:143–149.
43. Papadopoulos NG, Sanderson G, Hunter J, et al. Rhinoviruses replicate effectively at lower airway temperatures. *J Med Virol*. 1999;58:100–104.
44. Arruda E, Boyle TR, Winther B, et al. Localization of human rhinovirus replication in the upper respiratory tract by in situ hybridization. *J Infect Dis*. 1995;171:1329–1333.
45. Winther B, Brofeldt S, Christensen B, et al. Light and scanning electron microscopy of nasal biopsy material from patients with naturally acquired common colds. *Acta Otolaryngol*. 1984;97:309–318.
46. Turner RB, Hendley JO, Gwaltney JM Jr. Shedding of infected ciliated epithelial cells in rhinovirus colds. *J Infect Dis*. 1982;145:849–853.
47. Winther B, Farr B, Turner RB, et al. Histopathologic examination and enumeration of polymorphonuclear leukocytes in the nasal mucosa during experimental rhinovirus colds. *Acta Otolaryngol*. 1984;413:19–24.
48. Naclerio RM, Proud D, Lichtenstein LM, et al. Kinins are generated during experimental rhinovirus colds. *J Infect Dis*. 1988;157:133–142.
49. Douglas RG Jr, Alford RH, Cate TR, et al. The leukocyte response during viral respiratory illness in man. *Ann Intern Med*. 1966;64:521–530.
50. Winther B. Effects on the nasal mucosa of upper respiratory viruses (common cold). *Dan Med Bull*. 1994;41:193–204.
51. Levandowski RA, Weaver CW, Jackson GG. Nasal-secretion leukocyte populations determined by flow cytometry during acute rhinovirus infection. *J Med Virol*. 1988;25:423–432.
52. Griego SD, Weston CB, Adams JL, et al. Role of p38 mitogen-activated protein kinase in rhinovirus-induced cytokine production by bronchial epithelial cells. *J Immunol*. 2000;165:5211–5220.
53. Newcomb DC, Sajjan U, Nanua S, et al. Phosphatidylinositol 3-kinase is required for rhinovirus-induced airway epithelial cell interleukin-8 expression. *J Biol Chem*. 2005;280:36952–36961.
54. Grassme H, Riehle A, Wilker B, et al. Rhinoviruses infect human epithelial cells via ceramide-enriched membrane platforms. *J Biol Chem*. 2005;280:26256–26262.
55. Dumitru CA, Dreschers S, Gulbins E. Rhinoviral infections activate p38MAP-kinases via membrane rafts and RhoA. *Cell Physiol Biochem*. 2006;17:159–166.
56. Proud D, Naclerio RM, Gwaltney JM Jr, et al. Kinins are generated in nasal secretions during natural rhinovirus colds. *J Infect Dis*. 1990;161:120–123.
57. Higgins PG, Barrow GI, Tyrrell DA. A study of the efficacy of the bradykinin antagonist, NPC 567, in rhinovirus infections in human volunteers. *Antiviral Res*. 1990;14:339–344.
58. Gustafson LM, Proud D, Hendley JO, et al. Oral prednisone therapy in experimental rhinovirus infections. *J Allergy Clin Immunol*. 1996;97:1009–1014.
59. Muether PS, Gwaltney JM Jr. Variant effect of first- and second-generation antihistamines as clues to their mechanism of action on the sneeze reflex in the common cold. *Clin Infect Dis*. 2001;33:1483–1488.
60. Igarashi Y, Skoner DP, Doyle WJ, et al. Analysis of nasal secretions during experimental rhinovirus upper respiratory infections. *J Allergy Clin Immunol*. 1993;92:722–731.
61. Hendley JO, Gwaltney JM Jr. Mechanisms of transmission of rhinovirus infections. *Epidemiol Rev*. 1988;10:243–258.
62. Reed SE. An investigation of the possible transmission of rhinovirus colds through indirect contact. *J Hyg (Lond)*. 1975;75:249–258.
63. Dick EC, Jennings LC, Mink KA, et al. Aerosol transmission of rhinovirus colds. *J Infect Dis*. 1987;156:442–448.
64. Gwaltney JM Jr, Moskalski PB, Hendley JO. Hand-to-hand transmission of rhinovirus colds. *Ann Intern Med*. 1978;88:463–467.
65. D'Alessio DJ, Peterson JA, Dick CR, et al. Transmission of experimental rhinovirus colds in volunteer married couples. *J Infect Dis*. 1976;133:28–36.
66. Hendley JO, Gwaltney JM Jr, Jordan WS Jr. Rhinovirus infections in an industrial population. IV. Infections within families of employees during two fall peaks of respiratory illness. *Am J Epidemiol*. 1969;89:184–196.
67. Peltola V, Waris M, Osterback R, et al. Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. *J Infect Dis*. 2008;197:382–389.
68. D'Alessio DJ, Peterson JA, Dick CR, et al. Transmission of experimental rhinovirus colds in volunteer married couples. *J Infect Dis*. 1976;133:28–36.
69. Meschievitz CK, Schultz SB, Dick EC. A model for obtaining predictable natural transmission of rhinoviruses in human volunteers. *J Infect Dis*. 1984;150:195–201.
70. Jennings LC, Dick EC. Transmission and control of rhinovirus colds. *Eur J Epidemiol*. 1987;3:327–335.
71. Turner RB, Fuls JL, Rodgers ND, et al. A randomized trial of the efficacy of hand disinfection for prevention of rhinovirus infection. *Clin Infect Dis*. 2012;54:1422–1426.
72. Jennings LC, Dick EC, Mink KA, et al. Near disappearance of rhinovirus along a fomite transmission chain. *J Infect Dis*. 1988;158:888–892.
73. Mackay IM, Lambert SB, Faux CE, et al. Community-wide, contemporaneous circulation of a broad spectrum of human rhinoviruses in healthy Australian preschool-aged children during a 12-month period. *J Infect Dis*. 2013;207:1433–1441.
74. Gwaltney JM Jr, Hendley JO, Simon G, et al. Rhinovirus infections in an industrial population, II. Characteristics of illness and antibody response. *J Am Med Assoc*. 1967;202:494–500.
75. Pitkaranta A, Starck M, Savolainen S, et al. Rhinovirus RNA in the maxillary sinus epithelium of adult patients with acute sinusitis. *Clin Infect Dis*. 2001;33:909–911.
76. Puhakka T, Makela MJ, Alanen A, et al. Sinusitis in the common cold. *J Allergy Clin Immunol*. 1998;102:403–408.
77. Revai K, Dobbs LA, Nair S, et al. Incidence of acute otitis media and sinusitis complicating upper respiratory tract infection: the effect of age. *Pediatrics*. 2007;119:e1408–e1412.
78. McBride TP, Doyle WJ, Hayden FG, et al. Alterations of the eustachian tube, middle ear, and nose in rhinovirus infection. *Arch Otolaryngol Head Neck Surg*. 1989;115:1054–1059.
79. Moody SA, Alper CM, Doyle WJ. Daily tympanometry in children during the cold season: association of otitis media with upper respiratory tract infections. *Int J Pediatr Otorhinolaryngol*. 1998;45:143–150.
80. Pitkaranta A, Virolainen A, Jero J, et al. Detection of rhinovirus, respiratory syncytial virus, and coronavirus infections in acute otitis media by reverse transcriptase polymerase chain reaction. *Pediatrics*. 1998;102:291–295.
81. Wald ER, Guerra N, Byers C. Upper respiratory tract infections in young children: duration of and frequency of complications. *Pediatrics*. 1991;87:129–133.

82. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ*. 1995;310:1225–1229.
83. Rakes GP, Arruda E, Ingram JM, et al. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care: IgE and eosinophil analyses. *Am J Respir Crit Care Med*. 1999;159:785–790.
84. Duff AL, Pomeranz ES, Gelber LE, et al. Risk factors for acute wheezing in infants and children: viruses, passive smoke, and IgE antibodies to inhalant allergens. *Pediatrics*. 1993;92:535–540.
85. Zambrano JC, Carper HT, Rakes GP, et al. Experimental rhinovirus challenges in adults with mild asthma: response to infection in relation to IgE. *J Allergy Clin Immunol*. 2003;111:1008–1016.
86. Jackson DJ, Evans MD, Gangnon RE, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *Am J Respir Crit Care Med*. 2012;185:281–285.
87. Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. *Lancet*. 2010;376:826–834.
88. Caliskan M, Bochkov YA, Kreiner-Moller E, et al. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med*. 2013;368:1398–1407.
89. Calvo C, Pozo F, Garcia-Garcia ML, et al. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. *Acta Paediatr*. 2010;99:883–887.
90. Papadopoulos NG, Moustaki M, Tsolia M, et al. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. *Am J Respir Crit Care Med*. 2002;165:1285–1289.
91. Jacques J, Bouscambert-Duchamp M, Moret H, et al. Association of respiratory picornaviruses with acute bronchiolitis in French infants. *J Clin Virol*. 2006;35:463–466.
92. Miron D, Srugo I, Kra-Oz Z, et al. Sole pathogen in acute bronchiolitis: is there a role for other organisms apart from respiratory syncytial virus? *Pediatr Infect Dis J*. 2010;29:e7–e10.
93. Miller EK, Lu X, Erdman DD, et al. Rhinovirus-associated hospitalizations in young children. [see comment]. *J Infect Dis*. 2007;195:773–781.
94. Juven T, Mertsola J, Waris M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. *Pediatr Infect Dis J*. 2000;19:293–298.
95. Jain S, Self WH, Wunderink RG, et al. Community-acquired pneumonia requiring hospitalization among U.S. adults. *N Engl J Med*. 2015;373:415–427.
96. Holter JC, Müller F, Bjørang O, et al. Etiology of community-acquired pneumonia and diagnostic yields of microbiological methods: a 3-year prospective study in Norway. *BMC Infect Dis*. 2015;15:64.
97. Jennings LC, Anderson TP, Beynon KA, et al. Incidence and characteristics of viral community-acquired pneumonia in adults. *Thorax*. 2008;63:42–48.
98. Karhu J, Ala-Kokko TI, Vuorinen T, et al. Lower respiratory tract virus findings in mechanically ventilated patients with severe community-acquired pneumonia. *Clin Infect Dis*. 2014;59:62–70.
99. Choi SH, Hong SB, Ko GB, et al. Viral infection in patients with severe pneumonia requiring intensive care unit admission. *Am J Respir Crit Care Med*. 2012;186:325–332.
100. Ruuskanen O, Järvinen A. What is the real role of respiratory viruses in severe community-acquired pneumonia? *Clin Infect Dis*. 2014;59:71–73.
101. Piralla A, Zecca M, Comoli P, et al. Persistent rhinovirus infection in pediatric hematopoietic stem cell transplant recipients with impaired cellular immunity. *J Clin Virol*. 2015;67:38–42.
102. Engelmann I, Dewilde A, Lazrek M, et al. In vivo persistence of human rhinoviruses in immunosuppressed patients. *PLoS ONE*. 2017;12:e0170774.
103. Jacobs SE, Lamson DM, St George K, et al. Human rhinoviruses. *Clin Microbiol Rev*. 2013;26:135–162.
104. Jacobs SE, Lamson DM, Soave R, et al. Clinical and molecular epidemiology of human rhinovirus infections in patients with hematologic malignancy. *J Clin Virol*. 2015;71:51–58.
105. Kraft CS, Jacob JT, Sears MH, et al. Severity of human rhinovirus infection in immunocompromised adults is similar to that of 2009 H1N1 influenza. *J Clin Microbiol*. 2012;50:1061–1063.
106. Hung IF, Zhang AJ, To KK, et al. Unexpectedly higher morbidity and mortality of hospitalized elderly patients associated with rhinovirus compared with influenza virus respiratory tract infection. *Int J Mol Sci*. 2017;18:pii: E259.
107. Gama RE, Horsnell PR, Hughes PJ, et al. Amplification of rhinovirus specific nucleic acids from clinical samples using the polymerase chain reaction. *J Med Virol*. 1989;28:73–77.
108. Ireland DC, Kent J, Nicholson KG. Improved detection of rhinoviruses in nasal and throat swabs by seminested RT-PCR. *J Med Virol*. 1993;40:96–101.
109. Lee WM, Grindle K, Pappas T, et al. High-throughput, sensitive, and accurate multiplex PCR-microsphere flow cytometry system for large-scale comprehensive detection of respiratory viruses. *J Clin Microbiol*. 2007;45:2626–2634.
110. Faux CE, Arden KE, Lambert SB, et al. Usefulness of published PCR primers in detecting human rhinovirus infection. *Emerg Infect Dis*. 2011;17:296–298.
111. Cate TR, Rossen RD, Douglas R Jr, et al. The role of nasal secretion and serum antibody in the rhinovirus common cold. *Am J Epidemiol*. 1966;84:352–363.
112. Perkins JC, Tucker DN, Knopf HL, et al. Comparison of protective effect of neutralizing antibody in serum and nasal secretions in experimental rhinovirus type 13 illness. *Am J Epidemiol*. 1969;90:519–526.
113. Kaiser L, Crump CE, Hayden FG. In vitro activity of pleconaril and AG7088 against selected serotypes and clinical isolates of human rhinoviruses. *Antiviral Res*. 2000;47:215–220.
114. Hayden FG, Herrington DT, Coats TL, et al. Efficacy and safety of oral pleconaril for treatment of picornavirus colds in adults: results of two double-blind, randomized, placebo-controlled trials. *Clin Infect Dis*. 2003;36:1523–1532.
115. Pevear DC, Hayden FG, Demenczuk TM, et al. Relationship of pleconaril susceptibility and clinical outcomes in treatment of common colds caused by rhinoviruses. *Antimicrob Agents Chemother*. 2005;49:4492–4499.
116. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2006 Nov 6. Identifier NCT00394914, Effects of Pleconaril Nasal Spray on Common Cold Symptoms and Asthma Exacerbations Following Rhinovirus Exposure (Study P04295); 2013 Oct 31 [cited 2015 Jul 16]. https://clinicaltrials.gov/ct2/show/NCT00394914. Accessed March 21, 2018.
117. Lambert JN The Biota Research and Product Development Team. BTA798 is an orally bioavailable and potent inhibitor of human rhinovirus both in vitro and in an experimental challenge virus infection model in healthy volunteers Washington, DC, USA American society for Microbiology Abstracts of the Fifty-first Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL; 2011. Abstract V-1557.
118. Hayden FG, Turner RB, Gwaltney JM, et al. Phase II, randomized, double-blind, placebo-controlled studies of ruprintrivir nasal spray 2-percent suspension for prevention and treatment of experimentally induced rhinovirus colds in healthy volunteers. *Antimicrob Agents Chemother*. 2003;47:3907–3916.
119. Arruda E, Hayden FG. Clinical studies of antiviral agents for picornavirus infections. In: Jeffries DJ, De Clercq E, eds. *Antiviral Chemotherapy*. New York: Wiley; 1995.
120. Sattar SA, Abebe M, Bueti AJ, et al. Activity of an alcohol-based hand gel against human adeno-, rhino-, and rotaviruses using the fingerpad method. *Infect Control Hosp Epidemiol*. 2000;21:516–519.
121. Sandora TJ, Taveras EM, Shih MC, et al. A randomized, controlled trial of a multifaceted intervention including alcohol-based hand sanitizer and hand-hygiene education to reduce illness transmission in the home. *Pediatrics*. 2005;116:587–594.
122. Turner RB, Fuls JL, Rodgers ND. Effectiveness of hand sanitizers with and without organic acids for removal of rhinovirus from hands. *Antimicrob Agents Chemother*. 2010;54:1363–1364.
123. Farr BM, Hendley JO, Kaiser DL, et al. Two randomized controlled trials of virucidal nasal tissues in the prevention of natural upper respiratory infections. *Am J Epidemiol*. 1988;128:1162–1172.
124. Gern JE, Mosser AG, Swenson CA, et al. Inhibition of rhinovirus replication in vitro and in vivo by acid-buffered saline. *J Infect Dis*. 2007;195:1137–1143.
125. Turner RB, Wecker MT, Pohl G, et al. Efficacy of tremacamra, a soluble intercellular adhesion molecule 1, for experimental rhinovirus infection. *J Am Med Assoc*. 1999;281:1797–1804.
126. Glanville N, Johnston SL. Challenges in developing a cross-serotype rhinovirus vaccine. *Curr Opin Virol*. 2015;11:83–88.
127. Mitichison DA. Prevention of colds by vaccination against a rhinovirus: a report by the Scientific Committee on Common Cold Vaccines. *Br Med J*. 1965;1:1344–1349.
128. Perkins JC, Tucker DN, Knope HL, et al. Evidence for protective effect of an inactivated rhinovirus vaccine administered by the nasal route. *Am J Epidemiol*. 1969;90:319–326.
129. Katpally U, Fu TM, Freed DC, et al. Antibodies to the buried N terminus of rhinovirus VP4 exhibit cross-serotypic neutralization. *J Virol*. 2009;83:7040–7048.
130. Lee S, Nguyen MT2, Currier MG, et al. A polyvalent inactivated rhinovirus vaccine is broadly immunogenic in rhesus macaques. *Nat Commun*. 2016;7:12838.

176 Noroviruses and Sapoviruses (Caliciviruses)

Raphael Dolin and John J. Treanor

SHORT VIEW SUMMARY

Definition
- The genera *Norovirus* and *Sapovirus,* of the family Caliciviridae, cause acute gastroenteritis.

Epidemiology
- Noroviruses are the major worldwide cause of viral gastroenteritis, cause both foodborne and person-to-person outbreaks, and can be spread via fomites.
- Noroviruses account for 699 million illnesses worldwide annually, 21 million cases of gastroenteritis in the United States, and 219,000 deaths annually in the developing world, mostly among children younger than 5 years.
- Sapoviruses cause gastroenteritis less frequently than noroviruses.

Microbiology
- Noroviruses and sapoviruses are single-stranded, positive-sense RNA viruses, which are divided into 7 genogroups (noroviruses) and 14 genogroups (sapoviruses). Each genogroup has multiple genotypes.
- Human noroviruses have been cultured in vitro for the first time recently.

Clinical Manifestations
- Illness caused by noroviruses and sapoviruses consists of combinations of vomiting and diarrhea, often accompanied by abdominal cramps, nausea, and low-grade fever.
- Illness usually lasts 12 to 60 hours and remits spontaneously.
- More severe disease and even fatalities can occur in young children and in the elderly. Immunocompromised patients may have prolonged shedding of virus, along with severe and persistent gastrointestinal illness.

Diagnosis
- Noroviruses may be suspected as a cause of outbreaks of gastrointestinal illness if the aforementioned clinical features are present, and particularly if a large number of individuals are involved. However, the illness is not sufficiently distinctive to permit a diagnosis to be made on clinical grounds alone.
- Specific diagnosis is made by detection of virus in stool specimens with reverse-transcriptase polymerase chain reaction assay. Enzyme immunoassays are also available, but they are generally of lower sensitivity and specificity.

Therapy
- Only supportive therapy is available, and maintenance of hydration is particularly important.

Prevention
- The main control measures are to prevent contamination of food supplies and water through good hygiene and restriction of food handling by ill individuals until at least 2 to 3 days after gastrointestinal illness has resolved.
- Vigorous hand washing by ill individuals should be maintained.
- Decontamination of exposed surfaces should be carried out with Environmental Protection Agency–recommended disinfectants; for example, household bleach should be used at 1:10 to 1:50 dilutions.
- Vaccines against norovirus are under active development, including virus-like particles, which are undergoing clinical trial.

Acute infectious gastroenteritis is an exceedingly common and widespread illness throughout the world. Noroviruses are major causes of this illness,[1,2] which is generally self-limited but can result in hospitalization,[3] mortality in young children in the developing world,[4] and severe illness in elderly[5] and immunocompromised patients.[6] *Sapovirus,* another calicivirus, and *Astrovirus* (see Chapter 177) also cause gastroenteritis but are much less common than noroviruses.

HISTORY

The failure to isolate causative agents, bacterial or viral, from apparently infectious outbreaks of diarrhea or vomiting, or both, led to the widely held assumption that undetected viruses were responsible for such disease. In 1945, Reimann and coworkers[7] transmitted disease to volunteers by administering bacteria-free filtrates of throat washings, stool filtrates, or both from naturally occurring cases. Gordon[8] and Jordan[9] and their associates also induced disease in volunteers with bacteria-free material. These studies described two transmissible agents of subbacterial size, the Marcy and FS agents, which appeared to be antigenically distinct. However, these workers were unable to detect viral agents in vitro with techniques available at that time. Despite extensive virologic investigations in laboratories throughout the world, relatively little progress was made in this area until 1972, when the Norwalk virus, the prototype of this group, was described and partially characterized.[10,11] This virus was initially detected in diarrheal stools obtained during an outbreak of gastroenteritis in Norwalk, Ohio, that involved students in an elementary school and family contacts. Subsequently, additional viruses with similar properties were described, including the Hawaii,[12] Montgomery County (MC),[13] Taunton,[14] and Snow Mountain[15] viruses, also named for the geographic regions in which they were first recognized. All these viruses had a similar small, round, structured morphologic appearance with electron microscopy; were of a similar size and density; did not grow in any in vitro propagation system; and were responsible for acute gastroenteritis, commonly in epidemic form with high secondary attack rates.[16–18] At the same time, viruses with more readily identifiable morphologic features with electron microscopy, referred to as *human caliciviruses,*[19] were observed in the stools of individuals, primarily children, with gastroenteritis.

A major advance in this field occurred when polymerase chain reaction (PCR) techniques were applied to amplify the genome of the Norwalk virus from virion-containing stool samples.[20,21] These

studies identified the Norwalk virus as a member of the Caliciviridae family and allowed determination of the complete nucleotide sequence of this virus.[22] Subsequent molecular studies have clearly identified all these viruses as caliciviruses and established them as major causes of gastrointestinal disease in both adults and children worldwide.

VIROLOGY

Taxonomy

The name *calicivirus,* and hence the virus family Caliciviridae, is derived from the characteristic appearance of the viral particles on electron micrographs, which consists of a scalloped border with cuplike indentations on its surface (Fig. 176.1), from which the Latin name *chalice* or *calyx* is derived.[23,24] Caliciviruses have been detected in a variety of animal species, including marine mammals, swine, felines, and rabbits, in addition to humans. Five genera have been described: *Norovirus, Sapovirus, Vesivirus, Lagovirus,* and *Nebovirus.* Human infections are caused by *Norovirus*[25,26] and *Sapovirus.*[27] *Vesivirus* causes vesicular diseases in swine, cats, and marine mammals; *Lagovirus* causes hemorrhagic diseases in rabbits; and *Nebovirus* causes enteric disease in calves. The prototypical *Norovirus* is the Norwalk virus, and the prototypical *Sapovirus* is the Sapporo virus. In addition to sequence differences, the genera differ in minor details of genome organization. Whereas many animal caliciviruses replicate efficiently in cell culture, the propagation of human noroviruses or sapoviruses in vitro has been difficult to achieve, although transfection of viral RNA from norovirus in human embryonic cells[28,29] and infection of human intestinal organ cultures[30] have been reported. Thus, much of the initial microbiologic information on noroviruses was based on physical properties determined with electron microscopy or by means of physicochemical manipulation of infectious inocula. Because of the small numbers of virions characteristically found in stool samples, it is sometimes necessary to enhance electron microscopic visualization of the particles through the addition of immune serum, which obscures the typical morphologic features (Fig. 176.2). Recently a major breakthrough was reported, in which efficient culture of human norovirus was achieved in enterocytes obtained from stem cells in human intestinal epithelium.[31] The cultivation of noroviruses in vitro in a B-cell line has also been reported.[32,33] These advances should enable efficient, detailed molecular characterization of noroviruses to be carried out, along with facilitation of efforts to develop effective vaccines and therapeutics (see later).

Genome Organization

Characteristics of the noroviruses include a single-stranded, positive-sense RNA genome with a polyadenylated 3′ tail[22,34] and a single capsid polypeptide of 59- to 62-kDa molecular mass.[35] The virions are 26 to 34 nm in diameter, have cubic symmetry with a buoyant density in cesium chloride of 1.34 to 1.41 g/mL, and are relatively heat and acid stable and ether resistant.[11]

The genomic organization of noroviruses is shown in Fig. 176.3.[36] Noroviruses have a positive-sense, single-stranded RNA genome of approximately 7700 nucleotides, excluding the polyadenylated tail. Three long open reading frames (ORFs) are present. The first ORF encodes a polyprotein of about 57-kDa molecular weight, which includes and codes for seven nonstructural proteins, including the viral RNA polymerase, helicase, and protease functions.[37] The second ORF encodes the viral capsid protein (VP1) of 58-kDa molecular weight, which determines the antigenic phenotype and interacts with host cell receptors.[38] When expressed in eukaryotic cells, the capsid protein spontaneously assembles into virus-like particles (VLPs), which are immunogenic and react specifically with convalescent human sera.[38] The three-dimensional structure of these empty capsids has been studied with electron cryomicroscopy, which suggests that the capsid has icosahedral symmetry with T = 3.[39] The x-ray crystallographic structure of these VLPs shows that the capsid contains two domains, a shell (S) domain and a protruding (P) domain that may be involved in binding to susceptible cells.[36,40] Finally, the third ORF encodes a minor structural protein (VP2) of 12- to 29-kDa molecular weight,[22,41] which may add to particle stability and may also be involved in capsid assembly and genome encapsidation.[42] The overall genetic organization of sapoviruses is similar to that of noroviruses, except that sapoviruses contain two ORFs. ORF-1 encodes both nonstructural and the major structural protein (VP1), whereas ORF-2 encodes the minor structural protein.[27,43] A third ORF has been predicted in several sapoviruses, but its function is unknown.

Based on phylogenetic analyses, noroviruses have been subdivided into seven genogroups designated GI to GVII,[26,44,45] and the genogroups have been further divided into at least 34 genotypes. Individual genotypes are designated by Arabic numerals after the genogroup designation—for

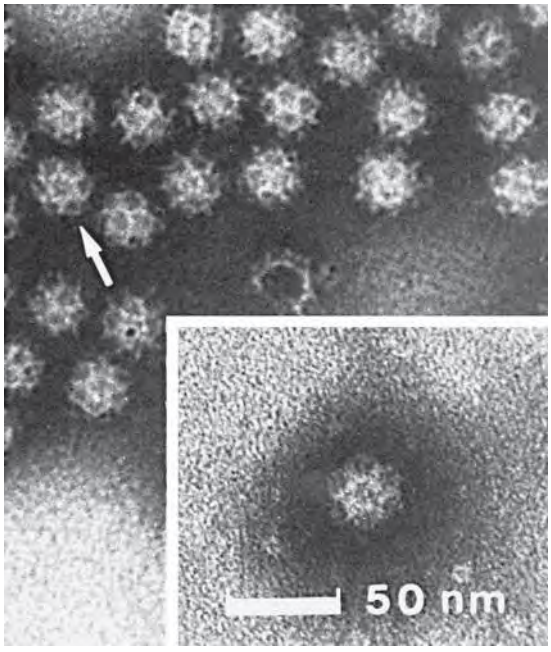

FIG. 176.1 Calicivirus particles *(arrow)* in a fecal extract from a child with gastroenteritis. *Inset* is higher magnification of particle indicated by *arrow. (From Chiba S, Sakuma Y, Kogasaka R, et al. An outbreak of gastroenteritis associated with calicivirus in an infant home.* J Med Virol. *1979;4:249–254.)*

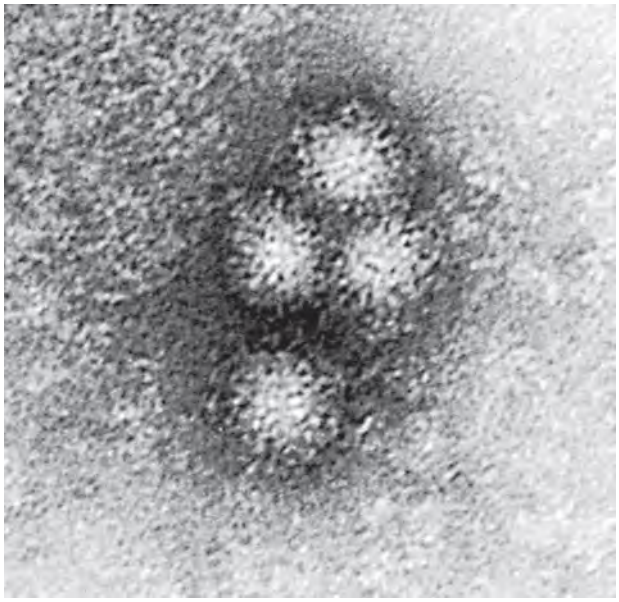

FIG. 176.2 Snow mountain virus in stool filtrate from a volunteer with experimentally induced disease as visualized with immune electron microscopy. Particles are 27 nm in diameter and are stained with 2% phosphotungstic acid.

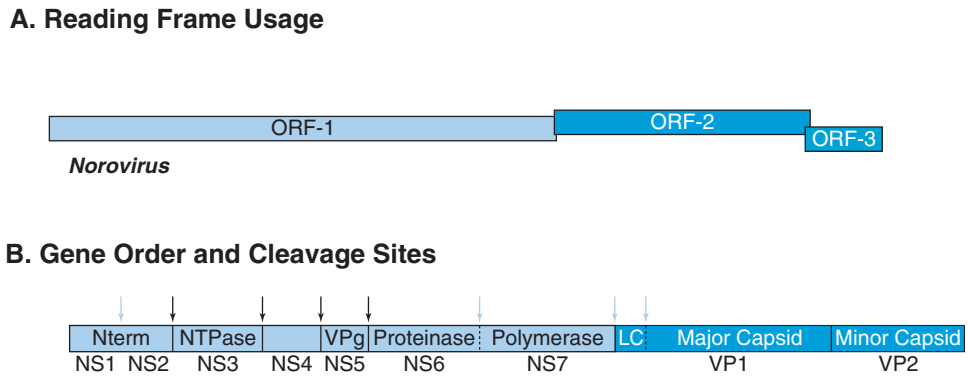

FIG. 176.3 Genome organization of norovirus. (A) Reading frames: Open reading frame 1 (ORF-1) codes for nonstructural proteins: ORF-2 codes for the major capsid VP1; ORF-3 codes for the minor capsid VP2. (B) Gene order and cleavage sites: ORF-1 codes a polyprotein that has seven distinct proteins. ORF-2 codes for a leader protein *(LC)* and for VP1. ORF-3 codes for VP2. *(Modified from Green KY. Caliciviridae: the noroviruses. In:* Fields Virology. *6th ed. Philadelphia: Lippincott; 2013.)*

example GI.1 or GII.1. Most of the strains implicated in human disease fall into genogroups GI and GII, but GIV strains also infect humans.[26] The GI genogroup includes the Norwalk virus, whereas the Snow Mountain and Hawaii viruses belong to genogroup GII.

Sapoviruses have also been further subdivided into 14 genogroups (GI to GXIV), of which GI, GII, GIV, and GV are known to infect humans.[46] Multiple genotypes within the genogroups have also been noted.[26]

Antigenic Characterization

Because of the lack of a convenient in vitro propagation system, antigenic characterization of these viruses has not been straightforward until now. Not unexpectedly, predicted amino-acid homology within the capsid region is less than that within the polymerase region.[44] Thus, phylogenetic trees based on capsid sequence have a slightly different structure than those based on polymerase structure.[47] VLPs have been generated by expression of the capsid regions of many of the noroviruses, including Norwalk[38] and Desert Shield[48] viruses (genogroup GI) and MX,[49] Lordsdale,[50] Snow Mountain,[47] Hawaii,[51] and Toronto[52] viruses (genogroup GII), in addition to the prototype sapovirus, Sapporo.[53] In general, hyperimmune animal sera raised against capsids are very specific. However, tests of postinfection human sera have suggested a significant degree of cross-reactivity among viruses within a genogroup.

The most clear-cut antigenic distinction among noroviruses is between the Norwalk and Hawaii viruses because these agents have been compared through cross-challenge experiments in human subjects.[54] In these studies, infection with the Norwalk virus provided short-term protection against rechallenge with the Norwalk virus but not against the Hawaii virus, and vice versa. Because this type of experiment is the closest analogue to virus neutralization that has been previously available, this is direct evidence that there are at least two distinct norovirus serotypes, roughly corresponding to the GI and GII genogroups described earlier.

EPIDEMIOLOGY

With the advent of efficient means for their detection (see later), noroviruses have emerged as major, worldwide causes of gastroenteritis in diverse populations and in both children and adults. Noroviruses are the most common cause of epidemic gastroenteritis and account for more than 90% of outbreaks of viral gastroenteritis and for about 50% of all-cause outbreaks worldwide.[18,26,55–60] Noroviruses are also important causes of sporadic cases of gastroenteritis and account for 4.4% to 30.7% of children younger than 5 years who are hospitalized with gastroenteritis throughout the world.[26,61] Overall, it has been estimated that noroviruses cause 699 million illnesses and 219,000 deaths annually worldwide. Mortality is believed to occur primarily in children younger than 5 years.[4,62] The Centers for Disease Control and Prevention (CDC) estimates that noroviruses cause 40% to 50% of foodborne outbreaks of gastroenteritis in the United States and overall account for 21 million cases of gastroenteritis, 2 million office visits, 70,000 hospitalizations, and up to 800 deaths annually in the United States.[26,63,64]

Antigenic Variation

The role of antigenic variation in the epidemiology of noroviruses remains an area of continued investigation. In general, antibody to noroviruses within genogroup II appears to be more common than to viruses within genogroup I. However, a number of diverse genotypes often cocirculate, including recombinants among different strains,[65–67] and there may be year-to-year variation in the predominant viruses associated with illnesses.[68,69] Despite this, viruses from a single genotype, GII.4, have been responsible for the majority of outbreaks worldwide since 1995.[26,70–72] Novel pandemic GII.4 variants have evolved every 2 to 3 years since the 1990s and replaced previously predominant GII.4 strains. GII.4 strains were the predominant strains associated worldwide with outbreaks from 2001 to 2012.[73,74] In 2014, GII.17 virus emerged and spread worldwide in 2014–2015.[75–79]

The epidemiology of sapoviruses has been less extensively studied than that of noroviruses, but sapoviruses appear to be substantially less frequent causes of outbreaks of gastroenteritis than noroviruses.[27] The original Sapporo virus was reported in Japan and appeared to affect young children primarily.[80] Subsequently, sapoviruses have been reported in outbreaks in adults in various settings, including long-term care facilities.[80–84] Reports have indicated that sapoviruses caused 1.8% to 8.0% of outbreaks that were studied in diverse geographic locales.[27]

Seroprevalence

In developed countries, serum antibody to the noroviruses is first noted at ages 3 to 4 years, with antibody prevalence gradually rising to greater than 50% by the fifth decade of life.[85–88] Studies using recombinant Norwalk antigen have suggested that significant increases in antibody prevalence occur in infancy, at entry into primary schools, and in young adulthood.[89] Seroepidemiologic studies of the sapoviruses carried out in Japan, Southeast Asia, and the United Kingdom have indicated that antibody is acquired in early childhood and can be detected in up to 90% of older children and adults.[90–92] Antibody appears to be acquired more rapidly in developing countries[85] and may be rare or nonexistent in some isolated populations.[85,93] Transmission of noroviruses occurs year round but with a higher incidence of disease in the winter months in temperate climates.[94]

Seroprevalence studies of sapovirus antibodies indicated that infection also occurs early in childhood and reaches >90% in school age children. Seroprevalence remained high (80%–100%) in pooled immunoglobulins obtained from adults.[27]

Transmission of Infection

The experimental induction of illness in volunteers suggests that the major route of person-to-person transmission is fecal-oral. Epidemiologic reports have also implicated vomitus as a vehicle of transmission,[95,96]

and virus has been detected in vomitus with electron microscopy[97] and PCR.[98] Airborne transmission has also occasionally been implicated,[99,100] but limited attempts to experimentally transmit virus with nasopharyngeal washings from an ill volunteer were unsuccessful.[11] It has been estimated that fewer than 100 viral particles are required for infection of a susceptible individual,[101] although studies have suggested that 1300 to 2800 particles may be necessary.[102]

Incubation periods are generally 24 to 48 hours, although ranges from 18 to 72 hours have been observed. Virus shedding in stools is maximal over the first 24 to 48 hours after illness.[13,15] In volunteer studies, virus has been rarely detected beyond 72 hours after the onset of vomiting or diarrhea[13,15] with immune electron microscopy. However, virus can be detected for 3 to 4 weeks after illness with use of sensitive enzyme-linked immunosorbent assay techniques[103] or PCR.[104–106] Prolonged shedding of norovirus for months may occur in immunocompromised indivduals.[107] The clinical significance of the prolonged detection of virus in stools is unclear, but epidemiologic data have implicated individuals who are not symptomatic in the transmission of illness.[108]

Noroviruses were first recognized in association with point-source outbreaks of gastroenteritis, and such outbreaks are a common situation in which noroviruses have been implicated as etiologic agents. Several features are characteristic of such outbreaks that may be useful in suspecting norovirus as a cause of the illness. These include a short-lived illness of 2 to 3 days' duration, with vomiting as a prominent symptom in most affected individuals; an incubation period of 24 to 48 hours; high secondary attack rates; and lack of other identifiable pathogens on routine examinations of stool samples.[109] The application of modern diagnostic techniques has shown that the frequency of norovirus infection in such outbreaks is extremely high.[65]

Outbreaks of norovirus gastroenteritis are particularly common in closed settings, such as in hospitals, nursing homes, ships, schools, and the military.[110–113] Secondary transmission is a prominent feature of such outbreaks. Although most of these outbreaks will terminate spontaneously after 1 to 2 weeks, some may be quite prolonged. For example, up to 12 recurrent outbreaks of norovirus gastroenteritis have been reported on cruise ships despite stringent attempts to determine the source and disinfect the ship between cruises.[114]

Environmental Contamination

Contamination of objects can result in transmission of infection via fomites, and such surfaces should be decontaminated using Environmental Protection Agency (EPA)–recommended disinfectants (see "Prevention").

Almost any type of food that has contact with contaminated water or is contaminated by food handlers may serve as a vehicle for outbreaks of norovirus gastroenteritis.[115] The most common foods implicated in norovirus outbreaks are sandwiches and salads, particularly those that require handling but not subsequent cooking. Contamination of lettuce and salad greens with noroviruses accounts for nearly 25% of all produce-associated outbreaks.[26,116] Outbreaks have also been associated with drinking contaminated water or even swimming in pools or lakes in which ill individuals have also been swimming,[117,118] which indicates the highly infectious nature of these viruses. Of note, these viruses appear to be relatively resistant to inactivation by chlorine.[96] Because such products as shellfish or contaminated commercial ice[119] can be distributed to multiple sites, these outbreaks can encompass a wide geographic area.[120] Contamination of foodstuffs has been traced to both presymptomatic[121] and postsymptomatic[108] food handlers, complicating infection-control recommendations.

Shellfish, such as clams and oysters, are filter feeders and efficiently concentrate microorganisms from contaminated water. When consumed, these foods are frequently implicated in the transmission of enteric viruses in general and of norovirus gastroenteritis in particular.[122] Because noroviruses are relatively resistant to heat inactivation, steaming of shellfish does not entirely eliminate the risk for transmission.[123,124]

Recommendations for evaluation and control of nosocomial outbreaks[101] include identification and elimination of common sources and the use of hand washing and barrier methods to prevent secondary transmission. Exclusion of ill employees may be important in limiting the spread of nosocomial outbreaks.[125] Health departments have recommended that individuals with norovirus-associated illness not be involved in food handling or preparation until 2 to 3 days after gastrointestinal signs and symptoms have resolved. These methods have generally been found to be more effective in limiting the spread of outbreaks from unit to unit within an institution than in terminating an outbreak in an individual unit once it has begun.[126,127] The Viral Gastroenteritis Section of the CDC is available for advice regarding such outbreaks.

Although noroviruses were initially recognized primarily in association with outbreaks of acute gastroenteritis mostly involving adults, there has been an increasing recognition of the role of these viruses as causes of sporadic gastroenteritis in children in various parts of the world.[19,23,128–132] Toronto virus has been reported to be the second most common virus detected in the stools of young children with gastroenteritis.[133] The frequency of norovirus gastroenteritis has been estimated at between 10% and 100% of that of rotavirus in children, when direct comparisons have been made.[134–136] In one study, 49% of prospectively followed Finnish infants and children seroresponded to norovirus over a 2-year period.[137] Sapoviruses and noroviruses have also been detected in community-wide, daycare,[138] and nosocomial outbreaks of gastroenteritis in children.[139] After the introduction of the rotavirus vaccines, norovirus has become the leading cause of medically attended acute gastroenteritis in US children, with nearly 1 million health care visits annually.[140]

Norovirus outbreaks occur throughout the year but tend to peak during cold weather months in temperate climates.[141,142] Frequency, seasonality, and geographic location of outbreaks may vary substantially from year to year, and the factors related to this variation are unclear. The markedly increased number of outbreaks reported in a number of years since 1995 have been associated with the emergence of novel GII.4 strains,[18,142] including the emergence of a novel strain in 2012, GII.4 Sydney.[72–74] In 2014, novel GII.17 viruses emerged and spread worldwide.[75,76,78,79]

PATHOGENESIS

Although norovirus infection of nonhuman primates and gnotobiotic piglets has been accomplished,[18] convenient animal models for gastroenteritis induced by the Norwalk viruses are not available, and therefore information about the pathogenesis of this illness is based largely on studies of experimentally induced disease in healthy volunteers. Acute infection with Norwalk and Hawaii viruses results in a reversible histopathologic lesion in the jejunum,[12,143–145] with apparent sparing of the stomach[146] and rectum (Fig. 176.4). The villi are blunted, but the mucosa is otherwise intact. Round cells and polymorphonuclear leukocytic infiltration are seen in the lamina propria. With electron microscopy, the epithelial cells are similarly intact, microvilli are shortened, and widened intercellular spaces are noted. These histopathologic changes

FIG. 176.4 Hawaii virus–induced disease. Light micrograph of a jejunal mucosal biopsy specimen from a volunteer with Hawaii virus–induced disease 48 hours after challenge. Blunted villi and inflammatory cell infiltrate in the lamina propria are present. (Hematoxylin-eosin stain, ×140.)

appear within 24 hours after virus challenge, are present at the height of illness, and persist for a variable period of time after the illness. The histopathologic changes have generally cleared within 2 weeks after the onset of illness, although some jejunal changes have been noted as late as 6 weeks after challenge. Histopathologic changes have been described in both clinical and subclinical cases of infection[144,145] and appear to be indistinguishable between Norwalk virus– and Hawaii virus–induced disease.

Diarrhea induced by the Norwalk virus is associated with a transient malabsorption of D-xylose and fat[147] and with decreased activity of brush-border enzymes, including alkaline phosphatase and trehalase.[143] Absorption and brush-border enzyme levels return to normal values within 2 weeks after challenge. During acute illness, a variable amount of intestinal fluid is produced, but infection with Norwalk and Hawaii agents has not been associated with detectable enterotoxin production. Adenylate cyclase levels in jejunal biopsy specimens appear to be normal during infection.[148] Thus, the precise mechanisms of virus-induced diarrhea, vomiting, or both remain unknown at the present. Calicivirus infections of animals have been associated with atrophy of the small intestinal mucosa, along with a mild inflammatory infiltrate in the lamina propria.[149,150]

The cellular receptor for infection with norovirus has not been characterized. In human challenge studies, susceptibility to infection with certain norovirus strains has been associated with the presence of H blood group carbohydrate antigens (HBGAs), which are also expressed on gastrointestinal epithelial cells.[151–156] These observations, along with in vitro studies demonstrating binding of noroviruses to these carbohydrate antigens within a pocket of the P2 domain of the viral capsid,[157] have suggested that these carbohydrates may represent or be related to cellular receptors for noroviruses. It is postulated that allelic variation in expression of HBGAs affects susceptibility to norovirus infection.[158] These findings may explain, at least in part, the observations of a poorly defined long-term resistance to norovirus infection seen in previous challenge studies, in which some individuals consistently remained well despite repeated challenge with virus.[159] Epidemiologic studies have supported the concept that expression of HBGAs increases susceptibility to norovirus infection, but they suggested that the effects may be strain specific.[106,160] Sapoviruses do not bind to HBGAs, and susceptibility to infection with sapoviruses does not appear related to HBGA phenotypes.[27]

IMMUNE RESPONSE

Infection with the Norwalk virus results in the induction of virus-specific serum immunoglobulin G (IgG), IgA, and IgM antibody,[161–164] even in the presence of previous exposure. IgA and IgM responses appear to be relatively short lived, whereas elevations in Norwalk-specific serum IgG persist for months.[163,164] In addition to recognizing the infecting strain of norovirus, serum antibody responses to infection may recognize other variants within the same genogroup, although generally to lower titer.[165] Such heterologous antibody responses are more common within a genogroup than between genogroups.[162,166] Serum IgM and IgA antibody may be more specific for the infecting strain of virus.[162,167] With use of baculovirus-expressed capsid proteins, it has been demonstrated that responses to viruses within genogroup GI may be more specific than responses to infection with viruses within genogroup GII.[168] Heterologous responses have also been seen in individuals infected with the sapoviruses.[91] These broad responses are in contrast to the extremely specific antibody response of animals hyperimmunized with capsid antigen[169] and may in part reflect the extensive prior exposure of most adults to related viruses. The significance of such heterologous responses with respect to protection against reinfection is not clear.

