Chapter 24

Coronaviridae

Chapter Outline

The family *Coronaviridae* is included with the families *Arteriviridae*, *Roniviridae*, and *Mesoniviridae* in the order *Nidovirales*; viruses in these four families share a distinctive replication strategy. The family *Coronaviridae* is comprised of two subfamilies. One, the subfamily *Coronavirinae*, 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 (Table 24.1). Coronavirus and coronavirus-like infections have been described in swine, cattle, horses, camels, cats, dogs, rodents, birds, bats, rabbits, ferrets, mink, and various wildlife species, although many coronavirus infections are subclinical. In humans, coronaviruses are included in the spectrum of viruses that cause the common cold as well as more severe respiratory disease—specifically, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), which are both zoonoses. The second subfamily, *Torovirinae*, contains pathogens of both terrestrial and aquatic animals. The genus *Torovirus* includes the type species, equine torovirus (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. White bream virus from fish is the type species of the genus *Bafinivirus*.

PROPERTIES OF CORONAVIRUSES

Classification

Despite profound differences in virion structure and genome size, coronaviruses, toroviruses, arteriviruses, roniviruses, and mesoniviruses exhibit remarkable similarities in their genome organization and replication strategy (Fig. 24.1; Table 24.2). In infected cells, these viruses

Fenner's Veterinary Virology. DOI: http://dx.doi.org/10.1016/B978-0-12-800946-8.00024-6

TABLE 24.1 Molecular Properties and Clinical Characteristics of Major Coronavirus Infections of Veterinary Significance

Virus	Disease/Symptoms	Transmission	Prevention/Control
Subfamily *Coronavirinae*			
***Genus* Alphacoronavirus**			
Feline coronavirus (Feline enteric coronavirus; Feline infectious peritonitis virus)	Feline enteric coronavirus: mild gastroenteritis and diarrhea Feline infectious peritonitis virus: peritonitis, pneumonia, CNS signs etc.	Feline enteric coronavirus: direct contact; fecal–oral route from maternal shedding Feline infectious peritonitis virus: blood, body fluids	Interruption of transmission cycle, quarantine, high-level hygiene
Canine coronavirus	Mild gastroenteritis and diarrhea Possible severe enteritis and systemic signs (leucopenia)	Fecal–oral route	Inactivated vaccine
Transmissible gastroenteritis (TGE) virus of swine	Gastroenteritis. Watery diarrhea, vomiting, dehydration	Fecal–oral route	Oral attenuated vaccine to pregnant sows. Good sanitation
Porcine respiratory coronavirus	Mild respiratory disease or subclinical	Aerosol Nasal swabs; trachea, lung sections	No vaccine available
Porcine epidemic diarrhea virus (PEDv)	Gastroenteritis. Watery diarrhea, vomiting, dehydration	Fecal–oral route	Inactivated and oral live-attenuated virus vaccine to pregnant sows. Good sanitation
Genus *Betacoronavirus*			
Group A			
Porcine hemagglutinating encephalomyelitis virus	Vomiting, wasting disease, encephalomyelitis. Anorexia, hyperesthesia, muscle tremors, emaciation	Aerosols, oronasal secretions	Good husbandry, maintain immune sows No vaccine available
Mouse hepatitis virus Rat sialodacryoadenitis virus	Enteritis, hepatitis, demyelinating encephalomyelitis Rhinitis, epiphora, pneumonia	Introduction of virus into a naïve colony: aerosols and direct contact Aerosols	Depopulation. Preventive quarantine
Bovine coronavirus Equine coronavirus Canine respiratory coronavirus	Gastroenteritis with profuse or bloody diarrhea, dehydration, decreased milk, or respiratory disease Gastroenteritis Respiratory disease	Fecal–oral route, aerosols, respiratory droplets Fecal–oral route Aerosols	Maternal immunization: inactivated or attenuated vaccines; no vaccine for winter dysentery
Group B			
Severe acute respiratory syndrome (SARS) coronavirus	Respiratory disease; zoonotic with bats as natural reservoir	Aerosols, oronasal secretions	No vaccines available; enhanced biosecurity for human cases
Group C			
Middle East respiratory syndrome (MERS) coronavirus	Respiratory disease; zoonotic with camels and bats as a likey reservoir	Aerosols, oronasal secretions	No vaccines available; enhanced biosecurity for human cases

