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Coronaviridae

The family Coronaviridae is included with the families Arteriviridae and Roniviridae in the order Nidovirales; viruses in these three families share a distinctive replication strategy. The family Coronaviridae comprises at least two genera. One, the genus Coronavirus, contains a substantial number of pathogens of mammals and birds that individually cause a remarkable variety of diseases, including pneumonia, reproductive disease, enteritis, polyserositis, sialodacryoadenitis, hepatitis, encephalomyelitis, nephritis, and various other disorders. Coronavirus and coronavirus-like infections have been described in swine, cattle, horses, cats, dogs, rats, birds, bats, rabbits, ferrets, mink, and various wildlife species, although many coronavirus infections are subclinical or asymptomatic.

In humans, coronaviruses are included in the spectrum of viruses that cause the common cold and, recently, severe acute respiratory syndrome (SARS), which is a zoonosis. The second genus, Torovirus, contains at least two viruses of animals: Berne virus, which was first isolated from a horse with diarrhea, and Breda virus, which was first isolated from neonatal calves with diarrhea. Berne virus neutralizing antibodies have been detected in sera of sheep, goats, rabbits, and mice, and torovirus-like particles have also been observed by electron microscopy in feces of swine, cats, turkeys, and humans. A nidovirus from fish—white bream virus, which is most closely related to the toroviruses—recently was proposed as the prototype member of a new genus, Bafinivirus.
PROPERTIES OF CORONAVIRUSES

Classification

Despite profound differences in virion structure and genome size, coronaviruses, toroviruses, arteriviruses, and roniviruses exhibit remarkable similarities in their genome organization and replication strategy. In infected cells, these viruses all utilize a distinctive “nested set” transcription strategy in which the expression of genes encoding structural viral proteins is mediated via a nested set of 3′ co-terminal subgenomic mRNAs. This unique strategy has been recognized by the establishment of the order Nidovirales (from the Latin nidus, nest), encompassing the family Coronaviridae, with two genera (Coronavirus and Torovirus), the family Arteriviridae, with one genus (Arterivirus), and the family Roniviridae containing invertebrate nidoviruses. Sequence analysis of the gene encoding portions of the viral RNA-dependent RNA polymerase (transcriptase) suggests that the member viruses of the order Nidovirales probably evolved from a common ancestor (Figure 24.1). Extensive genome rearrangements through heterologous RNA recombination have resulted in the variations seen—that is, viruses with similar replication and transcription strategies but disparate structural features.

The genus Coronavirus can be subdivided into at least three cluster groups on the basis of genetic and serologic properties, with subgroups in two of these (Table 24.1; Figure 24.2). Group 1a includes transmissible gastroenteritis virus of swine, porcine respiratory coronavirus, canine coronavirus, feline enteritis coronavirus (feline infectious peritonitis virus), ferret and mink coronaviruses, and spotted hyena coronavirus. Group 1b includes human coronaviruses, porcine epidemic diarrhea virus, and bat coronavirus. Group 2a includes mouse hepatitis virus, bovine coronavirus, sialodacryoadenitis virus of rats, porcine hemagglutinating encephalomyelitis virus, canine respiratory coronavirus, and other human coronaviruses. Group 2b includes human SARS coronavirus and civet cat, raccoon dog, and horseshoe bat coronaviruses. Group 3 includes avian infectious bronchitis virus, turkey coronavirus, and several potential but still largely uncharacterized new species from ducks, geese, and pigeons. Further taxonomic subdivision of these viruses is likely in the future.

Viruses in the genus Torovirus are all apparently closely related and genetically distinct from coronaviruses; however, many toroviruses have yet to be fully characterized.

Virion Properties

Member viruses of the family Coronaviridae are enveloped, 80–220 nm in size, pleomorphic although often spherical (coronaviruses), or 120–140 nm in size and disc, kidney, or rod shaped (toroviruses). Coronaviruses have large (20 nm long) club-shaped spikes (peplomers) enclosing
| Coronavirus or Torovirus | Disease / Symptoms | Transmission / Diagnostic Specimen | Prevention / Control |
|-------------------------|--------------------|-----------------------------------|----------------------|
| **Group 1a**            |                    |                                   |                      |
| Feline enteric coronavirus (formerly feline infectious peritonitis virus) | Peritonitis, pneumonia, meningoencephalitis, panophthalmitis, wasting syndrome Anorexia, chronic fever, malaise, weight loss, abdominal enlargement, CNS signs | Direct contact; fecal–oral route from maternal shedding Feces, blood, body fluids | Attenuated (TS) vaccine Interruption of transmission cycle, quarantine, high-level hygiene |
| Canine coronavirus | Mild gastroenteritis Mild diarrhea | Ingestion by fecal–oral route Acute feces; small intestinal sections or smears | Inactivated vaccine |
| Transmissible gastroenteritis virus of swine | Gastroenteritis Watery diarrhea, vomiting, dehydration | Fecal–oral route Acute feces; small intestinal sections or smears | Oral attenuated vaccine to pregnant sows Good sanitation |
| Porcine respiratory coronavirus | Interstitial pneumonia Mild respiratory disease or subclinical | Aerosols Nasal swabs; trachea, lung sections | No vaccine available |
| **Group 1b**            |                    |                                   |                      |
| Porcine epidemic diarrhea virus | Gastroenteritis Watery diarrhea, vomiting, dehydration | Fecal–oral route Acute feces; small intestinal sections or smears | Oral attenuated virus vaccine (Asia) to pregnant sows |
| **Group 2a**            |                    |                                   |                      |
| Porcine hemagglutinating encephalomyelitis virus | Vomiting, wasting disease, encephalomyelitis Anorexia, hyperesthesia, muscle tremors, emaciation | Aerosols, oronasal secretions Nasal swabs, tonsil, lung, brain | Good husbandry, maintain immune sows No vaccine available |
| Mouse hepatitis virus | Enteritis, hepatitis, nephritis, demyelinating encephalomyelitis Various | Introduction of virus into a naïve colony: aerosols and direct contact Target tissues, secretions | Depopulation Preventive quarantine |
| Sialodacryoadenitis virus of rats | Inflammation and necrosis of salivary and nasolacrimal glands Lacrimation, anorexia, weight loss, chromodacryorrhea | Direct contact, fomites, and aerosols Nasopharyngeal aspirates, respiratory tissues | Depopulation and repopulation, preventive quarantine |
| Bovine coronavirus | Gastroenteritis, winter dysentery, shipping fever Profuse or bloody diarrhea, dehydration, decreased milk, respiratory disease | Fecal–oral route, aerosols, respiratory droplets Feces, large intestinal sections or smears, nasal swabs, lung sections | Maternal immunization: inactivated or attenuated vaccines; no vaccine for winter dysentery |
| **Group 2b**            |                    |                                   |                      |
| SARS coronavirus (humans) | Severe acute respiratory syndrome (10% patients) Fever, myalgia, diarrhea, dyspnea | Aerosol droplets, ?fecal–oral route Nasopharyngeal aspirates, stools, serum | Quarantine, stringent isolation of patients |
| SARS coronavirus (civet cats, bats) | Subclinical? | Fecal–oral route Feces | Testing and depopulation of animals in live markets |

(Continued)
what appears to be an icosahedral internal core structure within which is a helical nucleocapsid (Figure 24.3). Some coronaviruses also have a second fringe of shorter (5 nm long) spikes (hemagglutinin). Toroviruses also have large club-shaped spikes, but the particles are more pleomorphic and have a tightly coiled tubular nucleocapsid bent into a doughnut shape. By thin-section electron microscopy, torovirus nucleocapsids appear as kidney-, disc-, or rod-shaped forms.

The genome of the family Coronaviridae consists of a single molecule of linear positive-sense, single-stranded RNA, 27.6–31 kb in size for coronaviruses and 25–30 kb for toroviruses, the largest known non-segmented RNA viral
genomes. The genomic RNA is 5’ capped and 3’ polyadenylated, and is infectious (Table 24.2).

The major virion proteins of the member viruses of the genus Coronavirus and Torovirus include a nucleocapsid protein (N, 50–60 kDa, 19 kDa for toroviruses) and several envelope/spike proteins: (1) the major spike glycoprotein (S), transmembrane glycoproteins (M and E), a nucleoprotein (N), and, in some viruses, a hemagglutinin esterase (HE). Toroviruses contain analogous proteins, but there is no E protein.

Viruses replicate in the cytoplasm; the genome is transcribed, forming a full-length complementary RNA from which is transcribed a 3’ co-terminal nested set of mRNAs, only the unique sequences of which are translated.

Virions are formed by budding into the endoplasmic reticulum and are released by exocytosis.

**TABLE 24.2 Properties of Coronaviruses and Toroviruses**

| Property                              | Value                                                                 |
|---------------------------------------|----------------------------------------------------------------------|
| Virions                               | Pleomorphic or spherical (genus Coronavirus) or disc, kidney, or rod-shaped (genus Torovirus) 80–220 nm (genus Coronavirus) or 120–140 nm (genus Torovirus) in diameter. Virions are enveloped, with large club-shaped spikes (peplomers) |
| Virions                                | Have an icosahedral internal core structure within which is a helical nucleocapsid (genus Coronavirus) or a tightly coiled tubular nucleocapsid bent into a doughnut shape (genus Torovirus) |
| The genome                            | Consists of a single molecule of linear positive-sense, single-stranded RNA, 25–31 kb in size; the genome is 5’ capped, 3’ polyadenylated, and infectious |
| Coronavirus virions                   | Contain three or four structural proteins: a major spike glycoprotein (S), transmembrane glycoproteins (M and E), a nucleoprotein (N), and, in some viruses, a hemagglutinin esterase (HE). Toroviruses contain analogous proteins, but there is no E protein |
| Viruses                               | Replicate in the cytoplasm; the genome is transcribed, forming a full-length complementary RNA from which is transcribed a 3’ co-terminal nested set of mRNAs, only the unique sequences of which are translated |
| Virions                                | Are formed by budding into the endoplasmic reticulum and are released by exocytosis |

Virus neutralizing antibodies generated during natural infections are directed at the surface glycoproteins of coronaviruses and toroviruses, with the majority being conformational epitopes located at the N-terminal portion of the S protein. Cellular immune responses are principally directed toward the S and N proteins. Besides the canonical structural proteins, coronaviruses are unique among nidoviruses because their genomes encode (within differing regions) variable numbers of accessory proteins (four or five in most; eight in the SARS coronavirus) that are dispensable for in-vitro virus replication, but which increase virus fitness in vivo. The accessory proteins encoded by the SARS virus open reading frames 3b and 6, for example, are antagonists of innate immune responses, specifically interfering with the development of type I interferon responses; the specific roles of other accessory proteins are still largely unknown. The accessory proteins have homologous versions within coronavirus groups, but lack similarity with proteins in different groups. In group 2 coronaviruses, for example, the HE protein is considered an accessory protein, and mouse hepatitis virus HE-deletion mutants replicate like wild-type virus in vitro, but in mice they have an attenuated phenotype.