Mucosal immune responses have not been studied extensively, but jejunal IgA synthesis has been shown to be elevated in biopsy specimens obtained 2 weeks after challenge with the Norwalk agent,[170] and fecal IgA responses after Norwalk infection have also been reported.[171] Limited studies of cell-mediated immune responses in these infections have indicated that acute illness is associated with a transient lymphopenia that involves thymus-derived, bone marrow–derived, and null cell subpopulations.[172] Antigen-specific cellular responses to the capsid have been demonstrated in peripheral blood after experimental infection and are predominantly of the Th1 type.[173] Such responses are also cross-reactive within a genogroup.

Parameters defining protective immunity to noroviruses are poorly understood. After infection with Norwalk virus, most individuals manifest resistance to reinfection that persists for at least 4 to 6 months.[159,174] Multiple exposure appears to increase this resistance.[174] This short-term resistance does not appear to extend to other antigenically distinct viruses.[54] Infection-induced resistance eventually wanes, and after 2 to 3 years, such individuals are susceptible to reinfection with the same virus.[159] As noted earlier, the absence of secretion of HBGAs is associated with resistance to infection with the Norwalk virus, and these antigens may represent or be related to cellular receptors for noroviruses.[18] Norwalk virus infections can induce serum antibodies that block binding of virus to HBGAs, and this may serve as a surrogate for neutralizing activity.[175]

Studies of the role of serum antibody in mediating protection have yielded conflicting results. In most studies in adults, infection and illness induced by Norwalk-like agents occur in the presence of a wide range of preexisting serum antibody levels, which thus correlate poorly with protection.[162,174] However, after repeated experimental exposure of adults[174] and in epidemiologic studies of noroviruses and sapoviruses in children,[176–178] there has been a better correlation between the presence of serum antibody and protection from illness. Studies in children have shown that high serum levels of norovirus genotype–specific blocking antibodies correlate with protection against infection with genotype-identified noroviruses.[179] Antibody activity has been assessed with a surrogate neutralizing assay using histo–blood group antigens.[180] Protection may also be related to other host defense factors such as local mucosal antibody. However, direct measurements of intestinal antibody have failed to show a correlation with protection from Norwalk-induced illness,[181] and the presence of prechallenge Norwalk-specific fecal IgA was also not protective against challenge.[104] Studies of related animal caliciviruses viruses have also suggested a role for innate immunity in resistance to norovirus infection.[182] The role of T cells in immunity to noroviruses remains poorly understood. Prolonged norovirus-associated diarrhea in an immunosuppressed patient was reported to improve when T-cell counts increased.[183]

CLINICAL MANIFESTATIONS

Clinical characteristics of illness induced by the noroviruses appear to be similar in both naturally occurring and experimentally induced disease (Fig. 176.5), and there are no apparent differences in clinical findings between genogroups. Illness induced by sapovirus infections appears to be similar to that induced by noroviruses, although earlier reports suggested that illness may be milder than that seen with norovirus infection.[136] The onset of symptoms can be either gradual or abrupt, and most persons complain first of abdominal cramps or nausea. In general, both vomiting and diarrhea occur, although either can be present alone. Myalgias, malaise, and occasional headaches are also seen. Low-grade fever, with temperatures of 101°F to 102°F, occurs in about half of cases. Diarrheal stool is generally moderate in amounts, with four to eight nonbloody stools usually being produced over a period of 24 hours. Disease manifestations generally last 48 to 72 hours and usually remit without sequelae.

More severe disease has been reported in elderly patients, including deaths that have been observed in outbreaks in long-term care facilities.[84,184–187] Cases in such outbreaks have shed high titers of virus in stools for up to 3 weeks.[188] These outbreaks often involve person-to-person transmission, although foodborne spread may also occur. Prolonged symptomatic infection and virus shedding of noroviruses have been reported in pediatric oncology patients[189] and in transplant recipients, both hematopoietic stem cell and solid organ transplants.[6,190–193] Prolonged diarrhea for months may be present, along with associated malnutrition, dehydration, graft failure, and kidney injury Chronic norovirus infection for periods as long as 8 years has also been described in patients with enteropathy and common variable immunodeficiency.[194]

DIAGNOSIS

Clinical Diagnosis

A clinical diagnosis of norovirus-related illness can be suspected on the basis of epidemiologic information and on the absence of other documented pathogens. Outbreaks of illness have a high likelihood of

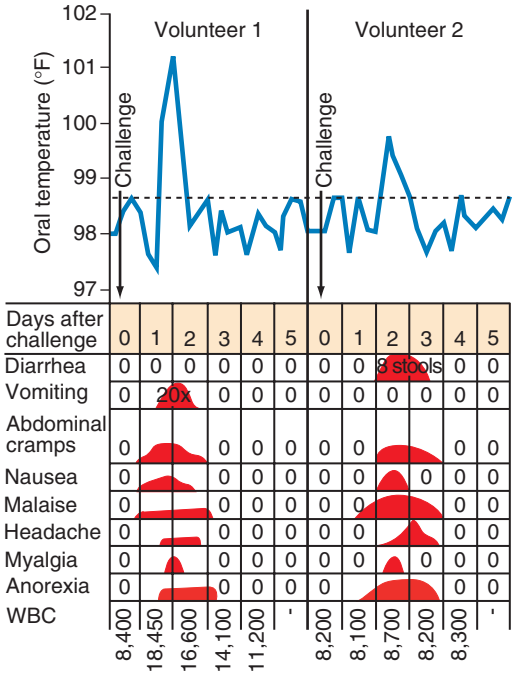

FIG. 176.5 Clinical response of two volunteers after the oral administration of the norwalk virus. The height of the shaded curve is proportional to the severity of the sign or symptom. *WBC,* White blood count. *(From Blacklow NR, Dolin R, Fedson DS, et al. Acute infectious non-bacterial gastroenteritis: etiology and pathogenesis.* Ann Intern Med. *1972;76:993–1008.)*

being caused by noroviruses if vomiting occurs in more than 50% of persons, if the incubation period is 24 to 48 hours, if illness lasts 12 to 60 hours, and if bacterial pathogens are not identified.[195] However, the signs and symptoms of illness are not sufficiently characteristic to enable a diagnosis in an individual case to be made on clinical grounds alone. Routine laboratory tests are also generally not helpful in making a specific diagnosis of norovirus infection. Peripheral white blood cell counts are normal or slightly elevated, with a relative polymorphonuclear leukocytosis and lymphopenia, but with otherwise unremarkable white cell morphology. The results of liver function tests, blood urea nitrogen and creatinine determinations, and urinalysis are generally within normal limits. The absence of fecal leukocytes, as determined by means of microscopic examination of stools stained with methylene blue,[196] is a useful tool with which to exclude infection with enteroinvasive pathogens such as *Shigella* spp.

Laboratory Diagnosis

Specific diagnosis requires laboratory confirmation. Because these agents currently cannot be cultivated in vitro in clinical diagnostic laboratories, a variety of methods have been developed to detect virus directly in stool samples.[197] Immune electron microscopy, in which immune sera are used to aggregate and highlight virions in stool suspensions (see Fig. 176.2), was the method used originally to identify these viruses[15,198] but is no longer commonly used.

The diagnostic tests of choice are highly sensitive and specific reverse-transcriptase polymerase chain reaction (RT-PCR) techniques that have been developed for detection of noroviruses and sapoviruses and are now widely available.[199–204] The success of this strategy depends on the ability to remove inhibitors of reverse transcription from the samples and on the choice of primers in relatively conserved regions of the genome, to widen the scope of viruses detected.[205,206] Conversely, carefully selected primers within relatively more divergent regions can be used to genotypically differentiate virus strains.[207] RT-PCR may be particularly useful for detecting contamination of food and environmental samples.[98,208–212]

Enzyme immunoassays (EIAs) to detect norovirus antigens in stool are commercially available, but are generally of lower sensitivity and specificity than PCR assays.[26,213–215] EIAs may be useful to assess specimens from outbreaks when multiple samples are available.

Techniques have also been adapted for the detection of antibody to noroviruses and may be useful for serologic diagnosis of infection, particularly when appropriate stool samples are not available. Serum antibody titer rises can be detected within 10 to 14 days after the onset of illness.[216–218]

THERAPY

Specific antiviral therapy is not available, and therefore treatment consists entirely of supportive measures. Oral fluid replacement with isotonic liquids is generally adequate to replace fluid losses. Rarely, parenteral intravenous therapy may be required if severe vomiting and diarrhea develop. Symptomatic treatment of headache, myalgias, and nausea with analgesics and antiemetics may provide relief. In one study, the administration of bismuth subsalicylate reduced gastrointestinal symptoms in Norwalk virus–induced disease in volunteers, but had no effect on the number or character of stools or on virus shedding.[219] Although antiperistaltic agents are frequently prescribed to control diarrhea, their effect on the disease course and on excretion of virus has not been rigorously evaluated.

The recent success in cultivation of human norovirus in vitro should facilitate the search for effective antivirals.[31] Previously, the cultivation of a murine norovirus in tissue culture had identified antiviral activities of nucleoside analogues.[220] Recently, 2′-C-methylcytidine has been reported to reduce shedding of murine norovirus in a mouse model and in vitro.[221–225] Rupintrivir, an enterovirus protease inhibitor, has been reported to have anti–human norovirus activity in vitro.[226] A small clinical study has suggested that nitazoxanide may result in modestly reduced symptoms in norovirus-associated illness.[227]

For norovirus infections in immunosuppressed patients, the most important therapeutic measure is reduction of immunosuppression when possible.[193] Therapies with nitazoxanide, ribavirin, or pooled immunoglobulins have been attempted, with uncertain results.[193,228]

PREVENTION

The primary control measures for norovirus outbreaks are prevention of contamination of water and food supplies by means of proper hygiene procedures, including restriction of the activity of symptomatic food handlers. Many health departments recommend that individuals with norovirus illness refrain from being involved in food handling or preparation until 2 to 3 days after illness has resolved.[229] Vigorous hand washing should be used during and after illness to reduce spread. The use of soap and water appears to be more effective than alcohol-based hand sanitizers.[229] Potential sources of contamination of fomites should be treated with appropriate cleaning solutions. Because of the resistance of noroviruses to freezing, heating, and standard cleaning solutions, decontamination procedures should include EPA-recommended disinfectants, such as chlorine bleach with a concentration of 1000 to 5000 ppm (1:50 to 1:10 dilution of household bleach).[229]

Vaccines

The availability of an effective vaccine against norovirus-associated illness would have significant economic impact. In the United States, it has been estimated that it could prevent more than 2 million episodes of gastroenteritis annually, and reduce health expenditures by $2.1 billion per year.[230] However, such vaccines face multiple challenges.[230–232] As described earlier, infection may not induce long-term protective immunity, although short-term protection has been demonstrated. The likely presence of multiple antigenic types is also a challenge. However, progress toward a candidate norovirus vaccine has been made using VLPs.[233,234] Norwalk VLPs have been shown to be immunogenic when administered orally to human volunteers, inducing serum IgG, mucosal IgA, and cellular responses that resemble those seen after infection, although of substantially lower magnitude.[235,236] Intranasally administered norovirus VLPs, administered in two doses 3 weeks apart, provided protection of 47% against gastroenteritis induced by challenge with a Norwalk virus.[237] Somewhat less protection (26%) was provided against infection with norovirus. Additional studies of intramuscularly administered VLPs have been carried out, which showed that such

administration was also well tolerated and immunogenic.[238] A vaccine regimen of two intramuscular injections of VLPs followed by an oral challenge of norovirus did not meet the predefined end point of reduction of cases of illness or infection, but it did result in reduced severity of illness.[239] A large-scale field trial of this VLP vaccine, sponsored by Takeda vaccines, has been undertaken. Additional candidate vaccines based on adenovirus serotype 5 vectors are also under development. In addition, Norwalk virus has proved to be an excellent model to evaluate expression of vaccine antigens in plants as a method for oral immunization.[240]

Key References

The complete reference list is available online at Expert Consult.

1. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. *N Engl J Med*. 2009;361:1776–1785.
2. Lopman BA, Steele D, Kirkwood CD, et al. The vast and varied global burden of norovirus: prospects for prevention and control. *PLoS Med*. 2016;13:e1001999.
3. Lopman BA, Hall AJ, Curns AT, et al. Increasing rates of gastroenteritis hospital discharges in US adults and the contribution of norovirus, 1996-2007. *Clin Infect Dis*. 2011;52:466–474.
4. Patel MM, Widdowson MA, Glass RI, et al. Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg Infect Dis*. 2008;14:1224–1231.
5. Hall AJ, Curns AT, McDonald LC, et al. The roles of *Clostridium difficile* and norovirus among gastroenteritis-associated deaths in the United States, 1999-2007. *Clin Infect Dis*. 2012;55:216–223.
6. Bok K, Green KY. Norovirus gastroenteritis in immunocompromised patients. *N Engl J Med*. 2012;367:2126–2132.
11. Dolin R, Blacklow NR, DuPont H, et al. Biological properties of Norwalk agent of acute infectious nonbacterial gastroenteritis. *Proc Soc Exp Biol Med*. 1972;140:578–583.
12. Dolin R, Levy AG, Wyatt RG, et al. Viral gastroenteritis induced by the Hawaii agent: jejunal histopathology and serologic response. *Am J Med*. 1975;59:768–771.
15. Dolin R, Reichman RC, Roessner KD, et al. Detection by immune electron microscopy of the Snow Mountain agent of acute viral gastroenteritis. *J Infect Dis*. 1982;146:184–189.
16. Dolin R, Treanor J, Madore HP. Novel agents of viral enteritis in humans. *J Infect Dis*. 1987;155:365–375.
17. Blacklow NR, Greenberg HB. Viral gastroenteritis. *N Engl J Med*. 1991;325:252–264.
18. Dolin R. Noroviruses—challenges to control. *N Engl J Med*. 2007;357:1072–1073.
22. Jiang X, Wang M, Wang K, et al. Sequence and genomic organization of Norwalk virus. *Virology*. 1993;195:51–61.
25. Patel MM, Hall AJ, Vinjé J, et al. Noroviruses: a comprehensive review. *J Clin Virol*. 2009;44:1–8.
26. Vinjé J. Advances in laboratory methods for detection and typing of norovirus. *J Clin Microbiol*. 2015;53:373–381.
27. Oka T, Wang Q, Katayama K, et al. Comprehensive review of human sapoviruses. *Clin Microbiol Rev*. 2015;28:32–53.
31. Ettayebi K, Crawford SE, Murakami K, et al. Replication of human noroviruses in stem cell-derived human enteroids. *Science*. 2016;353:1387–1393.
32. Karst SM. Identification of a novel cellular target and a co-factor for norovirus infection - B cells & commensal bacteria. *Gut Microbes*. 2015;6:266–271.
33. Jones MK, Grau KR, Costantini V, et al. Human norovirus culture in B cells. *Nat Protoc*. 2015;10:1939–1947.
36. Hardy ME. Norovirus protein structure and function. *FEMS Microbiol Lett*. 2005;253:1–8.
37. Green K. *Caliciviridae*: the noroviruses. In: Knipe DM, Howley PM, eds. *Fields Virology*. 5th ed. Philadelphia: Lippincott-Raven; 2007:949–979.
42. Vongpunsawad S, Venkataram Prasad BV, Estes MK. Norwalk virus minor capsid protein VP2 associates within the VP1 shell domain. *J Virol*. 2013;87:4818–4825.
43. Clarke IN, Lambden PR. The molecular biology of human caliciviruses. *Novartis Found Symp*. 2001;238:180–191, discussion 191–196.
44. Kroneman A, Vega E, Vennema H, et al. Proposal for a unified norovirus nomenclature and genotyping. *Arch Virol*. 2013;158:2059–2068.
45. Ando T, Noel JS, Fankhauser RL. Genetic classification of Norwalk-like viruses. *J Infect Dis*. 2000;181(suppl 2):S336–S348.
46. Hansman GS, Oka T, Kakayama K, et al. Human sapoviruses: genetic diversity, recombination, and classification. *Rev Med Virol*. 2007;17:133–141.
50. Dingle KE, Lambden PR, Caul EO, et al. Human enteric Caliciviridae: the complete genome sequence and expression of viruslike particles from a genetic group II small round structured virus. *J Gen Virol*. 1995;76:2349–2355.
51. Green KY, Kapikian AZ, Valdesuso J, et al. Expression and self-assembly of recombinant capsid protein from the antigenically distinct Hawaii human calicivirus. *J Clin Microbiol*. 1997;35:1909–1914.
55. Widdowson MA, Sulka A, Bulens SN, et al. Norovirus and foodborne disease, United States, 1991-2000. *Emerg Infect Dis*. 2005;11:95–102.
56. Ike AC, Brockmann SO, Hartelt K, et al. Molecular epidemiology of norovirus in outbreaks of gastroenteritis in southwest Germany from 2001-2004. *J Clin Microbiol*. 2006;44:1262–1267.
60. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis*. 2011;17:7–15.
61. Patel MM, Widdowson MA, Glass RI, et al. Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg Infect Dis*. 2008;14:1224–1231.
62. Ahmed SM, Hall AJ, Robinson AE, et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2014;14:725–730.
63. Hall AJ, Lopman BA, Payne DC, et al. Norovirus disease in the United States. *Emerg Infect Dis*. 2013;19:1198–1205.
70. Siebenga JJ, Vennema H, Zheng DP, et al. Norovirus illness is a global problem: emergence and spread of Norovirus GII.4 variants, 2001-2007. *J Infect Dis*. 2009;200:802–812.
72. Centers for Disease Control and Prevention. Notes from the field: emergence of new norovirus strain GII.4 Sydney—United States, 2012. *MMWR Morb Mortal Wkly Rep*. 2013;62:55.
73. Widdowson MA, Cramer EH, Hadley L, et al. Outbreaks of acute gastroenteritis on cruise ships and on land: identification of a predominant circulating strain of norovirus—United States, 2002. *J Infect Dis*. 2004;190:27–36.
74. Centers for Disease Control and Prevention. Emergence of new norovirus strain GII.4 Sydney-United States, 2012. *MMWR Morb Mortal Wkly Rep*. 2013;62:55.
75. de Graaf M, van Beek J, Vennema H, et al. Emergence of a novel GII.17 norovirus—end of the GII.4 era? *Euro Surveill*. 2015;20.
76. Lu J, Sun L, Fang L, et al. Gastroenteritis outbreaks caused by norovirus GII.17, Guangdong Province, China, 2014-2015. *Emerg Infect Dis*. 2015;21:1240–1242.
77. Wang HB, Wang Q, Zhao JH, et al. Complete nucleotide sequence analysis of the norovirus GII.17: a newly emerging and dominant variant in China, 2015. *Infect Genet Evol*. 2015;38:47–53.
78. Lindesmith LC, Kocher JF, Donaldson EF, et al. Emergence of novel human norovirus GII.17 strains correlates with changes in blockade antibody epitopes. *J Infect Dis*. 2017;216:1227–1234.
79. Chan MCW, Hu Y, Chen H, et al. Global spread of norovirus GII.17 Kawasaki 308, 2014-2016. *Emerg Infect Dis*. 2017;23:1350–1354.
80. Chiba S, Sakuma Y, Kogasaka R, et al. An outbreak of gastroenteritis associated with calicivirus in an infant home. *J Med Virol*. 1979;4:249–254.
81. Pang XL, Lee BE, Tyrrell GJ, et al. Epidemiology and genotype analysis of sapovirus associated with gastroenteritis outbreaks in Alberta, Canada: 2004-2007. *J Infect Dis*. 2009;199:547–551.
82. Mikula C, Springer B, Reichart S, et al. Sapovirus in adults in rehabilitation center, Upper Austria. *Emerg Infect Dis*. 2010;16:1186–1187.
83. Svraka S, Vennema H, van der Veer B, et al. Epidemiology and genotype analysis of emerging sapovirus-associated infections across Europe. *J Clin Microbiol*. 2010;48:2191–2198.
84. Lee LE, Cebelinski EA, Fuller C, et al. Sapovirus outbreaks in long-term care facilities, Oregon and Minnesota, USA, 2002-2009. *Emerg Infect Dis*. 2012;18:873–876.
85. Greenberg HB, Valdesuso J, Kapikian AZ, et al. Prevalence of antibody to the Norwalk virus in various countries. *Infect Immun*. 1979;26:270–273.
102. Atmar RL, Opekun AR, Gilger MA, et al. Determination of the 50% human infectious dose for Norwalk virus. *J Infect Dis*. 2014;209:1016–1022.
106. Costantini VP, Cooper EM, Hardaker HL, et al. Epidemiologic, virologic, and host genetic factors of norovirus outbreaks in long-term care facilities. *Clin Infect Dis*. 2016;62:1–10.
107. Teunis PF, Sukhrie FH, Vennema H, et al. Shedding of norovirus in symptomatic and asymptomatic infections. *Epidemiol Infect*. 2015;143:1710–1717.
109. Kaplan JE, Feldman R, Campbell DS, et al. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. *Am J Public Health*. 1982;72:1329–1332.
115. Bitler EJ, Matthews JE, Dickey BW, et al. Norovirus outbreaks: a systematic review of commonly implicated transmission routes and vehicles. *Epidemiol Infect*. 2013;141:1563–1571.
126. Augustin AK, Simor AE, Shorrock C, et al. Outbreaks of gastroenteritis due to Norwalk-like virus in two long-term care facilities for the elderly. *Can J Infect Control*. 1995;10:111–113.
136. Sakai Y, Nakata S, Honma S, et al. Clinical severity of Norwalk virus and Sapporo virus gastroenteritis in children in Hokkaido, Japan. *Pediatr Infect Dis J*. 2001;20:849–853.
140. Payne DC, Vinjé J, Szilagyi PG, et al. Norovirus and medically attended gastroenteritis in U.S. children. *N Engl J Med*. 2013;368:1121–1130.
142. Blanton LH, Adams SM, Beard RS, et al. Molecular and epidemiologic trends of caliciviruses associated with outbreaks of acute gastroenteritis in the United States, 2000-2004. *J Infect Dis*. 2006;193:413–421.
143. Agus SG, Dolin R, Wyatt RG, et al. Acute infectious nonbacterial gastroenteritis: intestinal histopathology. *Ann Intern Med*. 1973;79:18–25.
144. Schreiber DS, Blacklow NR, Trier JS. The mucosal lesion of the proximal small intestine in acute infectious nonbacterial gastroenteritis. *N Engl J Med*. 1973;288:1318–1323.
145. Schreiber DS, Blacklow NR, Trier JS. The small intestinal lesion induced by Hawaii agent acute infectious nonbacterial gastroenteritis. *J Infect Dis*. 1974;129:705–708.
151. Ramani S, Estes MK, Atmar RL. Correlates of protection against norovirus infection and disease—where are we now, where do we go? *PLoS Pathog*. 2016;12:e1005334.
153. Hutson AM, Atmar RL, Marcus DM, et al. Norwalk virus-like particle hemagglutination by binding to histo-blood group antigens. *J Virol*. 2003;77:405–415.
154. Huang P, Farkas T, Marionneau S, et al. Noroviruses bind to human ABO, Lewis, and secretor histo-blood group antigens: identification of 4 distinct strain-specific patterns. *J Infect Dis*. 2003;188:19–31.
155. Tan M, Huang P, Meller J, et al. Mutations within the P2 domain of Norovirus capsid affect binding to human histoblood group antigens: evidence for a binding pocket. *J Virol*. 2003;77:12562–12571.
156. Hutson AM, Atmar RL, Graham DY, et al. Norwalk virus infection and disease is associated with ABO histo-blood group type. *J Infect Dis*. 2002;185:1335–1337.
158. Tan M, Jiang X. Norovirus-host interaction: multi-selections by human histoblood group antigens. *Trends Microbiol*. 2011;19:382–388.
159. Parrino TA, Schreiber DS, Trier JS, et al. Clinical immunity in acute gastroenteritis caused by Norwalk agent. *N Engl J Med*. 1977;291:86–89.
160. Kambhampati A, Payne DC, Costantini V, et al. Host genetic susceptibility to enteric viruses: a systematic review and metaanalysis. *Clin Infect Dis*. 2016;62:11–18.
175. Lindesmith LC, Donaldson E, Leon J, et al. Heterotypic humoral and cellular immune responses following Norwalk virus infection. *J Virol*. 2010;84:1800–1815.
179. Malm M, Uusi-kerrtula H, Vesikari T, et al. High serum levels of norovirus genotype-specific blocking antibodies correlate with protection from infection in children. *J Infect Dis*. 2014;210:1755–1762.
180. Lee BE, Pang X. One more step toward understanding the immune response to norovirus. *J Infect Dis*. 2014;210:1691–1692.
183. Wingfield T, Gallimore C, Xerry J, et al. Chronic norovirus infection in an HIV-positive patient with persistent diarrhoea: a novel cause. *J Clin Virol*. 2010;49:219–222.
186. Kirk MD, Fullerton KE, Hall GV, et al. Surveillance for outbreaks of gastroenteritis in long-term care facilities, Australia, 2002-2008. *Clin Infect Dis*. 2010;51:907–914.
187. Jones TF. When diarrhea gets deadly: a look at gastroenteritis outbreaks in nursing homes. *Clin Infect Dis*. 2010;51:915–916.

188. Costantini VP, Cooper EM, Hardaker HL, et al. Epidemiologic, virologic, and host genetic factors of norovirus outbreaks in long-term care facilities. *Clin Infect Dis*. 2016;62:1–10.
191. Roddie C, Paul JP, Benjamin R, et al. Allogenic hematopoietic stem cell transplantation and norovirus gastroenteritis: a previously unrecognized cause of morbidity. *Clin Infect Dis*. 2009;49:1061–1068.
192. Koo HL, DuPont HL. Noroviruses as a potential cause of protracted and lethal disease in immunocompromised patients. *Clin Infect Dis*. 2009;49:1069–1071.
193. Angarone MP, Sheahan A, Kamboj M. Norovirus in transplantation. *Curr Infect Dis Rep*. 2016;18:17.
194. Woodward JM, Gkrania-Klotsas E, Cordero-Ng AY, et al. The role of chronic norovirus infection in the enteropathy associated with common variable immunodeficiency. *Am J Gastroenterol*. 2015;110:320–327.
195. Kaplan JE, Feldman R, Campbell DS, et al. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis [abstract]. *Am J Public Health*. 1982;72:1329–1332.
215. Centers for Disease Control and Prevention. Norovirus: Laboratory Diagnosis and Treatment. http://www.cdc.gov/norovirus/hcp/diagnosis-treatment.html. Accessed March 18, 2014.
221. Rocha-Pereira J, Van Dycke J, Neyts J. Treatment with a nucleoside polymerase inhibitor reduces shedding of murine norovirus in stool to undetectable levels without emergence of drug-resistant variants. *Antimicrob Agents Chemother*. 2015;60:1907–1911.
222. Rocha-Pereira J, Jochmans D, Dallmeier K, et al. Inhibition of norovirus replication by the nucleoside analogue 2'-C-methylcytidine. *Biochem Biophys Res Commun*. 2012;427:796–800.
223. Kolawole AO, Rocha-Pereira J, Elftman MD, et al. Inhibition of human norovirus by a viral polymerase inhibitor in the B cell culture system and in the mouse model. *Antiviral Res*. 2016;132:46–49.
224. Rocha-Pereira J, Jochmans D, Debing Y, et al. The viral polymerase inhibitor 2'-C-methylcytidine inhibits Norwalk virus replication and protects against norovirus-induced diarrhea and mortality in a mouse model. *J Virol*. 2013;87:11798–11805.
225. Rocha-Pereira J, Jochmans D, Neyts J. Prophylactic treatment with the nucleoside analogue 2'-C-methylcytidine completely prevents transmission of norovirus. *J Antimicrob Chemother*. 2015;70:190–197.
226. Rocha-Pereira J, Nascimento MS, Ma Q, et al. The enterovirus protease inhibitor rupintrivir exerts cross-genotypic anti-norovirus activity and clears cells from the norovirus replicon. *Antimicrob Agents Chemother*. 2014;58:4675–4681.
227. Rossignol JF, El-Gohary YM. Nitazoxanide in the treatment of viral gastroenteritis: a randomized double-blind placebo-controlled clinical trial. *Aliment Pharmacol Ther*. 2006;24:1423–1430.
228. Siddiq DM, Koo HL, Adachi JA, et al. Norovirus gastroenteritis successfully treated with nitazoxanide. *J Infect*. 2011;63:394–397.
229. Centers for Disease Control and Prevention. NoroSTAT Data. https://www.cdc.gov/norovirus/reporting/norostat/data.html. Accessed January 25, 2018.
230. Bartsch SM, Lopman BA, Hall AJ, et al. The potential economic value of a human norovirus vaccine for the United States. *Vaccine*. 2012;30:7097–7104.
231. Cortes-Penfield NW, Ramani S, Estes MK, et al. Prospects and challenges in the development of a norovirus vaccine. *Clin Ther*. 2017;39:1537–1549.
232. Baehner F, Bogaerts H, Goodwin R, et al. Vaccines against norovirus: state of the art trials in children and adults. *Clin Microbiol Infect*. 2016;22(suppl 5):S136–S139.
233. Baehner F, Bogaerts H, Goodwin R. Vaccines against norovirus: state of the art trials in children and adults. *Clin Microbiol Infect*. 2016;22(suppl 5):S136–S139.
234. Riddle MS, Walker RI. Status of vaccine research and development for norovirus. *Vaccine*. 2016;34:2895–2899.
237. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk virus illness. *N Engl J Med*. 2011;365:2178–2187.
238. Treanor JJ, Atmar RL, Frey SE, et al. A novel intramuscular bivalent norovirus-like particle vaccine candidate—reactogenicity, safety, and immunogenicity in a phase 1 trial in healthy adults. *J Infect Dis*. 2014;210:1763–1771.
239. Bernstein DI, Atmar RL, Lyon GM, et al. Norovirus vaccine against experimental human GII.4 virus illness: a challenge study in healthy adults. *J Infect Dis*. 2015;211:870–878.

References

1. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. *N Engl J Med*. 2009;361:1776–1785.
2. Lopman BA, Steele D, Kirkwood CD, et al. The vast and varied global burden of norovirus: prospects for prevention and control. *PLoS Med*. 2016;13:e1001999.
3. Lopman BA, Hall AJ, Curns AT, et al. Increasing rates of gastroenteritis hospital discharges in US adults and the contribution of norovirus, 1996-2007. *Clin Infect Dis*. 2011;52:466–474.
4. Patel MM, Widdowson MA, Glass RI, et al. Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg Infect Dis*. 2008;14:1224–1231.
5. Hall AJ, Curns AT, McDonald LC, et al. The roles of *Clostridium difficile* and norovirus among gastroenteritis-associated deaths in the United States, 1999-2007. *Clin Infect Dis*. 2012;55:216–223.
6. Bok K, Green KY. Norovirus gastroenteritis in immunocompromised patients. *N Engl J Med*. 2012;367:2126–2132.
7. Reimann HA, Price AH, Hodges JH. The cause of epidemic diarrhea, nausea, and vomiting (viral dysentery? *Proc Soc Exp Biol Med*. 1945;59:8–9.
8. Gordon I, Ingraham HS, Korns RF. Transmission of epidemic gastroenteritis to human volunteers by oral administration of fecal filtrate. *J Exp Med*. 1947;86:409–422.
9. Jordan WS, Gordon I, Dorrance WR. A study of illness in a group of Cleveland families. VII. Transmission of acute nonbacterial gastroenteritis to volunteers: evidence for two different etiologic agents. *J Exp Med*. 1953;98:461–475.
10. Kapikian AZ, Gerin JL, Wyatt RG, et al. Density in cesium chloride of the Norwalk agent: determination by ultracentrifugation and immune electron microscopy. *Proc Soc Exp Biol Med*. 1973;142:874–877.
11. Dolin R, Blacklow NR, DuPont H, et al. Biological properties of Norwalk agent of acute infectious nonbacterial gastroenteritis. *Proc Soc Exp Biol Med*. 1972;140:578–583.
12. Dolin R, Levy AG, Wyatt RG, et al. Viral gastroenteritis induced by the Hawaii agent: jejunal histopathology and serologic response. *Am J Med*. 1975;59:768–771.
13. Thornhill TS, Wyatt RG, Kalica AR, et al. Detection by immune electron microscopy of 26-27 nm virus-like particles associated with two family outbreaks of gastroenteritis. *J Infect Dis*. 1977;138:20–27.
14. Caul EO, Ashley C, Pether JVS. Norwalk-like particle in epidemic gastroenteritis in the U.K. *Lancet*. 1979;2:1292.
15. Dolin R, Reichman RC, Roessner KD, et al. Detection by immune electron microscopy of the Snow Mountain agent of acute viral gastroenteritis. *J Infect Dis*. 1982;146:184–189.
16. Dolin R, Treanor J, Madore HP. Novel agents of viral enteritis in humans. *J Infect Dis*. 1987;155:365–375.
17. Blacklow NR, Greenberg HB. Viral gastroenteritis. *N Engl J Med*. 1991;325:252–264.
18. Dolin R. Noroviruses—challenges to control. *N Engl J Med*. 2007;357:1072–1073.
19. Flewett TH, Davies H. Caliciviruses [letter]. *Lancet*. 1976;1:311.
20. Jiang X, Graham DY, Wang K, et al. Norwalk virus genome cloning and characterization. *Science*. 1990;250:1580–1583.
21. Matsui S, Kim JP, Greenberg HB, et al. The isolation and characterization of a Norwalk virus-specific cDNA. *J Clin Invest*. 1991;87:1456–1461.
22. Jiang X, Wang M, Wang K, et al. Sequence and genomic organization of Norwalk virus. *Virology*. 1993;195:51–61.
23. Chiba S, Sakuma Y, Kogasaka R, et al. An outbreak of gastroenteritis associated with calicivirus in an infant home. *J Med Virol*. 1979;4:249–254.
24. Chiba S, Sakuma Y, Kagasaka R, et al. Fecal shedding of virus in relation to the days of illness in infantile gastroenteritis due to calicivirus. *J Infect Dis*. 1980;142:247–249.
25. Patel MM, Hall AJ, Vinjé J, et al. Noroviruses: a comprehensive review. *J Clin Virol*. 2009;44:1–8.
26. Vinjé J. Advances in laboratory methods for detection and typing of norovirus. *J Clin Microbiol*. 2015;53:373–381.
27. Oka T, Wang Q, Katayama K, et al. Comprehensive review of human sapoviruses. *Clin Microbiol Rev*. 2015;28:32–53.
28. Asanaka M, Atmar RL, Ruvolo V, et al. Replication and packaging of Norwalk virus RNA in cultured mammalian cells. *Proc Natl Acad Sci USA*. 2005;102:10327–10332.
29. Katayama K, Hansman GS, Oka T, et al. Investigation of norovirus replication in a human cell line. *Arch Virol*. 2006;151:1291–1308.
30. Straub TM, Höner zu Bentrup K, Orosz-Coghlan P, et al. In vitro cell culture infectivity assay for human noroviruses. *Emerg Infect Dis*. 2007;13:396–403.
31. Ettayebi K, Crawford SE, Murakami K, et al. Replication of human noroviruses in stem cell-derived human enteroids. *Science*. 2016;353:1387–1393.
32. Karst SM. Identification of a novel cellular target and a co-factor for norovirus infection - B cells & commensal bacteria. *Gut Microbes*. 2015;6:266–271.
33. Jones MK, Grau KR, Costantini V, et al. Human norovirus culture in B cells. *Nat Protoc*. 2015;10:1939–1947.
34. Hardy ME, Estes MK. Completion of the Norwalk virus genome sequence. *Virus Genes*. 1996;12:287–290.
35. Greenberg HB, Valdesuso J, Kalica AR. Proteins of Norwalk virus. *J Virol*. 1981;37:994–999.
36. Hardy ME. Norovirus protein structure and function. *FEMS Microbiol Lett*. 2005;253:1–8.
37. Green K. *Caliciviridae*: the noroviruses. In: Knipe DM, Howley PM, eds. *Fields Virology*. 5th ed. Philadelphia: Lippincott-Raven; 2007:949–979.
38. Jiang X, Wang M, Graham DY, et al. Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. *J Virol*. 1992;66:6527–6532.
39. Prasad BV, Rothnagel R, Jiang X, et al. Three-dimensional structure of baculovirus-expressed Norwalk virus capsids. *J Virol*. 1994;68:5117–5125.
40. Prasad BV, Hardy ME, Dokland T, et al. X-ray crystallographic structure of the Norwalk virus capsid. *Science*. 1999;286:287–290.
41. Glass PJ, White LJ, Ball JM, et al. Norwalk virus open reading frame 3 encodes a minor structural protein. *J Virol*. 2000;74:6581–6591.
42. Vongpunsawad S, Venkataram Prasad BV, Estes MK. Norwalk virus minor capsid protein VP2 associates within the VP1 shell domain. *J Virol*. 2013;87:4818–4825.
43. Clarke IN, Lambden PR. The molecular biology of human caliciviruses. *Novartis Found Symp*. 2001;238:180–191, discussion 191–196.
44. Kroneman A, Vega E, Vennema H, et al. Proposal for a unified norovirus nomenclature and genotyping. *Arch Virol*. 2013;158:2059–2068.
45. Ando T, Noel JS, Fankhauser RL. Genetic classification of Norwalk-like viruses. *J Infect Dis*. 2000;181(suppl 2):S336–S348.
46. Hansman GS, Oka T, Kakayama K, et al. Human sapoviruses: genetic diversity, recombination, and classification. *Rev Med Virol*. 2007;17:133–141.
47. Hardy ME, Kramer SF, Treanor JJ, et al. Human calicivirus genogroup II capsid sequence diversity revealed by analysis of the prototype Snow Mountain agent. *Arch Virol*. 1997;142:1469–1479.
48. Lew JF, Kapikian AZ, Jiang X, et al. Molecular characterization and expression of the capsid protein of a Norwalk-like virus recovered from a Desert Shield troop with gastroenteritis. *Virology*. 1994;200:319–325.
49. Jiang X, Matson DO, Ruiz-Palacios GM, et al. Expression, self-assembly, and antigenicity of a Snow Mountain agent-like calicivirus capsid protein. *J Clin Microbiol*. 1995;33:1452–1455.
50. Dingle KE, Lambden PR, Caul EO, et al. Human enteric Caliciviridae: the complete genome sequence and expression of viruslike particles from a genetic group II small round structured virus. *J Gen Virol*. 1995;76:2349–2355.
51. Green KY, Kapikian AZ, Valdesuso J, et al. Expression and self-assembly of recombinant capsid protein from the antigenically distinct Hawaii human calicivirus. *J Clin Microbiol*. 1997;35:1909–1914.
52. Leite JP, Ando T, Noel JS, et al. Characterization of Toronto virus capsid protein expressed in baculovirus. *Arch Virol*. 1996;141:865–875.
53. Numata K, Hardy ME, Nakata S, et al. Molecular characterization of morphologically typical human calicivirus Sapporo. *Arch Virol*. 1997;142:1537–1552.
54. Wyatt RG, Dolin R, Blacklow NR, et al. Comparison of three agents of acute infectious nonbacterial gastroenteritis by virus challenge in volunteers. *J Infect Dis*. 1974;129:709–714.
55. Widdowson MA, Sulka A, Bulens SN, et al. Norovirus and foodborne disease, United States, 1991-2000. *Emerg Infect Dis*. 2005;11:95–102.
56. Ike AC, Brockmann SO, Hartelt K, et al. Molecular epidemiology of norovirus in outbreaks of gastroenteritis in southwest Germany from 2001-2004. *J Clin Microbiol*. 2006;44:1262–1267.
57. Lopman BA, Reacher MH, van Duijnhoven Y, et al. Viral gastroenteritis outbreaks in Europe, 1995-2000. *Emerg Infect Dis*. 2003;9:90–96.
58. Reuter G, Krisztalovics K, Vennema H, et al. Evidence of the etiological predominance of norovirus in gastroenteritis outbreaks-emerging new variant and recombinant noroviruses in Hungary. *J Med Virol*. 2005;76:598–607.
59. van Duynhoven YT, de Jager CM, Kortbeek LM, et al. A one-year intensified study of outbreaks of gastroenteritis in the Netherlands. *Epidemiol Infect*. 2005;133:9–21.
60. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis*. 2011;17:7–15.
61. Patel MM, Widdowson MA, Glass RI, et al. Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg Infect Dis*. 2008;14:1224–1231.
62. Ahmed SM, Hall AJ, Robinson AE, et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2014;14:725–730.
63. Hall AJ, Lopman BA, Payne DC, et al. Norovirus disease in the United States. *Emerg Infect Dis*. 2013;19:1198–1205.
64. Centers for Disease Control and Prevention. Norovirus: Trends and Outbreaks. http://www.cdc.gov/norovirus/trends-outbreaks.html. Accessed March 18, 2014.
65. Fankhauser RL, Noel JS, Monroe SS, et al. Molecular epidemiology of "Norwalk-like viruses" in outbreaks of gastroenteritis in the United States. *J Infect Dis*. 1998;178:1571–1578.
66. Fankhauser RL, Monroe SS, Noel JS, et al. Epidemiologic and molecular trends of "Norwalk-like viruses" associated with outbreaks of gastroenteritis in the United States. *J Infect Dis*. 2002;186:1–7.
67. Gonin P, Couillard M, d'Halewyn MA. Genetic diversity and molecular epidemiology of Norwalk-like viruses. *J Infect Dis*. 2000;182:691–697.
68. Lewis DC, Hale A, Jiang X, et al. Epidemiology of Mexico virus, a small round-structured virus in Yorkshire, United Kingdom, between January 1992 and March 1995. *J Infect Dis*. 1997;175:951–954.
69. Vinjé J, Altena SA, Koopmans MP. The incidence and genetic variability of small round-structured viruses in outbreaks of gastroenteritis in the Netherlands. *J Infect Dis*. 1997;176:1374–1378.
70. Siebenga JJ, Vennema H, Zheng DP, et al. Norovirus illness is a global problem: emergence and spread of Norovirus GII.4 variants, 2001-2007. *J Infect Dis*. 2009;200:802–812.
71. Noel JS, Fankhauser RL, Ando T, et al. Identification of a distinct common strain of "Norwalk-like viruses" having a global distribution. *J Infect Dis*. 1999;179:1334–1344.
72. Centers for Disease Control and Prevention. Notes from the field: emergence of new norovirus strain GII.4 Sydney—United States, 2012. *MMWR Morb Mortal Wkly Rep*. 2013;62:55.
73. Widdowson MA, Cramer EH, Hadley L, et al. Outbreaks of acute gastroenteritis on cruise ships and on land: identification of a predominant circulating strain of norovirus—United States, 2002. *J Infect Dis*. 2004;190:27–36.
74. Centers for Disease Control and Prevention. Emergence of new norovirus strain GII.4 Sydney-United States, 2012. *MMWR Morb Mortal Wkly Rep*. 2013;62:55.
75. de Graaf M, van Beek J, Vennema H, et al. Emergence of a novel GII.17 norovirus—end of the GII.4 era? *Euro Surveill*. 2015;20.
76. Lu J, Sun L, Fang L, et al. Gastroenteritis outbreaks caused by norovirus GII.17, Guangdong Province, China, 2014-2015. *Emerg Infect Dis*. 2015;21:1240–1242.
77. Wang HB, Wang Q, Zhao JH, et al. Complete nucleotide sequence analysis of the norovirus GII.17: a newly emerging and dominant variant in China, 2015. *Infect Genet Evol*. 2015;38:47–53.
78. Lindesmith LC, Kocher JF, Donaldson EF, et al. Emergence of novel human norovirus GII.17 strains correlates with changes in blockade antibody epitopes. *J Infect Dis*. 2017;216:1227–1234.
79. Chan MCW, Hu Y, Chen H, et al. Global spread of norovirus GII.17 Kawasaki 308, 2014-2016. *Emerg Infect Dis*. 2017;23:1350–1354.
80. Chiba S, Sakuma Y, Kogasaka R, et al. An outbreak of gastroenteritis associated with calicivirus in an infant home. *J Med Virol*. 1979;4:249–254.
81. Pang XL, Lee BE, Tyrrell GJ, et al. Epidemiology and genotype analysis of sapovirus associated with gastroenteritis outbreaks in Alberta, Canada: 2004-2007. *J Infect Dis*. 2009;199:547–551.
82. Mikula C, Springer B, Reichart S, et al. Sapovirus in adults in rehabilitation center, Upper Austria. *Emerg Infect Dis*. 2010;16:1186–1187.
83. Svraka S, Vennema H, van der Veer B, et al. Epidemiology and genotype analysis of emerging sapovirus-associated infections across Europe. *J Clin Microbiol*. 2010;48:2191–2198.
84. Lee LE, Cebelinski EA, Fuller C, et al. Sapovirus outbreaks in long-term care facilities, Oregon and Minnesota, USA, 2002-2009. *Emerg Infect Dis*. 2012;18:873–876.