(*Continued*)

TABLE 24.1 (Continued)

Virus	Disease/Symptoms	Transmission	Prevention/Control
Genus *Gammacoronavirus*			
Avian infectious bronchitis virus	Tracheobronchitis, nephritis Rales, decreased egg production	Aerosols and ingestion of food contaminated with feces	Multivalent attenuated and inactivated vaccines available. Good sanitation and testing
Turkey coronavirus, Bluecomb virus	Enteritis Diarrhea, depression, cyanotic skin	Fecal–oral route, aerosol	Inactivated virus vaccine
Genus *Deltacoronavirus*			
Porcine deltacoronavirus	Gastroenteritis in sows and nursing pigs; low mortality in nursing pigs; clinically indistinguishable from TGE and PEDv	Fecal–oral route	No vaccine; biosecurity
Subfamily *Torovirinae*			
***Genus* Torovirus**			
Breda virus (cattle)	Enteritis Diarrhea, dehydration	Fecal–oral route	No vaccine available
Genus *Bafinivirus*			
White bream virus Fathead minnow Necrotic nidovirus lesions in kidney, liver, and spleen	None observed Hemorrhages in the eyes and skin	Assumed horizontal via water Assumed horizontal	No control method proposed None available

CNS, central nervous system.

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′ coterminal 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 subfamilies (*Coronavirinae* and *Torovirinae*), and the families *Arteriviridae*, *Roniviridae*, and *Mesoniviridae* (Fig. 24.2A). Sequence analysis of the gene encoding portions of the viral RNA-dependent RNA polymerase (RdRp) suggests that the member viruses of the order *Nidovirales* probably evolved from a common ancestor. Extensive genome rearrangements through heterologous RNA recombination, along with accumulation of mutations over time, have resulted in the variations seen—that is, viruses with similar replication and transcription strategies but disparate structural features.

The subfamily *Coronavirinae* is subdivided into four genera on the basis of genetic and serologic properties, sometimes with subgroups within these (Table 24.1; Fig. 24.2). The genus *Alphacoronavirus* (previously group 1 coronaviruses) includes transmissible gastroenteritis virus of swine, porcine respiratory coronavirus, porcine epidemic diarrhea virus, canine coronavirus, feline coronavirus, ferret and mink coronaviruses, the human coronaviruses 229E and HKU1, as well as many viruses found in bats. The genus *Betacoronavirus* (previously group 2 coronaviruses) is divided into four groups; *Betacoronavirus* group A includes mouse hepatitis virus, rat (sialodacryoadenitis) coronavirus, bovine and equine coronaviruses, porcine hemagglutinating encephalomyelitis virus, canine respiratory coronavirus, and other human coronaviruses. *Betacoronavirus* group B includes human SARS coronavirus, civet cat, raccoon dog, and horseshoe bat coronaviruses. *Betacoronavirus* group C includes MERS coronavirus from both humans and camels, as well as closely related bat coronaviruses, and group D currently includes only coronaviruses of bats. The genus *Gammacoronavirus* (previously group 3 coronaviruses) includes avian infectious bronchitis virus, turkey coronavirus, and several potential but still largely uncharacterized new species from wild birds and marine mammals, including dolphins and whales. The more recently identified genus *Deltacoronavirus* includes viruses from pigs and a variety of wild birds, as well as a virus from a wild Asian leopard