**Virus Replication**

The host spectrum of individual coronaviruses appears to be largely determined by the S protein, portions of which mediate receptor binding and virus cell fusion that occur at either the plasma membrane or within endosomes of susceptible cells. Individual coronaviruses utilize a variety of cellular proteins as receptors. Aminopeptidase N serves as a receptor for several group 1 coronaviruses, including feline enteric coronavirus (formerly feline infectious peritonitis virus), canine coronavirus, transmissible gastroenteritis virus and human coronavirus 229E. SARS and some other human coronaviruses utilize angiotensin converting enzyme 2. Mouse hepatitis virus utilizes carinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1), and other group 2 coronaviruses utilize N-acetyl-9-O-acetyl neuraminic acid. The functional receptor for group 3 coronaviruses such as infectious bronchitis virus is undefined, although heparan sulfate and sialic acid residues may serve as non-specific attachment factors.

The strategy of expression of the coronavirus genome is complex (Figure 24.4). First, the viral RNA serves as messenger RNA (mRNA) for synthesis of the RNA-dependent RNA polymerase. The two large open reading frames (some 20 kb in total size) encoding the units of the polymerase are translated—the larger via ribosomal frameshifting—as a single polyprotein that is then cleaved. These proteins then assemble to form the active RNA polymerase. This enzyme is then used to transcribe full-length complementary (negative-sense) RNA, from which in turn are transcribed, not only full-length genomic RNA, but also a 3’ co-terminal nested set of subgenomic mRNAs. The nested set comprises up to 10 (differing in the various viruses) overlapping mRNAs that extend for different lengths from common 3’ ends and share a common 5’ leader sequence. They are
generated by a leader-primed mechanism of discontinuous transcription: the polymerase first transcribes the non-coding leader sequence from the 3' end of the complementary (negative-sense) RNA. The capped leader RNA then dissociates from the template and reassociates with a complementary sequence at the start of any one of the genes, to continue copying the template right through to its 5' end. Only the unique sequence that is not shared with the next smallest mRNA in the nested set is translated; this strategy yields the various viral proteins in regulated amounts. Intergenic sequences serve as promoters and attenuators of transcription.

Torovirus transcription and replication apparently are similar to those of coronaviruses, except that there are no common 5' leader sequences on the mRNAs. A puzzling finding is that subgenomic negative-sense RNAs complementary to the nested set of mRNAs are also present in torovirus-infected cells. The fact that these subgenomic RNAs contain 5'- and 3'-terminal sequences that are identical to those of genomic RNA implies that they may function as replicons.

The synthesis, processing, oligomerization, and transport of the several envelope glycoproteins of coronaviruses display some unusual features. For example, the envelope protein M, which in some coronaviruses contains O-linked rather than N-linked glycans, is directed exclusively to cisternae of the endoplasmic reticulum and other pre-Golgi membranes. As a result, virions bud only there and not from the plasma membrane. Virions are then transported in vesicles to the plasma membrane and are released by exocytosis (Figure 24.5). After their release, many of the mature enveloped virions remain adherent to the outside of the cell.

In addition to the accumulation of point mutations as a result of polymerase errors during transcription (genetic drift), genetic recombination occurs at high frequency between the genomes of different but related coronaviruses. This may be an important mechanism for the generation of the genetic diversity seen with these viruses in nature, and provides a constant potential source of new viruses with novel phenotypic properties, including host species tropism and virulence.

![Figure 24.4](image-url) **Figure 24.4** Structural relationship between mRNAs and the genomic RNA of coronaviruses. Thick lines represent the translated sequence. Thinner lines, untranslated sequences. The names below the boxes indicate the proteins encoded by the corresponding genes. An, poly A sequences; E, minor transmembrane envelope protein; HE, spike protein hemagglutinin esterase; M, transmembrane envelope protein; MHV, mouse hepatitis virus; N, nucleocapsid protein; S, spike glycoprotein. [From Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses (C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, L. A. Ball, eds.), p. 952. Copyright © Elsevier (2005), with permission.]

![Figure 24.5](image-url) **Figure 24.5** Mouse hepatitis virus infection in the duodenum of a 1-week-old mouse. Virions are transported to the plasma membrane from their site of formation in the endoplasmic reticulum in vesicles and are released by exocytosis. After their release, many virions remain adherent to the outside of the cell. Thin-section electron microscopy. Magnification: ×30,000.
MEMBERS OF THE GENUS
CORONAVIRUS

TRANSMISSIBLE GASTROENTERITIS VIRUS

Transmissible gastroenteritis is a highly contagious enteric disease of swine that occurs throughout much of the world. Porcine respiratory coronavirus arose from transmissible gastroenteritis virus through genetic deletions, and the respiratory virus now has superseded its enteric parent in many regions.

Clinical Features and Epidemiology

Clinical signs of transmissible gastroenteritis are most severe in very young piglets, and include vomiting, profuse watery yellow diarrhea, rapid weight loss, and dehydration. Most, often all, seronegative neonates succumb within a few days of infection with highly virulent strains of transmissible gastroenteritis virus, whereas death is uncommon in pigs infected after 2–3 weeks of age. Older growing and finishing swine often develop a transient, watery diarrhea, but vomiting is unusual. Infections of adult swine typically are asymptomatic, but in some outbreaks there is high mortality, and infected sows sometimes exhibit anorexia, fever, vomiting, diarrhea, and agalactia.

Transmissible gastroenteritis virus is highly contagious to swine of all ages. Dogs and cats have been experimentally infected with the virus, although their role in the epidemiology of infection is doubtful. Spread of transmissible gastroenteritis virus among farms occurs with the introduction of pigs excreting the virus or by mechanical vectors (fomites) such as contaminated vehicles, clothing, instruments, etc. Introduction of the virus into non-immune herds leads to explosive outbreaks, with epizootic spread among animals of all ages; mortality is very high in neonates. Disease is usually less severe in older animals. The epizootic terminates when no susceptible swine remain and no new animals are reintroduced, typically within a few weeks, although chronic or intermittent shedding has been described in some experimentally exposed sows. Another epidemiologic pattern occurs in intense production facilities where the farrowing system makes susceptible piglets available continuously. Enzootic infection and background immunity to transmissible gastroenteritis virus or related porcine respiratory coronavirus usually lead to low mortality and relatively mild disease that is most pronounced shortly after weaning, when maternally acquired immunoglobulin A (IgA)-based immunity has waned. Notably in Europe, virulent enteric transmissible gastroenteritis virus infections largely have been displaced by enzootic porcine respiratory coronavirus infections. Porcine respiratory coronavirus is a genetic variant of transmissible gastroenteritis virus with a deletion of variable size within the spike protein (see below), but which engenders strong immunity against transmissible gastroenteritis virus infection.

Pathogenesis and Pathology

Transmissible gastroenteritis virus enters the body by ingestion (fecal–oral transmission), and after an incubation period of 18–72 hours it causes clinical signs that vary according to the age of the animal infected. There are several reasons for the susceptibility of very young piglets: (1) their gastric secretions are less acidic than those of older animals and their milk diet buffers gastric acid, both of which are somewhat protective to the virus during its passage through the stomach; (2) renewal of enterocytes lining the intestinal villi from progenitor cells in the intestinal crypts is less rapid than in older pigs; (3) the neonatal immune system is naïve and not fully mature; (4) neonates are especially vulnerable to the electrolyte and fluid derangements that result from the malabsorption and severe malabsorption diarrhea that are characteristic of transmissible gastroenteritis in very young pigs. After virus passes through the stomach, the infection proceeds as a wave down the intestinal tract. The virus selectively infects and destroys the mature enterocytes lining the small intestinal villi, quickly resulting in profound shortening and blunting of villi, with consequent loss of the mucosal absorptive area (Figure 24.6). The destruction of enterocytes lining the villi leads to malabsorption because of the loss of critical digestive enzymes such as lactase and other disaccharidases, normally present in the microvillus brush border of villous enterocytes, that are responsible for digestion of milk. Thus destruction of villous enterocytes results in both malabsorption and malabsorption. The increased osmolarity of the intestinal contents from the presence of undigested milk results in further loss of water and electrolytes into the bowel lumen. The consequence is diarrhea, electrolyte imbalance leading to acidosis, and severe dehydration. Intestinal crypt epithelial cells remain uninfected, so recovery of the integrity and function of villi is rapid if the animal survives the infection; however, the proliferation of progenitor enterocytes in the crypts also increases intestinal secretion of fluid and electrolytes, which further exacerbates the diarrhea and metabolic perturbations that are characteristic of fulminant transmissible gastroenteritis.