85. Greenberg HB, Valdesuso J, Kapikian AZ, et al. Prevalence of antibody to the Norwalk virus in various countries. *Infect Immun*. 1979;26:270–273.
86. Dolin R, Roessner KD, Treanor J, et al. Radioimmunoassay for detection of Snow Mountain agent of viral gastroenteritis. *J Med Virol*. 1985;19:11–18.
87. Hinkula J, Ball JM, Lofgren S, et al. Antibody prevalence and immunoglobulin IgG subclass pattern to Norwalk virus in Sweden. *J Med Virol*. 1995;47:52–57.
88. Numata K, Nakata S, Jiang X, et al. Epidemiologic study of Norwalk virus infections in Japan and Southeast Asia by enzyme-linked immunosorbent assays with Norwalk virus capsid protein produced by the baculovirus expression system. *J Clin Microbiol*. 1994;32:121–126.
89. Gray JJ, Jiang X, Morgan-Capner P, et al. Prevalence of antibodies to Norwalk virus in England: detection by enzyme-linked immunosorbent assay using baculovirus-expressed Norwalk virus capsid antigen. *J Clin Microbiol*. 1993;31:1022–1025.
90. Sakuma Y, Chiba S, Kogasaka R, et al. Prevalence of antibody to human calicivirus in general population of northern Japan. *J Med Virol*. 1981;7:221–225.
91. Cubitt WD, Blacklow NR, Herrmann JE. Antigenic relationships between human caliciviruses and Norwalk virus. *J Infect Dis*. 1987;156:806–814.
92. Nakata S, Chiba S, Terashima H, et al. Prevalence of antibody to human calicivirus in Japan and Southeast Asia determined by radioimmunoassay. *J Clin Microbiol*. 1985;22:519–521.
93. Gabbay YB, Glass RI, Monroe SS, et al. Prevalence of antibodies to Norwalk virus among Amerindians in isolated Amazonian communities. *Am J Epidemiol*. 1994;139:728–733.
94. Mounts AW, Ando T, Koopmans M, et al. Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. *J Infect Dis*. 2000;181(suppl 2):S284–S287.
95. Chadwick PR, McCann R. Transmission of a small round structured virus by vomiting during a hospital outbreak of gastroenteritis. *J Hosp Infect*. 1994;26:251–259.
96. Patterson W, Haswell P, Fryers PT, et al. Outbreak of small round structured virus gastroenteritis arose after kitchen assistant vomited. *Commun Dis Rep CDR Rev*. 1997;7:R101–R103.
97. Greenberg HB, Wyatt RG, Kapikian AZ. Norwalk virus in vomitus. *Lancet*. 1979;1:55.
98. Kilgore PE, Belay ED, Hamlin DM, et al. A university outbreak of gastroenteritis due to a small round-structured virus. Application of molecular diagnostics to identify the etiologic agent and patterns of transmission. *J Infect Dis*. 1996;173:787–793.
99. Sawyer LA, Murphy JJ, Kaplan JE, et al. 25- to 30-nm virus particle associated with a hospital outbreak of acute gastroenteritis with evidence for airborne transmission. *Am J Epidemiol*. 1988;127:1261–1271.
100. Caul EO. Small round structured viruses: airborne transmission and hospital control. *Lancet*. 1994;343:1240–1242.
101. Centers for Disease Control and Prevention. Norwalk-like viruses: public health impact and outbreak management. *MMWR Morb Mortal Wkly Rep*. 2001;50:1–17.
102. Atmar RL, Opekun AR, Gilger MA, et al. Determination of the 50% human infectious dose for Norwalk virus. *J Infect Dis*. 2014;209:1016–1022.
103. Graham DY, Jian X, Tanaka T, et al. Norwalk virus infection of volunteers: new insights based on improved assays. *J Infect Dis*. 1994;170:34–43.
104. Okhuysen PC, Jiang X, Ye L, et al. Viral shedding and fecal IgA response after Norwalk virus infection. *J Infect Dis*. 1995;171:566–569.
105. Rockx B, De Wit M, Vennema H, et al. Natural history of human calicivirus infection: a prospective cohort study. *Clin Infect Dis*. 2002;35:246–253.
106. Costantini VP, Cooper EM, Hardaker HL, et al. Epidemiologic, virologic, and host genetic factors of norovirus outbreaks in long-term care facilities. *Clin Infect Dis*. 2016;62:1–10.
107. Teunis PF, Sukhrie FH, Vennema H, et al. Shedding of norovirus in symptomatic and asymptomatic infections. *Epidemiol Infect*. 2015;143:1710–1717.
108. Patterson T, Hutching P, Palmer S. Outbreak of SRSV gastroenteritis at an international conference traced to food handled by a post-symptomatic caterer. *Epidemiol Infect*. 1993;111:157–162.
109. Kaplan JE, Feldman R, Campbell DS, et al. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. *Am J Public Health*. 1982;72:1329–1332.
110. Khan AS, Moe CL, Glass RI, et al. Norwalk virus-associated gastroenteritis traced to ice consumption aboard a cruise ship in Hawaii: comparison and application of molecular method-based assays. *J Clin Microbiol*. 1994;31:318–322.
111. Bourgeois AL, Gardiner CH, Thornton SA, et al. Etiology of acute diarrhea among United States military personnel deployed to South America and West Africa. *Am J Trop Med Hyg*. 1993;48:243–248.
112. Hyams KC, Bourgeois AL, Merrell BR, et al. Diarrheal disease during Operation Desert Shield. *N Engl J Med*. 1991;325:1423–1428.
113. Centers for Disease Control and Prevention. Norovirus outbreak in an elementary school—District of Columbia, February 2007. *MMWR Morb Mortal Wkly Rep*. 2008;56:1340–1343.
114. Ho MS, Glass RI, Monroe SS. Viral gastroenteritis aboard a cruise ship. *Lancet*. 1989;2:961–965.
115. Bitler EJ, Matthews JE, Dickey BW, et al. Norovirus outbreaks: a systematic review of commonly implicated transmission routes and vehicles. *Epidemiol Infect*. 2013;141:1563–1571.
116. Hedlund KO, Rubilar-Abreu E, Svensson L. Epidemiology of calicivirus infections in Sweden, 1994-1998. *J Infect Dis*. 2000;181(suppl 2):S275–S280.
117. Baron RC, Murphy FD, Greenberg HB. Norwalk gastrointestinal illness: an outbreak associated with swimming in a recreational lake with secondary person-to-person transmission. *Am J Epidemiol*. 1982;115:163–172.
118. Koopman JS, Eckert EA, Greenberg HB. Norwalk virus enteric illness acquired by swimming exposure. *Am J Epidemiol*. 1982;115:173–177.
119. Cannon RO, Poliner JR, Hirschhorn RB, et al. A multistate outbreak of Norwalk virus gastroenteritis associated with consumption of commercial ice. *J Infect Dis*. 1991;164:860–863.
120. Hedberg CW, Osterholm MT. Outbreaks of food-borne and waterborne viral gastroenteritis. *Clin Microbiol Rev*. 1993;6:199–210.
121. Lo SV, Connolly AM, Palmer SR, et al. The role of the presymptomatic food handler in a common source outbreak of food-borne SRSV gastroenteritis in a group of hospitals. *Epidemiol Infect*. 1994;113:513–521.
122. Stafford R, Strain D, Heymer M, et al. An outbreak of Norwalk virus gastroenteritis following consumption of oysters. *Commun Dis Intell*. 1997;21:317–320.
123. McDonnell S, Kirkland KB, Hlady WG, et al. Failure of cooking to prevent shellfish-associated viral gastroenteritis. *Arch Intern Med*. 1997;157:111–116.
124. Kirkland KB, Meriwether RA, Leiss JK, et al. Steaming oysters does not prevent Norwalk-like gastroenteritis. *Public Health Rep*. 1996;111:527–530.
125. Rodriguez EM, Parrott C, Rolka H, et al. An outbreak of viral gastroenteritis in a nursing home: importance of excluding ill employees. *Infect Control Hosp Epidemiol*. 1996;17:587–592.
126. Augustin AK, Simor AE, Shorrock C, et al. Outbreaks of gastroenteritis due to Norwalk-like virus in two long-term care facilities for the elderly. *Can J Infect Control*. 1995;10:111–113.
127. Russo PL, Spelman DW, Harrington GA, et al. Hospital outbreak of Norwalk-like virus. *Infect Control Hosp Epidemiol*. 1997;18:576–579.
128. Kjeldsberg E. Small spherical viruses in faeces from gastroenteritis patients. *Acta Pathol Microbiol Scand [B]*. 1977;85:351–354.
129. Spatt HC, Marks MI, Gomersall M, et al. Nosocomial infantile gastroenteritis associated with mini-rotavirus and calicivirus. *J Pediatr*. 1978;93:922–926.
130. Oishi I, Maeda A, Yamazaki K, et al. Calicivirus detected in outbreaks of acute gastroenteritis in school children. *Biken J*. 1980;23:163–168.
131. Steele AD, Phillips J, Smit TK, et al. Snow mountain-like virus identified in young children with winter vomiting disease in South Africa. *J Diarrhoeal Dis Res*. 1997;15:177–182.
132. Levett PN, Gu M, Luan B, et al. Longitudinal study of molecular epidemiology of small round-structured viruses in a pediatric population. *J Clin Microbiol*. 1996;34:1497–1501.
133. Middleton PJ, Szymanski MT, Petric M. Viruses associated with acute gastroenteritis in young children. *Am J Dis Child*. 1977;131:733–737.
134. Wolfaardt M, Taylor MB, Booysen HF, et al. Incidence of human calicivirus and rotavirus infection in patients with gastroenteritis in South Africa. *J Med Virol*. 1997;51:290–296.
135. Pang XL, Honma S, Nakata S, et al. Human caliciviruses in acute gastroenteritis of young children in the community. *J Infect Dis*. 2000;181(suppl 2):S288–S294.
136. Sakai Y, Nakata S, Honma S, et al. Clinical severity of Norwalk virus and Sapporo virus gastroenteritis in children in Hokkaido, Japan. *Pediatr Infect Dis J*. 2001;20:849–853.
137. Lew JF, Valdesuso J, Vesikari T, et al. Detection of Norwalk virus or Norwalk-like virus infections in Finnish infants and young children. *J Infect Dis*. 1994;169:1364–1367.
138. Grohmann G, Glass RI, Gold J, et al. Outbreak of human calicivirus gastroenteritis in a day-care center in Sydney, Australia. *J Clin Microbiol*. 1991;29:544–550.
139. Struve J, Bennet R, Ehrnst AE, et al. Nosocomial calicivirus gastroenteritis in a pediatric hospital. *Pediatr Infect Dis J*. 1994;13:882–885.
140. Payne DC, Vinjé J, Szilagyi PG, et al. Norovirus and medically attended gastroenteritis in U.S. children. *N Engl J Med*. 2013;368:1121–1130.
141. Mounts AW, Ando T, Koopmans M, et al. Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. *J Infect Dis*. 2000;181(suppl 2):S284–S287.
142. Blanton LH, Adams SM, Beard RS, et al. Molecular and epidemiologic trends of caliciviruses associated with outbreaks of acute gastroenteritis in the United States, 2000-2004. *J Infect Dis*. 2006;193:413–421.
143. Agus SG, Dolin R, Wyatt RG, et al. Acute infectious nonbacterial gastroenteritis: intestinal histopathology. *Ann Intern Med*. 1973;79:18–25.
144. Schreiber DS, Blacklow NR, Trier JS. The mucosal lesion of the proximal small intestine in acute infectious nonbacterial gastroenteritis. *N Engl J Med*. 1973;288:1318–1323.
145. Schreiber DS, Blacklow NR, Trier JS. The small intestinal lesion induced by Hawaii agent acute infectious nonbacterial gastroenteritis. *J Infect Dis*. 1974;129:705–708.
146. Widerlite L, Trier J, Blacklow N, et al. Structure of the gastric mucosa in acute infectious nonbacterial gastroenteritis. *Gastroenterology*. 1975;70:321–325.
147. Blacklow NR, Dolin R, Feson DS, et al. Acute infectious non-bacterial gastroenteritis: etiology and pathogenesis. *Ann Intern Med*. 1972;76:993–1000.
148. Levy AG, Widerlite L, Schwartz CJ, et al. Jejunal adenylate cyclase activity in human subjects during viral gastroenteritis. *Gastroenterology*. 1976;70:321–325.
149. Woode GN, Bridger JC. Isolation of small viruses resembling astroviruses and caliciviruses from acute enteritis of calves. *J Med Microbiol*. 1978;11:441–452.
150. Saif LJ, Bohl EH, Theil KW, et al. Rotavirus-like, calicivirus-like, and 23-nm virus-like particles associated with diarrhea in young pigs. *J Clin Microbiol*. 1980;12:105–111.
151. Ramani S, Estes MK, Atmar RL. Correlates of protection against norovirus infection and disease—where are we now, where do we go? *PLoS Pathog*. 2016;12:e1005334.
152. Marionneau S, Ruvoen N, Le Moullac-Vaidye B, et al. Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals. *Gastroenterology*. 2002;122:1967–1977.
153. Hutson AM, Atmar RL, Marcus DM, et al. Norwalk virus-like particle hemagglutination by binding to histo-blood group antigens. *J Virol*. 2003;77:405–415.
154. Huang P, Farkas T, Marionneau S, et al. Noroviruses bind to human ABO, lewis, and secretor histo-blood group antigens: identification of 4 distinct strain-specific patterns. *J Infect Dis*. 2003;188:19–31.
155. Tan M, Huang P, Meller J, et al. Mutations within the P2 domain of Norovirus capsid affect binding to human histoblood group antigens: evidence for a binding pocket. *J Virol*. 2003;77:12562–12571.
156. Hutson AM, Atmar RL, Graham DY, et al. Norwalk virus infection and disease is associated with ABO histo-blood group type. *J Infect Dis*. 2002;185:1335–1337.
157. Lindesmith L, Moe C, Marionneau S, et al. Human susceptibility and resistance to Norwalk virus infection. *Nat Med*. 2003;9:548–553.
158. Tan M, Jiang X. Norovirus-host interaction: multi-selections by human histoblood group antigens. *Trends Microbiol*. 2011;19:382–388.
159. Parrino TA, Schreiber DS, Trier JS, et al. Clinical immunity in acute gastroenteritis caused by Norwalk agent. *N Engl J Med*. 1977;291:86–89.
160. Kambhampati A, Payne DC, Costantini V, et al. Host genetic susceptibility to enteric viruses: a systematic review and metaanalysis. *Clin Infect Dis*. 2016;62:11–18.
161. Cukor G, Nowak NA, Blacklow NR. Immunoglobulin M responses to the Norwalk virus of gastroenteritis. *Infect Immun*. 1982;37:463–468.
162. Treanor JJ, Jiang X, Madore HP, et al. Subclass-specific serum antibody responses to recombinant Norwalk virus capsid antigen (rNV) in adults infected with Norwalk, Snow Mountain, or Hawaii viruses. *J Clin Microbiol*. 1993;31:1630–1634.
163. Erdman DD, Gary GW, Anderson LJ. Development and evaluation of an IgM capture enzyme immunoassay for diagnosis of recent Norwalk virus infection. *J Virol Methods*. 1989;24:57–66.
164. Erdmann DD, Gary GW, Anderson LJ. Serum immunoglobulin A response to Norwalk virus infection. *J Clin Microbiol*. 1989;27:1417–1418.

165. Farkas T, Thornton SA, Wilton N, et al. Homologous versus heterologous immune responses to Norwalk-like viruses among crew members after acute gastroenteritis outbreaks on 2 US Navy vessels. *J Infect Dis*. 2003;187:187–193.
166. Madore HP, Treanor JJ, Buja R, et al. Antigenic relatedness among the Norwalk-like agents by serum antibody rises. *J Med Virol*. 1990;32:96–101.
167. Hale AD, Lewis DC, Jiang X, et al. Homotypic and heterotypic IgG and IgM antibody responses in adults infected with small round structured viruses. *J Med Virol*. 1998;54:305–312.
168. Noel JS, Ando T, Leite JP, et al. Correlation of patient immune responses with genetically characterized small round-structured viruses involved in outbreaks of nonbacterial acute gastroenteritis in the United States, 1990 to 1995. *J Med Virol*. 1997;53:372–383.
169. Jiang X, Wang J, Estes MK. Characterization of SRSVs using RT-PCR and a new antigen ELISA. *Arch Virol*. 1995;140:363–374.
170. Agus S, Falchuk ZM, Sessoms CS, et al. Increased jejunal IgA synthesis in vitro during acute infectious nonbacterial gastroenteritis. *Am J Dig Dis*. 1974;19:127–131.
171. Okhuysen P, Jiang X, Tenjaria G, et al. *Detection of Norwalk Specific Fecal IgA in Challenged Volunteers Utilizing ELISA With Baculovirus Expressed Norwalk Particles as Coating Antigens. Abstract 1392. 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy, 1992*. Anaheim, CA: American Society for Microbiology; 1992:343.
172. Dolin R, Reichman RC, Fauci AS. Lymphocyte populations in acute viral gastroenteritis. *Infect Immun*. 1976;14:422–428.
173. Lindesmith L, Moe C, Lependu J, et al. Cellular and humoral immunity following Snow Mountain virus challenge. *J Virol*. 2005;79:2900–2909.
174. Johnson PC, Mathewson JJ, DuPont HL, et al. Multiple-challenge study of host susceptibility to Norwalk gastroenteritis in US adults. *J Infect Dis*. 1990;161:18–21.
175. Lindesmith LC, Donaldson E, Leon J, et al. Heterotypic humoral and cellular immune responses following Norwalk virus infection. *J Virol*. 2010;84:1800–1815.
176. Black RE, Greenberg HB, Kapikian AZ, et al. Acquisition of serum antibody to Norwalk virus and rotavirus and relation to diarrhea in a longitudinal study of young children in rural Bangladesh. *J Infect Dis*. 1982;145:483–489.
177. Ryder RW, Singh N, Reeves WC, et al. Evidence of immunity induced by naturally acquired rotavirus and Norwalk virus infection on two remote Panamanian islands. *J Infect Dis*. 1985;135:20–27.
178. Nakata S, Chiba A, Terashima H, et al. Humoral immunity in infants with gastroenteritis caused by human calicivirus. *J Infect Dis*. 1985;152:274–279.
179. Malm M, Uusi-kerrtula H, Vesikari T, et al. High serum levels of norovirus genotype-specific blocking antibodies correlate with protection from infection in children. *J Infect Dis*. 2014;210:1755–1762.
180. Lee BE, Pang X. One more step toward understanding the immune response to norovirus. *J Infect Dis*. 2014;210:1691–1692.
181. Greenberg HB, Wyatt RG, Kalica AR, et al. New insights in viral gastroenteritis. *Perspect Virol*. 1981;11:163–187.
182. Karst SM, Wobus CE, Lay M, et al. STAT1-dependent innate immunity to a Norwalk-like virus. *Science*. 2003;299:1575–1578.
183. Wingfield T, Gallimore C, Xerry J, et al. Chronic norovirus infection in an HIV-positive patient with persistent diarrhoea: a novel cause. *J Clin Virol*. 2010;49:219–222.
184. Dedman D, Laurichesse H, Caul EO, et al. Surveillance of small round structured virus (SRSV) infection in England and Wales, 1990-5. *Epidemiol Infect*. 1998;121:139–149.
185. Chadwick PR, Beards G, Brown D, et al. Management of hospital outbreaks of gastro-enteritis due to small round-structured viruses. *J Hosp Infect*. 2000;45:1–10.
186. Kirk MD, Fullerton KE, Hall GV, et al. Surveillance for outbreaks of gastroenteritis in long-term care facilities, Australia, 2002-2008. *Clin Infect Dis*. 2010;51:907–914.
187. Jones TF. When diarrhea gets deadly: a look at gastroenteritis outbreaks in nursing homes. *Clin Infect Dis*. 2010;51:915–916.
188. Costantini VP, Cooper EM, Hardaker HL, et al. Epidemiologic, virologic, and host genetic factors of norovirus outbreaks in long-term care facilities. *Clin Infect Dis*. 2016;62:1–10.
189. Simon A, Schildgen O, Maria Eis-Hubinger A, et al. Norovirus outbreak in a pediatric oncology unit. *Scand J Gastroenterol*. 2006;41:693–699.
190. Westhoff TH, Vergoulidou M, Loddenkemper C, et al. Chronic norovirus infection in renal transplant recipients. *Nephrol Dial Transplant*. 2009;24:1051–1053.
191. Roddie C, Paul JP, Benjamin R, et al. Allogenic hematopoietic stem cell transplantation and norovirus gastroenteritis: a previously unrecognized cause of morbidity. *Clin Infect Dis*. 2009;49:1061–1068.
192. Koo HL, DuPont HL. Noroviruses as a potential cause of protracted and lethal disease in immunocompromised patients. *Clin Infect Dis*. 2009;49:1069–1071.
193. Angarone MP, Sheahan A, Kamboj M. Norovirus in transplantation. *Curr Infect Dis Rep*. 2016;18:17.
194. Woodward JM, Gkrania-Klotsas E, Cordero-Ng AY, et al. The role of chronic norovirus infection in the enteropathy associated with common variable immunodeficiency. *Am J Gastroenterol*. 2015;110:320–327.
195. Kaplan JE, Feldman R, Campbell DS, et al. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis [abstract]. *Am J Public Health*. 1982;72:1329–1332.
196. Harris JC, DuPont HL, Hornick RB. Fecal leukocytes in diarrheal illness. *Ann Intern Med*. 1972;76:697–703.
197. Atmar RL, Estes MK. Diagnosis of noncultivatable gastroenteritis viruses, the human caliciviruses. *Clin Microbiol Rev*. 2001;14:15–37.
198. Kapikian Z, Wyatt RG, Dolin R, et al. Visualization of 27 nm particle associated infectious nonbacterial gastroenteritis. *J Virol*. 1972;10:1075–1081.
199. Jiang X, Wang J, Graham DY, et al. Detection of Norwalk virus in stool by polymerase chain reaction. *J Clin Microbiol*. 1992;30:2529–2534.
200. De Leon R, Matsui SM, Baric RS, et al. Detection of Norwalk virus in stool specimens by reverse transcriptase-polymerase chain reaction and nonradioactive oligoprobes. *J Clin Microbiol*. 1992;30:3151–3157.
201. Willcocks MM, Silcock JG, Carter MJ. Detection of Norwalk virus in the UK by the polymerase chain reaction. *FEMS Microbiol Lett*. 1993;112:7–12.
202. Moe CL, Gentsch J, Ando T, et al. Application of PCR to detect Norwalk virus in fecal specimens from outbreaks of gastroenteritis. *J Clin Microbiol*. 1994;32:642–648.
203. Honma S, Nakata S, Sakai Y, et al. Sensitive detection and differentiation of Sapporo virus, a member of the family Caliciviridae, by standard and booster nested polymerase chain reaction. *J Med Virol*. 2001;65:413–417.
204. Vinje J, Vennema H, Maunula L, et al. International collaborative study to compare reverse transcriptase PCR assays for detection and genotyping of noroviruses. *J Clin Microbiol*. 2003;41:1423–1433.
205. Jiang X, Huang PW, Zhong WM, et al. Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-like caliciviruses by RT-PCR. *J Virol Methods*. 1999;83:145–154.
206. Kojima S, Kageyama T, Fukushi S, et al. Genogroup-specific PCR primers for detection of Norwalk-like viruses. *J Virol Methods*. 2002;100:107–114.
207. Ando T, Monroe SS, Gentsch JR, et al. Detection and differentiation of antigenically distinct small round-structured viruses (Norwalk-like viruses) by reverse transcription-PCR and southern hybridization. *J Clin Microbiol*. 1995;33:64–71.
208. Atmar RL, Metcalf TG, Neill FH, et al. Detection of enteric viruses in oysters by using the polymerase chain reaction. *Appl Environ Microbiol*. 1993;59:631–635.
209. Gouvea V, Santos N, Timenetsky M, et al. Identification of Norwalk virus in artificially seeded shellfish and selected foods. *J Virol Methods*. 1994;8:177–187.
210. Beller M, Ellis A, Lee SH, et al. Outbreak of viral gastroenteritis due to a contaminated well: international consequences. *JAMA*. 1997;278:563–568.
211. Le Guyader F, Neill FH, Estes MK, et al. Detection and analysis of a small round-structured virus strain in oysters implicated in an outbreak of acute gastroenteritis. *Appl Environ Microbiol*. 1996;62:4268–4272.
212. Shieh Y, Monroe SS, Fankhauser RL, et al. Detection of Norwalk-like virus in shellfish implicated in illness. *J Infect Dis*. 2000;181(suppl 2):S360–S366.
213. De Bruin E, Duizer E, Vennema H, et al. Diagnosis of norovirus outbreaks by commercial ELISA or RT-PCR. *J Virol Methods*. 2006;137:259–264.
214. Gray JJ, Kohli E, Ruggeri FM, et al. European multicenter evaluation of commercial enzyme immunoassays for detecting norovirus antigen in fecal samples. *Clin Vaccine Immunol*. 2007;14:1349–1355.
215. Centers for Disease Control and Prevention. Norovirus: Laboratory Diagnosis and Treatment. http://www.cdc.gov/norovirus/hcp/diagnosis-treatment.html. Accessed March 18, 2014.
216. Gary GW, Anderson LJ, Keswick BH, et al. Norwalk virus antigen and antibody response in an adult volunteer study. *J Clin Microbiol*. 1987;25:2001–2003.
217. Brinker JP, Blacklow NR, Estes MK, et al. Detection of Norwalk virus and other genogroup 1 human caliciviruses by a monoclonal antibody, recombinant-antigen-based immunoglobulin M capture enzyme immunoassay. *J Clin Microbiol*. 1998;36:1064–1069.
218. Brinker JP, Blacklow NR, Jiang X, et al. Immunoglobulin M antibody test to detect genogroup II Norwalk-like virus infection. *J Clin Microbiol*. 1999;37:2983–2986.
219. Steinhoff MC, Douglas RG Jr, Greenberg HB, et al. Bismuth subsalicylate therapy of viral gastroenteritis. *Gastroenterology*. 1980;78:1495–1499.
220. Wobus CE, Thackray LB, Virgin HWIV. Murine norovirus: a model system to study norovirus biology and pathogenesis. *J Virol*. 2006;80:5104–5112.
221. Rocha-Pereira J, Van Dycke J, Neyts J. Treatment with a nucleoside polymerase inhibitor reduces shedding of murine norovirus in stool to undetectable levels without emergence of drug-resistant variants. *Antimicrob Agents Chemother*. 2015;60:1907–1911.
222. Rocha-Pereira J, Jochmans D, Dallmeier K, et al. Inhibition of norovirus replication by the nucleoside analogue 2'-C-methylcytidine. *Biochem Biophys Res Commun*. 2012;427:796–800.
223. Kolawole AO, Rocha-Pereira J, Elftman MD, et al. Inhibition of human norovirus by a viral polymerase inhibitor in the B cell culture system and in the mouse model. *Antiviral Res*. 2016;132:46–49.
224. Rocha-Pereira J, Jochmans D, Debing Y, et al. The viral polymerase inhibitor 2'-C-methylcytidine inhibits Norwalk virus replication and protects against norovirus-induced diarrhea and mortality in a mouse model. *J Virol*. 2013;87:11798–11805.
225. Rocha-Pereira J, Jochmans D, Neyts J. Prophylactic treatment with the nucleoside analogue 2'-C-methylcytidine completely prevents transmission of norovirus. *J Antimicrob Chemother*. 2015;70:190–197.
226. Rocha-Pereira J, Nascimento MS, Ma Q, et al. The enterovirus protease inhibitor rupintrivir exerts cross-genotypic anti-norovirus activity and clears cells from the norovirus replicon. *Antimicrob Agents Chemother*. 2014;58:4675–4681.
227. Rossignol JF, El-Gohary YM. Nitazoxanide in the treatment of viral gastroenteritis: a randomized double-blind placebo-controlled clinical trial. *Aliment Pharmacol Ther*. 2006;24:1423–1430.
228. Siddiq DM, Koo HL, Adachi JA, et al. Norovirus gastroenteritis successfully treated with nitazoxanide. *J Infect*. 2011;63:394–397.
229. Centers for Disease Control and Prevention. NoroSTAT Data. https://www.cdc.gov/norovirus/reporting/norostat/data.html. Accessed January 25, 2018.
230. Bartsch SM, Lopman BA, Hall AJ, et al. The potential economic value of a human norovirus vaccine for the United States. *Vaccine*. 2012;30:7097–7104.
231. Cortes-Penfield NW, Ramani S, Estes MK, et al. Prospects and challenges in the development of a norovirus vaccine. *Clin Ther*. 2017;39:1537–1549.
232. Baehner F, Bogaerts H, Goodwin R, et al. Vaccines against norovirus: state of the art trials in children and adults. *Clin Microbiol Infect*. 2016;22(suppl 5):S136–S139.
233. Baehner F, Bogaerts H, Goodwin R. Vaccines against norovirus: state of the art trials in children and adults. *Clin Microbiol Infect*. 2016;22(suppl 5):S136–S139.
234. Riddle MS, Walker RI. Status of vaccine research and development for norovirus. *Vaccine*. 2016;34:2895–2899.
235. Ball JM, Graham DY, Opekun AR, et al. Recombinant Norwalk virus-like particles given orally to volunteers: phase I study. *Gastroenterology*. 1999;117:40–48.
236. Tacket CO, Sztein MB, Losonsky G, et al. Humoral, mucosal, and cellular immune responses to oral Norwalk virus-like particles in volunteers. *Clin Immunol*. 2003;108:241–247.
237. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk virus illness. *N Engl J Med*. 2011;365:2178–2187.
238. Treanor JJ, Atmar RL, Frey SE, et al. A novel intramuscular bivalent norovirus-like particle vaccine candidate—reactogenicity, safety, and immunogenicity in a phase 1 trial in healthy adults. *J Infect Dis*. 2014;210:1763–1771.
239. Bernstein DI, Atmar RL, Lyon GM, et al. Norovirus vaccine against experimental human GII.4 virus illness: a challenge study in healthy adults. *J Infect Dis*. 2015;211:870–878.
240. Tacket CO, Mason HS, Losonsky G, et al. Human immune responses to a novel Norwalk virus vaccine delivered in transgenic potatoes. *J Infect Dis*. 2000;182:302–305.

177 Astroviruses and Picobirnaviruses

Raphael Dolin and John J. Treanor

SHORT VIEW SUMMARY

Definition
- Astroviruses cause acute gastroenteritis. Picobirnaviruses have been detected in stools but have not been established as causes of illness in humans.

Epidemiology
- Astroviruses are worldwide in distribution; they cause disease most frequently in young children, but can also cause gastroenteritis in adults and in immunosuppressed patients. Transmission is likely by the fecal-oral route.

Microbiology
- Astroviruses are single-stranded RNA viruses composed of multiple genogroups and genotypes, with a characteristic star-shaped morphologic appearance on electron micrographs. Picobirnaviruses are double-stranded RNA viruses with a bisegmented genome.

Clinical Manifestations
- Illness induced by astroviruses consists of diarrhea, headache, malaise, nausea, low-grade fever, and, less commonly, vomiting. Illness may be somewhat less severe than that seen with noroviruses and rotaviruses.

Diagnosis
- Astroviruses in stools can be detected with electron microscopy, enzyme immunoassay, and reverse-transcriptase polymerase chain reaction assay, which is the most sensitive technique.

Therapy
- Treatment is supportive. Intravenous immune globulin has been used in immunosuppressed patients, but its efficacy is not established.

Prevention
- Although virus-like particles from astroviruses have been produced, vaccines have not been developed.

Viral gastroenteritis is caused largely by noroviruses and sapoviruses (see Chapter 176) and by rotaviruses (see Chapter 150). Astroviruses are also established as important causes of gastroenteritis, albeit substantially less frequently than the aforementioned viruses. Picobirnaviruses are more recently described viruses that have a possible, although not yet established, role as agents of gastroenteritis.

ASTROVIRUSES

Virology

Classification

Astroviruses are members of the Astroviridae family, which consists of two genera: *Mamastrovirus*, which infects mammals and includes the human astroviruses, and *Avastrovirus*, which includes avian viruses. Astroviruses were first detected by their characteristic starlike appearance on electron micrographs (Fig. 177.1) and have subsequently undergone extensive molecular virologic characterization.[1–3] The ability to grow astroviruses in cell culture, including primary HEK cells and a variety of monkey cell lines, has facilitated their study.[4–6]

Mamastroviruses are subdivided into two genogroups (GI and GII), which have at least 19 genotypes. The first clade of these includes eight human astroviruses (HAstV1 to HAstV8) that correspond to the eight serotypes first noted in human astrovirus infections.[1–3,7–11] Genotypes in two additional clades have been subsequently identified: HAstV-MLB and HAstV-VA/HMO.[3,12] HAstV serotypes can be distinguished with immunofluorescence or plaque neutralization techniques,[13,14] whereas considerable cross-reactivity exists with enzyme immunoassays (EIAs).[4]

Structure

Astroviruses are positive-stranded RNA viruses that are found in a wide variety of animal species, in addition to humans. The virions are nonenveloped, display icosahedral symmetry, and are about 28 to 30 nm in diameter. Under the electron microscope, the particles in stool samples have a characteristic morphologic appearance that consists of round smooth edges with multiple triangular electron-lucent areas and an electron-dense center that results in the appearance of a five- or six-pointed star from which the virus derives its name (see Fig. 177.1).[1–3,12] Analysis of virus grown in cell culture has shown the virus particles to exhibit a layer of 41-nm spikelike projections on the surface.[15] The human astroviruses have a density of 1.35 to 1.37 g/mL in cesium chloride and contain a positive-sense, single-stranded 35S RNA genome with a 3′ polyadenylated tail.

Genomic Organization

The genomic organization includes three open reading frames (ORFs) (Fig. 177.2). ORF1a encodes the protease region, ORF1b encodes the RNA-dependent RNA polymerase, and ORF2 encodes a polyprotein that is cleaved into the core capsid and virion spike proteins. A possible additional ORF has been detected (ORF-X) whose function is uncertain.[1–3,12,16–19] From the perspective of genetic sequences, ORF1b is the least divergent, and ORF2 is the most divergent.[20] The capsid is translated from a subgenomic polyadenylated RNA in infected cells.[3,21,22] The number of viral structural proteins appears to vary with the serotype (see later in this section), with one to three reported for the human viruses,[23,24] possibly reflecting differences in the processing of the capsid precursor.[25]

Epidemiology

Astrovirus infection is widely geographically[26] distributed, and HAstV[1–3,12,15–18] serotypes or genotypes have been detected throughout the world.[8–11] HAstV-1 appears to be the most common, based on both serotypic[27–29] and genotypic surveys.[11,30–33]

Astroviruses are a recognized cause of gastroenteritis worldwide, most commonly in children, usually younger than 2 years. They have been estimated to cause 2% to 9% of cases of nonbacterial gastroenteritis.[2,3,34] Astroviruses have been detected in the stools of children with diarrhea brought to medical attention in a variety of settings.[35] Rates of 2% to 16% in children with diarrhea have been found in hospital-based studies and 5% to 17% in community-based studies.[36–38] Outbreaks have been described in schools, daycare settings, and pediatric wards.[39–42] Gastroenteritis associated with astroviruses has also been reported in adults in a variety of settings.[2,43–46] These include outbreaks in healthy adults[44,45]; elderly populations[46]; immunosuppressed patients

with diarrhea, including human immunodeficiency virus (HIV)–infected patients[47]; patients who have undergone hematopoietic cell transplantation[48]; and children with primary immunodeficiencies[49] or hematologic malignancies.[50] Astrovirus infections in immunosuppressed patients were associated with prolonged shedding of virus and persistent diarrhea, but often other pathogens were also present in stool specimens. The severity of illness in HIV-infected children with astroviruses, however, was no greater than that seen in healthy children.[51,52] Astroviruses, which are phylogenetically divergent from HAstV-1 through HAstV-8 and are found in mink and cattle, have been associated with encephalitis in immunosuppressed children.[53,54]

A winter predilection has been noted in temperate climates in some studies,[24,55] whereas infection occurs throughout the year in tropical climates,[56] similar to the pattern reported for rotaviruses. Transmission is presumably by the fecal-oral route, and astrovirus infection has been induced by oral administration of stool filtrates to normal volunteers.[23] HAstV can be shed in high amounts in the stools of infected subjects, with up to 10^{23} genome copies/g, contributing to the spread of infection.[57]

Contamination of foods and water supplies appears to be important in astrovirus-associated outbreaks.[2]

Seroprevalence studies in worldwide sites, including in the United States, indicate that HAstV infections occur early in life, such that by age 5 more than 90% of children have antibody to at least one HAstV serotype.[12,27] Seroprevalence in healthy adults appears to be somewhat lower.[12]

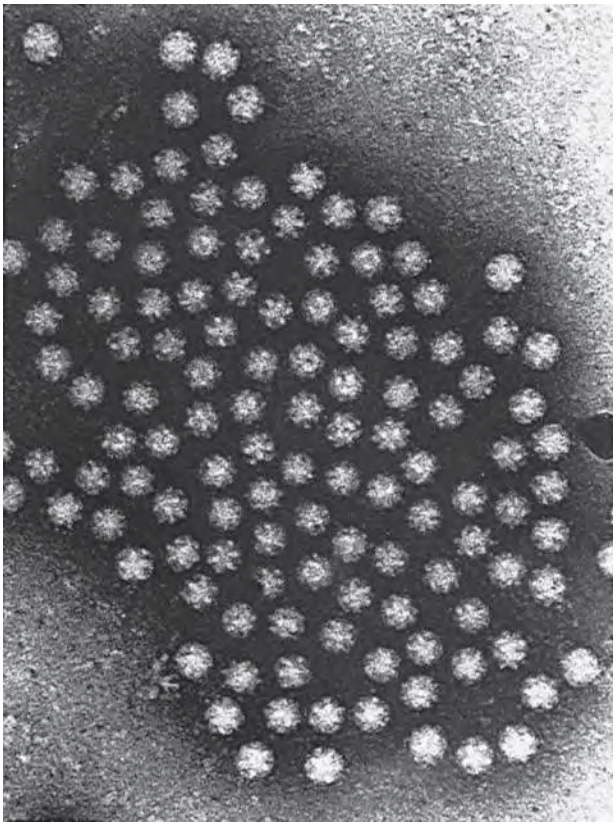

FIG. 177.1 Astrovirus in the intestinal contents of gnotobiotic lambs. Particles are 30 nm in diameter. *(From Snodgrass DR, Gray W. Detection and transmission of 30 nm virus particles [astroviruses] in the faeces of lambs with diarrhoea.* Arch Virol. *1977;55:287–291.)*

Pathogenesis

The pathogenesis of astrovirus-induced illness is not well understood, and studies have been limited by the lack of a convenient animal model for HAstV infection. Astrovirus infections in animals have been associated with small intestinal villus shortening and with mild inflammatory infiltrates in the lamina propria.[58,59] Astrovirus infection may result in decreased intestinal disaccharidase activity and subsequent osmotic diarrhea,[60] similar to the mechanism postulated for rotavirus. As noted previously, stool filtrates that contain astroviruses can infect volunteers after oral administration, but in comparison to noroviruses, astroviruses containing filtrates appear to induce illness less frequently when administered to healthy adults.[23,61]

Clinical Manifestations

Illness attributed to astroviruses consists primarily of diarrhea, headache, malaise, abdominal pain, and nausea; vomiting appears to be less common.[2,3] In general, the symptoms are similar to those seen in norovirus and rotavirus infection in children but are milder, and children with astrovirus gastroenteritis are less likely to become dehydrated.[2,43,62] Low-grade fever is frequently present. The incubation period of illness has been estimated to be 3 to 4 days, and in the absence of coexisting pathogens, disease manifestations usually last 5 days or less, with occasionally longer duration. The duration of virus shedding as assessed with EIA is generally short (mean of 1.5 days; range, 1–9 days).[63] Detection of shedding with polymerase chain reaction (PCR) is longer (mean of 4 days), with a prolonged range reported in one outbreak (1–35 days)[63]

Astrovirus infection may also occur in immunocompromised patients, in whom it can be more severe and prolonged, including multiorgan dissemination in severely immunocompromised children.[2,12,64,65]

Immune Responses

Immune responses to astroviruses and their role in protection from infection and illness are not well understood. Epidemiologic observations suggest that infection results in at least short-term protection against reinfection with the same serotype.[66]

In volunteer experiments, the ability to infect participants with astrovirus and the severity of subsequent illness were inversely correlated with the presence and magnitude of serum anti-HAstV.[23,61] Mucosal immunoglobulin A (IgA) antibodies are also induced by HAstV infection, but their role in protection is unclear.[67] T-cell responses to inactivated HAstV have been demonstrated in mucosal biopsy specimens from healthy adults.

Diagnosis

In contrast to caliciviruses, astroviruses are often shed in large amounts in stool and can be readily detected with electron microscopy even without immune aggregation. Detection of astroviruses has been carried out with immune electron microscopy (IEM) or with immunofluorescence, and an EIA technique[68] that detects the astrovirus group antigen has been used widely in epidemiologic studies. EIA is of comparable sensitivity (91%) and specificity (98%) to IEM.[2,3] Reverse-transcriptase polymerase chain reaction (RT-PCR) techniques have been developed and are the most widely used techniques to detect astroviruses, and they are significantly more sensitive than EIA or IEM.[2,63,69,70]

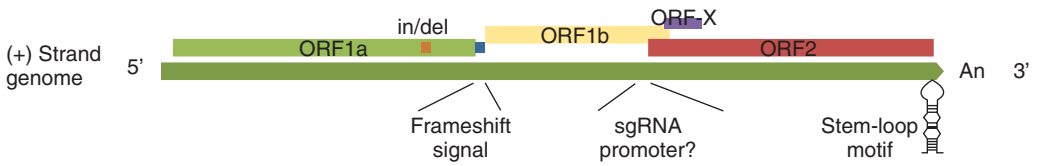

FIG. 177.2 Genome organization of human astrovirus. The genomic RNA contains three open reading frames *(ORF1a, ORF1b, ORF2),* and possibly a fourth *(ORF-X).* See "Genomic Organization" in text. *(Modified from Mendez E, Arias CF. Astroviruses. In:* Fields Virology. *6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013:611.)*

Therapy

Illness associated with astroviruses is generally self-limited, and treatment, if required at all, is supportive and directed at maintaining hydration and electrolyte balance. Intravenous immune globulin has been used in immunosuppressed patients with astrovirus gastroenteritis, but its efficacy, if any, is not established by clinical trials.[71] Antivirals against astroviruses are not available, but in vitro activity of flavonoids has been reported.[72]

Prevention

The major approaches to prevention of infection with HAstVs are avoidance of contamination of water and food supplies. Measures to avoid person-to-person transmission from active cases also appear prudent. Virus-like particles of astroviruses have been generated,[73] raising the possibility of vaccine approaches at some point in the future.

PICOBIRNAVIRUSES

Picobirnavirus is the only genus within the virus family Picobirnaviridae.[74,75] Picobirnaviruses are small, nonenveloped icosahedral viruses with a segmented double-stranded RNA genome consisting of two segments. The genomes have been characterized into two types or profiles[76,77]—a large profile (2.3–2.6 kbp for the large segment) and a small profile (1.75–1.55 kbp)—which are now being used for molecular epidemiologic studies.[78] Their exact taxonomic position is unclear, and they derive their name from the observation that they (usually) have a *bi*segmented double-stranded RNA genome, similar to the *Bi*rnaviridae, but are smaller *(pico),* with a virion size of 30 to 40 nm.[79] They were first observed in the stools of humans[80] in the course of studies using polyacrylamide gel electrophoresis to detect rotavirus. Picobirnavirus genomes have been found in the stools of a variety of animals[75] and in children and adults with diarrhea, including HIV-infected adults,[47,78,81–84] and in patients with hematopoietic stem cell transplants.[85] Two genogroups and a potential third one have been identified among human viruses.[86] However, the role of these viruses as causative agents of gastroenteritis remains unclear because the prevalence of picobirnaviruses in stools of individuals with and without diarrhea is similar.[87]

Key References

The complete reference list is available online at Expert Consult.