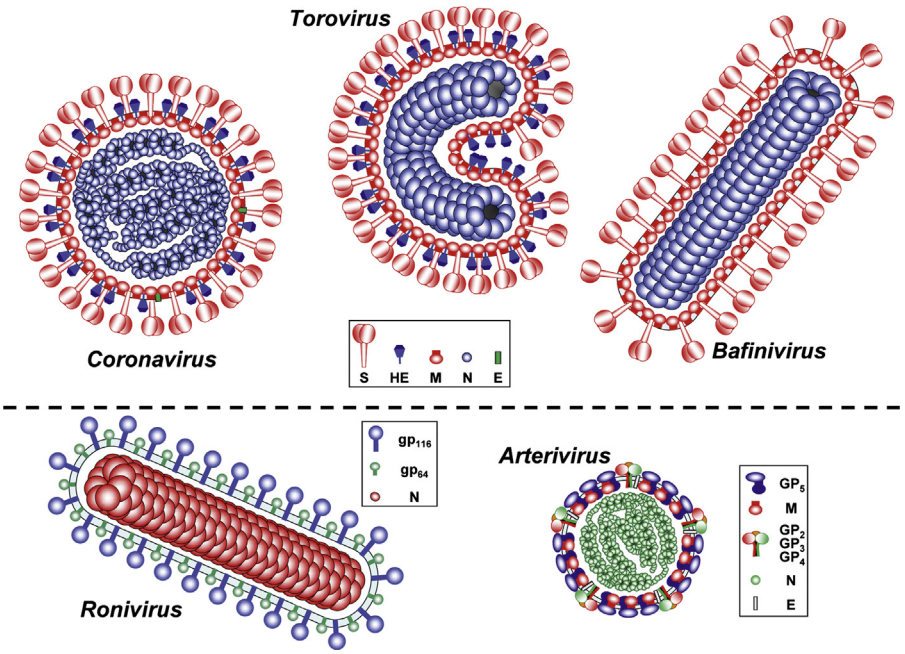

FIGURE 24.1 Schematic structure of particles of members of the order *Nidovirales*. *From King, A.M., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses, p. 785. Copyright © Elsevier (2012), with permission.*

TABLE 24.2 Structural Proteins of Nidoviruses: Acronyms and Sizes (in Amino Acid Residues). Boxed proteins are believed to be evolutionarily related

Protein[a]		Coronavirus	Torovirus	Bafinivirus	Okavirus	Arterivirus
Spike glycoprotein	S	1035–1472	1562–1584	1220	–	–
Large spike glycoprotein	gp116	–	–	–	873[c]–899	–
Small spike glycoprotein	gp64	–	–	–	539	––
Minor surface glycoprotein	GP2	–	–	–	–	227–249
	GP3	–	–	–	–	163–256
	GP4	–	–	–	–	152–183
Major surface glycoprotein	GP5	–	–	–	–	199–278
Membrane protein	M	218–263	233	227	–	162–174
Nucleocapsid protein	N	349–470	159–167	161	144–146	110–128
Envelope protein	E	74–109	–	–	–	67–80
Hemagglutinin-esterase protein	HE	386–440[b]	416–430	–	–	

[a]*Only proteins typical for each lineage are listed; for some CoVs additional, virus species-specific accessory envelope proteins have been described.*
[b]*Only found in a cluster of betacoronaviruses ("phylogroup A,"* Betacoronavirus 1, Murine coronavirus, Human coronavirus HKU1*).*
[c]*Size predicted for gill-associated virus gp116 protein.*

cat. Further taxonomic subdivision of these viruses is likely in the future. It is believed currently that warm-blooded flying vertebrates are the definitive hosts for the coronavirus gene pool, with alpha- and betacoronaviruses having their origin in bats, and gamma- and delta-coronaviruses having their origin in birds.

Viruses in the *Torovirinae* subfamily are all apparently closely related but genetically distinct from coronaviruses; however, many toroviruses have yet to be fully characterized. There are currently two genera within the family *Torovirinae*, specifically, the genera *Torovirus* and *Bafinivirus* (Fig. 24.2A).