Gross pathology (except for dehydration) is restricted to the gastrointestinal tract, and consists of a distended stomach that contains undigested milk, and flaccid, gas- and fluid-distended intestines. The destruction of villi, which can be seen when sections of intestine are submerged in isotonic buffer and viewed with a dissecting microscope, results in thinning of the intestinal wall (Figure 24.7).

Diagnosis

Mucosal impression smears or cryostat sections of intestine from neonatal piglets with acute disease can be stained for transmissible gastroenteritis virus by immunofluorescence or immunoperoxidase staining—these methods provide
rapid results. Antigen-capture enzyme-linked immunosorbent assay (ELISA) also can be used to detect transmissible gastroenteritis virus in the feces of infected pigs. Virus isolation can be done in porcine kidney, thyroid, or testicle cells; there is cytopathology, and isolates are identified with specific antisera, usually using an enzyme immunoassay. Serology using paired serum samples and either serum neutralization or enzyme immunoassay allows retrospective diagnosis and is also valuable in epidemiological investigations. However, none of these assays definitively differentiates transmissible gastroenteritis and porcine respiratory coronavirus infections; reverse-transcriptase-polymerase chain reaction (RT-PCR) assays using primers targeting the deletion region of the porcine respiratory coronavirus S gene can be used to detect and differentiate the two viruses. Serological discrimination of prior infection with these two viruses can be accomplished using a blocking (competitive) ELISA incorporating monoclonal antibodies that recognize an antigenic site present in the S protein of transmissible gastroenteritis virus that is deleted in porcine respiratory coronavirus.

**Immunity, Prevention, and Control**

Oral vaccines have not proven highly effective, and better protection has been obtained when virulent virus has been
orally administered to pregnant sows, thereby boosting lactogenic immunity in piglets. Maternal IgA antibodies, passed to piglets in colostrum and milk, provide protection against infection, whereas systemic IgG antibody does not. IgA antibodies are protected against proteolytic degradation in the intestine and provide immunity within the intestinal lumen. Lactogenic immunity is not stimulated by parenteral immunization, only by mucosal infection or immunization.

Control of transmissible gastroenteritis by exclusion of the virus from premises requires strict sanitation and management practices that eliminate all potential sources of the virus, including potentially infected or carrier animals, and which prevent reintroduction of the virus.

**PORCINE RESPIRATORY CORONAVIRUS**

The respiratory variant of transmissible gastroenteritis virus, porcine respiratory coronavirus, was discovered in 1986 when seroconversion was detected in swine herds in countries (e.g., Denmark) known to be free of transmissible gastroenteritis; the virus causing this disease pattern is a spike gene deletion mutant that has lost its enteric tropism. Instead, porcine respiratory coronavirus acquired a respiratory tropism and transmission pattern.

**Clinical Features and Epidemiology**

Porcine respiratory coronavirus infects piglets of all ages, causing subclinical or mild respiratory disease. Clinical signs may include mild fever with variable degrees of dyspnea, polypnea, and anorexia. Co-infection of pigs with other respiratory pathogens (bacteria, influenza virus, porcine reproductive and respiratory syndrome virus) or treatment with immunosuppressive agents accentuates porcine respiratory coronavirus infections and disease.

Porcine respiratory coronavirus now is enzootic in swine herds worldwide, spreading long distances by airborne respiratory transmission or directly by contact. Swine population density, distance between farms, and season all can influence the epidemiology of infection with this virus.

**Pathogenesis and Pathology**

The large 5’ region deletion (621–681 nt in size) in the spike gene of porcine respiratory coronavirus probably accounts for the reduced virulence and altered tropism of this virus. Porcine respiratory coronavirus is spread by respiratory droplets and aerosols and, after infection, replicates in the tonsils, the mucosal epithelium of the nasal mucosa and airways of the lungs, and in both type I and II pneumocytes in alveoli. Virus-induced inflammation and necrosis in the terminal airways and airspaces manifest as bronchointerstitial pneumonia that can affect 5–60% of the lung, even in asymptomatic pigs. The severity of clinical signs and lesions vary, but infection is subclinical in many infected herds.

**Diagnosis**

Porcine respiratory coronavirus replicates to high titers in the lungs of infected swine, and the virus can be detected readily in nasal swabs. Laboratory diagnosis of porcine respiratory coronavirus infection utilizes the same assays as those described for transmissible gastroenteritis virus, and the two related viruses are only distinguished by virus-specific RT-PCR assays or highly specific competitive ELISA. The virus also can be isolated and grown in pig kidney or testicle cells.

**Immunity, Prevention, and Control**

There currently are no vaccines for prevention of porcine respiratory coronavirus infection, probably because most infections are so mild that there is little perceived need for a vaccine. Experimental and field studies suggest that repeated exposure of swine to porcine respiratory coronavirus results in high levels of both passive and active immunity to transmissible gastroenteritis, such that the latter disease has largely disappeared from porcine respiratory coronavirus enzootic herds in some countries.

**PORCINE HEMAGGLUTINATING ENCEPHALOMYELITIS VIRUS**

Porcine hemagglutinating encephalitis virus causes vomiting and wasting disease in susceptible piglets, and neurological disease in others. Vomiting and wasting disease was first reported in Canada in 1958, and serologic surveys indicate that the causative virus is common in many countries; however, disease is relatively infrequent, because neonatal pigs are often passively protected by colostral antibodies and subsequently develop age-related resistance to the disease.

Infection of adult swine usually is inapparent, and vomiting and wasting disease is a disease of piglets under 3 weeks of age suckling non-immune sows. The disease is characterized by repeated vomiting after feeding, depression, progressive emaciation, and death. In contrast to transmissible gastroenteritis, in vomiting and wasting disease diarrhea is not common. Infection also can lead to neurological signs similar to those of porcine polioencephalomyelitis, which is caused by a picornavirus; specifically, affected piglets may show a dog-sitting posture, paddling movements, opisthotonus, paralysis or convulsions, and death.

Porcine hemagglutinating encephalitis virus is spread by respiratory aerosols and multiplies first in the nasal
mucosa, tonsils, lung, and small intestine; it then spreads to the central nervous system via peripheral nerves. Viremia is not important in the pathogenesis of this disease, neither is involvement of organs other than the nervous system. Infection of the vagal sensory ganglia is proposed to be responsible for the vomiting that characteristically occurs in affected animals, and the wasting component is attributed to viral infection of gastric myenteric plexuses leading to delayed emptying of the stomach.

A clinical diagnosis of porcine hemagglutinating virus encephalomyelitis may be confirmed by the isolation of virus in primary porcine kidney cell culture or in various pig cell lines; growth of the virus is detected by characteristic hemagglutination. Because no vaccines are available, good husbandry is essential for the prevention and control of the disease.

PORCINE EPIDEMIC DIARRHEA VIRUS

Porcine epidemic diarrhea is a diarrheal disease of piglets that has been described in Europe and Asia. The disease is clinically similar to transmissible gastroenteritis, but is caused by a different and less contagious coronavirus. Suckling piglets are unaffected in many outbreaks. The main clinical sign in young pigs is watery diarrhea, sometimes preceded by vomiting. Mortality can be very high (up to 80%). The virus also can cause diarrhea in growing and fattening pigs. Infection of adult swine is frequently asymptomatic, although diarrhea occurs occasionally. A diagnosis may be confirmed by the isolation of virus in primary porcine cell culture or Vero (African green monkey kidney) cells, by immunofluorescence or ELISA tests for porcine epidemic diarrhea virus antigens in intestine or feces, respectively, by RT-PCR assay to detect viral RNA, or by the demonstration of virus-specific antibodies in convalescent swine. Attenuated vaccines are available in some countries.

FELINE ENTERIC CORONAVIRUS AND FELINE INFECTIOUS PERITONITIS VIRUS

Feline infectious peritonitis was first described in the 1960s as a systemic and often fatal disease of cats. The pathogenesis of feline infectious peritonitis is complex and not fully characterized, despite intensive study. Feline enteric coronavirus infection is central to the pathogenesis of this disease, as the sporadic occurrence of feline infectious peritonitis is proposed to be the result of mutations of the enteric coronavirus during natural infection of cats, resulting in the emergence of a virus with an acquired tropism for macrophages. Although feline enteric coronavirus is classified in group 1a (Table 24.1), two serotypes of the virus have been identified, both being able to cause feline infectious peritonitis. The serotype 2 feline enteric coronavirus is a recombinant that includes portions of the genome of canine coronavirus. Both virus types can cause the two forms of feline infectious peritonitis, one that has a characteristic abdominal effusion (the “wet” form), and the other (the “dry” form) without abdominal effusion. Thus the pathologic manifestations are not solely a virus strain-specific property, as individual virus strains can cause either form of the disease in individual cats.

Clinical Features and Epidemiology

Feline infectious peritonitis is a common progressive, debilitating lethal disease of domestic and wild members of the family Felidae. Disease typically occurs in young or very old cats. The initial clinical signs are vague, and affected cats present with anorexia, chronic fever, malaise, and weight loss. Ocular and/or neurological manifestations occur in some individuals. In the classical wet or effusive form of feline infectious peritonitis, these signs are accompanied by progressive abdominal distention from the accumulation of a highly viscous fluid in the peritoneal cavity and rapid disease progression, with death typically within weeks to months. The dry or non-effusive form of the disease, with little or no peritoneal exudate, is more slowly progressive. The wet and dry forms of feline infectious peritonitis are different manifestations of the same infection, and both forms of the disease are characterized by foci of pyogranulomatous inflammation in several organs.

The following is a proposed scenario of fatal feline infectious peritonitis. A kitten suckling a seropositive queen is protected by colostral antibody against enteric coronavirus infection during the first few weeks of life. As maternal antibody wanes, the kitten becomes infected during an episode of maternal feline enteric coronavirus shedding. The kitten now develops an active immune response, but in most cases not a sterilizing response, and a persistent viral infection of the gut with chronic fecal shedding is established. Virus and antibodies co-exist in the kitten, but the infection is modulated by an efficient cellular immune response that keeps infected macrophages and monocytes in check. The animal may remain healthy, but becomes susceptible to development of feline infectious peritonitis should it become stressed or immunosuppressed. Viral mutants then emerge, with rapid selection and proliferation of macrophage-tropic mutants that cause the development of feline infectious peritonitis.