1. De Benedictis P, Schultz-Cherry S, Burnham A, et al. Astrovirus infections in humans and animals—molecular biology, genetic diversity, and interspecies transmissions. *Infect Genet Evol*. 2011;11:1529–1544.
2. Bosch A, Pintó RM, Guix S. Human astroviruses. *Clin Microbiol Rev*. 2014;27:1048–1074.
10. Chu DK, Chin AW, Smith GJ, et al. Detection of novel astroviruses in humans. *J Gen Virol*. 2010;91(Pt 10):2457–2462.
11. Gabbay YB, Linhares AC, Cavalcante-Pepino EL, et al. Prevalence of human astrovirus genotypes associated with acute gastroenteritis among children in Bélem, Brazil. *J Med Virol*. 2007;79:530–538.
12. Cortez V, Meliopoulos VA, Karlsson EA, et al. Astrovirus biology and pathogenesis. *Annu Rev Virol*. 2017;4:327–348.
15. Risco C, Carrascosa JL, Pedregosa AM, et al. Ultrastructure of human astrovirus serotype 2. *J Gen Virol*. 1995;76:2075–2080.
16. Jiang B, Monroe SS, Koonin EV, et al. RNA sequence of astrovirus: distinctive genomic organization and a putative retrovirus-like ribosomal frameshifting signal that directs the viral replicase synthesis. *Proc Natl Acad Sci USA*. 1993;90:10539–10543.
20. Strain E, Kelley LA, Schultz-Cherry S, et al. Genomic analysis of closely related astroviruses. *J Virol*. 2008;82:5099–5103.
21. Monroe SS, Stine SE, Gorelkin L, et al. Temporal synthesis of proteins and RNAs during human astrovirus infection of cultured cells. *J Virol*. 1991;65:641–648.
26. Malasao R, Khamrin P, Chaimongkol N, et al. Diversity of human astrovirus genotypes circulating in children with acute gastroenteritis in Thailand during 2000-2011. *J Med Virol*. 2012;84:1751–1756.
27. Mitchell DK, Matson DO, Cubitt WD, et al. Prevalence of antibodies to astrovirus types 1 and 3 in children and adolescents in Norfolk, Virginia. *Pediatr Infect Dis J*. 1999;18:249–254.
29. Koopmans MP, Bijen MH, Monroe SS, et al. Age-stratified seroprevalence of neutralizing antibodies to astrovirus types 1 to 7 in humans in the Netherlands. *Clin Diagn Lab Immunol*. 1998;5:33–37.
30. Espul A, Martinez N, Noel JS, et al. Prevalence and characterization of astroviruses in Argentinean children with acute gastroenteritis. *J Med Virol*. 2004;72:75–82.
31. Guix S, Caballero S, Villena C, et al. Molecular epidemiology of astrovirus infection in Barcelona, Spain. *J Clin Microbiol*. 2002;40:133–139.
34. Olortegui MP, Rouhani S, Yori PP, et al. Astrovirus infection and diarrhea in 8 countries. *Pediatrics*. 2018;141:pii: e20171326.
35. Wang Y, Li Y, Jin Y, et al. Recently identified novel human astroviruses in children with diarrhea, China. *Emerg Infect Dis*. 2013;19:1333–1335.
37. Walter JE, Mitchell DK. Role of astroviruses in childhood diarrhea. *Curr Opin Pediatr*. 2000;12:275–279.
41. Svraka S, Duizer E, Vennema H, et al. Etiological role of viruses in outbreaks of acute gastroenteritis in the Netherlands from 1994 through 2005. *J Clin Microbiol*. 2007;45:1389–1394.
42. Lyman WH, Walsh JF, Kotch JB, et al. Prospective study of etiologic agents of acute gastroenteritis outbreaks in child care centers. *J Pediatr*. 2009;154:253–257.
43. Jeong HS, Jeong A, Cheon DS. Epidemiology of astrovirus infection in children. *Korean J Pediatr*. 2012;55:77–82.
46. Gray JJ, Wreghitt TG, Cubitt WD, et al. An outbreak of gastroenteritis in a home for the elderly associated with astrovirus type 1 and human calicivirus. *J Med Virol*. 1987;23:377–381.
47. Grohmann GS, Glass RI, Pereira HG, et al. Enteric viruses and diarrhea in HIV-infected patients. Enteric Opportunistic Infections Working Group. *N Engl J Med*. 1993;329:14–20.
48. Cox GJ, Matsui SM, Lo RS, et al. Etiology and outcome of diarrhea after marrow transplantation: a prospective study. *Gastroenterology*. 1994;107:1398–1407.
49. Gallimore CI, Taylor C, Gennery AR, et al. Use of a heminested reverse transcriptase PCR assay for detection of astrovirus in environmental swabs from an outbreak of gastroenteritis in a pediatric primary immunodeficiency unit. *J Clin Microbiol*. 2005;43:3890–3894.
51. Liste MB, Natera I, Suarez JA, et al. Enteric virus infections and diarrhea in healthy and human immunodeficiency virus-infected children. *J Clin Microbiol*. 2000;38:2873–2877.
53. Quan PL, Wagner TA, Briese T, et al. Astrovirus encephalitis in boy with X-linked agammaglobulinemia. *Emerg Infect Dis*. 2010;16:918–925.
54. Brown JR, Morfopoulou S, Hubb J, et al. Astrovirus VA1/HMO-C: an increasingly recognized neurotropic pathogen in immunocompromised patients. *Clin Infect Dis*. 2015;60:881–888.
57. Caballero S, Guix S, El-Senousy WM, et al. Persistent gastroenteritis in children infected with astrovirus: association with serotype-3 strains. *J Med Virol*. 2003;71:245–250.
61. Kurtz JB, Lee TW, Craig JW, et al. Astrovirus infection in volunteers. *J Med Virol*. 1979;3:221–230.
64. González GG1, Pujol FH, Liprandi F, et al. Prevalence of enteric viruses in human immunodeficiency virus seropositive patients in Venezuela. *J Med Virol*. 1998;55:288–292.
65. Wunderli W, Meerbach A, Güngör T, et al. Astrovirus infection in hospitalized infants with severe combined immunodeficiency after allogeneic hematopoietic stem cell transplantation. *PLoS ONE*. 2011;6:e27483.
70. Al-Thani A, Baris M, Al-Lawati N, et al. Characterising the aetiology of severe acute gastroenteritis among patients visiting a hospital in Qatar using real-time polymerase chain reaction. *BMC Infect Dis*. 2013;13:329.
71. Björkholm M, Celsing F, Runarsson G, et al. Successful intravenous immunoglobulin therapy for severe and persistent astrovirus gastroenteritis after fludarabine treatment in a patient with Waldenström's macroglobulinemia. *Int J Hematol*. 1995;62:117–120.
72. Superti F, Seganti L, Orsi N, et al. In vitro effect of synthetic flavonoids on astrovirus infection. *Antiviral Res*. 1990;13:201–208.
73. Bosch A, Pintó RM, Guix S. Human astroviruses. *Clin Microbiol Rev*. 2014;27:1048–1074.
74. Ganesh B, Banyai K, Martella V, et al. Picobirnavirus infections: viral persistence and zoonotic potential. *Rev Med Virol*. 2012;22:245–256.
75. Ganesh B, Masachessi G, Mladenova Z. Animal picobirnavirus. *Virusdisease*. 2014;25:223–238.
76. Wakuda M, Pongsuwanna Y, Taniguchi K. Complete nucleotide sequences of two RNA segments of human picobirnaviruses. *J Virol Methods*. 2005;126:165–169.
77. Taniguchi K, Wakuda M. [Picobirnavirus]. [in Japanese]. *Uirusu*. 2005;55:297–302.
78. Bhattacharya R, Sahoo GC, Nayak MK, et al. Molecular epidemiology of human picobirnaviruses among children of a slum community in Kolkata, India. *Infect Genet Evol*. 2006;6:453–458.
79. Chandra R. Picobirnavirus, a novel group of undescribed viruses of mammals and birds: a minireview. *Acta Virol*. 1997;41:59–62.
81. Giordano MO, Masachessi G, Martinez LC, et al. Two instances of large genome profile picobirnavirus occurrence in Argentinian infants with diarrhea over a 26-year period (1977-2002). *J Infect*. 2008;56:371–375.
84. Ganesh B, Nagashima S, Ghosh S, et al. Detection and molecular characterization of multiple strains of Picobirnavirus causing mixed infection in a diarrheic child: emergence of prototype Genogroup II-like strain in Kolkata, India. *Int J Mol Epidemiol Genet*. 2011;2:61–72.
85. Legoff J, Resche-Rigon M, Bouquet J, et al. The eukaryotic gut virome in hematopoietic stem cell transplantation: new clues in enteric graft-versus-host disease. *Nat Med*. 2017;23:1080–1085.
86. Smits SL, Schapendonk CM, van Beek J, et al. New viruses in idiopathic human diarrhea cases, the Netherlands. *Emerg Infect Dis*. 2014;20:1218–1222.
87. Gallimore CI, Appleton H, Lewis D, et al. Detection and characterisation of bisegmented double-stranded RNA viruses (picobirnaviruses) in human faecal specimens. *J Med Virol*. 1995;45:135–140.

References

1. De Benedictis P, Schultz-Cherry S, Burnham A, et al. Astrovirus infections in humans and animals—molecular biology, genetic diversity, and interspecies transmissions. *Infect Genet Evol*. 2011;11:1529–1544.
2. Bosch A, Pintó RM, Guix S. Human astroviruses. *Clin Microbiol Rev*. 2014;27:1048–1074.
3. Méndez E, Arias CF. Astroviruses. In: Knipe DM, Howley PM, eds. *Fields Virology*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013:600–620.
4. Herrmann JE, Hudson RW, Perron-Henry DM, et al. Antigen characterization of cell-cultivated astrovirus serotypes and development of astrovirus-specific monoclonal antibodies. *J Infect Dis*. 1988;158:182–185.
5. Willcocks MM, Carter MJ, Laidler FR, et al. Growth and characterisation of human faecal astrovirus in a continuous cell line. *Arch Virol*. 1990;113:73–81.
6. Yamashita T, Kobayashi S, Sakae K, et al. Isolation of cytopathic small round viruses with BS-C-1 cells from patients with gastroenteritis. *J Infect Dis*. 1991;164: 954–957.
7. Lee TW, Kurtz JB. Prevalence of human astrovirus serotypes in the Oxford region 1976-1992, with evidence for two new serotypes. *Epidemiol Infect*. 1994;112:187–193.
8. Noel JS, Lee TW, Kurtz JB, et al. Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing. *J Clin Microbiol*. 1995;33: 797–801.
9. Taylor MB, Walter J, Berke T, et al. Characterisation of a South African human astrovirus as type 8 by antigenic and genetic analyses. *J Med Virol*. 2001;64:256–261.
10. Chu DK, Chin AW, Smith GJ, et al. Detection of novel astroviruses in humans. *J Gen Virol*. 2010;91(Pt 10):2457–2462.
11. Gabbay YB, Linhares AC, Cavalcante-Pepino EL, et al. Prevalence of human astrovirus genotypes associated with acute gastroenteritis among children in Bélem, Brazil. *J Med Virol*. 2007;79:530–538.
12. Cortez V, Meliopoulos VA, Karlsson EA, et al. Astrovirus biology and pathogenesis. *Annu Rev Virol*. 2017;4: 327–348.
13. Kurtz JB, Lee TW. Human astrovirus serotypes [letter]. *Lancet*. 1984;2:1405.
14. Hudson RW, Herrmann JE, Blacklow NR. Plaque quantitation and virus neutralization assays for human astroviruses. *Arch Virol*. 1989;108:33–38.
15. Risco C, Carrascosa JL, Pedregosa AM, et al. Ultrastructure of human astrovirus serotype 2. *J Gen Virol*. 1995;76:2075–2080.
16. Jiang B, Monroe SS, Koonin EV, et al. RNA sequence of astrovirus: distinctive genomic organization and a putative retrovirus-like ribosomal frameshifting signal that directs the viral replicase synthesis. *Proc Natl Acad Sci USA*. 1993;90:10539–10543.
17. Willcocks MM, Brown TDK, Madeley CR, et al. The complete sequence of a human astrovirus. *J Gen Virol*. 1994;75:1785–1788.
18. Caballero S, Guix S, Ribes E, et al. Structural requirements of astrovirus virus-like particles assembled in insect cells. *J Virol*. 2004;78:13285–13292.
19. Dalton RM, Pastrana EP, Sanchez-Fanquier A. Vaccinia virus recombinant expressing an 87-kilodalton polyprotein that is sufficient to form astrovirus-like particles. *J Virol*. 2003;77:9094–9098.
20. Strain E, Kelley LA, Schultz-Cherry S, et al. Genomic analysis of closely related astroviruses. *J Virol*. 2008;82:5099–5103.
21. Monroe SS, Stine SE, Gorelkin L, et al. Temporal synthesis of proteins and RNAs during human astrovirus infection of cultured cells. *J Virol*. 1991;65:641–648.
22. Matsui SM, Kim JP, Greenberg HB, et al. Cloning and characterization of human astrovirus immunoreactive epitopes. *J Virol*. 1993;67:1712–1715.
23. Midthun K, Greenberg HB, Kurtz JB, et al. Characterization and seroepidemiology of a type 5 astrovirus associated with an outbreak of gastroenteritis in Marin County, California. *J Clin Microbiol*. 1993;31: 955–962.
24. Monroe SS, Glass RI, Noah N, et al. Electron microscopic reporting of gastrointestinal viruses in the United Kingdom, 1985-1987. *J Med Virol*. 1991;33:193–198.
25. Belliot G, Laveran H, Monroe SS. Capsid protein composition of reference strains and wild isolates of human astroviruses. *Virus Res*. 1997;49:49–57.
26. Malasao R, Khamrin P, Chaimongkol N, et al. Diversity of human astrovirus genotypes circulating in children with acute gastroenteritis in Thailand during 2000-2011. *J Med Virol*. 2012;84:1751–1756.
27. Mitchell DK, Matson DO, Cubitt WD, et al. Prevalence of antibodies to astrovirus types 1 and 3 in children and adolescents in Norfolk, Virginia. *Pediatr Infect Dis J*. 1999;18:249–254.
28. Kriston S, Willcocks MM, Carter MJ, et al. Seroprevalence of astrovirus types 1 and 6 in London, determined using recombinant virus antigen. *Epidemiol Infect*. 1996;117:159–164.
29. Koopmans MP, Bijen MH, Monroe SS, et al. Age-stratified seroprevalence of neutralizing antibodies to astrovirus types 1 to 7 in humans in the Netherlands. *Clin Diagn Lab Immunol*. 1998;5:33–37.
30. Espul A, Martinez N, Noel JS, et al. Prevalence and characterization of astroviruses in Argentinean children with acute gastroenteritis. *J Med Virol*. 2004;72:75–82.
31. Guix S, Caballero S, Villena C, et al. Molecular epidemiology of astrovirus infection in Barcelona, Spain. *J Clin Microbiol*. 2002;40:133–139.
32. Resque HR, Munford V, Castilho JG, et al. Molecular characterization of astrovirus in stool samples from children in São Paulo, Brazil. *Mem Inst Oswaldo Cruz*. 2007;102:969–974.
33. Nguyen TA, Hoang L, Pham LD, et al. Identification of human astrovirus infections among children with acute gastroenteritis in the southern part of Vietnam during 2005-2006. *J Med Virol*. 2008;80:298–305.
34. Olortegui MP, Rouhani S, Yori PP, et al. Astrovirus infection and diarrhea in 8 countries. *Pediatrics*. 2018;141:pii: e20171326.
35. Wang Y, Li Y, Jin Y, et al. Recently identified novel human astroviruses in children with diarrhea, China. *Emerg Infect Dis*. 2013;19:1333–1335.
36. Polombo EA, Bishop RF. Annual incidence, serotype distribution, and genetic diversity of astrovirus isolates from hospitalized children in Melbourne, Australia. *J Clin Microbiol*. 1996;34:1750–1753.
37. Walter JE, Mitchell DK. Role of astroviruses in childhood diarrhea. *Curr Opin Pediatr*. 2000;12:275–279.
38. Méndez-Toss M, Griffin DD, Calva J, et al. Prevalence and genetic diversity of human astroviruses in Mexican children with symptomatic and asymptomatic infections. *J Clin Microbiol*. 2004;42:151–157.
39. Madeley CR, Cosgrove BP. 28 nm particles in faeces in infantile gastroenteritis [letter]. *Lancet*. 1975;2:451.
40. Esahli H, Breback K, Bennet R, et al. Astroviruses as a cause of nosocomial outbreaks of infant diarrhea. *Pediatr Infect Dis J*. 1991;10:511–515.
41. Svraka S, Duizer E, Vennema H, et al. Etiological role of viruses in outbreaks of acute gastroenteritis in the Netherlands from 1994 through 2005. *J Clin Microbiol*. 2007;45:1389–1394.
42. Lyman WH, Walsh JF, Kotch JB, et al. Prospective study of etiologic agents of acute gastroenteritis outbreaks in child care centers. *J Pediatr*. 2009;154:253–257.
43. Jeong HS, Jeong A, Cheon DS. Epidemiology of astrovirus infection in children. *Korean J Pediatr*. 2012;55:77–82.
44. Oishi I, Yamazaki K, Kimoto T, et al. A large outbreak of acute gastroenteritis associated with astrovirus among students and teachers in Osaka, Japan. *J Infect Dis*. 1994;170:439–443.
45. Belliot G, Laveran H, Monroe SS. Outbreak of gastroenteritis in military recruits associated with serotype 3 astrovirus infection. *J Med Virol*. 1997;51:101–106.
46. Gray JJ, Wreghitt TG, Cubitt WD, et al. An outbreak of gastroenteritis in a home for the elderly associated with astrovirus type 1 and human calicivirus. *J Med Virol*. 1987;23:377–381.
47. Grohmann GS, Glass RI, Pereira HG, et al. Enteric viruses and diarrhea in HIV-infected patients. Enteric Opportunistic Infections Working Group. *N Engl J Med*. 1993;329:14–20.
48. Cox GJ, Matsui SM, Lo RS, et al. Etiology and outcome of diarrhea after marrow transplantation: a prospective study. *Gastroenterology*. 1994;107:1398–1407.
49. Gallimore CI, Taylor C, Gennery AR, et al. Use of a heminested reverse transcriptase PCR assay for detection of astrovirus in environmental swabs from an outbreak of gastroenteritis in a pediatric primary immunodeficiency unit. *J Clin Microbiol*. 2005;43:3890–3894.
50. Coppo P, Scieux C, Ferchal F, et al. Astrovirus enteritis in a chronic lymphocytic leukemia patient treated with fludarabine monophosphate. *Ann Hematol*. 2000;79: 43–45.
51. Liste MB, Natera I, Suarez JA, et al. Enteric virus infections and diarrhea in healthy and human immunodeficiency virus-infected children. *J Clin Microbiol*. 2000;38: 2873–2877.
52. Giordano MO, Martinez LC, Rinaldi D, et al. Diarrhea and enteric emerging viruses in HIV-infected patients. *AIDS Res Hum Retrovir*. 1999;15:1427–1432.
53. Quan PL, Wagner TA, Briese T, et al. Astrovirus encephalitis in boy with X-linked agammaglobulinemia. *Emerg Infect Dis*. 2010;16:918–925.
54. Brown JR, Morfopoulou S, Hubb J, et al. Astrovirus VA1/HMO-C: an increasingly recognized neurotropic pathogen in immunocompromised patients. *Clin Infect Dis*. 2015;60:881–888.
55. Lew JF, Glass RI, Petric M, et al. Six-year retrospective surveillance of gastroenteritis viruses identified at ten electron microscopy centers in the United States and Canada. *Pediatr Infect Dis J*. 1990;9:709–714.
56. Cruz JR, Bartlett AV, Herrmann JE, et al. Astrovirus-associated diarrhea among Guatemalan ambulatory rural children. *J Clin Microbiol*. 1992;30:1140–1144.
57. Caballero S, Guix S, El-Senousy WM, et al. Persistent gastroenteritis in children infected with astrovirus: association with serotype-3 strains. *J Med Virol*. 2003;71:245–250.
58. Snodgrass DR, Angus KW, Gray EW, et al. Pathogenesis of diarrhoea caused by astrovirus infection in lambs. *Arch Virol*. 1979;60:217–226.
59. Woode GN, Bridger JC. Isolation of small viruses resembling astroviruses and caliciviruses from acute enteritis of calves. *J Med Microbiol*. 1978;11:441–452.
60. Thouvenelle ML, Haynes JS, Sell JL, et al. Astrovirus infection in hatchling turkeys: alterations in intestinal maltase activity. *Avian Dis*. 1995;39:343–348.
61. Kurtz JB, Lee TW, Craig JW, et al. Astrovirus infection in volunteers. *J Med Virol*. 1979;3:221–230.
62. Dennehy PH, Nelson SM, Spangenberger S, et al. A prospective case-control study of the role of astrovirus in acute diarrhea among hospitalized young children. *J Infect Dis*. 2001;184:10–15.
63. Mitchell DK, Monroe SS, Jiang X, et al. Virologic features of an astrovirus diarrhea outbreak in a day care center revealed by reverse transcriptase-polymerase chain reaction. *J Infect Dis*. 1995;172:1437–1444.
64. González GG1, Pujol FH, Liprandi F, et al. Prevalence of enteric viruses in human immunodeficiency virus seropositive patients in Venezuela. *J Med Virol*. 1998;55:288–292.
65. Wunderli W, Meerbach A, Güngör T, et al. Astrovirus infection in hospitalized infants with severe combined immunodeficiency after allogeneic hematopoietic stem cell transplantation. *PLoS ONE*. 2011;6:e27483.
66. Naficy AB, Rao MR, Holmes JL, et al. Astrovirus diarrhea in Egyptian children. *J Infect Dis*. 2000;182:685–690.
67. Molberg O, Nilsen EM, Sollid LM, et al. $CD4^+$ T cells with specific reactivity against astrovirus isolated from normal human small intestine [see comments]. *Gastroenterology*. 1998;114:115–122.
68. Herrmann JE, Nowak NA, Perron-Henry DM, et al. Diagnosis of astrovirus gastroenteritis by antigen detection with monoclonal antibodies. *J Infect Dis*. 1990;161: 226–229.
69. Major ME, Eglin RP, Easton AJ. 3′ terminal nucleotide sequence of human astrovirus type 1 and routine detection of astrovirus nucleic acid and antigens. *J Virol Methods*. 1992;3:217–225.
70. Al-Thani A, Baris M, Al-Lawati N, et al. Characterising the aetiology of severe acute gastroenteritis among patients visiting a hospital in Qatar using real-time polymerase chain reaction. *BMC Infect Dis*. 2013;13:329.
71. Björkholm M, Celsing F, Runarsson G, et al. Successful intravenous immunoglobulin therapy for severe and persistent astrovirus gastroenteritis after fludarabine treatment in a patient with Waldenström's macroglobulinemia. *Int J Hematol*. 1995;62:117–120.
72. Superti F, Seganti L, Orsi N, et al. In vitro effect of synthetic flavonoids on astrovirus infection. *Antiviral Res*. 1990;13:201–208.
73. Bosch A, Pintó RM, Guix S. Human astroviruses. *Clin Microbiol Rev*. 2014;27:1048–1074.
74. Ganesh B, Banyai K, Martella V, et al. Picobirnavirus infections: viral persistence and zoonotic potential. *Rev Med Virol*. 2012;22:245–256.
75. Ganesh B, Masachessi G, Mladenova Z. Animal picobirnavirus. *Virusdisease*. 2014;25:223–238.
76. Wakuda M, Pongsuwanna Y, Taniguchi K. Complete nucleotide sequences of two RNA segments of human picobirnaviruses. *J Virol Methods*. 2005;126:165–169.
77. Taniguchi K, Wakuda M. [Picobirnavirus]. [in Japanese]. *Uirusu*. 2005;55:297–302.
78. Bhattacharya R, Sahoo GC, Nayak MK, et al. Molecular epidemiology of human picobirnaviruses among children of a slum community in Kolkata, India. *Infect Genet Evol*. 2006;6:453–458.
79. Chandra R. Picobirnavirus, a novel group of undescribed viruses of mammals and birds: a minireview. *Acta Virol*. 1997;41:59–62.
80. Pereira HG, Fialho AM, Flewett TH, et al. Novel viruses in human faeces. *Lancet*. 1988;2:103–104.
81. Giordano MO, Masachessi G, Martinez LC, et al. Two instances of large genome profile picobirnavirus occurrence in Argentinian infants with diarrhea over a 26-year period (1977-2002). *J Infect*. 2008;56:371–375.
82. Giordano MO, Martinez LC, Rinaldi D, et al. Detection of picobirnavirus in HIV-infected patients with diarrhea in Argentina. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998;18:380–383.

83. Gonzalez GG, Pujol FH, Liprandi F, et al. Prevalence of enteric viruses in human immunodeficiency virus seropositive patients in Venezuela. *J Med Virol*. 1998;55:288–292.
84. Ganesh B, Nagashima S, Ghosh S, et al. Detection and molecular characterization of multiple strains of Picobirnavirus causing mixed infection in a diarrheic child: emergence of prototype Genogroup II-like strain in Kolkata, India. *Int J Mol Epidemiol Genet*. 2011;2:61–72.
85. Legoff J, Resche-Rigon M, Bouquet J, et al. The eukaryotic gut virome in hematopoietic stem cell transplantation: new clues in enteric graft-versus-host disease. *Nat Med*. 2017;23:1080–1085.
86. Smits SL, Schapendonk CM, van Beek J, et al. New viruses in idiopathic human diarrhea cases, the Netherlands. *Emerg Infect Dis*. 2014;20:1218–1222.
87. Gallimore CI, Appleton H, Lewis D, et al. Detection and characterisation of bisegmented double-stranded RNA viruses (picobirnaviruses) in human faecal specimens. *J Med Virol*. 1995;45:135–140.

178 Hepatitis E Virus

Hirsh D. Trivedi and Sanjiv Chopra

SHORT VIEW SUMMARY

Definition
- Hepatitis E virus (HEV) is an RNA virus which is a member of the Hepeviridae family, genus *Hepevirus,* composed of five genotypes, of which four are associated with human infection.

Epidemiology
- HEV was first recognized in the 1980s and has emerged as the leading cause of acute viral hepatitis worldwide. It can occur as outbreaks or in sporadic forms.
- Initially, HEV was thought to be present only in underdeveloped nations, but it has been noted in developed countries, where it is likely underdiagnosed.
- One-third of the world's population has positive seroprevalence for IgG antibodies against HEV, suggesting prior exposure.
- The most common forms of transmission are through fecal-oral contamination of drinking water supplies (genotypes 1 and 2) and through zoonoses (genotypes 3 and 4). (See Table 178.1.)
- Acute HEV infection is most often self-limiting. However, in women in the third trimester of pregnancy, it has a striking mortality of 20% to 25% (genotype 1). It can also be seen in immunocompromised patients.
- Chronic HEV has been described in organ-transplant recipients (genotype 2).

Microbiology
- HEV is a nonenveloped, icosahedral virus that measures 32 nm in diameter.
- It has a positive-sense, single-stranded RNA genome with three open reading frames.
- There are four genotypes that cause infection in humans. (See Table 178.1.)

Diagnosis
- Clinical characteristics are similar to those of other forms of acute viral hepatitis.
- Diagnosis with immunoglobulin M (IgM) antibodies to HEV is used in endemic areas.
- Reverse-transcriptase polymerase chain reaction (RT-PCR) is required for diagnosis in nonendemic areas. It is more sensitive and specific than serologic diagnosis.

Therapy
- Supportive care is the mainstay of therapy because most cases are self-limiting.
- Ribavirin treatment and interferon treatment have been used in severe acute cases and in immunosuppressed patients with encouraging results.

Prevention
- Maintaining clean water supplies is the cornerstone of prevention of HEV.
- A vaccine that is safe and effective has been approved in China but not yet in other countries.

Hepatitis E virus (HEV) is a ribonucleic acid (RNA) virus that causes a viral hepatitis that is indistinguishable from other forms of viral hepatitis. The World Health Organization (WHO) estimates a global incidence of 20 million cases of HEV infection annually, accounting for 3.3% of mortality from viral hepatitis.[1] HEV infection most often results in an acute, self-limited disease that does not necessitate hospitalization. Rarely, it can be a fatal cause of viral hepatitis. Certain individuals, such as those who are pregnant, are immunocompromised, or have coinfections, are at higher risk of significant hepatitis and death. The virus was first recognized in 1980 after several outbreaks of infection.[2–5] It has since emerged as an important and often missed cause of viral hepatitis. Initially thought to be limited only to underserved, underdeveloped nations, HEV has been increasingly documented in developed countries and is now the most common cause of acute viral hepatitis worldwide.

A BRIEF HISTORY

The disease was first recognized in the 1980s when sera collected during a large epidemic of viral hepatitis in New Delhi, India in 1955[6] was found to lack the typical serologic markers of hepatitis A or B virus.[5,7] This virus was therefore referred to as enterically transmitted, water-borne hepatitis non-A, non-B type virus.[5] Similar observations in serologic studies were made after an epidemic of viral hepatitis in 1978, in Kashmir, India.[5,8] During the Soviet occupation of Afghanistan in the 1980s, there was another unexplained outbreak of viral hepatitis at a military camp.[2] A member of the research team ingested a pool of fecal extract from the soldiers and became ill. The new virus was detected in the researcher's stool with electron microscopy. Electron microscopic diagnosis was also used in other epidemics to identify the virus (Fig. 178.1).[9] In the 18th and 19th centuries, several hepatitis outbreaks around the world had features that were similar to those of the HEV epidemics.[10] Endemic HEV infection was subsequently discovered in many underdeveloped countries, where it was thought to be more prevalent. More recently, HEV infection has been observed even in developed nations.

GENETICS

HEV is a small, icosahedral, nonenveloped single-stranded RNA virus with a positive-sense genome.[1] It measures 27 to 34 nm in diameter and is a member of the Hepeviridae family and *Hepevirus* genus. Large open reading frames (ORFs) of the RNA virus have been described; the largest one consists of 1693 codons.[11]

There are four genotypes of the virus that can infect humans (Table 178.1).[1] Genotypes 1 and 2 cause acute disease and are found only in humans, whereas genotypes 3 and 4 are found in animals (including pigs, deer wild boars, and others). Genotypes 3 and 4 can occasionally infect humans but do not cause acute disease.

NATURAL HISTORY OF HEPATITIS E

Burden of Disease

Hepatitis E is more common than previously realized. An estimated 20 million HEV infections annually occur worldwide.[1,12] About 3.3 million of these cases are symptomatic HEV infection.[1] WHO estimated

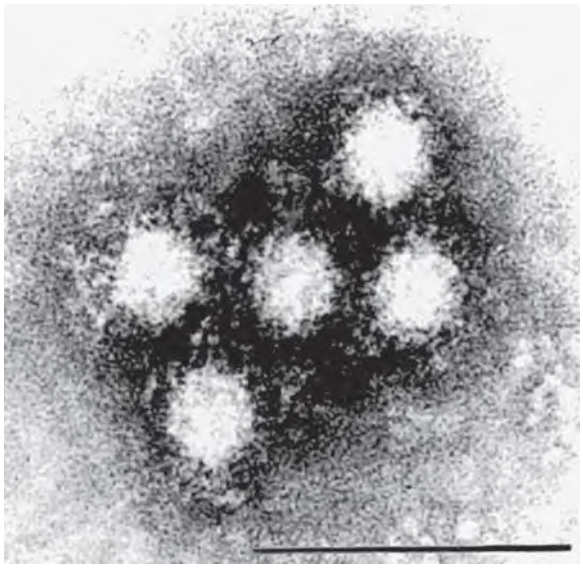

FIG. 178.1 Electron microscopy of antibody-coated hepatitis E virus particles in feces. *(Modified from Ticehurst J, Popkin TJ, Bryan JP, et al. Association of hepatitis E virus with an outbreak of hepatitis in Pakistan: serologic responses and pattern of virus excretion.* J Med Virol. *1992;36:84–92.)*

44,000 deaths due to HEV infection in 2015, accounting for 3.3% of the mortality cases of viral hepatitis.[1] Large outbreaks and sporadic cases occur frequently in endemic areas compared with nonendemic countries. Case-fatality rates in endemic areas range from 0.5% to 4% in hospital-based data and 0.07% to 0.6% in population surveys during outbreaks.[3,7]

Seroprevalence is characterized as positivity for immunoglobulin G (IgG) antibody against HEV without active infection. Data on the seroprevalence of HEV infection suggest a lifetime exposure risk in one-third of the world's population.[12] The prevalence of HEV antibody is 10% to 70% in developing countries, compared with 1% to 21% in developed countries.[13–15]

Geographic Distribution

HEV infection is a global health concern (Fig. 178.2). It is the most common cause of acute viral hepatitis worldwide. HEV was originally thought to occur only in underdeveloped nations with frequent water contamination but is now an emerging infectious disease in developed countries with safe drinking water supplies.[16] The highest prevalence of HEV infection is in East and South Asia.[1] It is the most common cause of acute hepatitis in highly endemic areas such as India, Egypt, and parts of China.[17] Sporadic cases of infection have been reported in South Africa,[18] Tunisia, Chad, and Morocco.[19] An epidemic of HEV has been noted in Mexico.[20] Cuba has been reported to have a high prevalence of clinically apparent HEV infection,[21,22] whereas only isolated cases of infection have been observed in South America.[23–25] Areas that

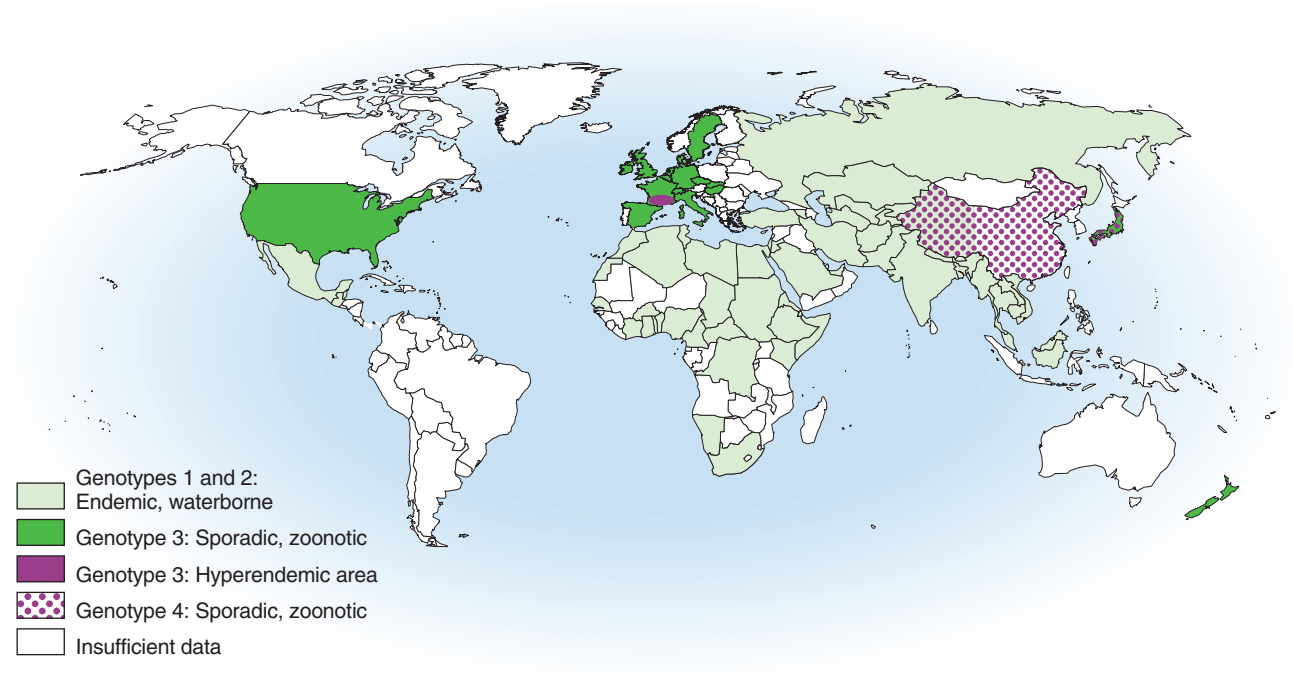

FIG. 178.2 Geographic distribution of clinical cases of hepatitis E virus infection. *(Modified from Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E.* Lancet. *2012;379:2477–2488.)*

TABLE 178.1 Genotype of Hepatitis E Virus

	GENOTYPE 1	GENOTYPE 2	GENOTYPE 3	GENOTYPE 4
Presentation	Acute	Acute	Chronic	Chronic
Source	Humans	Humans	Animals	Animals
Transmission	Contaminated water	Contaminated water	Undercooked meat	Undercooked meat
Geographic Distribution	Asia, India, North Africa	Asia, India, North and West Africa, Mexico	Western countries, Asia, North America	Asia, Europe

are commonly affected include hospitals,[26] war zones, and camps for refugees or displaced populations, where the water supply is often contaminated.[1] HEV has led to major epidemics in refugee settings such as Darfur, Sudan.[27,28] Developed countries do not experience such outbreaks but can encounter sporadic cases of HEV infection. The true incidence and prevalence of HEV infection in developed countries has not yet been systematically investigated.

Recent outbreaks of HEV infection have been reported. An outbreak in Am Timan, Chad involved 693 cases of HEV infection and led to 11 deaths from acute jaundice syndrome. In April 2017, WHO was informed of another outbreak of HEV infection in the Diffa region of Niger. Of the 282 cases reported, 27 resulted in death. All cases resulting in death, except 1, occurred in pregnant women.

Transmission

Fecal-oral spread is the most common route of transmission for HEV infection (Fig. 178.3). Infected individuals excrete the virus in their stool. Fecal contamination of drinking water supplies leads to widespread outbreaks of HEV in underdeveloped countries. The infected person excretes the virus in the stool starting a few days before to 3 to 4 weeks after the development of clinical symptoms. In developed nations, however, fecal contamination of food products and undercooked meats is more common, leading to sporadic cases of HEV infection.

Transmission through other routes does occur but is far less common.[1] These include vertical transmission from mother to child and transfusion of infected blood products. In addition, the ingestion of uncooked or raw shellfish can also transmit infection. Individuals with immunosuppression or chronic liver disease are susceptible to infection from the consumption of these foods.

CLINICAL PRESENTATION

Acute Hepatitis E

The more common form of HEV infection is acute viral hepatitis. It is usually caused by genotypes 1 and 2. Acute HEV clinically resembles other forms of viral hepatitis infection. Most cases of HEV infection are self-limiting. The average incubation period after exposure to HEV is 15 to 70 days. Early reports suggested an incubation period of 28 to 40 days between exposure and clinical onset of disease (Fig. 178.4).[29] Symptomatic infection within endemic areas is most common in young adults between 15 and 40 years of age; symptoms in children are less prevalent, often leading to the lack of diagnosis.

The clinical presentation of HEV infection is similar to that of other forms of acute viral hepatitis and often indistinguishable. HEV and hepatitis A virus share similar characteristics, making them difficult to differentiate (Table 178.2). The clinical presentation usually starts with a prodromal phase. The clinical symptoms include, but are not limited to, fever, anorexia, nausea, vomiting, abdominal pain, skin rash, and arthralgia. The prodromal phase usually lasts from 1 to 7 days and is followed by pruritus and jaundice, lasting up to a few weeks. Dark urine and pale-colored stools often accompany jaundice. Organomegaly,

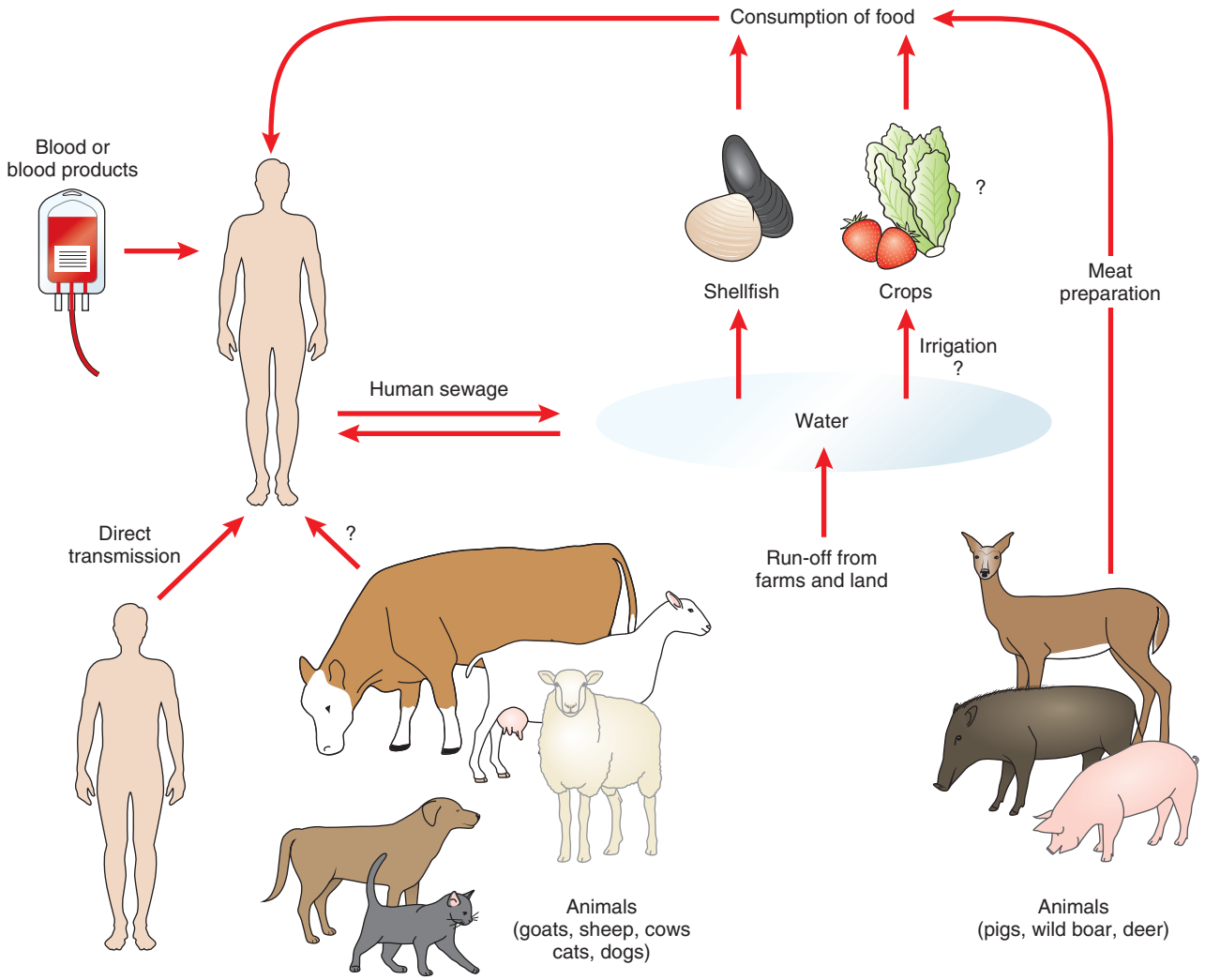

FIG. 178.3 Sources and routes of transmission of hepatitis E virus. *(Modified from Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E.* Lancet. *2012;379:2477–2488.)*

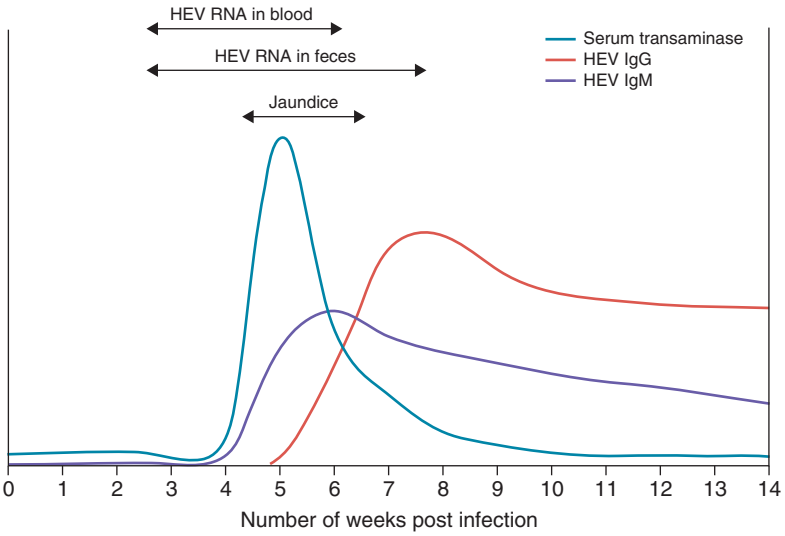

FIG. 178.4 Time course of hepatitis E virus infection including viral detection and shedding, clinical manifestations, and serologic response. HEV, Hepatitis E virus; IgG, immunoglobulin G; IgM, immunoglobulin M. *(Modified from Dalton HR, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries.* Lancet Infect Dis. *2008;8:698–709.)*

TABLE 178.2 Comparison Between Hepatitis A Virus and Hepatitis E Virus

CHARACTERISTICS	HEPATITIS A	HEPATITIS E
Genus and family	*Hepatovirus,* Picornaviridae	*Hepevirus,* Hepeviridae
Common mode of transmission	Fecal-oral route	Fecal-oral route
Incidence	1.4 million cases per year	20 million cases per year
Mortality rate	0.2%	1.0%
Clinical presentation	Self-limiting Fulminant hepatitis rare Cholestatic injury in <5%	Self-limiting Fulminant hepatitis is 20%–25% in third trimester of pregnancy Cholestatic liver injury in 60%
Geographic distribution	Developed and underdeveloped nations	Developed and underdeveloped nations
Diagnosis	HAV-IgM RT-PCR	Exclude HAV Endemic: HEV-IgM Nonendemic: RT-PCR
Treatment	Supportive care	Supportive care RBV or IFN in selected cases
Vaccination	Available	Available only in China

HAV, Hepatitis A virus; *HEV,* hepatitis E virus; *IFN,* interferon; *IgM,* immunoglobulin M; *RBV,* ribavirin; *RT-PCR,* reverse-transcriptase polymerase chain reaction.

abdominal tenderness, jaundice, and skin excoriations from itching can be found at clinical examination.