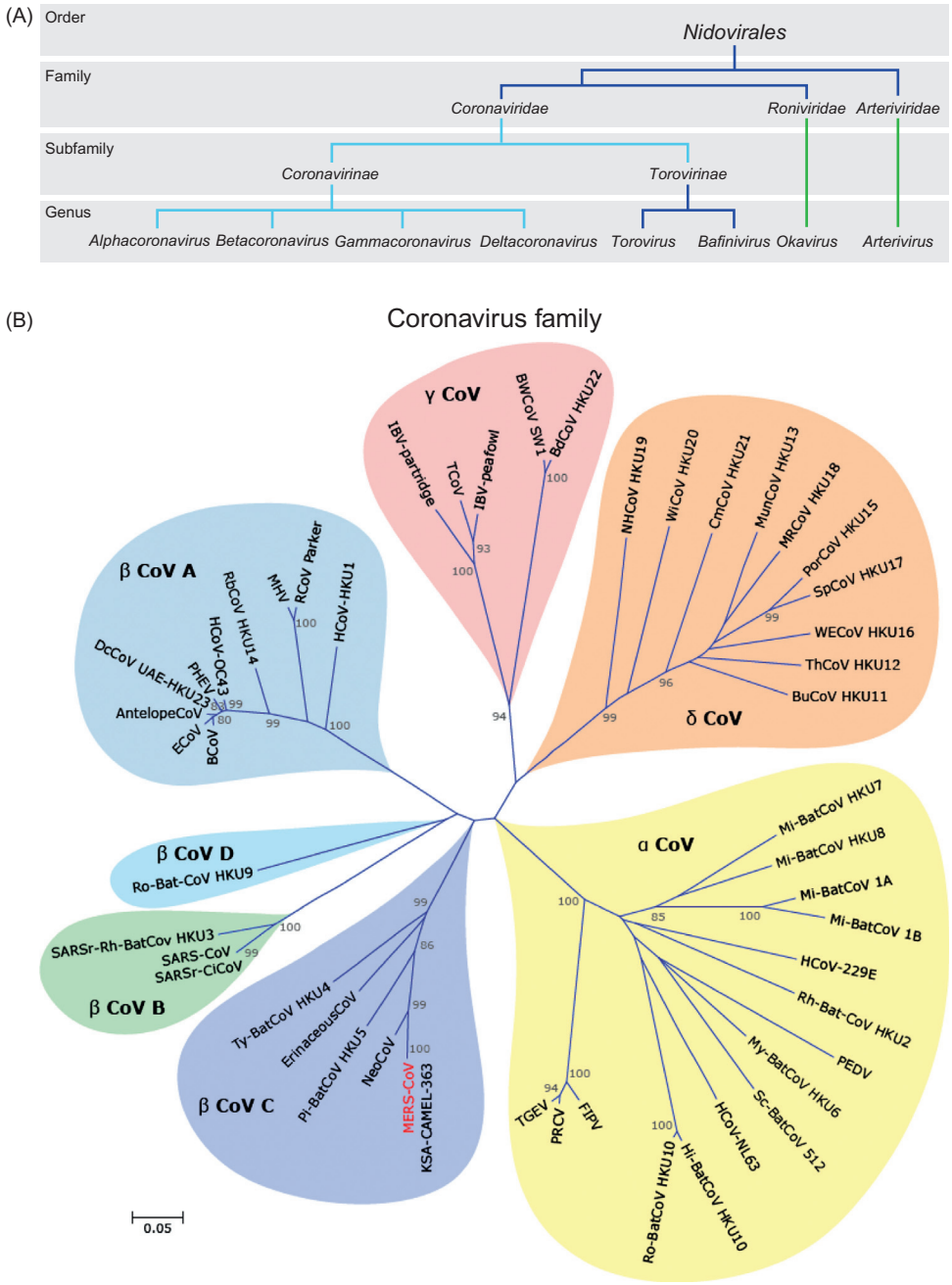

FIGURE 24.2 (A) Current taxonomy of *Coronaviridae* according to the International Committee on Taxonomy of Viruses. *Courtesy of G. Whittaker and R. Collins, Cornell University*. (B) Phylogenetic tree of 50 coronaviruses with partial nucleotide sequences of RNA-dependent RNA polymerase. The tree was constructed by the neighbor-joining method using MEGA 5.0. The scale bar indicates the estimated number of substitutions per 20 nucleotides. Abbreviations (accession numbers): *AntelopeCoV*, sable antelope coronavirus (EF424621); *BCoV*, bovine coronavirus (NC_003045); *BdCoV HKU22*, bottlenose dolphin coronavirus HKU22 (KF793826); *BuCoV HKU11*, bulbul coronavirus HKU11 (FJ376619); *BWCoV-SW1*, beluga whale coronavirus SW1 (NC_010646); *CMCoV HKU21*, common-moorhen coronavirus HKU21 (NC_016996); *DcCoV UAE-HKU23*, dromedary camel coronavirus UAE-HKU23 (KF906251); *ECoV*, equine coronavirus (NC_010327); *ErinaceousCoV*, betacoronavirus Erinaceus/VMC/DEU/2012 (NC_022643); *FIPV*, feline infectious peritonitis virus (AY994055); *HCoV-229E*, human coronavirus 229E (NC_002645); *HCoV-HKU1*, human coronavirus HKU1 (NC_006577); *HCoV-NL63*, human coronavirus NL63 (NC_005831); *HCoV-OC43*, human coronavirus OC43 (NC_005147); *Hi-BatCoV HKU10*, Hipposideros bat coronavirus HKU10 (JQ989269); *IBV-partridge*, avian infectious bronchitis virus partridge isolate (AY646283); *IBV-peafowl*, avian infectious bronchitis virus peafowl isolate (AY641576); *KSA-CAMEL-363*, KSA-CAMEL-363 isolate of Middle East respiratory syndrome coronavirus (KJ713298); *MERS-CoV*, Middle East respiratory syndrome coronavirus (NC_019843.3); *MHV*, murine hepatitis virus (NC_001846); *Mi-BatCoV 1A*, Miniopterus bat coronavirus 1A (NC_010437); *Mi-BatCoV 1B*, Miniopterus bat coronavirus 1B (NC_010436); *Mi-*

(*Continued*)