Pathogenesis and Pathology

The key initiating pathogenic event in feline infectious peritonitis is the productive infection of monocytes and macrophages by genetic variants (mutants) of the original enteric coronavirus. Experimentally, the virulence of strains of feline enteric coronavirus has been correlated with their capability
of productive infection of cultured peritoneal macrophages, with avirulent isolates infecting fewer macrophages and producing lower virus titers than virulent isolates. Avirulent isolates are also less able to sustain virus replication and spread between macrophages. Mutations within the spike (S) and, potentially, other proteins alter the tropism of the ubiquitous avirulent feline enteric coronavirus to macrophages, which then allows the virus to spread and to cause feline infectious peritonitis. Affected cats typically produce a strong antibody response that is ineffective in eliminating the virus, and cellular immune responses are unable to prevent virus replication in macrophages.

The lesions in feline infectious peritonitis are characteristically centered on small blood vessels, and vascular injury and leakage are central to the pathogenesis of the wet form of the disease. However, there is uncertainty regarding the pathogenetic mechanisms involved, as there is increasing evidence that vascular injury is not simply the result of immune complex deposition in the walls of the affected vessels, as was once proposed. The central role of viral infection of macrophages, however, is clear, and perivascular clusters of virus-infected macrophages are characteristically present in the tissues of cats with both the wet and dry forms of feline infectious peritonitis. Despite the inability of macrophages to prevent virus from replicating in them, viral infection of macrophages probably leads to their activation, with production of inflammatory mediators including cytokines and arachidonic acid derivatives (leukotrienes and prostaglandins). These mediators probably contribute substantially to the disease process, as these host-response molecules induce changes in vascular permeability and provide chemotactic stimuli for neutrophils and monocytes that further contribute to the inflammatory response. Both intravascular and recently emigrated monocytes and macrophages probably serve as new virus targets, thereby amplifying the infection further. The end result is enhanced local virus production, increased tissue damage, and a strong but ineffective host immune response.

Humoral immunity is not protective, but may actually enhance disease progression. Antibody-dependent enhancement of infection of macrophages is apparently mediated by neutralizing antibodies to the S protein, making vaccine development problematic. Cats that are seropositive to feline enteric coronavirus, either from natural infection or via purified IgG antibodies transfused into uninfected animals, develop an accelerated, fulminant disease when challenged experimentally with virulent feline enteric coronavirus (so-called feline infectious peritonitis virus). Clinical signs and lesions develop earlier, and the mean survival time is reduced as compared with seronegative cats.

The gross lesions of feline infectious peritonitis reflect one of the two forms of the disease. The wet form is characterized by the presence of variable quantities of thick, viscous, clear yellow peritoneal exudate, and the presence of extensive fibrinous plaque with numerous discrete gray-white nodules (from <1 to >10 mm in diameter) in the omentum and on the serosal surface of the liver, spleen, intestines, and kidneys (Figure 24.8). Microscopically, these nodules are composed of aggregates of macrophages and other inflammatory cells (granulomas or pyogranulomas) that characteristically are centered on blood vessels, sometimes with necrosis of the wall of involved vessels. These lesions can occur in many tissues, but omentum and peritoneal serosa, liver, kidney, lung and pleura, pericardium, meninges, brain,
and uvea are common sites. The lesions and pathogenesis of the dry form of feline infectious peritonitis are similar, but without the fibrinous polyserositis that characterizes the wet form, and discrete pyogranulomas form nodular masses within the parenchyma of affected organs. It is unknown what determines the form of feline infectious peritonitis that develops in an individual cat, neither is the relationship between the two forms well understood, as individual virus strains can cause either form in different animals.

**Diagnosis**

Serology utilizing indirect immunofluorescence or enzyme immunoassay generally shows cats with feline infectious peritonitis to have moderate to high antibody titers. Some cats with the disease remain seronegative or have only low antibody titers, however, whereas other cats with no clinical signs of disease may have high titers. Therefore, interpretation of serology data is frequently confusing, and surgical biopsy of affected organs not only confirms the diagnosis but also reveals the extent and stage of the disease. Immunohistochemistry can be used to obtain definitive confirmation of coronavirus infection of macrophages within the lesions in affected cats.

**Immunity, Prevention, and Control**

Feline infectious peritonitis is not controlled easily; control requires the elimination of the virus from the local environment, whether this is the household or the cattery. This requires a high level of hygiene, strict quarantine, and immunoprophylactic measures. Because kittens acquire the infection from their queens, early weaning programs have also been used in attempts to interrupt virus transmission. The development of a safe and highly effective vaccine remains elusive, even with the availability of bioengineering approaches. The only commercially available feline infectious peritonitis vaccine contains a temperature-sensitive mutant virus. The vaccine is applied to the nasal mucosa to reduce virus replication and antibody formation. Under these conditions, a cellular immune response is favored, and some protection putatively is achieved. Vaccination of infected, seropositive adult cats is not effective.

**CANINE CORONAVIRUS**

A canine coronavirus that usually produces only a mild gastroenteritis in infected dogs was originally identified in 1971. More recently, strains of canine coronavirus have been identified with different properties; these include canine respiratory coronavirus and pantropic strains of the virus. Constant, continuing evolution of canine coronavirus, through accumulation of point mutations within the genome and genetic insertions or deletions, leads to the regular emergence of viruses with altered properties, including their tropism and virulence.

Enteric canine coronavirus infection is common in dogs worldwide, and epidemics of coronavirus enteritis have also been recorded in wild dogs. Similar or identical group 1 coronaviruses have been identified in foxes, raccoon dogs, and cats. The intestinal disease caused by canine coronavirus is similar to that caused by enteric coronaviruses in other species, with destruction of mature enterocytes lining the intestinal villi causing maldigestion, malabsorption, and subsequent diarrhea. Because there are many causes of diarrhea in dogs, clinical suspicion of canine coronavirus infection should be confirmed by laboratory-based procedures. The virus may be visualized by electron microscopy, and some but not all virus strains can be isolated in primary canine cell culture. Highly sensitive and specific RT-PCR assays have now been developed. Detection of antibody in the sera of pups is of limited value, because it may be of maternal origin and unrelated to the cause of the diarrhea. An inactivated vaccine is available for the control of canine coronavirus diarrhea, but its protective value is controversial.

Pantropic strains of canine coronavirus have been described as the putative cause of severe systemic disease in dogs that is characterized by pyrexia, anorexia, depression, vomiting, diarrhea, leukopenia, and neurological signs of ataxia and seizures. Coronaviruses that are genetically distinct from enteric canine coronaviruses, but that resemble bovine coronaviruses, have been detected with some frequency in the respiratory tract of dogs from Europe, North America, and Asia, sometimes in association with respiratory disease.

**MOUSE HEPATITIS VIRUS**

Mouse hepatitis virus includes a spectrum of mouse coronaviruses that may not necessarily cause hepatitis. These viruses vary widely in their tissue tropism. One end of the spectrum consists of enteric coronaviruses, which have selective tropism for enteric epithelium. Historically, enterotropic mouse hepatitis virus was given the name “lethal intestinal virus of infant mice” (LIVIM). The other end of the spectrum consists of polytropic coronaviruses, which have primary tropism for upper respiratory epithelium, and secondary tropism for a wide variety of cells or tissues, particularly vascular endothelium, lymphoid tissue, hemopoietic tissue, liver, and central nervous system. These viruses received the nickname of “hepatitis viruses” because of their common property of inducing hepatitis in experimentally inoculated mice. Thanks to their polytropism, these mouse hepatitis virus types replicate readily in a wide variety of cell types in vitro, whereas enterotropic strains of the virus do not, and also tend not to induce hepatitis. Thus, for many years, LIVIM was considered to be distinct from mouse hepatitis virus.
There are numerous laboratory strains of polytropic mouse hepatitis virus that grow readily in vitro, including MHV-JHM, MHV-S, MHV-A59, and MHV-3. These polytropic viruses have been extensively studied as models of neurologic disease and hepatitis, and form the basis of an expansive scientific literature. The enterotropic viruses are far more common in contemporary mouse colonies, but have received less experimental scrutiny. Common enteric strains of mouse hepatitis virus include MHV-S/CDC, MHV-Y, MHV-RI, and MHV-D. Despite the fact that mouse hepatitis virus strains are often named, the nomenclature is meaningless, because of the inherent property of these viruses constantly to mutate and recombine within mouse populations. Furthermore, although the distinction between enterotropic and polytropic is useful for understanding the biology of the virus, there is considerable overlap among isolates, and one group probably served as a progenitor for the other.

Clinical Features and Epidemiology

Enterotropic strains of mouse hepatitis virus tend to be highly contagious, and cause devastating epizootics in naïve mouse populations, with mortality approaching 100% among infant mice. Clinical disease is limited to infant mice, because susceptibility is determined by enteric mucosal proliferative kinetics. Thus enterotropic mouse hepatitis virus infection follows the features of neonatal enteric coronavirus enteritides in other species. Disease course is rapid, with pups dying from dehydration within 24–48 hours after introduction of the virus to a naïve breeding population. Older pups may be runted, and bloated with poorly formed feces, but often recover. Adults are susceptible to infection, but do not manifest clinical disease. Once the virus is enzootic within a population, clinical disease is no longer apparent, as pups are protected by maternal antibody during the period of age-related susceptibility. Polytropic strains of mouse hepatitis virus are generally less contagious, and tend to spread by direct contact among naïve mice. Outcome of infection with these viruses is highly variable, and dependent upon age, mouse strain, and virulence of the virus. Infant mice are susceptible to disease, because of an immature immune system. Clinical disease is often inapparent, but tends to be manifest as running and neurologic signs, with reduced survival at weaning as a result of maternal cannibalism. When polytropic mouse hepatitis virus is enzootic within a population, clinical signs are absent among immunocompetent mice. In contrast, wasting disease, neurologic signs, and mortality may be observed in immunodeficient mice, particularly T cell deficient mice. A unique clinical presentation occurs in interferon-γ deficient mice, which develop abdominal distention as a result of polyserositis.