Acute hepatitis E can rarely result in severe, fulminant hepatitis and death. The results of laboratory and investigative tests are often abnormal and can help aid in the diagnosis. The abnormalities include elevations in alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), and direct bilirubin from a conjugated hyperbilirubinemia. In experimental studies, liver enzyme elevations peaked at 42 to 46 days after ingestion of the virus.[9] In animal studies of macaques administered intravenous challenge with a high dose inoculum of HEV, viremia was detected as early as 9 days after exposure, and disease became apparent as early as 2 weeks after exposure.[30–32] The serum ALT elevation may precede symptoms but does not correlate with severity of disease. Mild leukopenia and relative lymphocytosis can also be seen. An abdominal ultrasound examination is important in order to rule out biliary obstruction. Ultrasound findings in HEV infection can include an enlarged liver with increased echogenicity, enlarged spleen, edematous gallbladder wall, and prominence of portal venules. Most often, resolution of HEV infection occurs with normalization of serum ALT and bilirubin levels within 6 weeks. Histologic resolution lags behind and may take up to 6 months.

Chronic Hepatitis E

Hepatitis E was initially thought to cause only acute infection. However, chronic hepatitis E has been identified in areas with low endemicity and with genotype 3 and genotype 4 infection.[7] Hepatitis E viremia and fecal excretion can last for several months to years in patients who are immunosuppressed, such as organ-transplant recipients,[33–36] those receiving chemotherapy,[37–39] and those with human immunodeficiency virus (HIV) coinfection.[40–42] Individuals with chronic HEV infection can have a variable clinical presentation ranging from unremarkable symptoms to signs of liver disease with elevated serum laboratory parameters and progressive inflammation that can culminate in fibrosis and cirrhosis.[35] However, it is unclear how often this occurs.

Extrahepatic Manifestations

Extrahepatic complications of HEV infection are seemingly rare, but do occur. Neurologic manifestations have particularly been observed in several case reports and case series. Genotypes 1 and 3 of HEV infection have been implicated in causing neurologic consequences in case studies. Several cases were identified in India and in parts of Europe, such as France.[43] The observed neurologic manifestations include, but are not limited to, acute meningoencephalitis,[44] acute transverse myelitis,[45] brachial plexus neuritis,[46] Bell palsy,[47] and Guillain-Barré syndrome.[48,49] A case series of genotype 3 infections also included neurologic sequela with ataxia, proximal myopathy, bilateral brachial neuritis, encephalitis, Guillain-Barré syndrome, and inflammatory polyradiculopathy.[50] Some patients had detectable HEV RNA in the cerebrospinal fluid (CSF).[50,51] In general, the patients who cleared their HEV infection had resolution of their neurologic complaints.[50]

Other extrahepatic systemic involvement has been noted with HEV infection. Hematologic manifestations including severe thrombocytopenia[52] and hemolytic anemia in patients with glucose phosphate dehydrogenase deficiency have been reported during acute cases of infection.[53] Acute pancreatitis is rare but has also been found in acute HEV infection.[54] Chronic HEV genotype 3 infections have been associated with renal manifestations such as membranous glomerulonephritis and

TABLE 178.3 Extrahepatic Manifestations of Hepatitis E Virus

SYSTEM OR TYPE	MANIFESTATION
Central nervous system and peripheral nervous system	Meningoencephalitis Ataxia Transverse myelitis Pseudotumor cerebri Pyramidal syndrome Bell palsy Oculomotor nerve palsy Guillain-Barré syndrome Neuralgic amyotrophy
Gastroenterologic	Acute pancreatitis
Hematologic	Anemia (hemolytic, aplastic) G6PD deficiency Thrombocytopenia Pure red cell aplasia Hemophagocytic syndrome Monoclonal gammopathy of undetermined significance
Immune-mediated	Autoimmune disease Thyroiditis Myocarditis Henoch-Schönlein purpura Myasthenia gravis
Renal	IgA nephropathy Membranous glomerulonephritis Membranoproliferative glomerulonephritis Nephroangiosclerosis

G6PD, Glucose-6-phosphate dehydrogenase; *IgA,* immunoglobulin A.

membranoproliferative glomerulonephritis.[55] Table 178.3 lists the full spectrum of possible extrahepatic manifestations of HEV infection, with the majority being noted in isolated cases.

Special Considerations

Certain health states predispose to more aggressive forms of HEV infection. A heightened awareness of these conditions is imperative because their presence can result in significantly worse outcomes with significant morbidity and mortality.

Pregnancy

Fulminant hepatitis occurs more frequently with HEV infection in pregnancy and leads to an increased mortality.[1] In particular, those in the second and third trimesters are at an increased risk of developing acute liver failure, fetal loss, and death. Mortality rates as high as 20% to 25% have been reported in women in the third trimester of pregnancy.[56–58] HEV viral loads with genotype 1 infection in pregnant patients are higher compared with nonpregnant women.[59] The mechanism by which HEV infection increases mortality in pregnancy is not clearly understood.[19]

In the Kashmir, India epidemic of 1978 involving around 270 patients, HEV infection developed in 17.3% of pregnant women, compared with 2.1% in nonpregnant women and 2.8% in men of similar age.[8] Of the pregnant patients who were infected, 19.4% and 18.6% were in the second and third trimesters, respectively. Fulminant hepatic failure developed in 22% of the infected pregnant women, leading to abortions, stillbirths, and neonatal deaths. Vertical transmission of HEV with severe hepatitis in neonates has been reported.[60,61] It has not been associated with congenital abnormalities.

Solid Organ Transplantation

Immunocompromised individuals, particularly those with solid organ transplantation, are susceptible to HEV infection. The incidence of genotype 3 infections in France has been reported to be 3.2 cases per 100 person-years.[62] Transmission occurs with consumption of wild meat, pork, and mussels after transplantation.[63] Transmission of HEV infection via the transplanted organ is possible, but fortunately rare.[62,64] In a single patient in Germany, hepatic failure and death were reported in the transplant recipient.[64] HEV reactivation after organ transplantation in individuals who are seropositive for the virus is usually not a concern[62] but has been reported in a patient after allogeneic hematopoietic stem cell transplantation for acute lymphoblastic leukemia.[65]

The clinical presentation of organ transplant recipients with chronic HEV infection is variable. Most patients do not have clinical symptoms, whereas others can develop signs and symptoms of progressive liver disease and jaundice.[34] Laboratory parameters show mild-to-moderate elevations compared with acute hepatitis, with the ALT being approximately 300 IU/L or six times the upper limit of normal.[34] Diagnosis is challenging because their immunocompromised state prevents them from developing HEV IgG or IgM antibodies and seroconversion may never occur.[34] Genomic techniques to confirm diagnosis and measure therapeutic response to treatment are critical.[19]

Around 60% of solid organ transplant recipients with acute HEV infection fail to clear the virus and go on to develop chronic hepatitis.[34] Several factors have been associated with reduced clearance of the virus after acute infection. These factors include the degree of immunosuppression, time since transplantation, presence of leukopenia, and total and T-cell lymphopenia.[33,34] However, a study using multivariate analysis identified the use of tacrolimus and the presence of thrombocytopenia as the only two predictive factors for the development of chronic HEV infection in patients after solid organ transplantation.[34] Individuals with chronic hepatitis may develop fibrosis,[66] with 10% of cases progressing to cirrhosis.[34] Liver transplantation may be necessary to prevent death from decompensated liver failure.[35] Recurrence of chronic HEV infection is fortunately rare, having been reported in a single patient after a second liver transplantation.[67]

Coinfections

HEV infection can occur in patients who are HIV positive. Fortunately, coinfection is extremely rare; the estimated incidence is 0% to 0.9%.[41,68–70] All identified coinfections with HIV virus are genotype 3 HEV infections, whereas other genotypes have not been recognized.[40–42,68–72] Patients with coinfection usually have acute hepatitis,[40–42] but one patient with cirrhosis has been reported.[40] Although acute hepatitis is more common, the development of chronic HEV infection is associated with low CD4 counts.[2]

DIAGNOSTIC EVALUATION

General Principles

Timely identification and accurate diagnosis of HEV are essential. The implications are important for patient management, clinical outcomes, reduction of transmission, and optimization of disease control. The clinical manifestations of HEV infection are similar to those of other causes of viral hepatitis, making its diagnosis difficult.[73–75] Ruling out hepatitis A virus infection is important in the initial diagnostic work up of HEV infection because hepatitis A and HEV infections share many similarities in their characteristics (see Table 178.2). Hepatitis B and C should also be ruled out, particularly in HEV-nonendemic areas.

HEV should be suspected in the appropriate epidemiologic and clinical setting. However, the increasing detection of HEV in nonendemic areas suggests that it should be an important consideration in dealing with all cases of acute hepatitis regardless of location. Knowledge of HEV among clinicians is lacking compared with other forms of viral hepatitis. The diagnosis of HEV infection is often missed, and other causes, such as drug-induced liver injury, may be suspected. One analysis found that 2.2% of cases that expert hepatologists designated as drug-induced liver injury were actually acute HEV infection.[76] As a result, it is absolutely imperative that treating clinicians have knowledge about HEV infection.

Serologic Evaluation

The diagnosis of acute HEV infection is usually made serologically. However, comparison studies have shown significant differences in the sensitivities and specificities of the different serologic tests,[77,78] suggesting the need for improved diagnostic options. The detection of IgM antibodies to HEV is often adequate in endemic areas to make the diagnosis of acute infection.[1,7] The anti-HEV IgM antibodies occur in early phases of infection and usually last 4 to 5 months (see Fig. 178.4). These

antibodies can be detected in the majority of HEV outbreaks. The sensitivities of anti-HEV IgM antibody assays range from 72% to 98%; the specificities range from 78% to 96%.[78] The presence of IgG antibodies against HEV suggests a convalescent phase or past or chronic infection.[7] IgG antibodies develop a few days after IgM antibodies and can remain positive up to several years, but the exact duration is unknown. IgA antibodies to HEV infection have also been identified in 50% of infected patients.[79] These antibodies typically have a rapid decline to undetectable levels but can occasionally persist longer than IgM antibodies.[80,81] The role of IgA in HEV immunity is unknown, but it may help in establishing the diagnosis.

Molecular Diagnosis

Although serologic diagnosis is widely available, it is not currently licensed for use within the United States. Detecting HEV RNA in the blood or stool with reverse-transcriptase polymerase chain reaction (RT-PCR) has higher specificity and is used in areas where HEV is less common or in order to diagnose chronic HEV infection. It is an essential confirmatory test for IgM-positive individuals in nonendemic areas[82] and can be used to monitor infection.[2] RT-PCR is also popularly used for research purposes,[83–87] but is often impractical in the field.[19] It requires specialized laboratory facilities, which often leads to a longer time to diagnosis. It has poor sensitivity in diagnosing acute infection[88] and has interlaboratory variability,[89] further limiting its use. A viral antigen detection test also exists and is currently under investigation for its diagnostic usefulness in HEV infection. Genomic-based methods are important for the diagnosis of chronic HEV infection because many patients never have seroconversion.[40] Use of molecular methods with HEV viremia is vital to monitor response to antiviral treatment inpatients with chronic HEV infection.[90,91]

Histopathology

Liver biopsy for histopathologic diagnosis of HEV infection can be considered when dealing with fulminant hepatitis or when serologic and molecular tests are not ideal. The histologic changes in HEV infection can reveal a pattern of cholestatic injury or a typical acute hepatitis picture.[2,92] The characteristic findings in HEV infection (Fig. 178.5) include a lobular disarray with portal tract enlargement, focal hepatocyte and bridging necrosis, hepatocyte ballooning and acidophilic degeneration, Kupffer cell proliferation, and mononuclear cell infiltration.[19] There is also intrahepatocytic dilation of endoplasmic reticulum cisternae and an increase of cytoplasmic lysosomes. The mitochondrial matrix may appear condensed with dilation of the outer mitochondrial membrane. Prominent features of the cholestatic injury associated with HEV infection include bile stasis and glandular transformation of liver cell plates. Cholestatic changes persist until clinical recovery, whereas the other histopathologic features generally resolve over a 3- to 6-month period.[19] These histologic changes are not distinctive to HEV and should be used in conjunction with clinical parameters for appropriate and timely diagnosis.

In order to optimize outcomes of fulminant HEV infection, accurate diagnosis in a timely fashion is imperative. The histologic features of fulminant hepatitis include parenchymal necrosis with lobular collapse, foamy hepatocyte swelling, acinar pattern of hepatocyte arrangement, small bile ductular proliferation, portal and central hepatic vein phlebitis, and portal inflammation with neutrophilic and lymphocytic proliferation.[19,56,92,93] Involvement of other sites, such as biliary epithelial cells and extrahepatic sites, has been discovered in animal models[94,95] but has not been noted in humans.

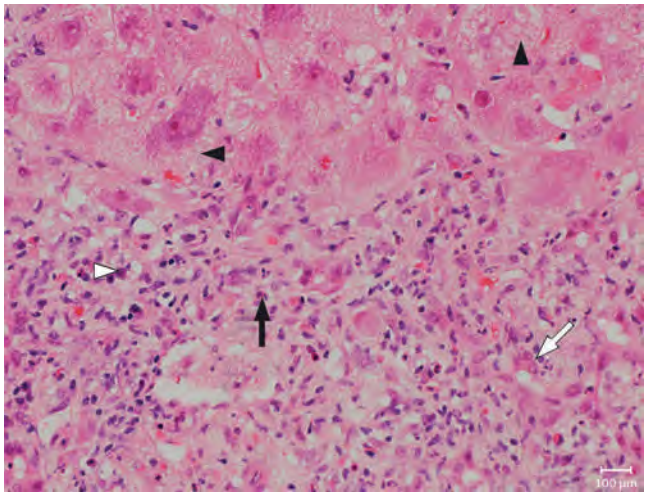

FIG. 178.5 Histologic appearance of liver of a patient with acute hepatitis E virus infection. In the hematoxylin and eosin–stained section, there is portal tract expansion with inflammatory infiltrate, occasional lymphoid aggregates, and bile ductile proliferation *(white arrow).* The inflammatory infiltrate consists of lymphocytes, including plasma cells *(white arrowhead),* neutrophils, and rare eosinophils *(black arrow).* The periportal hepatocytes show ballooning degeneration *(black arrowheads). (Modified from Dalton HR, Bendall R, Ijaz S, et al. Hepatitis E: an emerging infection in developed countries.* Lancet Infect Dis. *2008;8:698–709.)*

THERAPEUTIC STRATEGIES

Acute Hepatitis E

Acute HEV infection most commonly has a self-limiting course. Hospitalization is generally not required. Treatment revolves around supportive care, and specific therapy is rarely warranted. Patients with hepatic failure require admission to the intensive care unit, where measures to monitor for signs of cerebral edema can be used and evaluation for transplantation can be performed.[7] Pregnant patients, who are at higher risk of morbidity and mortality, also require hospitalization with close monitoring, although adequate therapeutic interventions for pregnant patients have yet to be established. The benefit of early termination of pregnancy has not been proven.[7] Patients with HEV genotype 3 infections have been noted to have a favorable response to ribavirin therapy, leading to reduced viral loads and improved clinical outcomes.[96,97] Ribavirin's teratogenicity contradicts its use in pregnancy, and alternative antiviral measures require investigation.[2]

Chronic Hepatitis E

The treatment of chronic HEV infection revolves around minimizing the use of immunosuppressive agents in organ transplant recipients. The reduction in immunosuppression can lead to clearance of HEV in about 30% of patients[66,90] and potentially decrease the risk of subsequent cirrhosis. Antiviral therapy should be considered for patients in whom a reduction of immunosuppression is not possible or in those who have not achieved viral clearance after decreased doses. Interferon-α and ribavirin either in combination or as monotherapy have been tried[90,91,98,99] and have been successful in certain cases.[39,99–102] Monotherapy with ribavirin can lead to viral clearance within a few weeks.[2] In a retrospective case series with a total of 59 solid-organ transplant recipients who received ribavirin monotherapy, 95% of patients were observed to have viral clearance with a median dose of 600 mg/day for a median duration of 3 months.[102] In addition, sofosbuvir, an RNA-dependent polymerase inhibitor used to treat patients with chronic hepatitis C, has been shown to inhibit replication of HEV and may provide additional antiviral effects.[103] Randomized trials have not been conducted, and treatments in other immunocompromised populations are less well studied.

PREVENTION

General Measures

Prevention of HEV infection is key in reducing the burden of disease. General measures of prevention should be based on the endemicity of the geographic area. In highly endemic areas, focus should be directed on maintaining clean drinking water supplies, protecting water from fecal contamination, and paying strict attention to sewage disposal.[7,19] In fact, many epidemics in underdeveloped countries occurred as a result of sewage pipes leaking into drinking water supplies, and hence maintaining a barrier between these two conduits is essential to prevent transmission of HEV infection.[19] Chlorination and filtration are usually

inadequate for cleaning a contaminated water supply.[28] Simple measures to improve the quality of water, such as boiling water, have led to a decline in new cases during epidemics.[7]

The history of previous outbreaks of HEV infection has led to the development of important preventive strategies. Often, extreme weather conditions can lead to massive contamination of water supplies in developing countries.[19] Certain environments such as refugee camps or other emergency settings are particularly susceptible to this type of contamination. In 2004, a significant outbreak of HEV in Darfur had a short 6-week interval from the first case of infection to rapidly reaching the peak of the epidemic.[27,28] Attempts at chlorination to sanitize water supplies were unsuccessful.[28] Timely detection of acute HEV infection in these settings is imperative.

In developed countries, strong public sanitation systems and low secondary HEV transmission rates lead to a low incidence of HEV infection. A study done in the United States in 1997 found no association between background HEV seroprevalence and risk factors for enterically transmitted viruses,[104] suggesting alternative routes of HEV transmission. In geographical locations with low endemicity of HEV infection, emphasis should be placed on avoiding undercooked meats and thoroughly cooking pork products to prevent zoonotic transmission.[7,19] These measures are particularly important in patients who are immunocompromised or have chronic liver disease

Travelers should take certain precautions when traveling to endemic areas of HEV infection. Measures should be geared toward avoiding the consumption of contaminated water, ice, and foods. Pregnant women should try to avoid travel to endemic areas altogether, and immunocompromised patients should pay attention to their food consumption.

Vaccination

Experimental studies in animal models have tested various vaccines containing recombinant HEV capsid proteins. Preclinical studies have demonstrated protection against hepatitis and viremia, but fecal excretion of virus was not prevented.[105] Cross-challenge studies in monkeys indicated that vaccines derived from one genotype could provide cross-protection against all four genotypes.[106]

Two vaccines have undergone trials in humans.[107] The first vaccine was with a recombinant truncated HEV capsid protein produced in insect cells as a virus-like particle (VLP) with aluminum hydroxide as an adjuvant.[107] In a phase II, double-blind, placebo-controlled safety and efficacy clinical trial in Nepal, nearly 2000 participants were randomly assigned to receive three doses of vaccine or a matched placebo at 0, 1, and 6 months.[107] Participants were followed for 2 years. There was a 95.5% protective effect against acute HEV infection after three doses of the vaccine.[107] The vaccinated individuals had high anti-HEV IgG levels at 1 month after the third dose of vaccine, but this effect had declined to 56% at the end of follow-up.[107]

A second vaccine with 239–amino acid truncated ORF2 protein is expressed in *Escherichia coli*.[108] This vaccine was studied in a large population-based trial in southern China with more than 110,000 subjects.[108] There was a robust protective effect against acute HEV infection at a rate of 100% during a 13-month follow-up period after three administered doses of the vaccine. The genotypes that were detected were primarily genotype 4 (92%) and only one case of genotype 1 (8%). Thus, although the vaccine is anticipated to provide pangenotypic protection, this has not yet been demonstrated. This vaccine is currently approved for use in China. Vaccination against HEV infection has not yet been approved for use in other countries.

Although passive immunization has shown beneficial results in preclinical studies,[109] the administration of immune globulin for pre-exposure or postexposure prophylaxis does not affect the incidence of HEV infection.[7] The administration of immune globulin in endemic and nonendemic regions has been ineffective and offers little to no protective function.[20,110] However, the identification of neutralizing monoclonal antibodies and their specific target epitopes provides a possible means for passive immunization in the future.[111–114]

The future of vaccination against HEV infection remains hopeful. Vaccines will eventually be available in countries where the infection is endemic. Vaccination is imperative to reduce disease burden and decrease morbidity and mortality in pregnant patients.[115] An ongoing uncertainty that requires further investigation is the duration of the protective role of the current investigational vaccines and whether booster immunization will be required. WHO identified the HEV-239 vaccine as a promising vaccine that has shown efficacy in 16- to 65-year-old healthy individuals in China,[116] but has not yet gathered enough information to recommend its use. DNA-based vaccines also show potential for the prevention of HEV infection.[117]

Key References

The complete reference list is available online at Expert Consult.

1. World Health Organization. Hepatitis E: Fact sheet. http://www.who.int/mediacentre/factsheets/fs280/en/.
2. Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E. *Lancet*. 2012;379:2477–2488.
3. Aggarwal R, Naik S. Epidemiology of hepatitis E: current status. *J Gastroenterol Hepatol*. 2009;24:1484–1493.
4. Purcell RH, Emerson SU. Hepatitis E: an emerging awareness of an old disease. *J Hepatol*. 2008;48:494–503.
5. Wong DC, Purcell RH, Sreenivasan MA, et al. Epidemic and endemic hepatitis in India: evidence for a non-A, non-B hepatitis virus aetiology. *Lancet*. 1980;2:876–879.
7. Aggarwal R, Krawczynski K. Hepatitis E. In: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease*. Vol 1. 10th ed. Philadelphia: Elsevier; 2016:1360–1365.
8. Khuroo MS. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. *Am J Med*. 1980;68:818–824.
12. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380:2095–2128.
13. Aggarwal R. Hepatitis E: historical, contemporary and future perspectives. *J Gastroenterol Hepatol*. 2011;26:72–82.
15. Rein DB, Stevens GA, Theaker J, et al. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology*. 2012;55:988–997.
16. Dalton HR, Bendall R, Ijaz S, et al. Hepatitis E: an emerging infection in developed countries. *Lancet Infect Dis*. 2008;8:698–709.
18. Tucker TJ, Kirsch RE, Louw SJ, et al. Hepatitis E in South Africa: evidence for sporadic spread and increased seroprevalence in rural areas. *J Med Virol*. 1996;50:117–119.
19. Walsh S. Hepatitis E virus. In: *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Disease*. 8th ed. Philadelphia: Elsevier; 2015:2131–2141.
20. Velazquez O, Stetler HC, Avila C, et al. Epidemic transmission of enterically transmitted non-A, non-B hepatitis in Mexico, 1986-1987. *JAMA*. 1990;263:3281–3285.
22. Rodriguez Lay Lde L, Quintana A, Villalba MC, et al. Dual infection with hepatitis A and E viruses in outbreaks and in sporadic clinical cases: Cuba 1998-2003. *J Med Virol*. 2008;80:798–802.
26. Robson SC, Adams S, Brink N, et al. Hospital outbreak of hepatitis E. *Lancet*. 1992;339:1424–1425.
29. Viswanathan R. Epidemiology. *Indian J Med Res*. 1957;45:1–29.
33. Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med*. 2008;358:811–817.
34. Kamar N, Garrouste C, Haagsma EB, et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology*. 2011;140:1481–1489.
35. Gerolami R, Moal V, Colson P. Chronic hepatitis E with cirrhosis in a kidney-transplant recipient. *N Engl J Med*. 2008;358:859–860.
36. Haagsma EB, Niesters HG, van den Berg AP, et al. Prevalence of hepatitis E virus infection in liver transplant recipients. *Liver Transpl*. 2009;15:1225–1228.
39. Alric L, Bonnet D, Laurent G, et al. Chronic hepatitis E virus infection: successful virologic response to pegylated interferon-alpha therapy. *Ann Intern Med*. 2010;153:135–136.
40. Dalton HR, Bendall RP, Keane FE, et al. Persistent carriage of hepatitis E virus in patients with HIV infection. *N Engl J Med*. 2009;361:1025–1027.
48. Sood A, Midha V, Sood N. Guillain-Barre syndrome with acute hepatitis E. *Am J Gastroenterol*. 2000;95:3667–3668.
50. Kamar N, Bendall RP, Peron JM, et al. Hepatitis E virus and neurologic disorders. *Emerg Infect Dis*. 2011;17:173–179.
53. Bazerbachi F, Haffar S, Garg SK, et al. Extra-hepatic manifestations associated with hepatitis E virus infection: a comprehensive review of the literature. *Gastroenterol Rep (Oxf)*. 2016;4:1–15.
55. Kamar N, Weclawiak H, Guilbeau-Frugier C, et al. Hepatitis E virus and the kidney in solid-organ transplant patients. *Transplantation*. 2012;93:617–623.
57. Hussaini SH, Skidmore SJ, Richardson P, et al. Severe hepatitis E infection during pregnancy. *J Viral Hepat*. 1997;4:51–54.
59. Kar P, Jilani N, Husain SA, et al. Does hepatitis E viral load and genotypes influence the final outcome of acute liver failure during pregnancy? *Am J Gastroenterol*. 2008;103:2495–2501.
60. Khuroo MS, Kamili S, Jameel S. Vertical transmission of hepatitis E virus. *Lancet*. 1995;345:1025–1026.
63. Legrand-Abravanel F, Kamar N, Sandres-Saune K, et al. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. *J Infect Dis*. 2010;202:835–844.
64. Schlosser B, Stein A, Neuhaus R, et al. Liver transplant from a donor with occult HEV infection induced chronic hepatitis and cirrhosis in the recipient. *J Hepatol*. 2012;56:500–502.
65. le Coutre P, Meisel H, Hofmann J, et al. Reactivation of hepatitis E infection in a patient with acute lymphoblastic leukaemia after allogeneic stem cell transplantation. *Gut*. 2009;58:699–702.
66. Kamar N, Abravanel F, Selves J, et al. Influence of immunosuppressive therapy on the natural history of genotype 3 hepatitis-E virus infection after organ transplantation. *Transplantation*. 2010;89:353–360.
73. Friedman LS, Lee SR, Nelson SB, et al. Case 36-2016. A 50-year-old man with acute liver injury. *N Engl J Med*. 2016;375:2082–2092.

75. Sherman KE. Hepatitis E virus infection: more common than previously realized? *Gastroenterol Hepatol (N Y)*. 2011;7:759–761.
76. Davern TJ, Chalasani N, Fontana RJ, et al. Acute hepatitis E infection accounts for some cases of suspected drug-induced liver injury. *Gastroenterology*. 2011;141:1665–1672, e1661–e1669.
77. Mast EE, Alter MJ, Holland PV, et al. Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E Virus Antibody Serum Panel Evaluation Group. *Hepatology*. 1998;27:857–861.
89. Baylis SA, Hanschmann KM, Blumel J, et al. Standardization of hepatitis E virus (HEV) nucleic acid amplification technique-based assays: an initial study to evaluate a panel of HEV strains and investigate laboratory performance. *J Clin Microbiol*. 2011;49:1234–1239.
90. Kamar N, Rostaing L, Abravanel F, et al. Ribavirin therapy inhibits viral replication on patients with chronic hepatitis E virus infection. *Gastroenterology*. 2010;139:1612–1618.
91. Kamar N, Rostaing L, Abravanel F, et al. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. *Clin Infect Dis*. 2010;50:e30–e33.
99. Mallet V, Nicand E, Sultanik P, et al. Brief communication: case reports of ribavirin treatment for chronic hepatitis E. *Ann Intern Med*. 2010;153:85–89.
102. Kamar N, Izopet J, Tripon S, et al. Ribavirin for chronic hepatitis E virus infection in transplant recipients. *N Engl J Med*. 2014;370:1111–1120.
103. Dao Thi VL, Debing Y, Wu X, et al. Sofosbuvir inhibits hepatitis E virus replication in vitro and results in an additive effect when combined with ribavirin. *Gastroenterology*. 2016;150:82–85.e84.
107. Shrestha MP, Scott RM, Joshi DM, et al. Safety and efficacy of a recombinant hepatitis E vaccine. *N Engl J Med*. 2007;356:895–903.
108. Zhu FC, Zhang J, Zhang XF, et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. *Lancet*. 2010;376:895–902.
109. Tsarev SA, Tsareva TS, Emerson SU, et al. Successful passive and active immunization of cynomolgus monkeys against hepatitis E. *Proc Natl Acad Sci USA*. 1994;91:10198–10202.
111. Emerson SU, Clemente-Casares P, Moiduddin N, et al. Putative neutralization epitopes and broad cross-genotype neutralization of Hepatitis E virus confirmed by a quantitative cell-culture assay. *J Gen Virol*. 2006;87:697–704.
112. Zhou YH, Purcell RH, Emerson SU. An ELISA for putative neutralizing antibodies to hepatitis E virus detects antibodies to genotypes 1, 2, 3, and 4. *Vaccine*. 2004;22:2578–2585.
116. WHO. Hepatitis E vaccine: WHO position paper, May 2015–Recommendations. *Vaccine*. 2016;34:304–305.

References

1. World Health Organization. Hepatitis E: Fact sheet. http://www.who.int/mediacentre/factsheets/fs280/en/.
2. Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E. *Lancet*. 2012;379:2477–2488.
3. Aggarwal R, Naik S. Epidemiology of hepatitis E: current status. *J Gastroenterol Hepatol*. 2009;24:1484–1493.
4. Purcell RH, Emerson SU. Hepatitis E: an emerging awareness of an old disease. *J Hepatol*. 2008;48:494–503.
5. Wong DC, Purcell RH, Sreenivasan MA, et al. Epidemic and endemic hepatitis in India: evidence for a non-A, non-B hepatitis virus aetiology. *Lancet*. 1980;2:876–879.
6. Demonte AJ, Jaggi OP. Infectious hepatitis (Delhi epidemic): bacteriological study. *Indian J Med Res*. 1957;45:141–144.
7. Aggarwal R, Krawczynski K. Hepatitis E. In: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease*. Vol 1. 10th ed. Philadelphia: Elsevier; 2016:1360–1365.
8. Khuroo MS. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. *Am J Med*. 1980;68:818–824.
9. Balayan MS, Andjaparidze AG, Savinskaya SS, et al. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirology*. 1983;20:23–31.
10. Teo CG. Fatal outbreaks of jaundice in pregnancy and the epidemic history of hepatitis E. *Epidemiol Infect*. 2012;140:767–787.
11. Koonin EV, Gorbalenya AE, Purdy MA, et al. Computer-assisted assignment of functional domains in the nonstructural polyprotein of hepatitis E virus: delineation of an additional group of positive-strand RNA plant and animal viruses. *Proc Natl Acad Sci USA*. 1992;89:8259–8263.
12. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012; 380:2095–2128.
13. Aggarwal R. Hepatitis E: historical, contemporary and future perspectives. *J Gastroenterol Hepatol*. 2011;26:72–82.
14. Kmush B, Wierzba T, Krain L, et al. Epidemiology of hepatitis E in low- and middle-income countries of Asia and Africa. *Semin Liver Dis*. 2013;33:15–29.
15. Rein DB, Stevens GA, Theaker J, et al. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology*. 2012;55:988–997.
16. Dalton HR, Bendall R, Ijaz S, et al. Hepatitis E: an emerging infection in developed countries. *Lancet Infect Dis*. 2008;8:698–709.
17. Kamar N, Mansuy JM, Cointault O, et al. Hepatitis E virus-related cirrhosis in kidney- and kidney-pancreas-transplant recipients. *Am J Transplant*. 2008;8:1744–1748.
18. Tucker TJ, Kirsch RE, Louw SJ, et al. Hepatitis E in South Africa: evidence for sporadic spread and increased seroprevalence in rural areas. *J Med Virol*. 1996;50: 117–119.
19. Walsh S. Hepatitis E virus. In: *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Disease*. 8th ed. Philadelphia: Elsevier; 2015:2131–2141.
20. Velazquez O, Stetler HC, Avila C, et al. Epidemic transmission of enterically transmitted non-A, non-B hepatitis in Mexico, 1986-1987. *JAMA*. 1990;263:3281–3285.
21. Quintana A, Sanchez L, Larralde O, et al. Prevalence of antibodies to hepatitis E virus in residents of a district in Havana, Cuba. *J Med Virol*. 2005;76:69–70.
22. Rodriguez Lay Lde L, Quintana A, Villalba MC, et al. Dual infection with hepatitis A and E viruses in outbreaks and in sporadic clinical cases: Cuba 1998-2003. *J Med Virol*. 2008;80:798–802.
23. Munne MS, Vladimirsky S, Otegui L, et al. Identification of the first strain of swine hepatitis E virus in South America and prevalence of anti-HEV antibodies in swine in Argentina. *J Med Virol*. 2006;78:1579–1583.
24. Pujol FH, Favorov MO, Marcano T, et al. Prevalence of antibodies against hepatitis E virus among urban and rural populations in Venezuela. *J Med Virol*. 1994;42: 234–236.
25. Schlauder GG, Frider B, Sookoian S, et al. Identification of 2 novel isolates of hepatitis E virus in Argentina. *J Infect Dis*. 2000;182:294–297.
26. Robson SC, Adams S, Brink N, et al. Hospital outbreak of hepatitis E. *Lancet*. 1992;339:1424–1425.
27. Boccia D, Guthmann JP, Klovstad H, et al. High mortality associated with an outbreak of hepatitis E among displaced persons in Darfur, Sudan. *Clin Infect Dis*. 2006;42:1679–1684.
28. Guthmann JP, Klovstad H, Boccia D, et al. A large outbreak of hepatitis E among a displaced population in Darfur, Sudan, 2004: the role of water treatment methods. *Clin Infect Dis*. 2006;42:1685–1691.
29. Viswanathan R. Epidemiology. *Indian J Med Res*. 1957;45:1–29.
30. Tsarev SA, Emerson SU, Tsareva TS, et al. Variation in course of hepatitis E in experimentally infected cynomolgus monkeys. *J Infect Dis*. 1993;167: 1302–1306.
31. Li F, Zhuang H, Kolivas S, et al. Persistent and transient antibody responses to hepatitis E virus detected by western immunoblot using open reading frame 2 and 3 and glutathione S-transferase fusion proteins. *J Clin Microbiol*. 1994;32:2060–2066.
32. Tsarev SA, Emerson SU, Reyes GR, et al. Characterization of a prototype strain of hepatitis E virus. *Proc Natl Acad Sci USA*. 1992;89:559–563.
33. Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med*. 2008;358:811–817.
34. Kamar N, Garrouste C, Haagsma EB, et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology*. 2011;140:1481–1489.
35. Gerolami R, Moal V, Colson P. Chronic hepatitis E with cirrhosis in a kidney-transplant recipient. *N Engl J Med*. 2008;358:859–860.
36. Haagsma EB, Niesters HG, van den Berg AP, et al. Prevalence of hepatitis E virus infection in liver transplant recipients. *Liver Transpl*. 2009;15:1225–1228.
37. Peron JM, Mansuy JM, Recher C, et al. Prolonged hepatitis E in an immunocompromised patient. *J Gastroenterol Hepatol*. 2006;21:1223–1224.
38. Tamura A, Shimizu YK, Tanaka T, et al. Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. *Hepatol Res*. 2007;37:113–120.
39. Alric L, Bonnet D, Laurent G, et al. Chronic hepatitis E virus infection: successful virologic response to pegylated interferon-alpha therapy. *Ann Intern Med*. 2010;153:135–136.
40. Dalton HR, Bendall RP, Keane FE, et al. Persistent carriage of hepatitis E virus in patients with HIV infection. *N Engl J Med*. 2009;361:1025–1027.
41. Kenfak-Foguena A, Schoni-Affolter F, Burgisser P, et al. Hepatitis E virus seroprevalence and chronic infections in patients with HIV, Switzerland. *Emerg Infect Dis*. 2011;17:1074–1078.
42. Colson P, Kaba M, Moreau J, et al. Hepatitis E in an HIV-infected patient. *J Clin Virol*. 2009;45:269–271.
43. Despierres LA, Kaphan E, Attarian S, et al. Neurologic disorders and hepatitis E, France, 2010. *Emerg Infect Dis*. 2011;17:1510–1512.
44. Kejariwal D, Roy S, Sarkar N. Seizure associated with acute hepatitis E. *Neurology*. 2001;57:1935.
45. Mandal K, Chopra N. Acute transverse myelitis following hepatitis E virus infection. *Indian Pediatr*. 2006;43:365–366.
46. Fong F, Illahi M. Neuralgic amyotrophy associated with hepatitis E virus. *Clin Neurol Neurosurg*. 2009;111:193–195.
47. Dixit VK, Abhilash VB, Kate MP, et al. Hepatitis E infection with Bell's palsy. *J Assoc Physicians India*. 2006;54:418.
48. Sood A, Midha V, Sood N. Guillain-Barre syndrome with acute hepatitis E. *Am J Gastroenterol*. 2000;95: 3667–3668.
49. Maurissen I, Jeurissen A, Strauven T, et al. First case of anti-ganglioside GM1-positive Guillain-Barre syndrome due to hepatitis E virus infection. *Infection*. 2012;40:323–326.
50. Kamar N, Bendall RP, Peron JM, et al. Hepatitis E virus and neurologic disorders. *Emerg Infect Dis*. 2011;17:173–179.
51. Kamar N, Izopet J, Cintas P, et al. Hepatitis E virus-induced neurological symptoms in a kidney-transplant patient with chronic hepatitis. *Am J Transplant*. 2010;10:1321–1324.
52. Fourquet E, Mansuy JM, Bureau C, et al. Severe thrombocytopenia associated with acute autochthonous hepatitis E. *J Clin Virol*. 2010;48:73–74.
53. Bazerbachi F, Haffar S, Garg SK, et al. Extra-hepatic manifestations associated with hepatitis E virus infection: a comprehensive review of the literature. *Gastroenterol Rep (Oxf)*. 2016;4:1–15.
54. Deniel C, Coton T, Brardjanian S, et al. Acute pancreatitis: a rare complication of acute hepatitis E. *J Clin Virol*. 2011;51:202–204.
55. Kamar N, Weclawiak H, Guilbeau-Frugier C, et al. Hepatitis E virus and the kidney in solid-organ transplant patients. *Transplantation*. 2012;93:617–623.
56. Hamid SS, Jafri SM, Khan H, et al. Fulminant hepatic failure in pregnant women: acute fatty liver or acute viral hepatitis? *J Hepatol*. 1996;25:20–27.
57. Hussaini SH, Skidmore SJ, Richardson P, et al. Severe hepatitis E infection during pregnancy. *J Viral Hepat*. 1997;4:51–54.
58. Tsega E, Krawczynski K, Hansson BG, et al. Hepatitis E virus infection in pregnancy in Ethiopia. *Ethiop Med J*. 1993;31:173–181.
59. Kar P, Jilani N, Husain SA, et al. Does hepatitis E viral load and genotypes influence the final outcome of acute liver failure during pregnancy? *Am J Gastroenterol*. 2008;103:2495–2501.
60. Khuroo MS, Kamili S, Jameel S. Vertical transmission of hepatitis E virus. *Lancet*. 1995;345:1025–1026.
61. Singh S, Mohanty A, Joshi YK, et al. Mother-to-child transmission of hepatitis E virus infection. *Indian J Pediatr*. 2003;70:37–39.
62. Legrand-Abravanel F, Kamar N, Sandres-Saune K, et al. Hepatitis E virus infection without reactivation in solid-organ transplant recipients, France. *Emerg Infect Dis*. 2011;17:30–37.
63. Legrand-Abravanel F, Kamar N, Sandres-Saune K, et al. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. *J Infect Dis*. 2010;202:835–844.
64. Schlosser B, Stein A, Neuhaus R, et al. Liver transplant from a donor with occult HEV infection induced chronic hepatitis and cirrhosis in the recipient. *J Hepatol*. 2012;56:500–502.
65. le Coutre P, Meisel H, Hofmann J, et al. Reactivation of hepatitis E infection in a patient with acute lymphoblastic leukaemia after allogeneic stem cell transplantation. *Gut*. 2009;58:699–702.
66. Kamar N, Abravanel F, Selves J, et al. Influence of immunosuppressive therapy on the natural history of genotype 3 hepatitis-E virus infection after organ transplantation. *Transplantation*. 2010;89:353–360.
67. Haagsma EB, van den Berg AP, Porte RJ, et al. Chronic hepatitis E virus infection in liver transplant recipients. *Liver Transpl*. 2008;14:547–553.
68. Keane F, Gompels M, Bendall R, et al. Hepatitis E virus coinfection in patients with HIV infection. *HIV Med*. 2012;13:83–88.
69. Renou C, Lafeuillade A, Cadranel JF, et al. Hepatitis E virus in HIV-infected patients. *AIDS*. 2010;24:1493–1499.
70. Kaba M, Richet H, Ravaux I, et al. Hepatitis E virus infection in patients infected with the human immunodeficiency virus. *J Med Virol*. 2011;83:1704–1716.
71. Colson P, Dhiver C, Poizot-Martin I, et al. Acute and chronic hepatitis E in patients infected with human immunodeficiency virus. *J Viral Hepat*. 2011;18:227–228.
72. Thoden J, Venhoff N, Miehle N, et al. Hepatitis E and jaundice in an HIV-positive pregnant woman. *AIDS*. 2008;22:909–910.
73. Friedman LS, Lee SR, Nelson SB, et al. Case 36-2016. A 50-year-old man with acute liver injury. *N Engl J Med*. 2016;375:2082–2092.
74. Calisti G, Irish DN, Ijaz S, et al. Acute hepatitis E mimicking a flare of disease in a patient with chronic autoimmune hepatitis. *Ann Hepatol*. 2017;16:160–163.
75. Sherman KE. Hepatitis E virus infection: more common than previously realized? *Gastroenterol Hepatol (N Y)*. 2011;7:759–761.
76. Davern TJ, Chalasani N, Fontana RJ, et al. Acute hepatitis E infection accounts for some cases of suspected drug-induced liver injury. *Gastroenterology*. 2011;141:1665–1672, e1661–e1669.
77. Mast EE, Alter MJ, Holland PV, et al. Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E Virus Antibody Serum Panel Evaluation Group. *Hepatology*. 1998;27:857–861.
78. Drobeniuc J, Meng J, Reuter G, et al. Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances. *Clin Infect Dis*. 2010;51:e24–e27.
79. Chau KH, Dawson GJ, Bile KM, et al. Detection of IgA class antibody to hepatitis E virus in serum samples from patients with hepatitis E virus infection. *J Med Virol*. 1993;40:334–338.
80. Tian DY, Chen Y, Xia NS. Significance of serum IgA in patients with acute hepatitis E virus infection. *World J Gastroenterol*. 2006;12:3919–3923.
81. Zhang S, Tian D, Zhang Z, et al. Clinical significance of anti-HEV IgA in diagnosis of acute genotype 4 hepatitis E virus infection negative for anti-HEV IgM. *Dig Dis Sci*. 2009;54:2512–2518.
82. Mushahwar IK. Hepatitis E virus: molecular virology, clinical features, diagnosis, transmission, epidemiology, and prevention. *J Med Virol*. 2008;80:646–658.
83. Schlauder GG, Desai SM, Zanetti AR, et al. Novel hepatitis E virus (HEV) isolates from Europe: evidence for additional genotypes of HEV. *J Med Virol*. 1999;57:243–251.
84. van Cuyck H, Juge F, Roques P. Phylogenetic analysis of the first complete hepatitis E virus (HEV) genome from

Africa. *FEMS Immunol Med Microbiol*. 2003;39:133–139.