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 and bafiniviruses) (Fig. 24.1). Coronaviruses have distinctive and large (20 nm long) club-shaped spikes (peplomers, composed of trimers of the spike protein). The association of the nucleocapsid (N) protein with the genomic RNA forms the helical nucleocapsid that is surrounded by an icosahedral structure composed of the viral membrane (M) protein. Some coronaviruses also have a second fringe of shorter (5 nm long) spikes (composed of the hemagglutinin-esterase (HE) protein), a particular characteristic of some betacoronaviruses. 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. Bafiniviruses appear as straight rods with a bacilliform morphology, which are surrounded by large peplomers.

The genome of viruses in 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 nonsegmented RNA viral genomes. The genomic RNA is 5′ capped and 3′ polyadenylated, and is infectious (Table 24.3; Fig. 24.3).

The major virion proteins of the member viruses of the subfamilies *Coronavirinae* and *Torovirinae* include a nucleocapsid protein (N, 50–60 kDa, 19 kDa for toroviruses) and several envelope proteins: (1) the spike glycoprotein trimer (S, 180–220 kDa per monomer); (2) a triple-spanning transmembrane protein (M, 23–35 kDa); (3) a minor transmembrane protein (E, 9–12 kDa), which together with the M protein is essential for coronavirus virion assembly and budding. Toroviruses lack a homolog of the coronavirus E protein, which may explain the structural differences between the coronaviruses and toroviruses (Fig. 24.1). The secondary, smaller spikes, seen in some betacoronaviruses and in toroviruses, consist of a dimer of a second class I membrane glycoprotein (65 kDa per monomer), a HE that shares 30% sequence identity with the N-terminal subunit of the HE fusion protein of influenza C virus. Sequence comparisons indicate that the *HE* genes of coronaviruses, toroviruses, and orthomyxoviruses were acquired by independent, nonhomologous recombination events (probably from the host cell). Although there is no sequence similarity between the torovirus proteins and their counterparts in coronaviruses, they are similar in structure and function, and are related phylogenetically. Bafiniviruses have only the S, M, and N structural proteins.