Host immunity to mouse hepatitis virus is virus-strain-specific, and directed toward the mutable S protein that constitutes the virion spikes. Immunocompetent mice mount an effective immune response to infection, with elimination of the virus and complete recovery. Duration of infection is therefore limited, except when mice with various types of immune perturbations are infected, in which case duration of infection varies. Mouse hepatitis virus has a reputation of being “latent” and “persistent,” but neither is the case. Latency does not occur, but signs of infection are often subclinical. Persistence occurs within the context of the population, with constantly evolving mutants arising that are capable of reinfesting immune mice, thereby maintaining the virus in the population. In laboratory animal housing contexts, commercially obtained mice free of mouse hepatitis virus tend to be introduced to infected colonies on a weekly basis, which is the perfect interval for maintaining infection and observing disease. Vertical transmission is not a practical concern, but the virus can be introduced into naïve mouse population through biological products (mouse serum, tissues, tumors, etc.). Polytropic mouse hepatitis virus can persistently infect cell lines, including ES cells, without cytopathic effect.

The significance of mouse hepatitis virus within laboratory mouse populations is not so much its overt pathogenicity; rather, it is its deleterious effects upon research. A wide variety of effects upon various physiologic parameters, particularly immune responses, have been documented. These research effects are often the only “clinical signs” of disease within an infected mouse population.

Pathogenesis and Pathology

Enterotropic strains of mouse hepatitis virus tend to selectively infect enterocytes, with minimal dissemination to other tissues, except mesenteric lymph nodes. The neonatal mouse bowel is poorly suited to deal with enterotropic mouse hepatitis virus infection, which induces rapid cytolysis of terminally differentiated enterocytes that line the intestinal villi. The intestinal mucosa of infant mice has shallow, slowly replicating crypt progenitors that are incapable of responding to the rapid cytolytic effects of the virus. Lesions consist of segmental epithelial necrosis, villus attenuation, and mucosal erosion. A diagnostic feature of enterotropic mouse hepatitis virus infection is prominent epithelial syncytia. Lesions are most likely to occur in the terminal small intestine, cecum, and proximal colon. As mice age, intestinal mucosal proliferative kinetics accelerate, allowing replacement of damaged mucosa. This is characterized by mucosal hyperplasia, which may contribute to clinical disease through malabsorption and increased mucosal secretion of fluid and electrolytes. Lesions are minimal in adult mice, which support ample virus replication, but the mucosa can compensate for the
damage. Under those circumstances, lesions are limited to an occasional syncytium in the surface mucosa. Disease susceptibility among immunodeficient mice varies with the nature of the immune defect, but is also dependent on age and mucosal kinetics. Infection of adult immunodeficient nude mice, for example, may be clinically silent, with minimal enteric disease limited to a few epithelial syncytia.

Polytropic virus strains initially replicate in nasal respiratory epithelium. Dissemination depends upon the age of the mouse, the strain of the mouse, the immune status of the mouse, and the virus strain. Neurotropic strains may extend from the olfactory epithelium to the olfactory tracts of the brain without dissemination to other organs. More commonly, the virus will disseminate hematogenously to the pulmonary vasculature, with secondary viremia to other organs, particularly liver, hemopoietic tissues, and lymphoid tissues. Gut-associated lymphoid tissue may be infected, but enteric mucosa is often spared. Depending upon the genetic background of the mouse, susceptibility to polytropic mouse hepatitis virus can be illustrated at the cellular level in vitro (intrinsic resistance) or in vivo, in which several host factors may determine susceptibility (extrinsic resistance). Susceptibility to the MHV-A59 and MHV-JHM strains of mouse hepatitis virus, for example, has been linked to allelic variation of carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1). SJL mice lack this susceptibility allele and are markedly resistant to infection with these virus strains. However, this explanation of susceptibility does not apply to all strains of mouse hepatitis virus or to all mouse genotypes.

Depending upon these various factors, lesions associated with polytropic mouse hepatitis virus are highly variable. Infection of adult immunocompetent mice with relatively avirulent strains of virus is often subclinical. When lesions are present, they consist of multiple foci of acute necrosis, and syncytia of parenchyma and vascular endothelium within lymphoid tissues, hemopoietic tissues (particularly spleen), liver, and brain. Lesions are particularly florid in immunodeficient mice, which develop progressively severe wasting disease with lesions that are strikingly apparent in liver, with foci of hemorrhage, necrosis, and nodular hyperplasia. Spleens are also enlarged as a result of extramedullary hemopoiesis. Central nervous system disease can arise directly through olfactory neural pathways (nasencephalitis) or hematogenous infection, with necrotizing encephalitis. Infection involves neurons, glia, and endothelium, and surviving mice progress to demyelinating disease, which may be manifest as posterior paresis. This is most apt to be observed in chronically infected immunodeficient mice. As previously noted, mice deficient in interferon-γ may develop chronic polyserositis, which features prominent synctia among infiltrating macrophages. Curiously, involvement of other organs or tissues (intestine, liver, etc.) may be absent, suggesting that mice are able to clear infection partially from those tissues, but not macrophages.

**Diagnosis**

Mouse hepatitis virus infection of a mouse population can be detected retrospectively by serology. Strains of the virus are highly cross-reactive serologically, so antigen is typically prepared from polytropic strains of virus propagated from cell culture. Active infections can be diagnosed at necropsy, and virus can be detected by RT-PCR or cultured (especially polytropic strains). There is no practical point in defining virus strain for diagnostic purposes.

**Immunity, Prevention, and Control**

Mouse hepatitis virus is generally controlled by exclusion from pathogen-free mouse populations, or acquisition of mice free of the virus from commercial vendors. Control is approached by periodic serology of sentinel animals, re-derivation of incoming mice through cesarean section or embryo transfer, or quarantine and testing of mice. Infectious disease quality control and building-, room-, and cage-level containment are major areas of emphasis in maintaining research mice. Infected immunocompetent mice can be rid of infection by selective quarantine of adults without breeding for several weeks, commencing breeding of seropositive animals, and testing progeny (which will be transiently seropositive from maternal antibody). Because of the mutability of mouse hepatitis virus, this approach is not feasible on a room or population basis. Alternatively, mice can be “re-derived” by cesarean section and foster nursing on or embryo transfer into virus-free dams. This is the only option with immunodeficient mice, and special care is needed in testing the progeny to assure virus-free status. Once a mouse population is re-established as free of mouse hepatitis virus, stringent effort is needed to prevent reintroduction of virus. Conventionally housed mice cannot be maintained free of mouse hepatitis virus unless they are completely isolated from all other mice, including feral and wild mice (which are commonly infected).

**SIALODACRYOADENITIS VIRUS**

Like mouse hepatitis virus in mice, sialodacryoadenitis virus is represented by many strains of rat coronaviruses. So-called Parker’s rat coronavirus is simply another isolate of sialodacryoadenitis virus. Although sialodacryoadenitis and mouse hepatitis viruses are closely related, they do not naturally cross the species barrier.

Sialodacryoadenitis virus is highly contagious within naïve rat populations. Primary tropism is to nasal respiratory epithelium, with secondary spread to lacrimal glands,
salivary glands, and lung. The virus can induce disease in all ages of rat, but disease is most severe in young rats. Mortality may occur in suckling rats, complicated by failure to nurse as a result of the destruction of olfactory epithelium. Clinical features include nasal and ocular discharge, cervical swelling, photophobia, keratitis, and dyspnea. Lacrimal secretions surrounding the eyes are tinted with porphyrin pigment derived from affected retro-orbital Harderian glands. Lesions consist of necrotizing rhinitis, necrosis of salivary glands (excluding the sublingual glands, which are not affected) and lacrimal glands, periglandular edema, and interstitial pneumonia. Resolving lesions often feature marked squamous metaplasia, particularly in the Harderian glands. Infections are acute, with complete recovery, but permanent damage to the eye can arise indirectly from dysfunction of lacrimal glands (keratitis sicca) and inflammation in the filtration angle of the eye, resulting in hyphema, megaloglobus, and corneal ulcerations. Infection may contribute to anesthetic deaths, and predispose rats to secondary respiratory bacterial diseases. Immune-deficient rats are uncommon, but chronic wasting syndrome may occur in athymic nude rats, which succumb to progressive pneumonia.

Although rats are immune to reinfection with the homologous strain, they can be reinfect ed with novel strains of the virus. Sialodacryoadenitis virus infection is diagnosed by clinical signs and lesions, and retrospective diagnosis is accomplished by serology, usually utilizing cross-reacting mouse hepatitis virus antigen. Virus isolation, RT-PCR, and immunohistochemistry are available, but seldom used for diagnostic purposes. Although sialodacryoadenitis virus and mouse hepatitis virus are closely related, they do not naturally cross species barriers.

GUINEA PIG AND RABBIT CORONAVIRUSES

In juvenile European (*Oryctolagus*) rabbits, enteric coronaviruses induce disease that is characterized by intestinal villus attenuation, malabsorption, and diarrhea. Infection may predispose rabbits to, or be obscured by, the enteritis complex (dysbiosis). Rabbit coronavirus has been isolated, but not characterized. Another coronavirus infects rabbits asymptomatically, but experimental inoculation induces serosal effusion, right-sided heart enlargement, mesenteric lymphadenopathy, and multifocal necrosis of multiple organs. The “pleural effusion virus” was discovered as a contaminant of *Treponema pallidum*, which is maintained by intratesticular inoculation of laboratory rats. Little is known about the prevalence of either rabbit coronavirus, but enteric coronavirus is probably common.