85. Hsieh SY, Yang PY, Ho YP, et al. Identification of a novel strain of hepatitis E virus responsible for sporadic acute hepatitis in Taiwan. *J Med Virol*. 1998;55:300–304.
86. Maila HT, Bowyer SM, Swanepoel R. Identification of a new strain of hepatitis E virus from an outbreak in Namibia in 1995. *J Gen Virol*. 2004;85:89–95.
87. Wang Y, Ling R, Erker JC, et al. A divergent genotype of hepatitis E virus in Chinese patients with acute hepatitis. *J Gen Virol*. 1999;80:169–177.
88. Clayson ET, Myint KS, Snitbhan R, et al. Viremia, fecal shedding, and IgM and IgG responses in patients with hepatitis E. *J Infect Dis*. 1995;172:927–933.
89. Baylis SA, Hanschmann KM, Blumel J, et al. Standardization of hepatitis E virus (HEV) nucleic acid amplification technique-based assays: an initial study to evaluate a panel of HEV strains and investigate laboratory performance. *J Clin Microbiol*. 2011;49:1234–1239.
90. Kamar N, Rostaing L, Abravanel F, et al. Ribavirin therapy inhibits viral replication on patients with chronic hepatitis E virus infection. *Gastroenterology*. 2010;139:1612–1618.
91. Kamar N, Rostaing L, Abravanel F, et al. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. *Clin Infect Dis*. 2010;50:e30–e33.
92. Krawczynski K. Hepatitis E. *Hepatology*. 1993;17:932–941.
93. Asher LV, Innis BL, Shrestha MP, et al. Virus-like particles in the liver of a patient with fulminant hepatitis and antibody to hepatitis E virus. *J Med Virol*. 1990;31:229–233.
94. Kawai HF, Koji T, Iida F, et al. Shift of hepatitis E virus RNA from hepatocytes to biliary epithelial cells during acute infection of rhesus monkey. *J Viral Hepat*. 1999;6:287–297.
95. Williams TP, Kasorndorkbua C, Halbur PG, et al. Evidence of extrahepatic sites of replication of the hepatitis E virus in a swine model. *J Clin Microbiol*. 2001;39:3040–3046.
96. Peron JM, Dalton H, Izopet J, et al. Acute autochthonous hepatitis E in western patients with underlying chronic liver disease: a role for ribavirin? *J Hepatol*. 2011;54:1323–1324, author reply 1324–1325.
97. Gerolami R, Borentain P, Raissouni F, et al. Treatment of severe acute hepatitis E by ribavirin. *J Clin Virol*. 2011;52:60–62.
98. Haagsma EB, Riezebos-Brilman A, van den Berg AP, et al. Treatment of chronic hepatitis E in liver transplant recipients with pegylated interferon alpha-2b. *Liver Transpl*. 2010;16:474–477.
99. Mallet V, Nicand E, Sultanik P, et al. Brief communication: case reports of ribavirin treatment for chronic hepatitis E. *Ann Intern Med*. 2010;153:85–89.
100. Alric L, Bonnet D, Beynes-Rauzy O, et al. Definitive clearance of a chronic hepatitis E virus infection with ribavirin treatment. *Am J Gastroenterol*. 2011;106:1562–1563.
101. Dalton HR, Keane FE, Bendall R, et al. Treatment of chronic hepatitis E in a patient with HIV infection. *Ann Intern Med*. 2011;155:479–480.
102. Kamar N, Izopet J, Tripon S, et al. Ribavirin for chronic hepatitis E virus infection in transplant recipients. *N Engl J Med*. 2014;370:1111–1120.
103. Dao Thi VL, Debing Y, Wu X, et al. Sofosbuvir inhibits hepatitis E virus replication in vitro and results in an additive effect when combined with ribavirin. *Gastroenterology*. 2016;150:82–85.e84.
104. Thomas DL, Yarbough PO, Vlahov D, et al. Seroreactivity to hepatitis E virus in areas where the disease is not endemic. *J Clin Microbiol*. 1997;35:1244–1247.
105. Purcell RH, Nguyen H, Shapiro M, et al. Pre-clinical immunogenicity and efficacy trial of a recombinant hepatitis E vaccine. *Vaccine*. 2003;21:2607–2615.
106. Emerson SU, Purcell RH. Hepatitis E virus. In: Knipe DM, Howley P, eds. *Fields Virology*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013:2242–2258.
107. Shrestha MP, Scott RM, Joshi DM, et al. Safety and efficacy of a recombinant hepatitis E vaccine. *N Engl J Med*. 2007;356:895–903.
108. Zhu FC, Zhang J, Zhang XF, et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. *Lancet*. 2010;376:895–902.
109. Tsarev SA, Tsareva TS, Emerson SU, et al. Successful passive and active immunization of cynomolgus monkeys against hepatitis E. *Proc Natl Acad Sci USA*. 1994;91:10198–10202.
110. Khuroo MS, Dar MY. Hepatitis E: evidence for person-to-person transmission and inability of low dose immune serum globulin from an Indian source to prevent it. *Indian J Gastroenterol*. 1992;11:113–116.
111. Emerson SU, Clemente-Casares P, Moiduddin N, et al. Putative neutralization epitopes and broad cross-genotype neutralization of Hepatitis E virus confirmed by a quantitative cell-culture assay. *J Gen Virol*. 2006;87:697–704.
112. Zhou YH, Purcell RH, Emerson SU. An ELISA for putative neutralizing antibodies to hepatitis E virus detects antibodies to genotypes 1, 2, 3, and 4. *Vaccine*. 2004;22:2578–2585.
113. Schofield DJ, Glamann J, Emerson SU, et al. Identification by phage display and characterization of two neutralizing chimpanzee monoclonal antibodies to the hepatitis E virus capsid protein. *J Virol*. 2000;74:5548–5555.
114. Schofield DJ, Purcell RH, Nguyen HT, et al. Monoclonal antibodies that neutralize HEV recognize an antigenic site at the carboxyterminus of an ORF2 protein vaccine. *Vaccine*. 2003;22:257–267.
115. Labrique AB, Sikder SS, Krain LJ, et al. Hepatitis E, a vaccine-preventable cause of maternal deaths. *Emerg Infect Dis*. 2012;18:1401–1404.
116. WHO. Hepatitis E vaccine: WHO position paper, May 2015–Recommendations. *Vaccine*. 2016;34:304–305.
117. Hong Y, Ruan B, Yang LH, et al. Hepatitis E virus chimeric DNA vaccine elicits immunologic response in mice. *World J Gastroenterol*. 2005;11:6713–6715.

B Prion Diseases

179 Prions and Prion Disease of the Central Nervous System (Transmissible Neurodegenerative Diseases)

Patrick J. Bosque and Kenneth L. Tyler

SHORT VIEW SUMMARY

Definition
- Prion diseases are transmissible neurodegenerative conditions of mammals characterized by a rapidly progressive dementia, usually with ataxia and other motor signs.
- Human prion diseases include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia, variably protease-sensitive prionopathy, and kuru.
- Important prion diseases of animals are scrapie of sheep and goats, chronic wasting disease of deer and related species, and bovine spongiform encephalopathy.

Epidemiology
- Annual incidence of prion disease is 1 to 2 per million annually.
- About 90% of cases are sporadic.
- About 10% of cases are genetic, caused by a mutation in the prion protein gene (*PRNP*).
- Infectious transmission accounts for less than 1% of human cases.

Microbiology
- The prion is composed primarily of an aggregate of misfolded forms of a normally produced protein, the prion protein (PrP).
- The aggregate serves as a catalytic template for the further aggregation of PrP, and thus the prion replicates.
- The prion contains no information-bearing nucleic acid.

Diagnosis
- Treatable mimics of prion disease should be excluded before making the diagnosis.
- A highly sensitive and specific test, real-time quaking-induced conversion (RT-QuIC), is now available to diagnose the most common form of prion disease from a sample of cerebrospinal fluid (CSF).

Therapy
- No effective therapy exists for prion infection.

Prevention
- Only standard precautions are needed for most procedures to prevent iatrogenic transmission of prion disease.
- Additional precautions are needed for neurosurgical cases and handling of CSF.
- Transfusion of red blood cells and plasma protein products can transmit one form of prion disease, variant CJD.
- Tissue from persons with prion disease must not be transplanted.
- Dietary exposure to ruminant animal prions should be avoided.

Prions are the transmissible agents of a class of neurodegenerative diseases of humans and other mammals. Prions differ from other infectious agents in that they contain no information-bearing nucleic acid. Rather, they are composed mainly, perhaps only, of abnormal conformations of a normally produced cell surface glycoprotein called the *prion protein* (PrP). These abnormal conformations of PrP are able to trigger the abnormal folding of previously normal conformers of PrP. It is the abnormal conformation of PrP, rather than the polypeptide itself, that propagates in prion diseases. The human forms of prion disease are most commonly referred to as Creutzfeldt-Jakob disease (CJD), although some specific clinical forms carry other names, such as kuru, Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI). The most important prion diseases of animals are scrapie of sheep, bovine spongiform encephalopathy (BSE) of cattle, and chronic wasting disease (CWD) of deer and related species.

BRIEF HISTORY OF PRION DISEASE RESEARCH

Scrapie, a disease of sheep and goats, is the prototypical prion disease. References to it are found at least as early as 1750 in Germany.[1] Studies on scrapie in the late 19th through mid-20th century[2] established that the disease was transmissible by a small filterable agent after incubation periods of up to 22 months. It was thus thought to be a virus or, because of the long latency between infection and the emergence of symptoms, a "slow virus." However, studies in the mid-20th century revealed the scrapie agent to be resistant to ionizing radiation and other physical and chemical treatments that ordinarily inactivate viruses. This led Alper and coworkers[3] to suggest that the agent might replicate despite lacking nucleic acid. The link between the agent of scrapie and human disease was established by Gajdusek and Zigas.[4] Gajdusek and coworkers then identified kuru, a neurodegenerative condition endemic in certain cannibalistic tribes in the highlands of New Guinea, as a human prion disease when they transmitted the condition to primates.[5] They were aided in this discovery by the insights of the veterinary neuropathologist Hadlow, who remarked on the histopathologic similarity of kuru to scrapie, triggering the search for the potential transmissibility of kuru.[6] The similarities between kuru and scrapie that first drew Hadlow's attention included a "soap bubble"–like vacuolation of the neuropil, profound neuronal loss, and intense reactive astrogliosis in the absence of an associated inflammatory response. Modern neuropathologists would add only the immunohistochemical detection of abnormal forms of PrP to this list of key pathologic features of prion diseases. Prusiner and coworkers[7,8] first purified the infectious agent. They found that a previously unidentified protein was the chief component of a highly infectious fraction isolated from brains of hamsters with scrapie.[8] In 1982, Prusiner proposed the name *prion* (pronounced PREE-on) for the agent responsible for scrapie and related neurodegenerative diseases.[9] The term *prion* initially was chosen to emphasize the hypothesis that the causative agents in these diseases were *p*roteinaceous *i*nfectious particles that could be distinguished from viruses and viroids by their apparent lack of nucleic acid. The protein that comprised the dominant component of the infection fraction was thus named the "prion protein."

MOLECULAR BIOLOGY AND PATHOPHYSIOLOGY OF PRION DISEASES

The Prion Protein

The process of prion propagation involves the conversion of a normally produced form of the prion protein (Fig. 179.1), usually designated PrP^{C} ("C" for cellular), to an abnormal disease-causing form, often designated PrP^{Sc} ("Sc" for scrapie). PrP^{C} is a cell surface glycoprotein, transcribed off a chromosomal gene, *PRNP,* located at 20p13 in humans.[10] The protein is modified during biosynthesis by the addition of a glycosylphosphatidylinositol moiety that anchors the carboxyl terminus of the mature protein to the external surface of the cell membrane.[11] It may be further modified by oligosaccharides (glycans) linked to asparagine at positions 181 and 197 in the polypeptide.[12] PrP^{C} is constitutively expressed at high levels in the normal brain.[13,14] The protein is also expressed at high levels in certain cells of the reticuloendothelial system, and this distribution contributes to the pathogenesis of epidemic prion disease (see "Transmission by Oral Exposure" later). The normal function of PrP^{C} remains obscure. Mice in which production of PrP is abolished by disruption of the *PRNP* gene survive to adulthood, and show no gross anatomic or behavioral abnormalities. The PrP sequence is highly conserved in evolution, so it presumably confers an as-yet unrecognized fitness benefit on the host. Candidate functions for PrP^{C} include cell adhesion, synaptic function, signal transduction, metabolism of amyloid β (the protein involved in Alzheimer disease pathogenesis), and a role in copper metabolism.[15,16] High-resolution structures for PrP^{C} have been obtained by nuclear magnetic resonance spectroscopy.[17] This shows PrP^{C} to be a globular protein composed of three α-helical segments and two short β strands that form a small antiparallel β sheet.

Infectious Prions

Prion diseases are marked by the appearance of a pathologic form of PrP, designated PrP^{Sc}. PrP^{Sc} has biochemical properties different from those of PrP^{C} (Table 179.1). In particular, the C-terminal portion of PrP^{Sc} is resistant to digestion by proteases, so that after treatment with the serine endopeptidase proteinase K, only the N-terminal portion of PrP^{Sc} is digested, whereas PrP^{C} is completely hydrolyzed. The generation of protease-resistant C-terminal fragments of characteristic size is a widely used assay for PrP^{Sc}. Spectroscopic studies indicate the PrP^{Sc} polypeptide has acquired extensive β sheet structure that is not present in PrP^{C}.[18] These and other data indicate that PrP^{Sc} is composed of aggregates of PrP tightly bound by interpeptide β sheets. In contrast, PrP^{C} exists as monomers or perhaps low-order multimers. The tertiary structure of PrP^{Sc} (i.e., the conformation of the polypeptide chain in aggregates) has proved difficult to determine, in part because available methods to determine high-resolution protein structure require that the protein be soluble, but the aggregates of which PrP^{Sc} is composed are insoluble. The conformation of PrP^{Sc} appears to be central to the process of prion propagation, so efforts to decipher this structure using advanced techniques are underway.[19,20]

Propagation of Prions

The precise mechanism of prion propagation is not completely understood. A popular model views prion propagation as similar to the "seeded polymerization" mechanism of amyloid formation. "Amyloid" is defined as a fibrillary protein aggregate, in which the protein monomers are bound together in β sheets oriented perpendicularly to the long axis of the fibril. Such aggregates may form the large "plaques" that are histologically identifiable by apple green birefringence under polarized light when stained with the dye Congo red (the classical definition of amyloid), but need not do so. The kinetics of amyloid fibril formation in vitro suggests a mechanism for prion propagation. Typically, a pure solution of monomers of an amyloidogenic peptide will exhibit a lag phase, often days in duration, before the rapid conversion of monomers into amyloid fibrils. If a small "seed" fibril is introduced into a solution of monomeric peptides, the lag phase is eliminated and the monomers rapidly polymerize. By analogy, an inoculum of PrP^{Sc} acts as seed to trigger aggregation of PrP^{C}. PrP amyloid of the classical type is seen in some forms of human and animal prion disease. Uncertainty persists over whether amyloid fibrils or a protofibrillary state may be the actual infectious moiety of prion diseases.[20,21] A conceptual model of prion propagation is presented in Fig. 179.2.

In vitro Synthesis of Prions

The seeded aggregation approach has been used to synthesize infectious prions in vitro. In the simplest application of this in vitro method, termed *prion misfolding cyclic amplification,* a small inoculum of infectious prions is added to a larger volume of brain homogenate.[22] This mixture is incubated at a warm temperature and subject to cycles of sonication. In this process, PrP^{C} in the brain homogenate is converted to PrP^{Sc}. A small amount of this treated mixture is then inoculated into another volume of brain homogenate. The process is repeated through many cycles. Infectious prions and PrP^{Sc} are present in the final mixture, yet the original inoculum has been diluted to nothingness. This method has been further refined to produce infectious prions using only defined components, confirming the prion hypothesis.[23,24] Further modifications of this method have been exploited to develop sensitive and specific minimally invasive (typically lumbar puncture) tests for prion disease (see "Laboratory Diagnosis of Prion Disease" later).

Other Macromolecules Contributing to Prion Propagation

Whether proteins or macromolecules other than PrP play a role in prion propagation is uncertain. In the yeast prion state [PSI+] (see

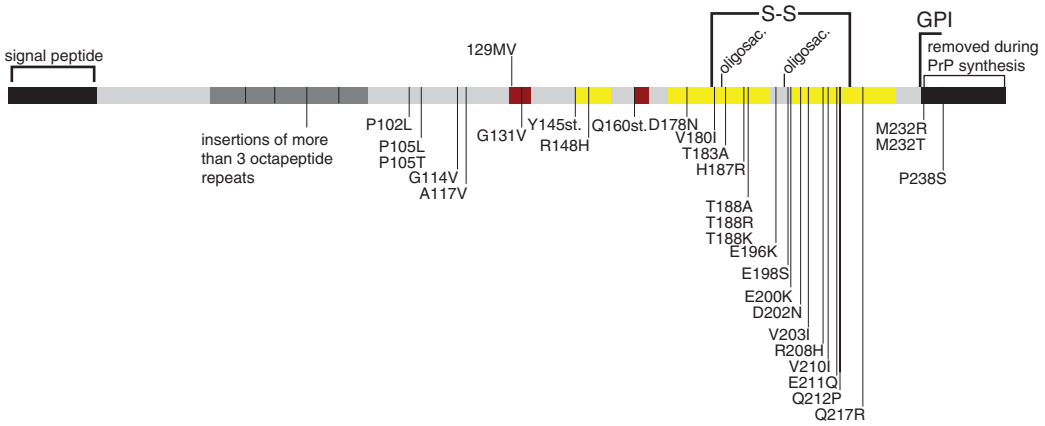

FIG. 179.1 Structural elements of prion protein (PrP) and mutations associated with genetic human prion disease. The PrP polypeptide is depicted as a horizontal bar running from the amino terminus on the left to the carboxyl terminus on the right. Amino and carboxyl termini removed in posttranslational processing are shown in black, and the locations of the five octapeptide repeats are shown in dark gray. The elements of regular secondary structure in recombinant cellular PrP (PrP^{C}) are the α-helix *(yellow)* and two short β strands *(red).* The sites of covalent modifications are shown above the bar, as is the location of the common codon 129MV polymorphism. PrP mutations associated with human prion disease are shown below the bar. Not shown are rare, apparently nonpathogenic polymorphisms. *GPI,* Glycosylphosphatidylinositol.

TABLE 179.1 The Properties of the Normal and Scrapie-Associated PrP Isoforms

FEATURE	PrP^{C} (NORMAL ISOFORM)	PrP^{Sc} (SCRAPIE ISOFORM)
Present in normal brain	Yes	No
Present in scrapie-infected brain	Yes	Yes
Covalent modifications	GPI anchor, Asn-linked polysaccharides, single intramolecular disulfide bond	Probably identical to PrP^{C}
Soluble in mild detergent	Yes	No
Effect of protease	Hydrolyzed to small peptides	Protease resistant C-terminal portion of variable length
Conformation		
Secondary structure: Local conformation of the polypeptide chain	About 40% α-helical, little β sheet	30% β sheet, 20% α-helix
Tertiary structure: Overall fold of the polypeptide	3 α-helical regions, unstructured N-terminus	Not determined
Quaternary structure: Association with other polypeptides	Monomeric or few-subunit oligomer	Aggregated

Asn, Asparagine; *GPI,* glycosylphosphatidylinositol.
Modified from Reisner D. Biochemistry and structure of PrP(C) and PrP(Sc). Br Med Bull. *2003;66:21–33.*

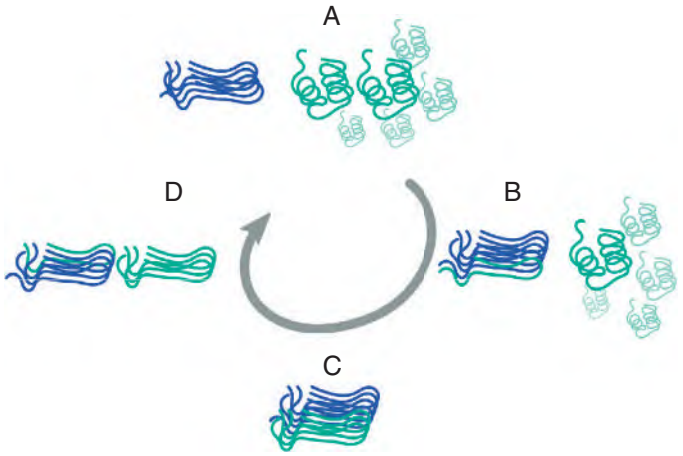

FIG. 179.2 A conceptual model of prion replication. (A) PrP^{C} *(green)* is formed of a nonaggregated polypeptide that contains α-helical domains and little β sheet, whereas PrP^{Sc} *(blue)* exists in aggregates of PrP polypeptides joined by intramolecular β sheet. (B) PrP^{Sc} and PrP^{C} interact and in the process a PrP^{C} adopts the conformation of PrP^{Sc} in the aggregate and joins the aggregate. (C) This process repeats and the aggregate grows. (D) At some point the aggregate fractures, creating two (or more) aggregates, each of which can serve as the nidus for further recruitment of PrP^{C} into PrP^{Sc} aggregates; the prions thus replicate. The process requires a source of PrP^{C} to continue, because it is the abnormal conformation of PrP that is propagating, not the PrP polypeptide itself.

"Yeast Prions" later), protein folding chaperones such as heat shock proteins can influence the rate of prion propagation.[25,26] The evidence of similar effects for mammalian prion disease is less clear.[27,28] To date, no protein other than PrP has been unequivocally demonstrated to participate in mammalian prion propagation. Genome-wide surveys of human populations have suggested that genes other than *PRNP* may contribute to a risk for prion disease, but conclusive evidence is lacking.[29–31] In vitro studies of prion replication indicate that phosphatidylethanolamine and RNA (of any sequence) may be important cofactors to produce infectious prions.[32]

Species Barrier to Transmission of Prion Diseases

Prion diseases are enzootic in several species of ruminants to which humans are exposed through food consumption and other routes, raising concerns of transmission of these conditions to humans (see "Variant Creutzfeldt-Jakob Disease" later). The amino-acid sequence of PrP can have a dramatic influence on prion propagation. The transmission of prion diseases across species is generally less efficient than transmission within the species. This phenomenon is known as the "species barrier." A crucial determinant of the species barrier is differences in the amino-acid sequence of PrP between species. Studies in transgenic mice demonstrate that the species barrier can be abrogated by the introduction of a gene directing expression of PrP of the prion donor species into the host.[33] Thus mice, which are usually not susceptible to human sporadic CJD (sCJD), are readily infected if the mouse *Prnp* gene has been replaced by a transgene coding for the human PrP sequence. This phenomenon has been exploited to generate transgenic mice that can be used to model prion diseases of several species.[34] The efficiency of cross-species transmission of prions is also determined by the strain of prion involved, as discussed in the next section.

Prion Strains

One of the most enigmatic features of prion diseases is the existence of what are termed *prion strains* (Table 179.2).[35] In experimental animals, prion strains can be distinguished by clinical features such as disease incubation time in animals or the pattern of clinical signs at disease onset.[36] Pathologically, strains can be characterized by the region of the brain in which PrP^{Sc} accumulates, by the degree of brain vacuolization, and by the morphology and distribution of PrP amyloid accumulation. Of particular importance to human health in the presence of animal prion disease epidemics, the species barrier to prion disease transmission may vary considerably between prion strains.[35] Thus the resistance of humans to prions of other species cannot be predicted simply from a comparison of *PRNP* sequences.

Because the prion consists of host-derived PrP, the means by which the properties of individual strains are maintained is puzzling. The biochemical basis of prion strain variety appears to reflect differences in PrP^{Sc} conformation between various strains (Fig. 179.2). Evidence for this includes differences in size of the protease-resistant carboxyl-terminal fragment between strains, different stabilities of the PrP aggregate to denaturation with chaotropic salts, and differential binding of antibodies to PrP^{Sc} of different strains.[37,38] Taken together, these and other observations suggest that subtle conformational differences between the PrP^{Sc} associated with the various strains somehow dictate the clinical and pathologic differences.

It appears that differences in the clinical manifestation of certain human prion diseases (e.g., sCJD, sporadic fatal insomnia, variant CJD [vCJD], and so-called variable protease-sensitive prionopathy) may relate to differences in the strain of the prion involved.

Transmission by Oral Exposure

Only a small proportion of cases of human prion disease are infectiously transmitted, but intraspecies transmission through the oral route is the major cause of epidemic prion disease in animals. After dietary exposure to prions, the reticuloendothelial system plays a major role in the initial propagation of prions and in carrying infection to the central nervous

TABLE 179.2 Comparison of Two Hamster Prion Strains

	STRAIN	
PROPERTY	**HYPER**	**DROWSY**
Incubation time[212]	65 days	165 days
Clinical signs[212]	Hyperexcitability and ataxia	Lethargy
Size of protease-resistant fragment (nonglycosylated band)[213]	21 kDa	20 kDa
Sensitivity of resistant carboxyl-terminal fragment to prolonged exposure to protease[213]	Present after 24 h of digestion	Hydrolyzed after 12 h
Resistance to denaturation (concentration of guanidine HCl that denatures 50% of PrP^{Sc})[214]	1.5 M	1.1 M
Distribution of PrP^{Sc} in the brains of clinically affected hamsters[215]	Most in medial geniculate nucleus and deep cerebellar nuclei	Most in regions of hippocampus, cerebellar granular layer, and occipital cortex
Distribution of PrP^{Sc} outside the CNS[216]	In spleen and other lymphoreticular organs	Not found in lymphoreticular system
Species barrier[212]	Nonpathogenic in mink	Pathogenic in mink

[a]Perhaps the two most well-studied prion strains are "hyper" and "drowsy," which are adapted to hamsters from transmissible mink encephalopathy, a prion disease of mink. The two strains are typically propagated in Syrian golden hamsters. Some characteristic properties are compared in the table. The number of potential strains that can exist on a single genetic background is not known, but evidence indicates that it is more than two.[213]
CNS, Central nervous system; *PrP^{Sc},* scrapie prion protein.

system (CNS). Prion titers rise first in gut-associated lymphoid tissue. Mice deficient in the number of functional Peyer patches show increased resistance to oral prion challenge.[39] Similarly, a number of studies have demonstrated that follicular dendritic cells are necessary for mice inoculated intraperitoneally with mouse-adapted scrapie to propagate prions to the brain.[40] Prion infection is carried to the brain from lymphoid tissue by axoplasmic transport in neurons of the sympathetic nervous system.[41–44]

Neurodegeneration in Prion Disease

How propagation of PrP^{Sc} leads to neurodegeneration remains unknown. Although the phenotypic expression of yeast prions, as discussed in the next section, is in several instances due to the loss of the protein's normal function, this does not appear to be the case with mammalian prions. Rather, the conversion of PrP^{C} to PrP^{Sc} causes a toxic "gain of function."[45] The nature of this toxic function remains poorly understood. Toxicity requires expression of PrP, and this PrP must be glycosylphosphatidylinositol anchored.[45,46] Substantial evidence suggests that the toxic species of PrP associated with prion infection may be different from the infectious species.[47] As with other neurodegenerative diseases associated with protein aggregates, dysfunction of the ubiquitin-proteasome system has been invoked as a cause of neurodegeneration in prion disease.[48]

Yeast Prions

Wickner first proposed that certain epigenetic traits of yeast could be manifestations of a process analogous to that which occurs in mammalian prion diseases.[49] To date, at least 10 yeast prions and 1 prion of the filamentous fungus *Podospora anserina* ([Het-s]) have been identified.[50] These traits are transmitted through exchange of cytoplasm but are not linked to the mitochondrial genome. Each particular prion trait is linked to a different protein, encoded by the nuclear genome, that is found to be in an aggregated state when the prion trait is expressed. Unlike the mammalian PrP prion, the phenotype of at least some yeast prions is equivalent to an inactivating mutation in the cognate protein. The [Het-s] prion is particularly interesting in that it appears to convey a useful property, mating incompatibility, upon its host. Yeast prions are intensively studied as models of mammalian prions and the processes of protein folding and aggregation in general. Yeast prions do not play any role in the initiation or transmission of mammalian prion diseases.

Prion-Like Behavior of Other Neurodegenerative Diseases

Prions formed of PrP remain the only proven mammalian prions, but there is some evidence that prion-like behavior of other proteins may play a role in neurodegenerative diseases such as Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis.[51] Limited evidence suggests that under unusual circumstances these conditions might be iatrogenically transmitted.[52,53]

HUMAN PRION DISEASES

Classification and Epidemiology of Human Prion Disease

Traditionally, human prion diseases have been classified by a combination of epidemiology, clinical features, histopathology, and family history. This has led to a proliferation of named syndromes that fundamentally share a similar pathophysiology. A more useful and consistent classification considers the various manifestations of human prion disease in terms of origin: sporadic, genetic, or infectiously transmitted. Most human prion disease is sporadic, that is, there is no determined infectious or genetic cause. In general, the term *Creutzfeldt-Jakob disease* refers to human prion disease and includes sporadic, genetic, and infectiously acquired forms that have not been given another name. The substantial clinical and pathologic diversity of human prion disease may be the manifestations of different strains of prions. However, typing of human prion strains is an emerging technology, and the basis of prion strain differences is incompletely understood. It is not clear whether the diverse manifestations of human prion disease can be attributed to differences in the properties of the initiating prion strains or result from other as-yet unidentified factors.

Epidemiology of Creutzfeldt-Jakob Disease

The term *Creutzfeldt-Jakob disease* was first used by Spielmeyer in 1922 to refer to a puzzling, rapidly progressive neurodegenerative syndrome initially described separately by the German neurologists Creutzfeldt and Jakob.[54–56] CJD is rare, with an annual prevalence and incidence typically said to be 1 case per 1 million population worldwide. In several countries, the rate of CJD has increased since the mid-1990s.[57] This most likely is the result of improved surveillance in response to the outbreak of BSE and vCJD. Based on these studies, the actual incidence of human prion disease may approach 2 cases per million annually.[58] In the United States, studies find a substantially lower rate of CJD among African Americans and other nonwhites than among whites.[59] Whether this reflects reduced ascertainment in these groups or a truly reduced incidence is not certain.

Sporadic Creutzfeldt-Jakob Disease Epidemiology

sCJD comprises approximately 85% to 94% of all cases of human prion disease.[60,61] It shows no gender predilection. Mean age at onset is 57 to 66 years, although patients as young as 17 years and older than 80 years

with sCJD have been reported.[61] Several studies found a peak incidence, approaching 6 per 1 million population, in the eighth decade and then a distinct decline in incidence in those older than 80 years.[59–61]

Clinical Features

The most distinctive clinical feature of sCJD is the pace of its progression, typically described as "rapid" or "subacute." In the context of neurodegenerative conditions, these terms refer to perceptible declines in cognitive and motor function that are obvious over a period of a few weeks. In contrast, in more common neurodegenerative conditions, such as Alzheimer or Parkinson disease, decline is typically only apparent over periods ranging from months to years. Some observers have noted that the pace of decline in sCJD accelerates until the later stages of akinetic mutism, when neurologic dysfunction is so severe that it is difficult to appreciate further decline. A second distinctive feature of sCJD is the prominent involvement of multiple brain systems in which motor signs, such as ataxia, bradykinesia, or spasticity, are combined with memory and other cognitive deficits. There is a great deal of variability in the clinical manifestations of sCJD, and this has led to attempts to describe a variety of clinical subtypes, including those with predominance of visual,[62] cerebellar,[63] thalamic,[64] and striatal[65,66] features. The existence of these syndromes indicates that sCJD may affect particular brain regions disproportionately. In some cases, the particular regional predominance of pathology may reflect the strain of prion involved.

In many patients with sCJD, there is a prodromal phase of psychiatric disturbance before the onset of neurologic signs. Of 126 mostly sporadic cases of CJD, 26% of patients had psychiatric signs in the prodromal or presenting phase.[67] Most common were sleep disturbance, depression, and anxiety. In approximately one-third of patients, initially prominent visual or cerebellar symptoms may overshadow dementia. Mental deterioration typically is rapidly progressive, and the average duration of illness from onset of symptoms to death is 7 to 9 months. In addition to profound and rapidly progressive mental deterioration, another very common feature is involuntary twitches or jerks of muscles, known as myoclonus. However, myoclonus in demented patients is not pathognomonic of sCJD. It can occasionally occur in Alzheimer disease and is common in Lewy body dementia. Extrapyramidal and cerebellar signs, including bradykinesia, rigidity, ataxia, nystagmus, and tremor, ultimately develop in approximately two-thirds of patients. Approximately 40% to 80% of patients have signs of corticospinal tract dysfunction, including hyperreflexia, spasticity, and extensor plantar responses. Prominent visual disturbances, which can include visual field cuts, cortical blindness, and visual agnosia, occur in 50% of sCJD patients.

Some patients have vague sensory complaints, including pruritus and aching limbs. It is unclear if these sensations are of peripheral or central origin.[68] Signs of a motor or sensory peripheral neuropathy are occasionally found in sCJD, although these signs are almost always overshadowed by the dramatic signs of CNS dysfunction. One study found clinical evidence of peripheral neuropathy in approximately 20% of cases examined and electrophysiologic abnormalities in 14 of 16 surveyed cases of sCJD.[69] On occasion, signs of sensory or motor neuropathy may dominate the early disease course. Rarely, fasciculations and muscle wasting will be so prominent as to suggest amyotrophic lateral sclerosis.[70,71] Some cases of sCJD also have been reported in which the clinical features indicated prominent autonomic nervous system involvement. These features included hypohidrosis, bowel dysfunction, abnormal pupillary responses to autonomic drugs, abnormal diurnal blood pressure variation, and electrocardiogram abnormalities. Such findings are cardinal features of FFI.

Certain neurologic disturbances occur only rarely as prominent features in sCJD, and their presence should prompt clinicians to consider other diagnostic possibilities. Although seizures occur in 10% to 20% of cases, they are rarely a dominant feature and typically are amenable to therapy. Cranial nerve involvement is never prominent, although isolated cases have been reported with involvement of the pupils,[72] extraocular movements,[73] and involvement of the auditory[74] and vestibular systems.[75]

Strains

At least two distinctive sporadic forms of sCJD bear the hallmarks of discrete prion strains. First, very rare sporadic cases of sporadic fatal insomnia, a clinical and pathologic syndrome indistinguishable from FFI (see "Familial Prion Disease" next), will be encountered in patients without *PRNP* mutations.[76,77] This form of sporadic prion disease maintains a biochemical signature identical to that of FFI upon serial transmission in transgenic mice expressing human PrP. It thus behaves as a distinct human prion strain.

Second, variably protease-sensitive prionopathy, a recently described condition, is a form of sCJD with fewer and less prominent motor and sensory signs and a pattern of cognitive impairment said to resemble frontotemporal dementia.[78,79] Progression is slower than typical sCJD, with a mean duration of 30 months. PrP^{Sc} from these patients is more sensitive to protease digestion than in typical sCJD. The predominant protease-resistant fragment is 8 kDa and cleaved at both the amino and carboxyl termini, whereas in sCJD the predominant fragment is cleaved only at the amino terminus and is 19 or 21 kDa in size. This 8-kDa fragment is also observed in the genetic prion disease GSS (see "Gerstmann-Sträussler-Scheinker Syndrome" later) leading some to speculate that variably protease-sensitive prionopathy is a sporadic form of GSS. The disease is quite rare; 13 cases were identified by the main US prion disease pathology center over an 8-year period, and 5 cases were identified in Britain in a retrospective review of 20 years.[79,80] Ignoring ascertainment problems, which may be significant, these translate into an annual incidence of approximately 0.5 per 100 million in either population. Attempts to transmit the condition to rodents, including transgenic mice expressing only human PrP, have been minimally successful, with some evidence of prion replication on first passage, but no propagation on later passages.[81,82]

Familial Prion Disease

Overview and Epidemiology

Approximately 10% of cases of human prion disease are caused by some amino-acid sequence–changing mutation in *PRNP*, although the precise proportion varies among countries.[60,83] At least 30 distinct mutations in the *PRNP* gene have been associated with the inherited prion diseases (see Fig. 179.1).[84] *PRNP* mutations are found in all cases from families with a history of inherited prion disease, but not all patients with genetic forms of prion disease present with a family history of dementia. In a large European survey of prion disease, a family history of a CJD-like dementia was elicited in only half of genetic cases.[83] Familial prion diseases are transmitted in an autosomal-dominant pattern, usually with high penetrance. Recent analysis of large data sets of the expressed genome in humans has discovered that some *PRNP* mutations previously reported only in association with prion disease or atypical dementia are found in persons in the general population without symptoms or a family history of prion disease.[85] This indicates that for these mutations penetrance is low, and in some cases that the association with prion disease may be coincidental. For example, the frequency of the V180I mutation in control populations without a history of prion disease suggests that the lifetime risk of prion disease in a carrier is around 1%.[85]

Specific mutations in the *PRNP* gene tend to be associated with particular clinical and pathologic disease phenotypes.[83] However, significant variability is seen both within and between kindreds harboring identical mutations. The disease associated with many mutations is essentially indistinguishable from sCJD. Among these is the most common mutation worldwide, a lysine-for–glutamic acid substitution in codon 200 (E200K).[86] This mutation has been found in geographic clusters of familial CJD in Slovakia and Chile and among Sephardic Jews in Greece, Libya, Tunisia, and Israel. Before the mutation was discovered to be prevalent in Libyan Jews, the high rate of CJD in this group was misattributed to the consumption of sheep brains or eyes.[87] The median age of onset is 58 years. The mutation is highly but incompletely penetrant so that approximately 80% of carriers will develop CJD by the eighth decade of life.[88] Because the mutation is found in inbred populations, rare carriers are homozygous for the mutation. Surprisingly, these people seem to develop illness only slightly earlier than heterozygotes (50 vs. 59 years).[89]

Some *PRNP* mutations are associated with syndromes markedly different from sCJD.

Gerstmann-Sträussler-Scheinker Syndrome

In some families with inherited prion disease, most victims develop prominent early ataxia and signs of corticospinal tract degeneration.

This picture is often associated with accumulations of PrP in large amyloid plaques in the CNS.[90,91] This clinicopathologic picture is known as *Gerstmann-Sträussler-Scheinker syndrome.* GSS is linked to several different mutations in *PRNP,* including P102L, P105L, A117V, Q187H, F198S, D202N, Q212P, Q217R, Y145STOP, and an insertion of eight or nine octapeptide repeats.[92] The duration of illness ranges from 3 months to 13 years, with a mean of 5 or 6 years.[92] Overt dementia occurs late in the disease.

The characteristic neuropathologic finding of GSS is widespread amyloid plaques composed of PrP. Within the cerebellum, where the concentration is typically most dense, plaques are found in the molecular layer and are often multicentric. The multicentric morphology of GSS plaques distinguishes them from plaques seen in kuru, which are typically unicentric.[93] The degree of spongiform change is variable, ranging from substantial and severe to completely absent. Western blot analysis of protease-resistant PrP finds mainly an 8-kDa fragment, as opposed to the 21-kDa or 19-kDa fragments associated with most forms of human prion disease. GSS is inconsistently transmitted to mice expressing human PrP, but transmission to another rodent, the bank vole, appears to be more consistent.[94]

Fatal Familial Insomnia

FFI first was reported as a human disease in 1986.[95] Subsequently, the immunohistochemical detection of abnormal PrP followed by the discovery of a D178N mutation in *PRNP* marked FFI as a prion disease with unusual features.[96] The D178N mutation can occur in two different *PRNP* allele forms, with either a methionine or a valine at the polymorphic codon 129. The D178N/129M allele associates with FFI, whereas a syndrome more closely resembling typical sCJD occurs in patients carrying the D178N/129V allele.[97] In the typical form of FFI, patients develop insomnia as a prominent and early complaint, along with signs of autonomic hyperactivity (increased sweating, tearing, salivation, mild nocturnal hyperthermia, tachycardia, and hypertension). Motor disturbances develop later and can include ataxia, myoclonus, spasticity, hyperreflexia, and dysarthria.[98,99] Marked memory impairment is not prominent early in the disease, although a delirium-like hallucinosis may occur. The mean age of onset in FFI is 50 to 56 years, somewhat younger than in sCJD, but cases in patients as young as 19 years and as old as 83 years of age have been reported.[83,100,101] Series of patients with the FFI genotype display substantial clinical heterogeneity. Insomnia is not always noted by the patient, family, or clinicians.[100,102] Polysomnography is a sensitive test for FFI, being abnormal in almost all cases, but investigations have shown this is true for sCJD as well.[103] Neuropathologic changes, including neuronal loss and reactive gliosis, are found consistently in the anterior ventral and mediodorsal nuclei of the thalamus and the inferior olives.[98] Immunostaining of brain material for PrP^{Sc} is positive, although the concentration of protein is 5 to 10 times less than that seen in sCJD.

As mentioned earlier, a rare sporadic form of human prion disease—sporadic fatal insomnia—presents with clinical and histopathologic features identical to those of FFI.[76,77]

Long-Duration Genetic Prion Disease

Certain *PRNP* mutations frequently cause disease with exceptionally slow progression. These include large expansions in the octapeptide repeat region and the missense mutations T183A and H187R.[104–107] Some mutations may be associated with lifelong psychiatric disturbances that precede the onset of progressive dementia by decades.[108]

Polymorphisms in *PRNP*

Polymorphisms at codon 129 of *PRNP* play a role in CJD expression and susceptibility. In European populations, approximately 60% to 70% of alleles have methionine at this position, and the rest have valine. The alleles are in Hardy-Weinberg equilibrium, so genotypes in the general population are approximately 42% MM, 46% MV, and 12% VV at codon 129.[109] In contrast, 95% of patients who develop sCJD exhibit homozygosity (either MM or VV) at this locus.[110–112] Codon 129 allele distributions vary across ethnic groups, but the tendency for overrepresentation of homozygotes in CJD cases holds.[113] Genome-wide association surveys have demonstrated that the codon 129 polymorphism genotype is a significant risk factor for both sCJD and acquired CJD.[84] A polymorphism at codon 219 is found mostly in Asian populations. Persons heterozygous at this codon appear to be resistant to sCJD, but may have increased susceptibility to vCJD.[113a]

Infectiously Acquired Human Prion Disease

Infectiously acquired prion disease is quite rare in humans, constituting less than 1% of cases in most countries. Nonetheless, the risk of transmission of these incurable conditions is a significant concern. Humans have acquired prion disease both through dietary exposure and iatrogenic routes.

Kuru

Kuru is a neurodegenerative disease that was endemic within the Fore linguistic tribal group of the Eastern Highlands of Papua New Guinea.[114] It arose in the early 20th century and was transmitted through the practice of ritual cannibalism at funeral feasts.[115] No one born since the cessation of this practice has developed kuru. The mean incubation period was 10 to 13 years, with 90% of cases occurring within 21 to 27 years of exposure. The incubation period was likely related to exposure dose and was shorter in women than in men and shorter in older women than in younger women, reflecting the likelihood of participation in cannibalistic practices.[116] The last known case of kuru occurred in 2009, likely as a result of exposure before cannibalism ceased in 1960. Thus in some cases the incubation period for kuru approached 50 years.[117]

Although it has disappeared as a clinical entity, kuru remains important conceptually as the largest outbreak of human-to-human transmission of a prion disease and as an example of human prion disease transmitted via the alimentary route. In its clinical and pathologic manifestations, it is distinct from most types of human prion disease. Kuru typically begins insidiously with a prodrome of headaches and aching limbs that may last for several months.[118] This prodrome is followed by the development of an inexorably progressive neurologic disease resulting in death within 3 to 24 months of onset, usually from intercurrent pneumonia and malnutrition. Typical disease duration is 12 months. Prominent clinical features include cerebellar ataxia, action tremor, and involuntary movements (choreoathetosis, myoclonic jerks, and coarse fasciculations). Dementia is usually not noted until late in the illness, 8 or more months after the onset of ataxia.

Neuropathologic examination of kuru brains shows neuronal loss, astrogliosis, and the accumulation of PrP^{Sc}, all findings typical of prion disease.[119,120] The pathologic hallmark of kuru is the presence of PrP amyloid plaques, predominantly in cerebellar tissue. These plaques are usually unicentric (in contrast to the multicentric plaques of GSS), located in the granular layer of the cerebellum, and often associated with microglial cells. The genetics of the *PRNP* gene in the kuru epidemic region show the powerful selection pressure that the disease exerted. In the kuru epidemic region, older women who potentially were exposed to infected brain material during the era of cannibalism, but who survived without developing kuru, show a higher-than-expected frequency of heterozygosity at codon 129.[121] Thus this heterozygosity may have played a protective role against the transmission of prion diseases among the Fore. More strikingly, a *PRNP* G127V mutation is found only among survivors of the kuru epidemic and their descendants, and in no other population in the world.[122] This mutation appears to have conferred resistance to kuru and to have been strongly selected for during the epidemic.