TABLE 24.3 Properties of Coronaviruses and Toroviruses

Virions are pleomorphic or spherical (Subfamily *Coronavirinae*) or disc-, kidney-, or rod-shaped (Subfamily *Torovirinae*); 80–220 nm (coronaviruses) or 120–140 nm (toroviruses) in diameter. Virions are enveloped, with large club-shaped spikes (peplomers)
Virions have an icosahedral core structure within which is a helical nucleocapsid (coronaviruses) or a tightly coiled tubular nucleocapsid in a doughnut (toroviruses) or bacilliform (bafiniviruses) shape
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). Torovirus virions contain analogous proteins, but there is no E protein. Bafiniviruses have only three structural proteins (S, M and N)
Viruses replicate in the cytoplasm; the genome is transcribed, forming a full-length complementary RNA from which is transcribed a 3′ coterminal 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. Cell–cell fusion may occur

◀ *BatCoV HKU7*, Miniopterus bat coronavirus HKU7 (DQ249226); *Mi-BatCoV HKU8*, Miniopterus bat coronavirus HKU8 (NC_010438); *MRCoV HKU18*, magpie robin coronavirus HKU18 (NC_016993); *MunCoV HKU13*, munia coronavirus HKU13 (FJ376622); *My-BatCoV HKU6*, Myotis bat coronavirus HKU6 (DQ249224); *NeoCoV*, coronavirus Neoromicia/PML-PHE1/RSA/2011 (KC869678); *NHCoV HKU19*, night heron coronavirus HKU19 (NC_016994); *PEDV*, porcine epidemic diarrhea virus (NC_003436); *PHEV*, porcine hemagglutinating encephalomyelitis virus (NC_007732); *Pi-BatCoV-HKU5*, Pipistrellus bat coronavirus HKU5 (NC_009020); *PorCoV HKU15*, porcine coronavirus HKU15 (NC_016990); *PRCV*, porcine respiratory coronavirus (DQ811787); *RbCoV HKU14*, rabbit coronavirus HKU14 (NC_017083); *RCoV parker*, rat coronavirus Parker (NC_012936); *Rh-BatCoV HKU2*, Rhinolophus bat coronavirus HKU2 (EF203064); *Ro-BatCoV-HKU9*, Rousettus bat coronavirusHKU9 (NC_009021); *Ro-BatCoV HKU10*, Rousettus bat coronavirus HKU10 (JQ989270); *SARS-CoV*, SARS coronavirus (NC_004718); *SARSr-CiCoV*, SARS-related palm civet coronavirus (AY304488); *SARSr-Rh-BatCoV HKU3*, SARS-related Rhinolophus bat coronavirus HKU3 (DQ022305); *Sc-BatCoV 512*, Scotophilus bat coronavirus 512 (NC_009657); *SpCoV HKU17*, sparrow coronavirus HKU17 (NC_016992); *TCoV*, turkey coronavirus (NC_010800); *TGEV*, transmissible gastroenteritis virus (DQ443743.1); *ThCoV HKU12*, thrush coronavirus HKU12 (FJ376621); *Ty-BatCoV-HKU4*, Tylonycteris bat coronavirus HKU4 (NC_009019); *WECoV HKU16*, white-eye coronavirus HKU16 (NC_016991); *WiCoV HKU20*, wigeon coronavirus HKU20 (NC_016995). *From Chan, J.F., Lau, S.K., To, K.K., Cheng, V.C., Woo, P.C., Yuen, K-W., 2015. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. Clin. Microbiol. Rev. 28, 465–522, with permission.*