Diarrhea and enteritis caused by a coronavirus has been reported in young guinea pigs, but its prevalence among guinea pig populations and its relationship to other coronaviruses are not known.

BOVINE CORONAVIRUS

Bovine coronavirus infections are associated with three distinct clinical syndromes in cattle: calf diarrhea, winter dysentery (hemorrhagic diarrhea) in adult cattle, and respiratory infections in cattle of various ages, including the bovine respiratory disease complex (shipping fever) in feedlot cattle. Coronaviruses were first reported as a cause of diarrhea in calves in the United States in 1973, and since then they have been recognized worldwide in association with the three clinical syndromes. The economic impact of respiratory disease and calf diarrhea is considerable.

Although many coronaviruses have restricted host ranges, group 2 coronaviruses such as bovine and SARS coronaviruses (Table 24.1) can infect other animal species, including wildlife. Bovine coronavirus is also closely related to the group 2 human coronavirus-OC43 that causes the common cold, and bovine coronavirus has been shown experimentally to infect dogs subclinically and to infect turkey poults, leading to fecal virus shedding, diarrhea, seroconversion, and transmission to contact controls. Genetically and/or antigenically related bovine coronavirus variants have been isolated from dogs with respiratory disease, humans with diarrhea, and captive or wild ruminants with intestinal disease similar to winter dysentery of cattle. The latter include Sambar deer (*Cervus unicolor*), water-buck (*Kobus ellipsiprymnus*), giraffe (*Giraffa camelopardalis*), and white-tailed deer (*Odocoileus virginianus*). Bovine coronavirus has also been linked to enteric disease in South American camelids. Interestingly, the human enteric coronavirus and wild ruminant coronaviruses both infected and caused diarrhea in experimentally exposed gnotobiotic calves, and the inoculated calves were subsequently immune to infection with bovine coronavirus.

Despite the different disease syndromes and apparent interspecies transmission of bovine coronavirus and its variants, only a single serotype of bovine coronavirus is recognized, and there is little sequence diversity between the wild ruminant coronaviruses and coronaviruses associated with the different disease syndromes in cattle. Furthermore, there are few common sequence differences to explain differences in host or tissue tropism.

Clinical Features and Epidemiology

Coronavirus-induced diarrhea commonly occurs in calves under 3 weeks of age after the decline of passively acquired antibodies, but disease can occur in calves up to 3 months of age. The severity of diarrhea and dehydration depends on the infecting dose as well as the age and immune status of the calf. Co-infections with other enteric pathogens such as rotavirus, torovirus, cryptosporidia, and enterotoxigenic *Escherichia coli* are common; their additive or synergistic effects increase the severity of diarrhea. Calf coronavirus
diarrhea is often seasonal, being more common in winter in part because of the increased stability of the virus in the cold.

Bovine coronavirus has also been implicated as a cause of winter dysentery, a sporadic acute disease of adult cattle worldwide that is especially prevalent during winter months, as the name implies. Winter dysentery is characterized by explosive, often bloody diarrhea, accompanied by decreased milk production, depression, anorexia, and frequent respiratory signs. Morbidity rates range from 20 to 100% in affected herds, but mortality rates are usually low (1–2%). A similar winter dysentery syndrome associated with bovine coronavirus variants occurs in captive and wild ruminants. This finding suggests that certain wild ruminants (deer, elk, caribou, etc.) that share common grazing areas with cattle could be a reservoir for coronavirus strains transmissible to cattle, or vice versa.

Bovine coronavirus causes mild respiratory disease (coughing, rhinitis) or pneumonia in 2–6-month-old calves. An epidemiologic study of calves from birth to 20 weeks of age confirmed both fecal and nasal shedding of coronavirus, with diarrhea prominent upon initial infection. The calves subsequently shed virus intermittently via the respiratory route, with or without signs of disease, suggesting that long-term mucosal immunity in the upper respiratory tract is ineffective in mediating virus clearance. As a consequence, coronavirus may recycle among cattle of all ages and regardless of their immune status, with sporadic nasal or fecal shedding from individual animals. Alternatively, new virus strains may be introduced when cattle from different sources are co-mingled, or from cohabiting wild ruminants.

Since 1993, bovine coronavirus has been incriminated as a precipitating cause of the bovine respiratory disease (shipping fever) complex. Both respiratory and enteric shedding of bovine coronavirus are common in affected feedlot cattle, peaking shortly after arrival at feedlots. Since its discovery, bovine coronavirus repeatedly has been identified in the lungs of feedlot cattle that died with bovine respiratory disease complex. Most feedlot cattle also seroconvert to bovine coronavirus within 3 weeks of arrival. Importantly, studies suggest that cattle arriving at feedlots with high serum titers of bovine coronavirus antibody were less likely to shed virus or to develop shipping fever. This observation suggests a role for serum antibodies in protection, or as an indicator of recent infection and active immunity.

Pathogenesis and Pathology

Concurrent fecal and nasal virus shedding persists for up to 10 days after coronavirus infection of calves. Coronavirus antigen is commonly detected in epithelial cells of both the upper respiratory and intestinal tracts, and occasionally in the lung. The pathogenesis of coronavirus enteritis in calves is similar to that caused by rotavirus, with the notable exception of extensive involvement of the large intestine by coronavirus. Disease occurs most commonly in calves at about 1–3 weeks of age, when virus exposure increases and antibody titers in the dam’s milk begin to wane. The pathogenesis and consequences of enteric coronavirus infection of calves are similar to those previously described for transmissible gastroenteritis in piglets. The destruction of the mature absorptive cells lining the intestinal villi and mucosal surface in the large intestine leads to malabsorption, often with rapid loss of water and electrolytes. The resultant hypoglycemia, acidosis, and hypovolemia can progress to circulatory failure and death, especially in very young animals.

The pathogenesis and lesions of winter dysentery of dairy and beef cattle resemble those of calf diarrhea, but often with marked intestinal hemorrhage and extensive necrosis of cells within the crypts of the large intestinal mucosa. Nasal and fecal shedding is more transient (up to 4–5 days). The anorexia and depression seen in dairy cattle with winter dysentery may explain the precipitous and sometimes prolonged decrease in milk production. The cause of the acute and often voluminous bloody diarrhea in some cattle is unexplained.

Both nasal and fecal shedding of bovine coronavirus can occur soon after cattle are transported to feedlots. Coronavirus infection is probably important in predisposing cattle entering feedlots to secondary bacterial infection that results in the characteristic shipping fever pneumonia—a severe, often fatal fibrinous bronchopneumonia caused by *Mannheimia haemolytica* biotype A, serotype 1 infection. Bovine coronavirus antigen also has been detected in epithelial cells of the upper (trachea, bronchi) and lower (terminal bronchioles and alveoli) respiratory tract of some affected cattle, but the precise role of coronavirus in precipitating the bovine respiratory disease complex awaits definitive characterization.

Diagnosis

Initially, the diagnosis of enteric bovine coronavirus infections was based on the detection of virus by electron microscopy, but cell culture isolation became a viable option when it was discovered that the virus could be grown when trypsin was added to the medium. For most bovine coronavirus strains, HRT-18 cells are optimal for primary isolation. Viral growth may be recognized by hemadsorption or cytopathogenic effects, and the presence of coronavirus confirmed by diagnostic tests. An array of assays is now available for detection of bovine (or variant) coronaviruses in cell culture or diagnostic specimens such as feces or nasal swabs, including ELISAs that incorporate monoclonal antibodies for antigen capture, immune electron microscopy using hyperimmune antiserum, and RT-PCR using...
bovine coronavirus or pan-coronavirus-specific primers to detect viral RNA. Post-mortem diagnosis is performed on acute fresh or fixed respiratory or intestinal tissues using hyperimmune antisera or monoclonal antibodies for immuno-fluorescence or immunohistochemical tissue staining.

**Immunity, Prevention, and Control**

**Passive Immunity to Enteric Bovine Coronavirus Infections in Calves**

Because coronavirus diarrhea occurs in young calves during the nursing period, maternal vaccination is required to provide immediate passive (lactogenic) immunity. Passive immunity to enteric viral infections in calves correlates with high levels of IgG1 antibodies in colostrum and milk. In ruminants, IgG1 antibodies are dominant in colostrum and milk and are selectively transported from serum. Most the antibody isotype (IgG1, IgG2, IgA) were correlated neutralizing or enzyme immunoassay antibody titer and feedlot cattle exposed upon entry, the serum magnitude of antibody to bovine coronavirus may be a marker for res-
edemiological studies suggest that the serum titer of infections in cattle are not clearly defined. Data from The correlates of immunity to respiratory coronavirus infections in calves correlates with high levels of IgG1 antibodies in colostrum and milk. In ruminants, IgG1 antibodies are dominant in colostrum and milk and are selectively transported from serum. Most adult cattle are seropositive for antibodies to bovine coronavirus. Therefore, parenteral vaccination of mothers with advuanted inactivated bovine coronavirus vaccines effectively boosts IgG1 antibody titer in serum and mammary secretions, to provide enhanced passive immunity to calves.

**Immunity to Respiratory Bovine Coronavirus Infections**

The correlates of immunity to respiratory coronavirus infections in cattle are not clearly defined. Data from epidemiological studies suggest that the serum titer of antibody to bovine coronavirus may be a marker for respiratory protection. In calves exposed in the field, and in feedlot cattle exposed upon entry, the serum magnitude of neutralizing or enzyme immunoassay antibody titer and the antibody isotype (IgG1, IgG2, IgA) were correlated with protection against respiratory disease, pneumonia, or coronavirus respiratory shedding. It is uncertain whether serum antibodies are correlates of protection, or whether they merely reflect prior enteric or respiratory coronavirus infection.