The properties of kuru prions on transmission to transgenic mice expressing human PrP suggest that it is the same strain as that most commonly found in sCJD.[123] This finding is consistent with the hypothesis that the kuru epidemic arose out of a naturally occurring case of sCJD that was then spread by cannibalism. It is puzzling that the clinical and pathologic features of kuru are quite distinct from those of typical sCJD. It is possible that the oral route of exposure alters the clinical and pathologic presentation, or that current methods of typing strains are insensitive to differences between kuru and sCJD.

Variant Creutzfeldt-Jakob Disease

Epidemiology

Beginning in 1995, cases of a new variant of CJD were reported from the United Kingdom.[124–126] As of April 2018, a total of 178 cases have

been reported to the UK National CJD Research & Surveillance Unit (see http://www.cjd.ed.ac.uk/surveillance for latest case totals). This includes three symptomatic "secondary" cases transmitted through non–leukocyte-depleted red blood cell transfusion and two asymptomatic cases—one from red blood cell transfusion and the other probably transmitted through pooled plasma-derived factor VIII.[127–129] An additional 53 cases have occurred outside the United Kingdom, with 27 of these in France and the remainder in the Republic of Ireland, Italy, the Netherlands, Portugal, Spain, Japan, Saudi Arabia, the United States, and Canada. Of the five North American cases, four appear to be due to exposure of the victims to infected beef products in the United Kingdom or Saudi Arabia.[130] Retrospective review of available autopsy material suggests that vCJD did emerge as a new disease entity in 1995; no current evidence suggests that earlier cases occurred.[131,132] It is likely that millions of people were exposed to BSE-infected food.[133] However, fears of a large epidemic of vCJD have not been realized. The largest number of annual cases (28) was reported in 2000.[134] The incidence of vCJD is declining; since 2012 there have been only two deaths from vCJD in Great Britain.[134]

Clinical Features

The epidemiologic, clinical, and pathologic features of the vCJD cases set them apart from typical sCJD.[126,135,136] Patients with vCJD have been considerably younger than patients with sCJD, with a mean age at onset of 26 years (range, 12–74 years) compared with 65 years for sCJD. Ninety percent of vCJD patients are younger than 40 years at the onset of their disease. The reason for this striking age dependence is not clear, but it appears to be due to greater susceptibility in the young rather than greater exposure to BSE-contaminated foodstuffs.[137] The duration of illness in vCJD is longer (average, 14 months) than in sCJD (average, 4.5 months). This longer duration may be a reflection of the younger age of the patients because younger patients with sCJD have a similar duration of illness.[138,139]

Patients with vCJD frequently present with sensory disturbances and psychiatric manifestations. Such symptoms are not unusual in young-onset sCJD, but in vCJD they are more prominent, perhaps because dementia and more obvious neurologic signs supervene later than in sCJD.[138] Among the sensory symptoms are vague pain, cold sensation, or paresthesias involving the face and limbs or in a hemisensory distribution. Most studied patients have had normal electromyography or mild abnormalities that do not point to a peripheral origin for the sensory disturbance.[68] Psychiatric manifestations frequently include dysphoria, withdrawal, anxiety, irritability, insomnia, and loss of interest in usual activities.[140,141] These symptoms often prompt an initial diagnosis of psychiatric illness. Neurologic signs and symptoms are uncommon within 4 months of onset. As disease progresses, the most frequent neurologic signs include dysarthria and gait disturbance. In the later stages of disease (>6 months after onset), prominent neurologic signs include hyperreflexia, myoclonus, incoordination, and other cerebellar signs.

Transmission by Blood Products

Blood products have very likely transmitted vCJD on at least five occasions. Among a group of 66 individuals identified as having received blood components from donors who later developed vCJD, only 33 of the transfusion recipients survived more than 5 years after transfusion, with the rest dying of conditions other than CJD or dementia.[142] Three of the remaining 33 recipients developed vCJD 6 to 8 years after transfusion. The donors had given blood 16 months to 3.5 years before the onset of vCJD. One donor was linked to vCJD cases in two recipients. All cases received non–leukocyte-depleted red blood cells. Remarkably, in an autopsied case, PrP^{Sc} was found in the tonsils, suggesting that the accumulation of PrP^{Sc} within tonsils that is found in most vCJD cases is not due to the alimentary route of exposure but, rather, is a tropism inherent in the vCJD prion strain.[127] Another of these 33 recipients died of causes unrelated to vCJD but was found at autopsy to have PrP^{Sc} in the spleen.[143] The rate of vCJD in transfusion recipients from donors who later developed vCJD (4 per 66, or 6%) is about 10,000-fold greater than the rate of vCJD in the British population, conclusively implicating the transfusion as the cause of disease. Finally, a survey of tissue obtained at autopsy from 11 persons with hemophilia, known to have received UK-sourced, pooled, plasma-derived clotting factor concentrates, found PrP^{Sc} in a single spleen.[144] Both cases of asymptomatic infection occurred in blood product recipients who were heterozygous (M/V) at *PRNP* codon 129, whereas that codon was homozygous (M/M) in the three symptomatic cases.

Neuropathology

The neuropathologic features of vCJD are strikingly different than those of sCJD.[126] The neuropathology of vCJD includes prominent involvement of the cerebellum in almost all cases, whereas only in a subset of sCJD brains, the Brownell-Oppenheimer variant and GSS cases, is the cerebellum prominently affected. vCJD cases have prominent amyloid plaques distributed throughout the cerebrum and cerebellum and, to a lesser extent, the basal ganglia and thalamus. These plaques have a dense eosinophilic center and pale periphery and are surrounded by vacuoles in the neuropil that are arranged around the plaque like petals on a flower (hence, the name "florid plaques"). This flower-like morphology is common in plaques from vCJD but is infrequently seen in other plaque-forming types of human prion disease. vCJD cases show typical spongiform change, neuronal loss, and astrogliosis in the cortex, basal ganglia, and thalamus, but these do not distinguish vCJD from sCJD. Patients with vCJD also have a consistent pattern of electrophoretic mobility of PrP^{Sc} protein (type 4 isoform) that is distinct from the mobility patterns encountered in sCJD and is similar to the pattern seen in BSE PrP^{Sc}.[145]

vCJD Caused by Exposure to BSE

Compelling evidence indicates that vCJD is the result of bovine-to-human transmission of BSE.[146–150] From an epidemiologic standpoint, cases of vCJD followed a massive epidemic of BSE in the United Kingdom, with a lag period that is consistent with the known incubation period of prions. During the BSE epidemic, it was estimated that several hundred thousand BSE-infected cattle might have entered the human food chain.[133] The number of BSE-infected cattle peaked during 1992 and 1993 and subsequently declined steadily. This decline has been attributed to bans on using ruminant protein for ruminant feeds (July 1988) and on using bovine brain, spinal cord, and other specified offal as feed for nonruminant animals and poultry (September 1990). Another ban prohibited use of certain bovine tissues for human consumption (November 1989). It has been suggested that the BSE epidemic was triggered by changes in the rendering process, particularly the abandonment of the use of organic solvents.[151]

Additional evidence for a BSE-vCJD link comes from the close neuropathologic similarities between the two diseases. Transgenic mice expressing bovine PrP develop indistinguishable neurologic illness and neuropathologic changes after a similar incubation period when injected with brain material from either cattle with BSE or humans with vCJD, and this differs from the pattern and incubation time seen after inoculation with scrapie.[150] In addition, the PrP^{Sc} protein isoforms isolated from the brains of the BSE-inoculated or vCJD-inoculated mice show an identical fragment size and glycosylation pattern, which differs from that seen after scrapie inoculation.[148] Furthermore, macaques inoculated with BSE prions develop florid plaques histologically indistinguishable from those seen in vCJD, whereas those inoculated with sporadic CJD prions do not develop plaques.[149] Finally, the susceptibility of mice expressing murine PrP, or transgenic mice expressing PrP of humans, to vCJD more closely resembles the susceptibility of these same mice to BSE than to other forms of human prion disease.[152]

Genetics of vCJD Susceptibility

With two possible exceptions, all vCJD patients tested to date are homozygous for methionine at polymorphic codon 129 (129M). One young patient in Great Britain with the 129VV genotype developed CJD with clinical and pathologic features not typical of sCJD or vCJD. Protease-resistant PrP^{Sc} from this patient showed a size and glycoform pattern identical to that found in vCJD.[153] Whether this was a sporadic case or, like vCJD, was caused by exposure to BSE prions is undetermined. Recently, a 36-year-old man died of prion disease in Great Britain. The molecular, but not the clinical, features of the disease were

consistent with vCJD.[154] It is fortunate, though puzzling, that despite the widespread exposure of populations to BSE, comparatively few persons have developed vCJD. As mentioned earlier, genome-wide association studies have sought loci that might confer exceptional susceptibility to vCJD upon some people. No convincing evidence for genetic risk factors, except for homozygosity for methionine at *PRNP* codon 129, has been found.[30,31]

Subclinical vCJD

The wide exposure of the British population to BSE-contaminated food products and the sometimes very long incubation period of prion diseases have raised concerns that human infection with BSE prions may be more prevalent than is apparent from the number of clinical cases. In this regard, in certain animal models of transmission of prions across species, animals can be efficiently infected, as indicated by demonstrable prion replication in the spleen and other organs of the reticuloendothelial system, but never show clinical signs of disease. Immunohistochemical surveys of large numbers of archived tonsils and appendices have been undertaken in Great Britain to look for subclinical prion infection. A survey of approximately 15,000 appendices collected between 1995 and 1999 found 3 with immunohistochemically detected PrP^{Sc}.[155] The study was repeated on approximately 32,000 appendices collected between 2000 and 2012, and 16 were found to harbor PrP^{Sc}.[156] Together, these studies suggest that about 1 in 2000 Britons may be subclinically infected with prions. However, as yet, no study has been performed on a control group, for instance, a population not known to have exposure to BSE. Therefore, it is uncertain whether these findings are related to BSE exposure, represent some other aspect of prion biology, or are artifactual. Interestingly, similar investigations of more than 60,000 tonsillectomy specimens failed to find any conclusive evidence of PrP^{Sc}.[155,157] If the PrP^{Sc} detected in appendices is the result of BSE prions propagating in human lymphoid tissue (i.e., subclinical vCJD), then it is possible that a large number of symptomatic cases could eventually occur and that donated blood products, organs, or tissues from subclinically infected Britons might transmit prion disease to recipients.

Diagnosis of vCJD should be considered when patients with a history of residence in a BSE-endemic area, such as the United Kingdom, develop a progressive neurodegenerative disease. Table 179.3 lists the World Health Organization criteria for vCJD. These criteria identified vCJD cases with 77% sensitivity and 100% specificity.[136] The newly developed real-time quaking-induced conversion (RT-QuIC) assays for prions in CSF (see "Specific Testing for Prion Disease [RT-QuIC]" later) appear to have limited sensitivity to detect vCJD prions.[158]

TABLE 179.3 World Health Organization Case Definitions for Sporadic and Variant Creutzfeldt-Jakob Disease (CJD)

Sporadic CJD

Possible CJD

Progressive dementia *and*
EEG atypical or not known *and*
Duration <2 years *and*
At least two of the following clinical features:
- Myoclonus
- Visual or cerebellar disturbance
- Pyramidal/extrapyramidal dysfunction
- Akinetic mutism

Probable CJD (in the Absence of an Alternative Diagnosis From Routine Investigation)

Progressive dementia *and*
At least two of the following four clinical features:
- Myoclonus
- [a]A typical EEG, whatever the clinical duration of the disease, *and/or*
- [a]A positive 14-3-3 assay for CSF, *and*
- A clinical duration to death <2 years

Definite CJD

Neuropathologic confirmation *and/or*
Confirmation of protease-resistant prion protein (immunocytochemistry or Western blot) *and/or*
The presence of scrapie-associated fibrils

Variant CJD

I. A. Progressive neuropsychiatric disorder
B. Duration of illness >6 months
C. Routine investigations do not suggest an alternative diagnosis
D. No history of potential iatrogenic exposure
E. No evidence of a familial form of TSE
II. A. Early psychiatric symptoms
B. Persistent painful sensory symptoms
C. Ataxia
D. Myoclonus or chorea or dystonia
E. Dementia
III. A. EEG does not show the typical appearance of sporadic CJD (or no EEG performed)
B. MRI brain scan shows bilateral symmetrical pulvinar high signal
IV. A. Positive tonsil biopsy

Definite CJD: I A *and* neuropathologic confirmation of variant CJD
Probable CJD: I *and* 4/5 of II *and* III A *and* III B *OR* I *and* IV A
Possible CJD: I and 4/5 of II and III A

[a]Note: These criteria have not been revised since the development of minimally invasive assays such as real-time quaking-induced conversion (RT-QuIC) of CSF. In cases of sporadic CJD, the RT-QuIC assay is superior to EEG or a 14-3-3 assay, and should be taken as evidence of probable or definite CJD.
CSF, Cerebrospinal fluid; *EEG,* electroencephalography; *MRI,* magnetic resonance imaging; *TSE,* transmissible spongiform encephalopathy.
From World Health Organization. *WHO Manual for Surveillance of Human Transmissible Spongiform Encephalopathies Including Variant Creutzfeldt-Jakob Disease.* Geneva: World Health Organization; 2003.

Iatrogenic Creutzfeldt-Jakob Disease

Examples of iatrogenic prion disease are exceedingly rare, but cases have occurred after transplantation of dura mater grafts,[159] corneal transplantation,[159] liver transplantation, use of dura mater material in radiographic embolization procedures,[160,161] use of contaminated neurosurgical instruments or stereotactic depth electrodes,[159] and, in cases of vCJD, blood and plasma concentrate transfusions.[142] Although the numbers are evolving constantly as new cases are identified, a review in 2012 identified 230 cases of iatrogenic CJD (iCJD) associated with human pituitary hormone administration (226, growth hormone; 4, gonadotropin), 228 cases associated with cadaver lyophilized dura mater grafts, 6 cases associated with contaminated neurosurgical instruments or intracortically implanted electroencephalography (EEG) depth electrodes, and 2 cases associated with cadaver corneal transplantation.[159]

Transmission Through Dura Mater Grafts

In the case of dura mater grafts, most implicated grafts have been Lyodura, the product of a single German commercial producer, manufactured before 1987. More cases (at least 154 by 2018) have been reported from Japan than from all other countries worldwide. [159,162] Cases have occurred in at least 17 additional countries, including at least 8 cases from Canada and the United States. The incubation period in these cases has ranged from 16 months to 30 years (median, approximately 12 years). In Japan, two distinct clinicopathologic forms of dura mater graft–associated CJD have been reported.[163] Approximately 75% of cases present very similarly to typical sCJD, with rapid progression, myoclonus, periodic sharp waves on the EEG, and a diffuse cortical distribution of PrP^{Sc}. About one-third of cases present with a picture reminiscent of GSS, with slower progression, absent myoclonus, absent or late appearance of sharp waves on the EEG, and deposition of PrP in amyloid plaques, some with a "florid" pattern similar to that seen in vCJD cases.

Transmission Through Cadaver-Derived Hormones

At least 226 cases of iCJD have been reported in young patients who received cadaver-derived human growth hormone for the treatment of endocrine disorders, with at least 4 additional cases in women receiving cadaver pituitary gonadotropin for infertility, practices that were discontinued when recombinant forms of these hormones became available.[159] Patients with human growth hormone–associated CJD typically received injections of growth hormone prepared from pools of 15,000 pituitary glands, several times weekly for several years. The risk of developing CJD differs among countries and cannot be absolutely determined because new cases continue to occur. As with kuru, incubation times can be extraordinarily long, with cases documented up to

40 years after exposure.[164] In 1977 a column chromatography step was introduced into the purification process for growth hormone produced under the National Hormone and Pituitary Program in the United States, and all but 1 of the US cases of growth hormone–associated CJD have occurred in the approximately 2700 patients who received treatment before that year.[159] The exception is a person who received commercially produced growth hormone from 1983 to 1985, and who developed prion disease in 2010.[165] France has an unusually high rate of iCJD among growth hormone recipients, in part because patients continued to receive the pituitary-derived product for several years after other countries abandoned the practice.[166]

Transmission Through Corneal Transplants

iCJD has been reported in 10 recipients of corneal transplants from 1974 to 2006 worldwide. However, in only two of those cases was the donor known to have had CJD. When the large number of corneal transplants performed annually is taken into account, it has been estimated that sCJD might coincidentally occur in a corneal graft recipient every 1.5 years in the United States alone.[167] Thus most of the 10 reported cases are likely coincidental.

Transmission Through Surgical Instruments

Five cases of iCJD have followed surgery with contaminated neurosurgical instruments, four from the United Kingdom and one from France.[168,169] The incubation period after surgery ranged from 12 to 28 months (median, 17 months). In two cases from Switzerland, intracortically implanted stereotactic depth electrodes for EEG monitoring were implicated.[170] The incubation period was 16 and 20 months in the two affected patients. Documented cases of neurosurgical transmission are remarkably rare, despite the large number of neurosurgical procedures performed. Epidemiologic studies have been mixed, but several show an increased risk of iCJD in those patients who have undergone *any* major surgical procedure.[171] Many of these studies may have been contaminated by recall and other biases, but a case-control study based on national hospital discharge registries in Sweden and Denmark also found an approximately twofold risk of CJD in those who had undergone major surgery, again of various sorts.[172] Odds ratios were mostly in the range of 3 or less. A modestly increased risk for a rare disease such as CJD translates into only a small increase in absolute risk, and the increased risk observed after surgery, even if real, does not necessarily implicate an infectious etiology.

Inadvertent Surgical Exposure

Several hospitals in the United States, Canada, and the United Kingdom have reported incidents in which neurosurgical instruments were inadvertently reused in other patients after having been used initially for diagnostic brain biopsies in patients who subsequently were found to have CJD. Despite these medical mishaps, no patient so exposed is known to have developed iCJD, although the time since surgery in most of these instances is insufficient to exclude absolutely the possibility that this may occur in the future. The Joint Commission on Accreditation of Healthcare Organizations has issued a sentinel event alert concerning this risk.[173] Hospitals should be aware of this potential and should establish guidelines for the handling, quarantine, and tracking of neurosurgical instruments in patients undergoing brain biopsy for dementia or other unknown neurodegenerative illnesses. In such cases, disposable instruments can be used if practicable, reducing the need for quarantine and tracking.

Blood Products and Sporadic CJD

As discussed previously, five cases of human-to-human transmission of vCJD prions through blood transfusion have occurred. In contrast, many epidemiologic studies have failed to find a convincing link between blood transfusions and any other form of human prion disease,[142,174] and a history of preceding transfusion does not seem to increase the risk for CJD in epidemiologic studies.[175] The reasons for this remarkable divergence are unknown. Animal transmission studies suggest that whole blood, serum, or buffy coat derived from patients with vCJD, cattle with BSE, deer with CWD, or animals experimentally inoculated with prions can transmit prion disease to at least some intravenously inoculated animals.[175] Recently, two cases of CJD occurred in Great Britain in patients with hemophilia. It is uncertain whether there is a causal connection between the lifelong receipt of clotting factors in these patients, or whether the occurrence of CJD was coincidental.[176]

CJD in Health Professionals

Cases of CJD occasionally occur in physicians, nurses, and other health care professionals. Despite the natural concern these reports produce, the incidence of CJD in this group does not seem to exceed what would be expected by chance alone.[177] There have been no documented reports of clear-cut transmission of disease from patients to hospital or mortuary staff. Similarly, although isolated cases of conjugal CJD have been reported, there does not seem to be any increased risk to spouses or other family members from exposure to CJD. As noted previously, the presence of familial cases of CJD seems invariably to result from genetic factors, rather than from person-to-person spread of illness.

Nosocomial and Iatrogenic Infection Risk

No special precautions need be taken in the routine care of patients with prion disease. Most bodily fluids, including blood, can be safely handled using standard precautions.[178] WHO recommends that clinical laboratories employ special precautions in the handling of CSF. Neurosurgical instruments utilized on patients with possible CJD should be sterilized by prolonged autoclaving (steam sterilization) or by using a combination of sodium hydroxide and autoclaving. (See Chapter 299 for further discussion.)

Prion Disease in Ruminants

Overwhelming evidence implicates the transmission of BSE from cattle to humans as the cause of vCJD. Prion disease is also endemic or epidemic in other ruminant species consumed by humans, including sheep and goats (caprinae), and deer, elk, and related species (cervidae). Recently, prion disease has been discovered in camels. The risk that prions in these species pose to humans appears low but is under active study. Intensive surveillance for prion disease in cattle and sheep has uncovered what appear to be strains of prion disease other than classic BSE and scrapie, and the risk of transmission of these forms to humans is especially uncertain.

Atypical Forms of Bovine Spongiform Encephalopathy

Two atypical forms of bovine prion disease, known as BSE-L (sometimes called BASE) and BSE-H, were first described in 2004.[179] They are uncommon, the rate being less than 0.5 per million adult cattle in France.[179,180] These forms are histopathologically distinct from classic BSE, and the protease-resistant fragments migrate differently from classic BSE on Western immunoblots. This, and data from transmission studies in various lines of transgenic and nontransgenic mice, supports the notion that these forms are strains of prions different from that which causes classic BSE.[180] These conditions are diagnosed mostly in aged cattle and seem to be found at a similar rate wherever cattle are intensively surveyed for prion disease. It is therefore suspected that these represent spontaneous cattle prion disease, rather than an exogenously acquired condition. A concern with these recently discovered strains of BSE prions is that they may be more pathogenic for humans than the classic BSE strain. Indeed, BSE-L has been transmitted both to transgenic mice expressing human PrP and to macaques, with incubation times suggesting the condition might more readily transmit to humans than does classic BSE.[181,182]

Atypical Forms of Scrapie

Scrapie has long been endemic in sheep, and epidemiologic evidence indicates that the disease either is not transmitted to humans or transmits at a very low rate.[183] As with BSE, intensive surveillance in recent years has revealed previously unrecognized strains of scrapie in domesticated sheep. Nor98 is the most distinctive of these strains, with an unusual 12-kDa protease-resistant core and a characteristic neuropathologic profile in sheep and in transgenic mice expressing ovine PrP.[184] Unlike classic scrapie, it is not clear if Nor98 is horizontally transmitted within flocks. Inoculation of transgenic mice expressing human PrP with either

classic scrapie or atypical forms such as Nor98 failed to cause disease, suggesting a substantial transmission barrier of sheep scrapie to humans.[185]

Chronic Wasting Disease

CWD was first discovered in captive mule deer in Colorado,[186] but now appears to be spreading worldwide.[187] It is found in free ranging and captive populations of deer, elk, and moose in North America. Recently it has been discovered in reindeer and moose in Norway. CWD is remarkable for the high rate of horizontal transmission. In northern Colorado, as many as 5% of all mule deer are infected, and, in captive populations, infection rates have exceeded 30%.[188] Human hunters and others have certainly consumed infected animals, but fortunately it appears that CWD is not readily transmissible to humans.[189] Nonetheless, the dynamics of interspecies transmission of prion diseases are poorly understood, so humans should avoid consuming CWD-contaminated deer and elk.

Camel Prion Disease

A prion disease has recently been discovered in more than 3% of dromedary camels sent to an abattoir in Algeria.[190] There is evidence that camels in this region may have been affected since the 1980s. Camels are a source of milk and meat for humans in northern and eastern Africa, the Middle East, and part of Asia. No information is currently available on the potential transmissibility of camel prion disease to humans.

LABORATORY DIAGNOSIS OF PRION DISEASE

In most cases, diagnosing human prion disease is approached by first excluding some more common conditions that might mimic prion disease, and then, if prion disease remains a diagnostic possibility, pursuing specific testing for these conditions. Typically, when prion disease is considered, the patient presents with a rapidly progressive dementia often with additional signs such as ataxia, parkinsonism, myoclonus, and spasticity. (See descriptions of the clinical presentations of these signs in the previous section, "Human Prion Diseases".) This is a relatively rare presentation, and certain conditions figure prominently in the differential diagnosis (Table 179.4). Many of these conditions are less common than prion disease, but some are treatable, so effort should be devoted to diagnosing them promptly.

Prion Disease Mimics

Suggested initial laboratory tests for a patient with rapidly progressive dementia are listed in Table 179.5. Common blood tests are typically normal in prion disease, so abnormalities might suggest other diagnoses. In prion disease, the CSF protein is usually normal or mildly elevated while cell counts and glucose concentration are usually normal. Rare reports exist of an inflammatory CSF profile in prion disease, but the presence of a significant pleocytosis or hypoglycorrhachia should prompt a search for other diagnostic possibilities.[191] These include infections, CNS neoplasms, and autoimmune encephalitis. In regard to this last possibility, an increasing number of autoantibodies against neural antigens are found to be associated with subacutely progressive neurologic illnesses, many of which share clinical features with prion diseases. A more detailed description of these syndromes is beyond the scope of this chapter, but they have been recently reviewed.[192,193] Typically signs of inflammation (elevated white blood cell counts and protein) are present in CSF, and areas of increased T2 signal may be seen in magnetic resonance imaging (MRI) sequences, but this is not always the case. Medication intoxications and nutritional deficiencies, particularly Wernicke encephalopathy, are encountered surprisingly often as prion disease mimics.

Brain MRI is useful both to suggest prion disease and to look for alternative causes of rapidly progressive dementia. Enhancing lesions and patterns of diffusion-weighted imaging (DWI) abnormality indicating infarction or white matter involvement suggest a diagnosis other than prion disease. The MRI findings in prion disease are discussed under "Brain Imaging" later.

Specific Testing for Prion Disease (RT-QuIC)

If the initial evaluation fails to suggest an alternative diagnosis, specific testing for prion disease should be pursued. Until very recently, the only highly reliable diagnosis of sporadic human prion disease was the demonstration of PrP^{Sc} in brain tissue obtained by biopsy or autopsy. Now, however, very specific and, as the technology develops, increasingly sensitive tests can be performed on CSF or other fairly easily obtained specimens to identify prions. These assays look for the ability of the specimen to cause the in vitro aggregation of recombinant PrP.[194] The precise techniques differ and are evolving, but the RT-QuIC method currently available in the United States through the National Prion Disease Pathology Surveillance Center has a 92% sensitivity and about a 99% specificity when performed on CSF.[195] Some forms of prion disease

TABLE 179.4 Differential Diagnosis of Prion Disease

CLASS	EXAMPLES[a]	COMMENT
Neurodegenerative	Alzheimer disease Diffuse Lewy body disease Frontotemporal dementia	The slower pace of progression usually distinguishes these from prion disease, but atypical rapidly progressive forms may occur.
Autoimmune encephalitis	SREAT Anti-NMDA Lupus cerebritis	Many treatable mimics of prion disease fall in this category.
Vascular	Primary angiitis of CNS Lupus-associated vasculitis CADASIL	
Nutritional/Metabolic	Thiamine deficiency Vitamin B_{12} deficiency	
Toxins	Serotonin syndrome Bismuth intoxication	
Neoplasms	Intravascular lymphoma Meningeal carcinomatosis	
Infections	Syphilis Lyme disease HIV	
Psychiatric	Factitious Catatonia	
Endocrine	Hyper- and hypothyroidism	

[a]Lists are not complete.
CADASIL, Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy; *CNS,* central nervous system; *HIV,* human immunodeficiency virus; *NMDA, N*-methyl-D-aspartate; *SREAT,* steroid-responsive encephalopathy with antithyroid antibodies.

may not be reliably detected by this method.[158] Fig. 179.3 depicts the RT-QuIC methodology. With the availability of these sensitive and specific minimally invasive tests, older diagnostic criteria for sporadic CJD are obsolete (see Table 179.3).

Supportive Testing for Prion Disease

CSF Proteins

Until recently, measuring elevated levels of certain proteins in CSF was important in diagnosis of sCJD. Such tests still may be useful in suggesting the diagnosis when RT-QuIC assay is negative. The intracellular neuronal protein 14-3-3 is found at elevated levels in the CSF of many patients with CJD. Testing for CSF 14-3-3 protein is available through commercial laboratories, but interpretation of 14-3-3 results is not standardized, so more reliable results may be available through the large central prion disease laboratories of the United States (National Prion Disease Pathology Surveillance Center, at https://case.edu/medicine/pathology/divisions/prion-center/), the United Kingdom, and the European Union. Although early studies reported a very high sensitivity and specificity for an elevated CSF 14-3-3 level, in subsequent practice the specificity has proved considerably lower. The American Academy of Neurology recently issued a guideline, based on a literature review, on the use of 14-3-3 CSF testing in the diagnosis of human prion disease. They reported a sensitivity of 92% and a specificity of 80% for the diagnosis of CJD. The guideline concluded that a negative 14-3-3 may reduce the suspicion of CJD but that a positive test can be found in potentially treatable conditions that mimic CJD.[196]

TABLE 179.5 Initial Evaluation of Rapidly Progressive Dementia

Blood
Complete blood count
Complete metabolic panel
Vitamin B12
Thyroid-stimulating hormone
Erythrocyte sedimentation rate
C-reactive protein
Antinuclear antibody
Syphilis and HIV serology
CSF[a]
Glucose
Protein
immunoglobulin G index
Oligoclonal bands
Cytology
Herpes simplex virus type 1
Imaging
Brain magnetic resonance imaging with and without contrast

[a]If prion disease is considered, instruct the laboratory to hold 2–5 mL frozen at –80°C. If initial evaluation fails to find an alternative diagnosis, this sample should be sent for real-time quaking-induced conversion testing.

Brain Imaging

Brain MRI is the most useful imaging technique in the diagnosis of CJD (Fig. 179.4). DWI is probably the most sensitive MRI sequence for detecting characteristic abnormalities associated with sporadic CJD.[197] In sporadic CJD the most common abnormality seen on standard MRI sequences is increased T2, DWI, or fluid-attenuated inversion recovery (FLAIR) signal in the striatum. Ribbon-like increased signal intensity in the cerebral cortex is also common. Increased signal intensity in the basal ganglia has been reported to have 67% sensitivity and 93% specificity for diagnosis of sCJD. In a study of 193 consecutive cases of suspected CJD, sensitivity of MRI changes ranged from 50% to 70%, depending on the evaluator, whereas specificity was approximately 80%.[198] The neuropathologic substrate for DWI abnormalities has not been characterized fully, although these abnormalities correlate with areas of severe neuropathologic change, including spongiform degeneration.[199] MRI is particularly useful in the diagnosis of vCJD.[200] In a prospective study of 368 MRI scans of patients with clinical histories suspicious for CJD, the presence of the "pulvinar sign" identified 74 of 82 patients eventually proven by neuropathologic studies to have vCJD. The pulvinar sign consists of increased signal in the posterior thalamus, usually seen on

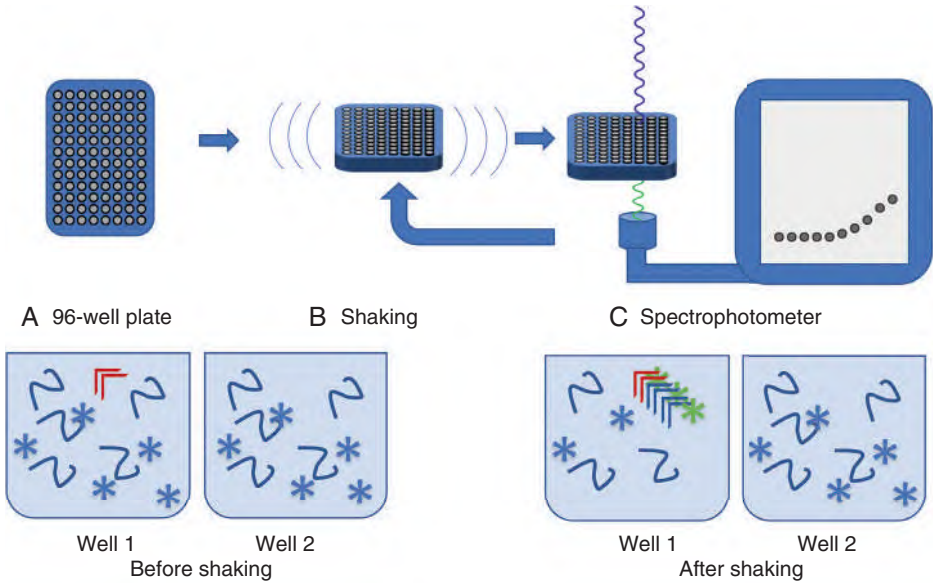

FIG. 179.3 The real-time quaking-induced conversion (RT-QuIC) method. RT-QuIC is an automated technique for amplifying and detecting seeded aggregation of PrP in vitro. The process is performed in 96-well plates, which allow for efficient processing of many samples and the inclusion of controls. The figure depicts the overall process above, and the contents of two hypothetical 96-well plates below. (A) The substrate consists of recombinant PrP *(blue lines)* in a solution containing thioflavin T *(stars)*. This compound undergoes a shift in fluorescence when it binds to amyloid fibrils. A sample to be tested, typically cerebrospinal fluid, is added to the substrate solution. (B) The plate is then shaken at a warm temperature. If PrP^{Sc} *(red)* is in the sample (well 1), recombinant PrP is incorporated to the fibril, and thioflavin T binds, undergoing a shift in fluorescence (depicted as a change to *green stars*). If there is no PrP^{Sc} in the sample (well 2), no fibrils are formed. (C) After shaking, the amount of bound thioflavin T is determined by spectrophotometry. The cycles of shaking and spectrophotometry are repeated, and samples containing PrP^{Sc} show a steady increase in the amount of bound thioflavin T, reflecting the increase in amyloid fibrils. This method has approximately 99% specificity and greater than 90% sensitivity for the diagnosis of sporadic Creutzfeldt-Jakob disease.

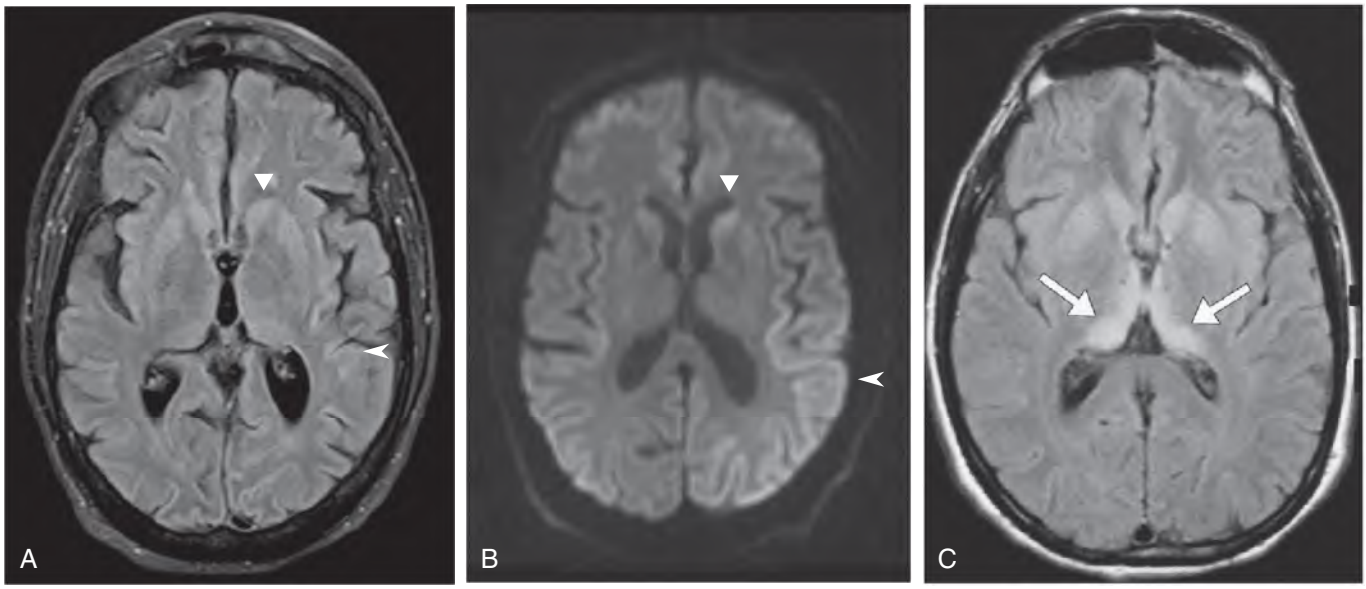

FIG. 179.4 The magnetic resonance imaging appearance of prion disease. (A and B) Fluid-attenuated inversion recovery (FLAIR) image (A) and diffusion-weighted image (DWI) (B) from a 63-year-old man with sporadic Creutzfeldt-Jakob disease (CJD). There is subtle hyperintensity in the cortical ribbon *(arrowheads)* and head of the caudate *(triangles),* seen more clearly on DWI than on FLAIR. (C) FLAIR image from a patient with variant CJD displays hyperintensity in the posterior thalamus *(arrows),* the so-called pulvinar sign that is characteristic of this form of CJD. *(C, From Tschampa HJ, Zerr I, Urbach H. Radiological assessment of Creutzfeldt-Jakob disease.* Eur Radiol. *2007;17:1200–1211.)*

image sequences sensitive to T2 effects (T2, FLAIR, DWI, etc.). Rarely, T1 hyperintensity is recognized in the pulvinar as well. When strictly defined as greater hyperintensity in the posterior thalamus relative to other deep brain nuclei, the pulvinar sign is specific for vCJD. Increased pulvinar and other deep gray matter hyperintensity occurs in some cases of sCJD; however, in these cases the signal in other nuclei, especially the anterior putamen, is greater than in the pulvinar.

Computed tomography is usually not helpful in the diagnosis of CJD, except that it may exclude some other conditions. Studies evaluating other neuroimaging techniques, such as positron emission tomography and single-photon emission computed tomography, are too few to determine the sensitivity or specificity of these tests.

Electroencephalography in Creutzfeldt-Jakob Disease

The EEG formerly played an important role in the diagnosis of CJD. The classic EEG pattern, which ultimately appears in 67% to 95% of patients, consists of a slow background interrupted by generalized, bilaterally synchronous biphasic or triphasic periodic sharp wave complexes (PSWCs).[201,202] These occur at intervals of 0.5 to 2.5 seconds and have a duration of 100 to 600 msec. PSWCs may be absent early in disease and may disappear in the terminal stages. They are more dramatic during periods of alertness, and may disappear during sleep or under the influence of certain drugs. Given the availability now of more specific and sensitive tests, the role of the EEG in the diagnosis of prion disease is uncertain.

Histology of Creutzfeldt-Jakob Disease

Brain histology remains the gold standard for diagnosis of prion diseases. The classic neuropathologic features of neuronal loss, reactive gliosis, and vacuolation of the neuropil (spongiform change), with an absence of inflammatory changes, typically are present in such cases and are consistent with the diagnosis. However, the modern diagnosis of prion disease rests on the immunohistochemical demonstration of abnormal forms of PrP. A variety of immunohistochemical techniques have been developed to detect PrP^{Sc} in paraffin-embedded brain tissue. The general strategy is to hydrolyze PrP^{C} while enhancing the antigenicity of PrP^{Sc} by partially denaturing the aggregates. A widely used protocol includes autoclaving tissue sections in water (hydrolytic autoclaving), followed by treatment with formic acid and then a chaotropic salt (guanidine thiocyanate).[203] With these techniques, a variety of PrP staining patterns have been identified in CJD brain tissue.[204] This tissue may show positive PrP staining limited to plaques or a more diffuse staining pattern that colocalizes with synaptic markers throughout the gray matter, or a combination of both patterns. In the United States, the National Prion Disease Pathology Surveillance Center at Case Western Reserve University in Cleveland, Ohio, assists clinicians and pathologists in analyzing fixed brain material for characteristic histopathology and the presence of PrP^{Sc}, and performs PrP^{Sc} immunohistochemistry and prion isoform analysis on frozen brain tissue from patients with suspected prion diseases. Instructions for shipping material are available at the center's website (https://case.edu/medicine/pathology/divisions/prion-center/).

Tissue Biochemical Tests

If frozen tissue is available, analysis of protease-resistant forms of PrP by Western immunoblot assay is generally performed. Two similar classification schemes correlate the clinical features of sporadic human prion disease with the biochemical properties of the PrP^{Sc} that accumulate in these cases. In these schemes, patients are classified by (1) the size of the protease-resistant fragment of PrP^{Sc} isolated from the brain and (2) the genotype at the polymorphic codon 129 of PrP (MM, MV, or VV). These "molecular" types are then correlated to the patients' clinical syndromes. In the scheme of Parchi and coworkers the protease-resistant fragment is either 21 kDa (type 1) or 19 kDa (type 2) in size.[205] Thus there are six possible molecular types of human prion disease under this scheme (MM1, MM2, MV1, etc.). In the scheme originally proposed by Collinge and coworkers,[146] three PrP^{Sc} size variants are recognized (with type 1 and type 2 being close in size to Parchi and coworkers' type 1). The subtle differences between the schemes may be an artifact.[206] At any rate, the molecular types do correlate to clinical syndromes, with distinct differences in age of onset, duration, and dominant clinical symptoms between the types. For example, under the Parchi scheme, patients with the MM1 molecular type have a mean age of onset of 66 years and a duration of 4 months, whereas those with the rare VV1 type have a mean onset of 40 years and a duration of 15 months. These molecular types likely reflect, in part, the same sorts of biochemical differences in the PrP^{Sc} seen between well-characterized animal prion strains, with an additional, incompletely understood influence from the PrP genotype.

Genetic Testing and Creutzfeldt-Jakob Disease

In a symptomatic patient with a family history of prion disease, finding a *PRNP* gene mutation can be taken as definitive evidence of CJD. A

family history of unusual dementia is absent in as many as 46% of patients with a genetic prion disease.[83] Such cases can be due to new mutations, incomplete family history, incomplete penetrance, or misidentified paternity. Thus it may be worth obtaining the sequence of the *PRNP* coding region in patients with undiagnosed subacute or otherwise unusual dementias. Genetic techniques are highly specific but insensitive when used for diagnosis of isolated cases of prion disease because approximately 90% of prion disease patients will have no *PRNP* mutation.

WHO has published criteria for the clinical diagnosis of CJD (see Table 179.3). The definition of "probable CJD" is obsolete, and needs to be revised to account for the availability now of highly specific and sensitive tests such as RT-QuIC.

PRION DISEASE THERAPY

All prion diseases remain incurable and uniformly fatal; however, the availability of excellent animal models of prion disease has encouraged investigations of a large number of different therapeutic strategies. These strategies include immunotherapies, such as antibodies and immunization against PrP, various small molecule inhibitors of PrP^{Sc} propagation identified by in vitro screening methods, polyanionic and polycationic compounds, and small interfering RNA and other inhibitors of PrP expression.[207,208] Several of these treatments are able to abolish scrapie infection in cultured cells. A number can delay the onset of clinical illness in rodents when administered before inoculation with prions and, in some cases, after inoculation but before the emergence of clinical signs. However, no treatment significantly alters the course of disease after the appearance of obvious clinical signs—the situation that would be most useful for treatment of sporadic human prion disease. Five compounds have been tried in series of humans symptomatic with prion disease.[209,210] Pentosan polysulfate by intraventricular infusion may have slowed disease progression in some patients, but the studies are uncontrolled. Studies of orally administered quinacrine treatment generally suggest no substantial benefit. Amantadine may have transiently improved symptoms in some patients, but it probably provides no clinically important benefit. In a placebo-controlled trial of flupirtine, the drug may have slowed cognitive decline.[211] A mostly observational trial of tetracycline suggested that it might slightly increase survival time.[210]

Key References

The complete reference list is available online at Expert Consult.

20. Singh J, Udgaonkar JB. Molecular Mechanism of the Misfolding and Oligomerization of the Prion Protein: current Understanding and Its Implications. *Biochemistry Mosc*. 2015;54:4431–4442.
29. Jeong B-H, Kim Y-S. Genetic studies in human prion diseases. *J Korean Med Sci*. 2014;29:623–632.
30. Sanchez-Juan P, Bishop MT, Kovacs GG, et al. A genome wide association study links glutamate receptor pathway to sporadic Creutzfeldt-Jakob disease risk. *PLoS ONE*. 2014;10:e0123654.
32. Supattapone S. Synthesis of high titer infectious prions with cofactor molecules. *J Biol Chem*. 2014;289:19850–19854.
38. Ushiki-Kaku Y, Shimizu Y, Tabeta N, et al. Heterogeneity of abnormal prion protein (PrP(Sc)) in murine scrapie prions determined by PrP(Sc)-specific monoclonal antibodies. *J Vet Med Sci*. 2014;76:285–288.
47. Sandberg MK, Al-Doujaily H, Sharps B, et al. Prion neuropathology follows the accumulation of alternate prion protein isoforms after infective titre has peaked. *Nat Commun*. 2014;5:4347.
50. Wickner RB, Shewmaker FP, Bateman DA, et al. Yeast prions: structure, biology, and prion-handling systems. *Microbiol Mol Biol Rev*. 2015;79:1–17.
74. Salazar R, Cerghet M, Ramachandran V. Bilateral hearing loss heralding sporadic Creutzfeldt-Jakob disease: a case report and literature review. *Otol Neurotol*. 2014;35:1327–1329.
75. Mantokoudis G, Saber Tehrani AS, Newman-Toker DE. An unusual stroke-like clinical presentation of Creutzfeldt-Jakob disease: acute vestibular syndrome. *Neurologist*. 2015;19:96–98.
81. Notari S, Xiao X, Espinosa JC, et al. Transmission characteristics of variably protease-sensitive prionopathy. *Emerg Infect Dis*. 2014;20:2006–2014.
82. Diack AB, Ritchie DL, Peden AH, et al. Variably protease-sensitive prionopathy, a unique prion variant with inefficient transmission properties. *Emerg Infect Dis*. 2014;20:1969–1979.
85. Minikel EV, Vallabh SM, Lek M, et al. Quantifying prion disease penetrance using large population control cohorts. *Sci Transl Med*. 2016;8:322ra9.
94. Nonno R, Angelo Di Bari M, Agrimi U, et al. Transmissibility of Gerstmann-Sträussler-Scheinker syndrome in rodent models: new insights into the molecular underpinnings of prion infectivity. *Prion*. 2016;10:421–433.
130. Maheshwari A, Fischer M, Gambetti P, et al. Recent US case of variant Creutzfeldt-Jakob disease—global implications. *Emerg Infect Dis*. 2015;21:750–759.
133. Chen C-C, Wang Y-H. Estimation of the exposure of the UK population to the bovine spongiform encephalopathy agent through dietary intake during the period 1980 to 1996. *PLoS ONE*. 2014;9:e94020.
142. Urwin PJM, Mackenzie JM, Llewelyn CA, et al. Creutzfeldt-Jakob disease and blood transfusion: updated results of the UK Transfusion Medicine Epidemiology Review Study. *Vox Sang*. 2016;110:310–316.
162. Ae R. Update: dura mater graft–associated Creutzfeldt-Jakob disease — Japan, 1975–2017. *MMWR Morb Mortal Wkly Rep*. 2018;67.
164. Rudge P, Jaunmuktane Z, Adlard P, et al. Iatrogenic CJD due to pituitary-derived growth hormone with genetically determined incubation times of up to 40 years. *Brain*. 2015;138(Pt 11):3386–3399.
171. López FJG, Ruiz-Tovar M, Almazán-Isla J, et al. Risk of transmission of sporadic Creutzfeldt-Jakob disease by surgical procedures: systematic reviews and quality of evidence. *Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull*. 2017;22.
174. Crowder LA, Schonberger LB, Dodd RY, et al. Creutzfeldt-Jakob disease lookback study: 21 years of surveillance for transfusion transmission risk. *Transfusion (Paris)*. 2017;57:1875–1878.
176. Urwin P, Thanigaikumar K, Ironside JW, et al. Sporadic Creutzfeldt-Jakob disease in 2 plasma product recipients, United Kingdom. *Emerg Infect Dis*. 2017;23.
178. World Health Organization. WHO infection control guidelines for transmissible spongiform encephalopathies. Report of a WHO consultation, Geneva, Switzerland, 23-26 March 1999. http://www.who.int/csr/resources/publications/bse/WHO_CDS_CSR_APH_2000_3/en/. Accessed April 30, 2018.
187. Herbst A, Velásquez CD, Triscott E, et al. Chronic wasting disease prion strain emergence and host range expansion. *Emerg Infect Dis*. 2017;23:1598–1600.
189. Waddell L, Greig J, Mascarenhas M, et al Current evidence on the transmissibility of chronic wasting disease prions to humans-A systematic review. Transbound Emerg Dis. January 2017.
190. Babelhadj B, Di Bari MA, Pirisinu L, et al. Prion disease in dromedary camels, Algeria. *Emerg Infect Dis*. 2018;24.
192. Dalmau J, Geis C, Graus F. Autoantibodies to synaptic receptors and neuronal cell surface proteins in autoimmune diseases of the central nervous system. *Physiol Rev*. 2017;97:839–887.
194. Zanusso G, Monaco S, Pocchiari M, et al. Advanced tests for early and accurate diagnosis of Creutzfeldt-Jakob disease. *Nat Rev Neurol*. 2016;12:325–333.
195. Foutz A, Appleby BS, Hamlin C, et al. Diagnostic and prognostic value of human prion detection in cerebrospinal fluid. *Ann Neurol*. 2017;81:79–92.
197. Fragoso DC, Gonçalves Filho AL da M, Pacheco FT, et al. Imaging of Creutzfeldt-Jakob disease: imaging patterns and their differential diagnosis. *Radiographics*. 2017;37:234–257.
208. Aguzzi A, Lakkaraju AKK, Frontzek K. Toward therapy of human prion diseases. *Annu Rev Pharmacol Toxicol*. 2018;58:331–351.
210. Varges D, Manthey H, Heinemann U, et al. Doxycycline in early CJD: a double-blinded randomised phase II and observational study. *J Neurol Neurosurg Psychiatry*. 2017;88:119–125.

References

1. Schneider K, Fangerau H, Michaelsen B, et al. The early history of the transmissible spongiform encephalopathies exemplified by scrapie. *Brain Res Bull*. 2008;77:343–355.
2. Cuille J, Chelle P. Experimental transmission of trembling to the goat. *Comptes Rendus Académie Sci*. 1939;208:1058–1060.
3. Alper T, Haig DA, Clarke MC. The exceptionally small size of the scrapie agent. *Biochem Biophys Res Commun*. 1966;22:278–284.
4. Gajdusek DC, Zigas V. Kuru; clinical, pathological and epidemiological study of an acute progressive degenerative disease of the central nervous system among natives of the Eastern Highlands of New Guinea. *Am J Med*. 1959;26:442–469.
5. Gajdusek DC, Gibbs CJ, Alpers M. Experimental transmission of a Kuru-like syndrome to chimpanzees. *Nature*. 1966;209:794–796.
6. Hadlow W. Scrapie and kuru. *Lancet*. 1959;274:289–290.
7. Prusiner SB, Hadlow WJ, Eklund CM, et al. Sedimentation properties of the scrapie agent. *Proc Natl Acad Sci USA*. 1977;74:4656–4660.
8. Prusiner SB, Groth DF, Cochran SP, et al. Molecular properties, partial purification, and assay by incubation period measurements of the hamster scrapie agent. *Biochemistry Mosc*. 1980;19:4883–4891.
9. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science*. 1982;216:136–144.
10. Oesch B, Westaway D, Wälchli M, et al. A cellular gene encodes scrapie PrP 27-30 protein. *Cell*. 1985;40:735–746.
11. Stahl N, Borchelt DR, Hsiao K, et al. Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell*. 1987;51:229–240.
12. Bolton DC, Meyer RK, Prusiner SB. Scrapie PrP 27-30 is a sialoglycoprotein. *J Virol*. 1985;53:596–606.
13. Manson J, West JD, Thomson V, et al. The prion protein gene: a role in mouse embryogenesis? *Development*. 1992;115:117–122.
14. Moser M, Colello RJ, Pott U, et al. Developmental expression of the prion protein gene in glial cells. *Neuron*. 1995;14:509–517.
15. Linden R, Martins VR, Prado MAM, et al. Physiology of the prion protein. *Physiol Rev*. 2008;88:673–728.
16. Laurén J, Gimbel DA, Nygaard HB, et al. Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature*. 2009;457:1128–1132.
17. Riek R, Hornemann S, Wider G, et al. NMR characterization of the full-length recombinant murine prion protein, mPrP(23-231). *FEBS Lett*. 1997;413:282–288.
18. Pan KM, Baldwin M, Nguyen J, et al. Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci USA*. 1993;90:10962–10966.
19. Diaz-Espinoza R, Soto C. High-resolution structure of infectious prion protein: the final frontier. *Nat Struct Mol Biol*. 2012;19:370–377.
20. Singh J, Udgaonkar JB. Molecular Mechanism of the Misfolding and Oligomerization of the Prion Protein: current Understanding and Its Implications. *Biochemistry Mosc*. 2015;54:4431–4442.
21. Silveira JR, Raymond GJ, Hughson AG, et al. The most infectious prion protein particles. *Nature*. 2005;437:257–261.
22. Saborio GP, Permanne B, Soto C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature*. 2001;411:810–813.
23. Deleault NR, Harris BT, Rees JR, et al. Formation of native prions from minimal components in vitro. *Proc Natl Acad Sci USA*. 2007;104:9741–9746.
24. Wang F, Wang X, Yuan C-G, et al. Generating a prion with bacterially expressed recombinant prion protein. *Science*. 2010;327:1132–1135.
25. Sadlish H, Rampelt H, Shorter J, et al. Hsp110 chaperones regulate prion formation and propagation in S. cerevisiae by two discrete activities. *PLoS ONE*. 2008;3:e1763.
26. Shorter J, Lindquist S. Hsp104, Hsp70 and Hsp40 interplay regulates formation, growth and elimination of Sup35 prions. *EMBO J*. 2008;27:2712–2724.
27. Kaneko K, Zulianello L, Scott M, et al. Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. *Proc Natl Acad Sci USA*. 1997;94:10069–10074.
28. Tamgüney G, Giles K, Glidden DV, et al. Genes contributing to prion pathogenesis. *J Gen Virol*. 2008;89(Pt 7):1777–1788.
29. Jeong B-H, Kim Y-S. Genetic studies in human prion diseases. *J Korean Med Sci*. 2014;29:623–632.
30. Sanchez-Juan P, Bishop MT, Kovacs GG, et al. A genome wide association study links glutamate receptor pathway to sporadic Creutzfeldt-Jakob disease risk. *PLoS ONE*. 2014;10:e0123654.
31. Balendra R, Uphill J, Collinson C, et al. Variants of PLCXD3 are not associated with variant or sporadic Creutzfeldt-Jakob disease in a large international study. *BMC Med Genet*. 2016;17:28.
32. Supattapone S. Synthesis of high titer infectious prions with cofactor molecules. *J Biol Chem*. 2014;289:19850–19854.
33. Scott M, Foster D, Mirenda C, et al. Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. *Cell*. 1989;59:847–857.
34. Moreno JA, Telling GC. Insights into Mechanisms of Transmission and Pathogenesis from Transgenic Mouse Models of Prion Diseases. *Methods Mol Biol*. 2017;1658:219–252.
35. Collinge J, Clarke AR. A general model of prion strains and their pathogenicity. *Science*. 2007;318:930–936.
36. Bruce ME. TSE strain variation. *Br Med Bull*. 2003;66:99–108.
37. Safar J, Wille H, Itri V, et al. Eight prion strains have PrP(Sc) molecules with different conformations. *Nat Med*. 1998;4:1157–1165.
38. Ushiki-Kaku Y, Shimizu Y, Tabeta N, et al. Heterogeneity of abnormal prion protein (PrP(Sc)) in murine scrapie prions determined by PrP(Sc)-specific monoclonal antibodies. *J Vet Med Sci*. 2014;76:285–288.
39. Prinz M, Huber G, Macpherson AJS, et al. Oral prion infection requires normal numbers of Peyer's patches but not of enteric lymphocytes. *Am J Pathol*. 2003;162:1103–1111.
40. Klein MA, Frigg R, Flechsig E, et al. A crucial role for B cells in neuroinvasive scrapie. *Nature*. 1997;390:687–690.
41. Chiocchetti R, Mazzuoli G, Albanese V, et al. Anatomical evidence for ileal Peyer's patches innervation by enteric nervous system: a potential route for prion neuroinvasion? *Cell Tissue Res*. 2008;332:185–194.
42. Hoffmann C, Ziegler U, Buschmann A, et al. Prions spread via the autonomic nervous system from the gut to the central nervous system in cattle incubating bovine spongiform encephalopathy. *J Gen Virol*. 2007;88(Pt 3):1048–1055.
43. McBride PA, Beekes M. Pathological PrP is abundant in sympathetic and sensory ganglia of hamsters fed with scrapie. *Neurosci Lett*. 1999;265:135–138.
44. Glatzel M, Heppner FL, Albers KM, et al. Sympathetic innervation of lymphoreticular organs is rate limiting for prion neuroinvasion. *Neuron*. 2001;31:25–34.
45. Aguzzi A, Sigurdson C, Heikenwaelder M. Molecular mechanisms of prion pathogenesis. *Annu Rev Pathol*. 2008;3:11–40.
46. Chesebro B, Trifilo M, Race R, et al. Anchorless prion protein results in infectious amyloid disease without clinical scrapie. *Science*. 2005;308:1435–1439.
47. Sandberg MK, Al-Doujaily H, Sharps B, et al. Prion neuropathology follows the accumulation of alternate prion protein isoforms after infective titre has peaked. *Nat Commun*. 2014;5:4347.
48. Andre R, Tabrizi SJ. Misfolded PrP and a novel mechanism of proteasome inhibition. *Prion*. 2012;6:32–36.
49. Wickner RB. [URE3] as an altered URE2 protein: evidence for a prion analog in Saccharomyces cerevisiae. *Science*. 1994;264:566–569.
50. Wickner RB, Shewmaker FP, Bateman DA, et al. Yeast prions: structure, biology, and Prion-Handling systems. *Microbiol Mol Biol Rev*. 2015;79:1–17.
51. Prusiner SB. Cell biology. A unifying role for prions in neurodegenerative diseases. *Science*. 2012;336:1511–1513.
52. Ritchie DL, Adlard P, Peden AH, et al. Amyloid-β accumulation in the CNS in human growth hormone recipients in the UK. *Acta Neuropathol (Berl)*. 2017;134:221–240.
53. Li J-Y, Englund E, Holton JL, et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med*. 2008;14:501–503.
54. Spielmeyer W. Die histopathologische Forschung in der Psychiatrie. Klin Wochenschr. 1922:1817–1819.
55. Creutzfeldt H. Über eine eigenartige herdförmige Erkrankung des Zentralnervensystems. *Z Ges Eurol Psychiat*. 1920;57:1–18.
56. Jakob A. Über eine der multiplen Sklerose klinisch nahestehende Erkrankung des Zentralnervensystems (spastische Pseudosklerose) mit bemerkenswertem anatomischem Befunde. Mitteilung eines vier ten Falles. *Med Klin*. 1921;17:372–376.
57. The National CJD Research & Surveillance Unit. 25th Annual Report. Creutzfeldt-Jakob disease surveillance in the UK; 2017. http://www.cjd.ed.ac.uk/surveillance/data-and-reports. Accessed November 22, 2017.
58. Klug GMJA, Wand H, Simpson M, et al. Intensity of human prion disease surveillance predicts observed disease incidence. *J Neurol Neurosurg Psychiatry*. 2013;84:1372–1377.
59. Holman RC, Belay ED, Christensen KY, et al. Human prion diseases in the United States. *PLoS ONE*. 2010;5:e8521.
60. Ladogana A, Puopolo M, Croes EA, et al. Mortality from Creutzfeldt-Jakob disease and related disorders in Europe, Australia, and Canada. *Neurology*. 2005;64:1586–1591.
61. Heinemann U, Krasnianski A, Meissner B, et al. Creutzfeldt-Jakob disease in Germany: a prospective 12-year surveillance. *Brain*. 2007;130(Pt 5):1350–1359.
62. Heidenhain A. Klinische und anatomische Untersuchungen Über eine eigenartige Erkrankung des Zentralnervensystems im Praesenium. *Z Ges Neurol Psychiat*. 1929;118:49–114.
63. Brownell B, Oppenheimer DR. An ataxic form of subacute presinle polioencephalopathy (Creutzfeldt-Jakob disease). *J Neurol Neurosurg Psychiatry*. 1965;28:350–361.
64. Stern K. Severe dementia associated with bilateral symmetrical degeneration of the thalamus. *Brain*. 1939;62:157–171.
65. Garcin R, Brion S, Khochneviss A. Le syndrome de Creutzfeldt-Jakob et les syndromes corticostries du presenium (a l'occasion de 5 observations anatomo-cliniques). *Rev Neurol (Paris)*. 1963;109:419–441.
66. Kirschbaum W. *Jakob-Creutzfeldt Disease*. New York: Elsevier; 1968.
67. Wall CA, Rummans TA, Aksamit AJ, et al. Psychiatric manifestations of Creutzfeldt-Jakob disease: a 25-year analysis. *J Neuropsychiatry Clin Neurosci*. 2005;17:489–495.
68. Macleod M-A, Stewart GE, Zeidler M, et al. Sensory features of variant Creutzfeldt-Jakob disease. *J Neurol*. 2002;249:706–711.
69. Niewiadomska M, Kulczycki J, Wochnik-Dyjas D, et al. Impairment of the peripheral nervous system in Creutzfeldt-Jakob disease. *Arch Neurol*. 2002;59:1430–1436.
70. Kovács T, Arányi Z, Szirmai I, et al. Creutzfeldt-Jakob disease with amyotrophy and demyelinating polyneuropathy. *Arch Neurol*. 2002;59:1811–1814.
71. Worrall BB, Rowland LP, Chin SS, et al. Amyotrophy in prion diseases. *Arch Neurol*. 2000;57:33–38.
72. Nagasaka K, Ohta E, Nagasaka T, et al. Rhythmic pupillary oscillation in Creutzfeldt-Jakob disease associated with the Glu/Lys mutation of prion protein codon 200. *Mov Disord*. 2010;25:111–116.
73. Huber FM, Bour F, Sazdovitch V, et al. Creutzfeldt-Jakob disease with slow progression. A mimickry of progressive supranuclear palsy. *Bull Soc Sci Med Grand Duche Luxemb*. 2007;2:125–130.
74. Salazar R, Cerghet M, Ramachandran V. Bilateral hearing loss heralding sporadic Creutzfeldt-Jakob disease: a case report and literature review. *Otol Neurotol*. 2014;35:1327–1329.
75. Mantokoudis G, Saber Tehrani AS, Newman-Toker DE. An unusual stroke-like clinical presentation of Creutzfeldt-Jakob disease: acute vestibular syndrome. *Neurologist*. 2015;19:96–98.
76. Parchi P, Capellari S, Chin S, et al. A subtype of sporadic prion disease mimicking fatal familial insomnia. *Neurology*. 1999;52:1757–1763.
77. Mastrianni JA, Nixon R, Layzer R, et al. Prion protein conformation in a patient with sporadic fatal insomnia. *N Engl J Med*. 1999;340:1630–1638.
78. Zou W-Q, Puoti G, Xiao X, et al. Variably protease-sensitive prionopathy: a new sporadic disease of the prion protein. *Ann Neurol*. 2010;68:162–172.
79. Zou W-Q, Gambetti P, Xiao X, et al. Prions in variably protease-sensitive prionopathy: an update. *Pathogens*. 2013;2:457–471.
80. Head MW, Yull HM, Ritchie DL, et al. Variably protease-sensitive prionopathy in the UK: a retrospective review 1991-2008. *Brain*. 2013;136(Pt 4):1102–1115.
81. Notari S, Xiao X, Espinosa JC, et al. Transmission characteristics of variably protease-sensitive prionopathy. *Emerg Infect Dis*. 2014;20:2006–2014.
82. Diack AB, Ritchie DL, Peden AH, et al. Variably protease-sensitive prionopathy, a unique prion variant with inefficient transmission properties. *Emerg Infect Dis*. 2014;20:1969–1979.
83. Kovács GG, Puopolo M, Ladogana A, et al. Genetic prion disease: the EUROCJD experience. *Hum Genet*. 2005;118:166–174.
84. Lloyd SE, Mead S, Collinge J. Genetics of prion diseases. *Curr Opin Genet Dev*. 2013;23:345–351.
85. Minikel EV, Vallabh SM, Lek M, et al. Quantifying prion disease penetrance using large population control cohorts. *Sci Transl Med*. 2016;8:322ra9.
86. Mead S. Prion disease genetics. *Eur J Hum Genet*. 2006;14:273–281.
87. Herzberg L, Herzberg BN, Gibbs CJ, et al. Letter: Creutzfeldt-Jakob disease: hypothesis for high incidence in Libyan Jews in Israel. *Science*. 1974;186:848.
88. Spudich S, Mastrianni JA, Wrensch M, et al. Complete penetrance of Creutzfeldt-Jakob disease in Libyan Jews

carrying the E200K mutation in the prion protein gene. *Mol Med*. 1995;1:607–613.

89. Simon ES, Kahana E, Chapman J, et al. Creutzfeldt-Jakob disease profile in patients homozygous for the PRNP E200K mutation. *Ann Neurol*. 2000;47:257–260.
90. Tagliavini F, Prelli F, Ghiso J, et al. Amyloid protein of Gerstmann-Sträussler-Scheinker disease (Indiana kindred) is an 11 kd fragment of prion protein with an N-terminal glycine at codon 58. *EMBO J*. 1991;10:513–519.
91. Giaccone G, Verga L, Bugiani O, et al. Prion protein preamyloid and amyloid deposits in Gerstmann-Sträussler-Scheinker disease, Indiana kindred. *Proc Natl Acad Sci USA*. 1992;89:9349–9353.
92. Collins S, McLean CA, Masters CL. Gerstmann-Sträussler-Scheinker syndrome,fatal familial insomnia, and kuru: a review of these less common human transmissible spongiform encephalopathies. *J Clin Neurosci*. 2001;8:387–397.
93. Liberski PP, Bratosiewicz J, Waliś A, et al. A special report I. Prion protein (PrP)–amyloid plaques in the transmissible spongiform encephalopathies, or prion diseases revisited. *Folia Neuropathol*. 2001;39:217–235.
94. Nonno R, Angelo Di Bari M, Agrimi U, et al. Transmissibility of Gerstmann-Sträussler-Scheinker syndrome in rodent models: new insights into the molecular underpinnings of prion infectivity. *Prion*. 2016;10:421–433.
95. Lugaresi E, Medori R, Montagna P, et al. Fatal familial insomnia and dysautonomia with selective degeneration of thalamic nuclei. *N Engl J Med*. 1986;315:997–1003.
96. Manetto V, Medori R, Cortelli P, et al. Fatal familial insomnia: clinical and pathologic study of five new cases. *Neurology*. 1992;42:312–319.
97. Goldfarb LG, Petersen RB, Tabaton M, et al. Fatal familial insomnia and familial Creutzfeldt-Jakob disease: disease phenotype determined by a DNA polymorphism. *Science*. 1992;258:806–808.
98. Medori R, Tritschler HJ, LeBlanc A, et al. Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. *N Engl J Med*. 1992;326:444–449.
99. Montagna P, Gambetti P, Cortelli P, et al. Familial and sporadic fatal insomnia. *Lancet Neurol*. 2003;2:167–176.
100. Zarranz JJ, Digon A, Atarés B, et al. Phenotypic variability in familial prion diseases due to the D178N mutation. *J Neurol Neurosurg Psychiatry*. 2005;76:1491–1496.
101. Krasnianski A, Bartl M, Sanchez Juan PJ, et al. Fatal familial insomnia: clinical features and early identification. *Ann Neurol*. 2008;63:658–661.
102. McLean CA, Storey E, Gardner RJ, et al. The D178N (cis-129M) "fatal familial insomnia" mutation associated with diverse clinicopathologic phenotypes in an Australian kindred. *Neurology*. 1997;49:552–558.
103. Landolt H-P, Glatzel M, Blättler T, et al. Sleep-wake disturbances in sporadic Creutzfeldt-Jakob disease. *Neurology*. 2006;66:1418–1424.
104. Bütefisch CM, Gambetti P, Cervenakova L, et al. Inherited prion encephalopathy associated with the novel PRNP H187R mutation: a clinical study. *Neurology*. 2000;55:517–522.
105. Cochran EJ, Bennett DA, Cervenáková L, et al. Familial Creutzfeldt-Jakob disease with a five-repeat octapeptide insert mutation. *Neurology*. 1996;47:727–733.
106. Laplanche JL, Hachimi KH, Durieux I, et al. Prominent psychiatric features and early onset in an inherited prion disease with a new insertional mutation in the prion protein gene. *Brain*. 1999;122(Pt 12):2375–2386.
107. Collinge J, Brown J, Hardy J, et al. Inherited prion disease with 144 base pair gene insertion. 2. Clinical and pathological features. *Brain*. 1992;115(Pt 3):687–710.
108. Hall DA, Leehey MA, Filley CM, et al. PRNP H187R mutation associated with neuropsychiatric disorders in childhood and dementia. *Neurology*. 2005;64:1304–1306.
109. Dyrbye H, Broholm H, Dziegiel MH, et al. The M129V polymorphism of codon 129 in the prion gene (PRNP) in the Danish population. *Eur J Epidemiol*. 2008;23:23–27.
110. Palmer MS, Dryden AJ, Hughes JT, et al. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. *Nature*. 1991;352:340–342.
111. Windl O, Dempster M, Estibeiro JP, et al. Genetic basis of Creutzfeldt-Jakob disease in the United Kingdom: a systematic analysis of predisposing mutations and allelic variation in the PRNP gene. *Hum Genet*. 1996;98:259–264.
112. Alperovitch A, Zerr I, Pocchiari M, et al. Codon 129 prion protein genotype and sporadic Creutzfeldt-Jakob disease. *Lancet*. 1999;353:1673–1674.
113. Jeong B-H, Lee K-H, Kim N-H, et al. Association of sporadic Creutzfeldt-Jakob disease with homozygous genotypes at PRNP codons 129 and 219 in the Korean population. *Neurogenetics*. 2005;6:229–232.

113a. Lukic A, Beck J, Joiner S, et al. Heterozygosity at polymorphic codon 219 in variant Creutzfeldt-Jakob disease. *Arch Neurol*. 2010;67:1021–1023.

114. Gajdusek DC, Zigas V. Degenerative disease of the central nervous system in New Guinea; the endemic occurrence of kuru in the native population. *N Engl J Med*. 1957;257:974–978.
115. Collinge J, Whitfield J, McKintosh E, et al. Kuru in the 21st century–an acquired human prion disease with very long incubation periods. *Lancet*. 2006;367:2068–2074.
116. Huillard d'Aignaux JN, Cousens SN, Maccario J, et al. The incubation period of kuru. *Epidemiol Camb Mass*. 2002;13:402–408.
117. Whitfield JT, Pako WH, Collinge J, et al. Cultural factors that affected the spatial and temporal epidemiology of kuru. *R Soc Open Sci*. 2017;4:160789.
118. Collinge J, Whitfield J, McKintosh E, et al. A clinical study of kuru patients with long incubation periods at the end of the epidemic in Papua New Guinea. *Philos Trans R Soc Lond B Biol Sci*. 2008;363:3725–3739.
119. McLean CA. Review. The neuropathology of kuru and variant Creutzfeldt-Jakob disease. *Philos Trans R Soc Lond B Biol Sci*. 2008;363:3685–3687.
120. Brandner S, Whitfield J, Boone K, et al. Central and peripheral pathology of kuru: pathological analysis of a recent case and comparison with other forms of human prion disease. *Philos Trans R Soc Lond B Biol Sci*. 2008;363:3755–3763.
121. Mead S, Whitfield J, Poulter M, et al. Genetic susceptibility, evolution and the kuru epidemic. *Philos Trans R Soc Lond B Biol Sci*. 2008;363:3741–3746.
122. Mead S, Whitfield J, Poulter M, et al. A novel protective prion protein variant that colocalizes with kuru exposure. *N Engl J Med*. 2009;361:2056–2065.
123. Wadsworth JDF, Joiner S, Linehan JM, et al. Review. The origin of the prion agent of kuru: molecular and biological strain typing. *Philos Trans R Soc Lond B Biol Sci*. 2008;363:3747–3753.
124. Britton TC, al-Sarraj S, Shaw C, et al. Sporadic Creutzfeldt-Jakob disease in a 16-year-old in the UK. *Lancet*. 1995;346:1155.
125. Bateman D, Hilton D, Love S, et al. Sporadic Creutzfeldt-Jakob disease in a 18-year-old in the UK. *Lancet*. 1995;346:1155–1156.
126. Will RG, Ironside JW, Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet*. 1996;347:921–925.
127. Wroe SJ, Pal S, Siddique D, et al. Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. *Lancet*. 2006;368:2061–2067.
128. Hewitt PE, Llewelyn CA, Mackenzie J, et al. Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study. *Vox Sang*. 2006;91:221–230.
129. Health Protection Agency. 4th case of variant CJD infection associated with blood transfusion. Published January 19, 2007. http://webarchive.nationalarchives.gov.uk/20100418085653/http://www.hpa.nhs.uk/hpr/archives/2007/hpr0307.pdf.
130. Maheshwari A, Fischer M, Gambetti P, et al. Recent US case of variant Creutzfeldt-Jakob disease—global implications. *Emerg Infect Dis*. 2015;21:750–759.
131. Hillier CEM, Salmon RL, Neal JW, et al. Possible underascertainment of variant Creutzfeldt-Jakob disease: a systematic study. *J Neurol Neurosurg Psychiatry*. 2002;72:304–309.
132. Will RG, Knight RSG, Ward HJT, et al. vCJD: the epidemic that never was. New variant Creutzfeldt-Jakob disease: the critique that never was. *BMJ*. 2002; 325:102.
133. Chen C-C, Wang Y-H. Estimation of the exposure of the UK population to the bovine spongiform encephalopathy agent through dietary intake during the period 1980 to 1996. *PLoS ONE*. 2014;9:e94020.
134. Latest CJD Figures. May 2017. http://www.cjd.ed.ac.uk/data-and-reports/latest-cjd-figures. Accessed June 3, 2017.
135. Ironside JW, Head MW. Neuropathology and molecular biology of variant Creutzfeldt-Jakob disease. *Curr Top Microbiol Immunol*. 2004;284:133–159.
136. Will RG, Zeidler M, Stewart GE, et al. Diagnosis of new variant Creutzfeldt-Jakob disease. *Ann Neurol*. 2000;47:575–582.
137. Boëlle P-Y, Cesbron J-Y, Valleron A-J. Epidemiological evidence of higher susceptibility to vCJD in the young. *BMC Infect Dis*. 2004;4:26.
138. Boesenberg C, Schulz-Schaeffer WJ, Meissner B, et al. Clinical course in young patients with sporadic Creutzfeldt-Jakob disease. *Ann Neurol*. 2005;58:533–543.
139. Pocchiari M, Puopolo M, Croes EA, et al. Predictors of survival in sporadic Creutzfeldt-Jakob disease and other human transmissible spongiform encephalopathies. *Brain*. 2004;127(Pt 10):2348–2359.
140. Spencer MD, Knight RSG, Will RG. First hundred cases of variant Creutzfeldt-Jakob disease: retrospective case note review of early psychiatric and neurological features. *BMJ*. 2002;324:1479–1482.
141. Zeidler M, Johnstone EC, Bamber RW, et al. New variant Creutzfeldt-Jakob disease: psychiatric features. *Lancet*. 1997;350:908–910.
142. Urwin PJM, Mackenzie JM, Llewelyn CA, et al. Creutzfeldt-Jakob disease and blood transfusion: updated results of the UK Transfusion Medicine Epidemiology Review Study. *Vox Sang*. 2016;110:310–316.
143. Peden AH, Head MW, Ritchie DL, et al. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet*. 2004;364:527–529.
144. Peden A, McCardle L, Head MW, et al. Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. *Haemophilia*. 2010;16:296–304.
145. Hill AF, Joiner S, Wadsworth JDF, et al. Molecular classification of sporadic Creutzfeldt-Jakob disease. *Brain*. 2003;126(Pt 6):1333–1346.
146. Collinge J, Sidle KC, Meads J, et al. Molecular analysis of prion strain variation and the aetiology of "new variant" CJD. *Nature*. 1996;383:685–690.
147. Hill AF, Desbruslais M, Joiner S, et al. The same prion strain causes vCJD and BSE. *Nature*. 1997;389:448–450, 526.
148. Scott MR, Will R, Ironside J, et al. Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. *Proc Natl Acad Sci USA*. 1999;96:15137–15142.
149. Lasmézas CI, Deslys JP, Demaimay R, et al. BSE transmission to macaques. *Nature*. 1996;381:743–744.
150. Bruce ME, Will RG, Ironside JW, et al. Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent. *Nature*. 1997;389:498–501.
151. Nathanson N, Wilesmith J, Griot C. Bovine spongiform encephalopathy (BSE): causes and consequences of a common source epidemic. *Am J Epidemiol*. 1997;145:959–969.
152. Lasmézas CI, Fournier JG, Nouvel V, et al. Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt– Jakob disease: implications for human health. *Proc Natl Acad Sci USA*. 2001;98:4142–4147.
153. Mead S, Joiner S, Desbruslais M, et al. Creutzfeldt-Jakob disease, prion protein gene codon 129VV, and a novel PrPSc type in a young British woman. *Arch Neurol*. 2007;64:1780–1784.
154. Mok T, Jaunmuktane Z, Joiner S, et al. Variant Creutzfeldt-Jakob disease in a patient with heterozygosity at PRNP codon 129. *N Engl J Med*. 2017;376:292–294.
155. Hilton DA, Ghani AC, Conyers L, et al. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol*. 2004;203:733–739.
156. Gill ON, Spencer Y, Richard-Loendt A, et al. Prevalent abnormal prion protein in human appendixes after bovine spongiform encephalopathy epizootic: large scale survey. *BMJ*. 2013;347:f5675.
157. Clewley JP, Kelly CM, Andrews N, et al. Prevalence of disease related prion protein in anonymous tonsil specimens in Britain: cross sectional opportunistic survey. *BMJ*. 2009;338:b1442.
158. Franceschini A, Baiardi S, Hughson AG, et al. High diagnostic value of second generation CSF RT-QuIC across the wide spectrum of CJD prions. *Sci Rep*. 2017;7:10655.
159. Brown P, Brandel J-P, Sato T, et al. Iatrogenic Creutzfeldt-Jakob disease, final assessment. *Emerg Infect Dis*. 2012;18:901–907.
160. Antoine JC, Michel D, Bertholon P, et al. Creutzfeldt-Jakob disease after extracranial dura mater embolization for a nasopharyngeal angiofibroma. *Neurology*. 1997;48:1451–1453.
161. Defebvre L, Destée A, Caron J, et al. Creutzfeldt-Jakob disease after an embolization of intercostal arteries with cadaveric dura mater suggesting a systemic transmission of the prion agent. *Neurology*. 1997;48:1470–1471.
162. Ae R. Update: dura mater graft–associated Creutzfeldt-Jakob disease — Japan, 1975–2017. *MMWR Morb Mortal Wkly Rep*. 2018;67.
163. Noguchi-Shinohara M, Hamaguchi T, Kitamoto T, et al. Clinical features and diagnosis of dura mater graft associated Creutzfeldt Jakob disease. *Neurology*. 2007;69:360–367.
164. Rudge P, Jaunmuktane Z, Adlard P, et al. Iatrogenic CJD due to pituitary-derived growth hormone with genetically determined incubation times of up to 40 years. *Brain*. 2015;138(Pt 11):3386–3399.
165. Appleby BS, Lu M, Bizzi A, et al. Iatrogenic Creutzfeldt-Jakob disease from commercial cadaveric human growth hormone. *Emerg Infect Dis*. 2013;19:682–684.
166. Spurgeon B. French doctors are tried for treating children with infected growth hormone. *BMJ*. 2008;336:348–349.

167. Maddox RA, Belay ED, Curns AT, et al. Creutzfeldt-Jakob disease in recipients of corneal transplants. *Cornea*. 2008;27:851–854.
168. Will RG, Matthews WB. Evidence for case-to-case transmission of Creutzfeldt-Jakob disease. *J Neurol Neurosurg Psychiatry*. 1982;45:235–238.
169. el Hachimi KH, Chaunu MP, Cervenakova L, et al. Putative neurosurgical transmission of Creutzfeldt-Jakob disease with analysis of donor and recipient: agent strains. *C R Acad Sci III*. 1997;320:319–328.
170. Bernoulli C, Siegfried J, Baumgartner G, et al. Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. *Lancet*. 1977;1:478–479.
171. López FJG, Ruiz-Tovar M, Almazán-Isla J, et al. Risk of transmission of sporadic Creutzfeldt-Jakob disease by surgical procedures: systematic reviews and quality of evidence. *Euro Surveill*. 2017;22.
172. de Pedro-Cuesta J, Mahillo-Fernández I, Rábano A, et al. Nosocomial transmission of sporadic Creutzfeldt-Jakob disease: results from a risk-based assessment of surgical interventions. *J Neurol Neurosurg Psychiatry*. 2011;82:204–212.
173. Exposure to Creutzfeldt-Jakob disease. *Sentinel Event Alert*. 2001;1–3.
174. Crowder LA, Schonberger LB, Dodd RY, et al. Creutzfeldt-Jakob disease lookback study: 21 years of surveillance for transfusion transmission risk. *Transfusion (Paris)*. 2017;57:1875–1878.
175. Zou S, Fang CT, Schonberger LB. Transfusion transmission of human prion diseases. *Transfus Med Rev*. 2008;22:58–69.
176. Urwin P, Thanigaikumar K, Ironside JW, et al. Sporadic Creutzfeldt-Jakob disease in 2 plasma product recipients, United Kingdom. *Emerg Infect Dis*. 2017;23.
177. Alcalde-Cabero E, Almazan-Isla J, Brandel JP, et al. Health professions and risk of sporadic Creutzfeldt-Jakob disease, 1965 to 2010. *Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull*. 2012;17.
178. World Health Organization. WHO infection control guidelines for transmissible spongiform encephalopathies. Report of a WHO consultation, Geneva, Switzerland, 23-26 March 1999. http://www.who.int/csr/resources/publications/bse/WHO_CDS_CSR_APH_2000_3/en/. Accessed April 30, 2018.
179. Biacabe A-G, Morignat E, Vulin J, et al. Atypical bovine spongiform encephalopathies, France, 2001-2007. *Emerg Infect Dis*. 2008;14:298–300.
180. Béringue V, Bencsik A, Le Dur A, et al. Isolation from cattle of a prion strain distinct from that causing bovine spongiform encephalopathy. *PLoS Pathog*. 2006;2:e112.
181. Ono F, Tase N, Kurosawa A, et al. Atypical L-type bovine spongiform encephalopathy (L-BSE) transmission to cynomolgus macaques, a non-human primate. *Jpn J Infect Dis*. 2011;64:81–84.
182. Comoy EE, Casalone C, Lescoutra-Etchegaray N, et al. Atypical BSE (BASE) transmitted from asymptomatic aging cattle to a primate. *PLoS ONE*. 2008;3:e3017.
183. Chatelain J, Cathala F, Brown P, et al. Epidemiologic comparisons between Creutzfeldt-Jakob disease and scrapie in France during the 12-year period 1968-1979. *J Neurol Sci*. 1981;51:329–337.
184. Benestad SL, Arsac J-N, Goldmann W, et al. Atypical/Nor98 scrapie: properties of the agent, genetics, and epidemiology. *Vet Res*. 2008;39:19.
185. Wadsworth JDF, Joiner S, Linehan JM, et al. Atypical scrapie prions from sheep and lack of disease in transgenic mice overexpressing human prion protein. *Emerg Infect Dis*. 2013;19:1731–1739.
186. Williams ES, Young S. Chronic wasting disease of captive mule deer: a spongiform encephalopathy. *J Wildl Dis*. 1980;16:89–98.
187. Herbst A, Velásquez CD, Triscott E, et al. Chronic wasting disease prion strain emergence and host range expansion. *Emerg Infect Dis*. 2017;23:1598–1600.
188. Williams ES. Chronic wasting disease. *Vet Pathol*. 2005;42:530–549.
189. Waddell L, Greig J, Mascarenhas M, et al Current evidence on the transmissibility of chronic wasting disease prions to humans-A systematic review. Transbound Emerg Dis. January 2017.
190. Babelhadj B, Di Bari MA, Pirisinu L, et al. Prion disease in dromedary camels, Algeria. *Emerg Infect Dis*. 2018;24.
191. Bui E, Ehrensperger E, Sahlas DJ, et al. Inflammatory cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. *Can J Neurol Sci*. 2008;35:625–629.
192. Dalmau J, Geis C, Graus F. Autoantibodies to synaptic receptors and neuronal cell surface proteins in autoimmune diseases of the central nervous system. *Physiol Rev*. 2017;97:839–887.
193. Leypoldt F, Wandinger K-P, Bien CG, et al. Autoimmune encephalitis. *Eur Neurol Rev*. 2013;8:31–37.
194. Zanusso G, Monaco S, Pocchiari M, et al. Advanced tests for early and accurate diagnosis of Creutzfeldt-Jakob disease. *Nat Rev Neurol*. 2016;12:325–333.
195. Foutz A, Appleby BS, Hamlin C, et al. Diagnostic and prognostic value of human prion detection in cerebrospinal fluid. *Ann Neurol*. 2017;81:79–92.
196. Muayqil T, Gronseth G, Camicioli R. Evidence-based guideline: diagnostic accuracy of CSF 14-3-3 protein in sporadic Creutzfeldt-Jakob disease: report of the guideline development subcommittee of the American Academy of Neurology. *Neurology*. 2012;79:1499–1506.
197. Fragoso DC, Gonçalves Filho AL da M, Pacheco FT, et al. Imaging of Creutzfeldt-Jakob disease: imaging patterns and their differential diagnosis. *Radiographics*. 2017;37:234–257.
198. Tschampa HJ, Kallenberg K, Urbach H, et al. MRI in the diagnosis of sporadic Creutzfeldt-Jakob disease: a study on inter-observer agreement. *Brain*. 2005;128(Pt 9):2026–2033.
199. Macfarlane RG, Wroe SJ, Collinge J, et al. Neuroimaging findings in human prion disease. *J Neurol Neurosurg Psychiatry*. 2007;78:664–670.
200. Collie DA, Summers DM, Sellar RJ, et al. Diagnosing variant Creutzfeldt-Jakob disease with the pulvinar sign: MR imaging findings in 86 neuropathologically confirmed cases. *AJNR Am J Neuroradiol*. 2003;24:1560–1569.
201. Aguglia U, Farnarier G, Tinuper P, et al. Subacute spongiform encephalopathy with periodic paroxysmal activities: clinical evolution and serial EEG findings in 20 cases. *Clin Electroencephalogr*. 1987;18:147–158.
202. Steinhoff BJ, Räcker S, Herrendorf G, et al. Accuracy and reliability of periodic sharp wave complexes in Creutzfeldt-Jakob disease. *Arch Neurol*. 1996;53:162–166.
203. Bell JE, Gentleman SM, Ironside JW, et al. Prion protein immunocytochemistry–UK five centre consensus report. *Neuropathol Appl Neurobiol*. 1997;23:26–35.
204. Budka H, Aguzzi A, Brown P, et al. Neuropathological diagnostic criteria for Creutzfeldt-Jakob disease (CJD) and other human spongiform encephalopathies (prion diseases). *Brain Pathol*. 1995;5:459–466.
205. Parchi P, Giese A, Capellari S, et al. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Ann Neurol*. 1999;46:224–233.
206. Cali I, Castellani R, Yuan J, et al. Classification of sporadic Creutzfeldt-Jakob disease revisited. *Brain*. 2006;129(Pt 9):2266–2277.
207. Trevitt CR, Collinge J. A systematic review of prion therapeutics in experimental models. *Brain*. 2006;129(Pt 9):2241–2265.
208. Aguzzi A, Lakkaraju AKK, Frontzek K. Toward therapy of human prion diseases. *Annu Rev Pharmacol Toxicol*. 2018;58:331–351.
209. Stewart LA, Rydzewska LHM, Keogh GF, et al. Systematic review of therapeutic interventions in human prion disease. *Neurology*. 2008;70:1272–1281.
210. Varges D, Manthey H, Heinemann U, et al. Doxycycline in early CJD: a double-blinded randomised phase II and observational study. *J Neurol Neurosurg Psychiatry*. 2017;88:119–125.
211. Otto M, Cepek L, Ratzka P, et al. Efficacy of flupirtine on cognitive function in patients with CJD: a double-blind study. *Neurology*. 2004;62:714–718.
212. Bessen RA, Marsh RF. Identification of two biologically distinct strains of transmissible mink encephalopathy in hamsters. *J Gen Virol*. 1992;73:329–334.
213. Bessen RA, Marsh RF. Biochemical and physical properties of the prion protein from two strains of the transmissible mink encephalopathy agent. *J Virol*. 1992;66:2096–2101.
214. Peretz D, Scott M, Groth D, et al. Strain-specified relative conformational stability of the scrapie prion protein. *Protein Sci*. 2001;10:854–863.
215. Bessen RA, Marsh RF. Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. *J Virol*. 1994;68:7859–7868.
216. Bartz JC, Aiken JM, Bessen RA. Delay in onset of prion disease for the HY strain of transmissible mink encephalopathy as a result of prior peripheral inoculation with the replication-deficient DY strain. *J Gen Virol*. 2004;85:265–273.