Available attenuated oral vaccines are not highly effective in preventing coronavirus-induced diarrhea in calves, because of interference by maternal antibodies and because vaccines typically do not have sufficient time to evoke protection of calves before the time of maximum risk. Attenuated or inactivated commercial vaccines are available to immunize the dam, thereby promoting increased antibody levels in colostrum and milk. Another alternative for dairy calves is to feed them colostrum and milk from hyperimmunized cows.

Currently, no vaccines are available to prevent winter dysentery or respiratory coronavirus infections. However, there are indications that the risk of shipping fever in cattle entering a feedlot is reduced if they receive intranasal vaccination with an attenuated enteric coronavirus vaccine.

**SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS**

In 2002, a new coronavirus emerged in China, associated with a severe acute respiratory syndrome (SARS) and substantial mortality in humans. The disease quickly spread globally before the epidemic was contained, in 2003, after more than 8000 cases and some 800 deaths in 29 countries. Patients infected with SARS virus initially presented with fever, general malaise, chills, and dry cough that progressed to diarrhea with fecal virus shedding, and about 30% of patients developed severe respiratory disease with interstitial pneumonia. Viral loads in nasopharynx, serum, and feces increased progressively to peak about day 10, and especially high viral loads in aerosols from some patients were correlated to superspreading events, an important but unexplained means of SARS virus transmission. Consistent with the clinical signs, SARS virus was detected mainly in intestine and lung, with infection of pulmonary type I pneumocytes and macrophages. The epidemic was contained by strict quarantine and sanitation methods, without the availability of vaccines or effective antiviral therapy.

A considerable and coordinated international effort led to the rapid cell culture isolation, genetic sequencing, and identification of an apparently new coronavirus as the causative agent of SARS. Both epidemiologic and genetic data suggest that SARS in humans is a zoonosis, and that SARS coronavirus evolved from a coronavirus that naturally infects a wildlife reservoir host. Individuals who were closely associated with live-animal markets in China were over-represented in initial cases of SARS, and SARS-like coronaviruses were isolated from clinically normal Himalayan palm civets (Paguma larvata) and a raccoon dog (Nyctereutes procyonoides) from live-animal markets. Although civets are susceptible to experimental infection with human SARS coronavirus, this virus was not detected in civets raised on farms, or in wild civets. Thus it was proposed that civets and raccoon dogs may amplify virus in wild-animal markets as intermediate hosts, but they probably are not the natural host reservoir for SARS coronavirus. Bats are now proposed to be the definitive reservoir hosts of SARS coronavirus, as enzootic infection of Chinese horseshoe bats with a remarkable genetic spectrum of SARS-like coronaviruses has now been established.

Changes in three genes were identified during the adaptation of SARS coronavirus to humans, including the S gene, as related to adaptation to the human cell receptor (ACE 2) and in the accessory proteins encoded by open reading frames 3a and 8, which are of uncertain biologic significance. In 2004, SARS re-emerged in China, and, judged by sequence data, the re-emerged SARS virus strains were more like civet viruses, suggesting that these cases represented new introductions from animals to humans.
The emergence of SARS was a sobering but timely reminder to the global biomedical community of the potential ramifications of “species jumping” of coronaviruses. It had been clearly shown previously that some animal coronaviruses were promiscuous in terms of their species specificity, but it was only when a zoonotic disease as devastating as SARS emerged that serious attention was given to the importance of this phenomenon. Importantly, SARS appears to have a relatively broad host range, in common with group 2a bovine coronaviruses, and experimental SARS coronavirus infection has now been described in rhesus macaques, ferrets, mice, cats, and hamsters. Despite their obvious importance, the determinants of host range specificity and interspecies transmission among coronaviruses remain largely undefined.

**INFECTIOUS BRONCHITIS VIRUS**

Infectious bronchitis was the term coined in 1931 to describe the principal clinical-pathological feature of a transmissible respiratory disease of poultry in the United States first reported in North Dakota. Infectious bronchitis virus was identified retrospectively as the cause of a disease that had been misidentified as high-pathogenicity avian influenza in New England and the upper Midwest during 1924–1925. The disease has now been identified worldwide and is one of the most important viral diseases of chickens. The virus is the prototype of the family *Coronaviridae*; there are many antigenic variants and serotypes as a consequence of mutations in its large genome.

**Clinical Features and Epidemiology**

The clinical presentation of infectious bronchitis depends on age, genetic background, and immune status of the bird at the time of infection, route of exposure, nutritional factors (especially levels of calcium in the diet), virulence of the virus strain, and the presence of stressors such as cold temperatures or secondary bacterial pathogens. Outbreaks may be explosive, with the virus spreading rapidly to involve the entire flock within a few days. The incubation period is typically brief: 18–48 hours. In chicks 1–4 weeks of age, virulent virus strains produce severe respiratory disease, with gasping, coughing, tracheal rales, sneezing, nasal exudate, wet eyes, respiratory distress, and, occasionally, swollen sinuses. Mortality in young chicks is usually 25–30%, but in some outbreaks can be as high as 75%. Less virulent strains cause fewer and milder respiratory signs, and lower morbidity and mortality rates. Infection of young female chicks may result in permanent hypoplasia of the oviduct that is evident later in life as reduced egg production and inferior quality eggs.

When the disease is uncomplicated by opportunistic bacterial superinfection, respiratory signs last for 5–7 days and disappear from the flock in 10–14 days. High mortality can occur in broilers as a result of secondary infection with *Escherichia coli* or pathogenic mycoplasmas. Egg-laying chickens usually present with reproductive tract involvement that is manifest as a decline or cessation in egg production or, less consistently, respiratory disease. When laying resumes, many eggs are abnormal, including lack of calcified shell, thin shells, and shells with stipples, distortions, dimples, depressions, or ridging; eggs that should be colored are often pale or white, and egg albumen may be watery. In acutely infected birds, the kidneys can be pale and swollen, with urates distending the ureters, and in the chronic phase there can be atrophy of kidney lobules, with large calculi within the ureters (urolithiasis).

Infectious bronchitis virus spreads between birds by aerosol and by ingestion of food contaminated with feces. In the environment, the virus can survive on fomites for several days and possibly for weeks, especially at low environmental temperatures. Outbreaks of infectious bronchitis have declined in recent years as a result of the extensive use of vaccines; however, the disease may occur even in vaccinated flocks when immunity is waning, or upon exposure to variant viral serotypes. To minimize this risk, most poultry producers obtain 1-day-old chicks from maternal antibody-positive breeders and then spray-vaccinate them with live attenuated vaccine in the hatchery.

**Pathogenesis and Pathology**

The virus replicates to high titer first in the respiratory tract (ciliated epithelial cells); this is followed by viremia (within 1–2 days of infection), which distributes the virus to many organs. The virus can cause extensive damage to the ovaries, oviduct, and the kidneys. The intestinal tract is another site of primary infection, but damage usually is minimal.

Infecitivity declines rapidly, and isolation of virus beyond 7 days after infection is uncommon (except from chicks). Rarely, virus has been reported to persist for up to 14 weeks in cecal tonsils, and has been recovered from the feces for up to 20 weeks after infection. Kidney and intestine are the likely sites of virus persistence.

The most frequent gross pathological finding is mucosal thickening, with serous or catarrhal exudate in the nasal passages, trachea, bronchi, and airsacs. In very young chicks, the main bronchi may be blocked with caseous yellow casts. Pneumonia and conjunctivitis are sometimes seen. In laying birds, ova can be congested and sometimes ruptured, with free yolk in the abdominal cavity. Desquamation of respiratory epithelium, edema, epithelial hyperplasia, mononuclear cell infiltration of the submucosa, and regeneration are seen in various combinations. Repair processes begin after 6–10 days, and are complete in 14–21 days. Some virus strains affect the kidney, causing interstitial nephritis, and some Asian strains of
the virus cause enlargement of the glandular stomach (proven- triculus), with ulceration and inflammation.

**Diagnosis**

Direct immunofluorescence staining of tracheal tissue smears is useful in the diagnosis of early cases before secondary bacterial infection has occurred. For virus isolation, embryonated eggs are inoculated via the allantoic sac route. Changes suggestive of the presence of a coronavirus include congestion of the main blood vessels in the chorioallantoic membrane and embryo stunting, curling, clubbing of down, or urate deposits in the mesonephros. Identification of virus in the chorioallantoic membrane is usually done by immunofluorescence or immunohistochemical staining, or in allantoic fluid by serological methods, nucleic acid analysis, or electron microscopy. Isolates are usually typed and subtyped by serological methods and nucleic acid analyses such as restriction length polymorphism, or genotype-specific RT-PCR assays.

**Immunity, Prevention, and Control**

Infection induces IgM, IgG, and IgA antibodies. In immune laying hens, the ovum begins to acquire IgG antibody (some of it virus specific) from the blood about 5 days before the egg is laid. As it becomes surrounded with albumen during passage down the oviduct, the ovum acquires both IgM and IgA antibodies, which are transferred into the amniotic fluid about halfway through development. During the last third of embryonation, IgG enters the circulation from the yolk; antibody can inhibit virus replication at this time. The chick hatches with a circulating IgG level similar to that of the hen. IgG antibody is metabolized with a half-life of approximately 3 days and may persist for 3–4 weeks. The virus may survive until passive immunity declines to a level at which it can replicate again, at which time the chicken mounts an active immune response. However, the correlates of active immunity to infectious bursal disease virus are less certain. Neutralizing antibodies can prevent virus dissemination from the respiratory tract and block secondary infection of the reproductive tract and kidneys. The adaptive transfer of CD8 T lymphocytes protects chicks against infectious bronchitis virus challenge, suggesting a role for cellular immunity as well in protection.

Attenuated virus vaccines are widely used to protect meat chickens. These vaccine viruses are derived by serial passage in embryonated eggs. They are administered in drinking water, by coarse spray, or by deposition on the conjunctiva (eye drops). The first vaccination is typically given in the hatchery when birds are 1 day old, and booster vaccination is given at 10–18 days. Passively acquired maternal immunity prevents respiratory infection and disease for the first 7 days. For layers or breeders, attenuated vaccines are used for priming, followed by killed oil-adjuvanted booster vaccines, often given repeatedly during the laying cycle. Vaccination breaks occur because of the variable presence of new antigenic variants and existence of several serotypes. Such variants will continue to emerge and spread, posing continuing problems for poultry producers.

Control of infectious bronchitis is difficult because of the presence of persistently infected chickens in some flocks and the continuing emergence of antigenically variant viruses. The domestic chicken is the primary and most important host, but infections and disease have been described in pheasants infected with a closely related coronavirus. Sporadic or individual cases of avian infectious bronchitis virus infection also have been described in peafowl, teal, partridge, and guinea fowl. Group 3 avian coronaviruses have been infrequently identified in graylag geese, mallards, pigeons, green-cheeked Amazon parrots, and Manx shearwater.

**TURKEY CORONAVIRUS**

Coronaviruses were first recognized in turkeys in the United States in 1951 and were associated with various enteric disease syndromes, variously termed “blue comb disease,” “mud fever,” “transmissible enteritis,” and “coronaviral enteritis.” The disease is present throughout the world, essentially wherever turkeys are raised. The virus can infect turkeys of all ages, but the most severe enteric disease is evident within the first few weeks of life. The onset is characterized by loss of appetite, watery diarrhea, dehydration, hypothermia, weight loss, and depression. Younger poults may die. The duodenum and jejunum are pale and flaccid, and the ceca filled with frothy, watery contents. The feces may be green to brown, watery, and may contain mucus and urates. The cloacal bursa is small (atrophic). Some turkeys may shed virus in their feces for up to 7 weeks, with virus transmission by the fecal–oral route. Turkey coronavirus infections also result in reduced egg production in breeder hens, and eggs may lack normal pig- ment and have a chalky shell surface. Interaction between turkey coronavirus and other agents (E. coli, astrovirus, etc.) accentuate the disease.

Only one serotype of turkey coronavirus is recognized. Turkey coronavirus is classified, along with other avian coronaviruses, in antigenic group 3. Although there is high sequence identity (85–90%) in the three major viral proteins (polymerase, M, and N) of turkey coronavirus and avian infectious bronchitis virus, their S proteins are quite different. Whether the latter divergence reflects altered enteric tropism, or adaptation to the turkey, is unclear. Recently, bovine coronavirus was shown experimentally to infect turkey poults, but natural cases have not been identified.

Turkey coronavirus can be isolated in embryonated eggs of turkeys and chickens using the amniotic route of inoculation. No licensed vaccines for turkey coronavirus
are available. Treatment involves supportive care, and is not specific.

OTHER CORONAVIRUSES

Coronavirus infections have been described in a wide variety of other species, including humans, horses, bats, wild carnivores, rabbits, numerous species of birds, and wildlife, sometimes in association with enteric or respiratory diseases. An enteric coronavirus occurs in ferrets and mink, in association with outbreaks of enteritis. A related virus recently was incriminated as the cause of systemic pyogranulomatous inflammation resembling feline infectious peritonitis amongst ferrets in both Europe and North America.

MEMBERS OF THE GENUS TOROVIRUS

Toroviruses have been described in the horse (Berne virus), cattle (Breda virus), and turkeys. The equine and bovine toroviruses are serologically related. A torovirus of swine (porcine torovirus) that is genetically closely related to the equine and bovine viruses has been demonstrated only by molecular techniques, and has yet to be propagated in cell culture.

At least two serotypes of Breda virus are recognized (defined by hemagglutination-inhibition assays), with a third genotype suggested on the basis of sequence heterogeneity; there are two distinct genotypes of porcine toroviruses. A surprising feature of toroviruses is their sequence divergence and the presence of interspecies sequence homology, presumably acquired via homologous RNA recombination events. For instance, the M protein and S2 subunit (stalk) sequences are highly conserved (10–15% maximum divergence) among toroviruses, whereas the S1 subunit (globular top of the S protein involved in receptor binding) is more divergent (maximum 38% divergence), presumably as a consequence of selection pressure. The hemagglutinin esterase (HE) proteins that are also subject to immune pressure are the most highly divergent. The Berne virus lacks this protein, which is largely deleted. The N protein, which is usually highly conserved within coronavirus groups, shows less sequence divergence (20%) between Berne and Breda viruses and more divergence (35–37%) with porcine torovirus (genotype 2). Furthermore, the N protein genes of genotypes 2 and 3 Breda viruses appear to have been acquired from porcine torovirus genotype 1 strains, presumably through an RNA recombination event.

Clinical Features and Epidemiology

Little is known of the disease potential of Berne virus in horses, as only a single case has been described—this in a horse with diarrhea. Breda virus causes diarrhea in calves, and can be a serious problem in some herds. In swine, torovirus infection has been associated with post-weaning diarrhea. Torovirus infections of turkeys cause diarrhea, poor feed conversion, reduced weight gain (stunting), listlessness, and litter eating.

Torovirus infections are common. In cattle, 90–95% of randomly sampled cattle have antibodies. Antibody-positive cattle have been identified in every country in which tests have been done. Most adult horses in Switzerland possess neutralizing antibodies to Berne virus, which is also true for goats, sheep, pigs, rabbits, and some species of wild mice. Epidemiological surveys have indicated that torovirus infections are involved in two disease entities in cattle: diarrhea in calves up to 2 months of age, and winter dysentery of adult cattle in the Netherlands and Costa Rica. Nasal shedding of Breda virus in feedlot cattle has been reported, but without any clear association with respiratory disease in the infected animals.

Human toroviruses have been detected in stool samples, most commonly from diarrheic children, with prevalence rates of 22–35%. Their detection was based largely on the detection by electron microscopy of virus particles with characteristic torovirus morphology, but, more recently, viral antigen or RNA was detected by ELISA or RT-PCR, respectively, using Berne or Breda virus-specific reagents. Berne virus neutralizing antibodies are also detected in human sera. Sequence analysis of torovirus amplicons from human stool specimens revealed essentially identical sequences in the corresponding 3’-untranslated region with Berne virus and 9% divergence with Breda virus. However, the sequence of the torovirus HE gene from human stool samples was unique and divergent from that of other toroviruses. Additional studies of human toroviruses are needed to clarify their prevalence and relationships to animal toroviruses.

Pathogenesis and Pathology

Breda virus, the bovine torovirus, is pathogenic for newborn gnotobiotic and non-immune conventional calves; these animals develop watery diarrhea lasting for 4–5 days, with virus shedding occurring for another 3–4 days. Diarrhea is more severe in calves with a normal intestinal flora than in gnotobiotic calves. Histological lesions include necrosis of enterocytes with subsequent villous contraction (atrophy) from mid-jejunum to distal ileum, in addition to enterocyte necrosis in the large intestine. Epithelial cells lining both the intestinal crypts and villi are infected. Infection of the former may affect the severity and duration of diarrhea, as mucosal regeneration begins by division of crypt enterocytes. The germinal centers of the Peyer’s patches become depleted of lymphocytes. There also is necrosis of dome epithelial cells, including M cells.
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Diagnosis

Berne virus was originally isolated and then propagated in vitro using several types of equine cell, with subsequent manifestation of cytopathic effects. Recently, a bovine torovirus (Aichi/2004 strain) has been isolated in human rectal tumor (HRT-18) cells—the same cell line used for bovine coronavirus primary isolation.

Using immunofluorescence, Breda virus antigen can be detected in epithelial cells of the small intestine. Fluorescence is cytoplasmic, and is generally most intense in areas of the intestines with the least tissue damage. The mid-jejunum is the first site to be infected, with viral infection progressing down the small intestine and eventually reaching the large intestine. Given this course of the infection, tissue specimens must be obtained at several levels, and as early after the onset of diarrhea as possible. Torovirus particles also can be directly visualized in feces or intestinal contents, using electron microscopy. However, immune electron microscopy using hyperimmune antiserum is preferred for definitive identification of torovirus–antibody complexes, and to avoid potential confusion (misidentification) with coronaviruses or cellular debris. Serum neutralization, ELISA, and hemagglutination-inhibition assays (for bovine or porcine torovirus only) are available, using bovine torovirus or Berne virus from infected cell cultures as antigen, or Breda virus purified from the feces or intestinal contents of gnotobiotic calves. RT-PCR with primers targeting the S protein has been used to diagnose field infections in cattle, using nasal or rectal swab specimens or feces.

The turkey torovirus can be isolated in turkey embryos via the amniotic route of inoculation.

Immunity, Prevention, and Control

The seroprevalence of antibodies to Breda virus in adult cattle and colostrum-fed young calves (approximately 1 month old) is high (up to 90%). In the latter, this presumably reflects maternally acquired passive antibodies that have been shown to protect at least partially against Breda virus diarrhea, but not infection during the initial month of life. Maternal antibodies may delay active immune responses of calves to Breda virus, with late or low IgM and IgG serum antibody responses. Passive antibodies decline and calves become seronegative or exhibit low antibody titers by 4–7 months of age. At 6–8 months of age, all seronegative (100%) but fewer seropositive (57%) feedlot calves were susceptible to Breda virus infection, as demonstrated by fecal and nasal virus shedding and seroconversion. A surprising aspect of Breda virus infection in one study was a lack of IgA seroconversion. The authors attributed this to infection of M cells interfering with an active mucosal antibody response.

In view of the variable role of toroviruses as pathogens, vaccines have not been developed against them. For Breda virus, symptomatic treatment (electrolytes) may be needed to control dehydration in severely affected calves. Colostrum containing bovine torovirus antibodies may be used for prophylaxis. General hygiene, biosecurity, and good calf management practices (colostrum feeding immediately after birth) may reduce outbreaks or adverse effects of Breda virus infections in cattle.