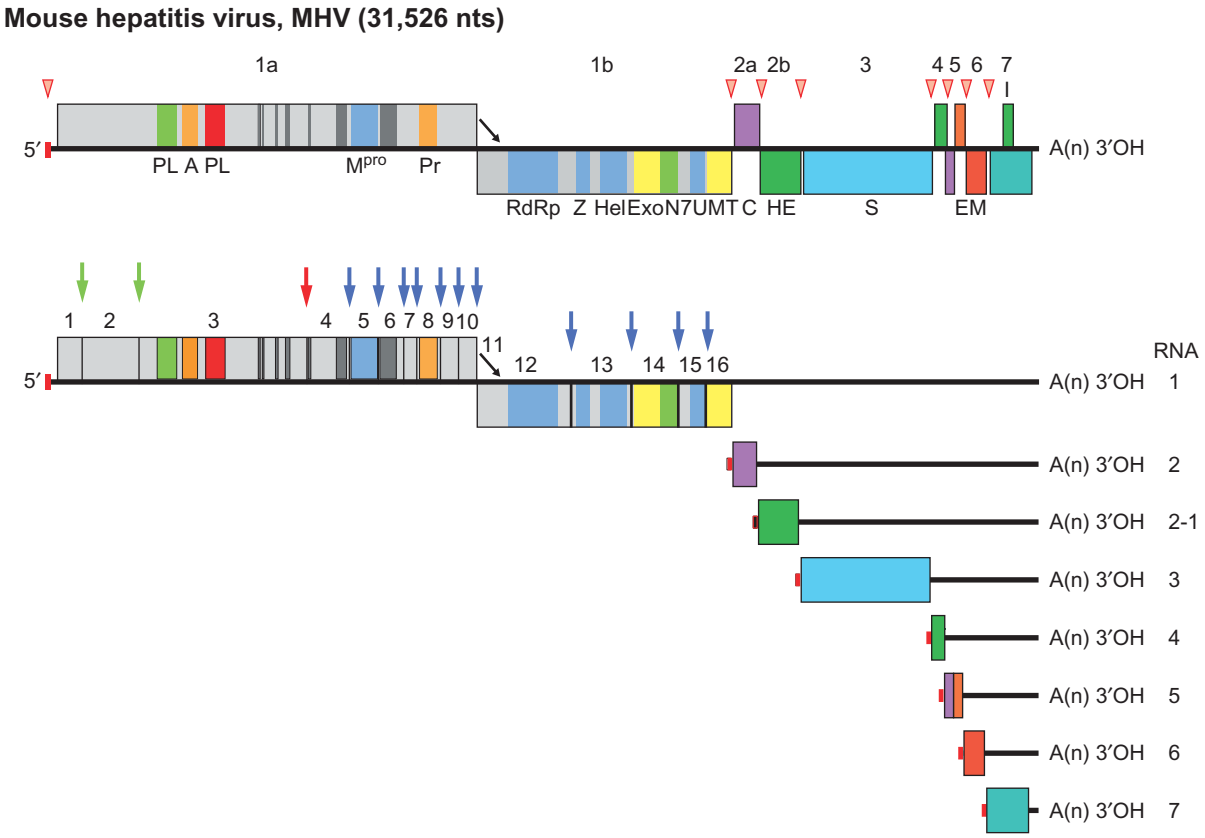

FIGURE 24.3 Coronavirus genome organization and expression. (Upper panel) Schematic representation of the genome of mouse hepatitis virus (MHV) shown as an example. Open reading frames (ORFs) are represented by boxes, indicated by number (above) and encoded protein (acronyms below). Regions encoding key domains in replicase polyproteins pp1a and pp1ab are color-coded with hydrophobic segments shown in dark grey. The 5′ leader sequence is depicted by a small red box. The arrow between ORF 1a and 1b represents the ribosomal frameshifting site. The poly (A) tail is indicated by "A(n)." Red arrowheads indicate the locations of transcription-regulating sequences (TSRs). PL (green) papain-like proteinase 1 (PL1pro); *PL (red)*, papain-like proteinase 2 (PL2pro); *A*, ADP-reibose-1" phosphatase (macrodomain); *Mpro*, 3C-like main protease; *Pr*, noncanonical RNA-dependent RNA polymerase, putative primase; *RdRp*, RNA-dependent RNA polymerase; *Z*, zinc-binding domain; *Hel*, helicase domain; *Exo*, 3′−5′ exoribonuclease domain; *N7*, guanine-N7-methyltransferase; *U*, nidoviral uridylate-specific endoribonuclease (NendoU); *MT*, ribose-2′-*O*-methyltransferase domain; *HE*, hemagglutinin-esterase; *S*, spike protein; *E*, envelope protein; *M*, membrane protein; *N*, nucleocapsid protein; *I*, internal ORF. (Lower panel) Processing of the replicase polyproteins and structural relationship between the genomic RNA and subgenomic mRNAs of coronaviruses. Arrows indicate cleavage sites for PL1pro (green), PL2pro (red) and Mpro (blue). The locations of the nonstructural proteins (nsp's) are indicated by their number. mRNA species are numbered as by convention on the basis of their size, from large to small, with the genome designated as RNA1. For the sg mRNAs only ORFs that are translated are shown. *From King, A.M., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses, p. 808. Copyright © Elsevier (2012), with permission.*

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 coronavirus open reading frames 3b and 6, for example, are antagonists of innate immune responses, specifically interfering with the development of type I interferon responses (see Chapter 4: Antiviral Immunity and Virus Vaccines); 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 the betacoronaviruses, 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/tropism 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 (APN or CD13) serves as a receptor for several alphacoronaviruses, including feline coronavirus, canine coronavirus, transmissible gastroenteritis virus, porcine epidemic diarrhea virus, and human coronavirus 229E. SARS coronavirus and human coronavirus NL63 utilize angiotensin converting enzyme 2 (ACE2). MERS coronavirus utilizes dipeptidyl-peptidase 4 (DPP4 or CD26). Some strains of mouse hepatitis virus utilizes carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1). Other betacoronaviruses utilize sialic acids as a primary receptor (eg, *N*-acetyl-9-*O*-acetyl neuraminic acid). In some cases, eg, transmissible gastroenteritis virus, the spike protein can bind to both specific and nonspecific receptors (eg, APN and sialic acids) via distinct subdomains. The functional receptor for gammacoronaviruses such as infectious bronchitis virus is undefined, although sialic acid residues may serve as nonspecific attachment factors. Many coronavirus spike proteins also interact with C-type lectins [such as liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN or CD 209L) and dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN or CD 209)], which may serve as nonspecific attachment factors in a complex with a primary receptor. In addition to receptor binding, the activation of virus fusion via the action of host cell-specific proteases that cleave spike is likely to be a powerful means of regulating coronavirus infection and host- or tissue-tropism.

Virus replication and transcription, as for many RNA viruses, takes place within an extensive membranous network of virus-modified endoplasmic reticulum-derived vesicles. The strategy of viral replication and transcription of the coronavirus genome is complex (Figs. 24.3 and 24.4; see also chapter: *Arteriviridae* and *Roniviridae*, Fig. 25.4 which depicts the replication of another member of the Order *Nidovirales*). First, the viral RNA serves as messenger RNA (mRNA) for synthesis of the RNA dependent RNA polymerase (RdRp). The two large 5′-most open reading frames, ORF1a and ORF1b (some 20 kb in total size) encoding the subunits of the polymerase are translated—the larger via ribosomal frameshifting—as a single polyprotein (pp1a or pp1ab) that is then cleaved by virus-encoded proteases found within the polyprotein, resulting in the production of mature products that are termed nsp1 to nsp16 (nsp, nonstructural protein). These proteins then assemble

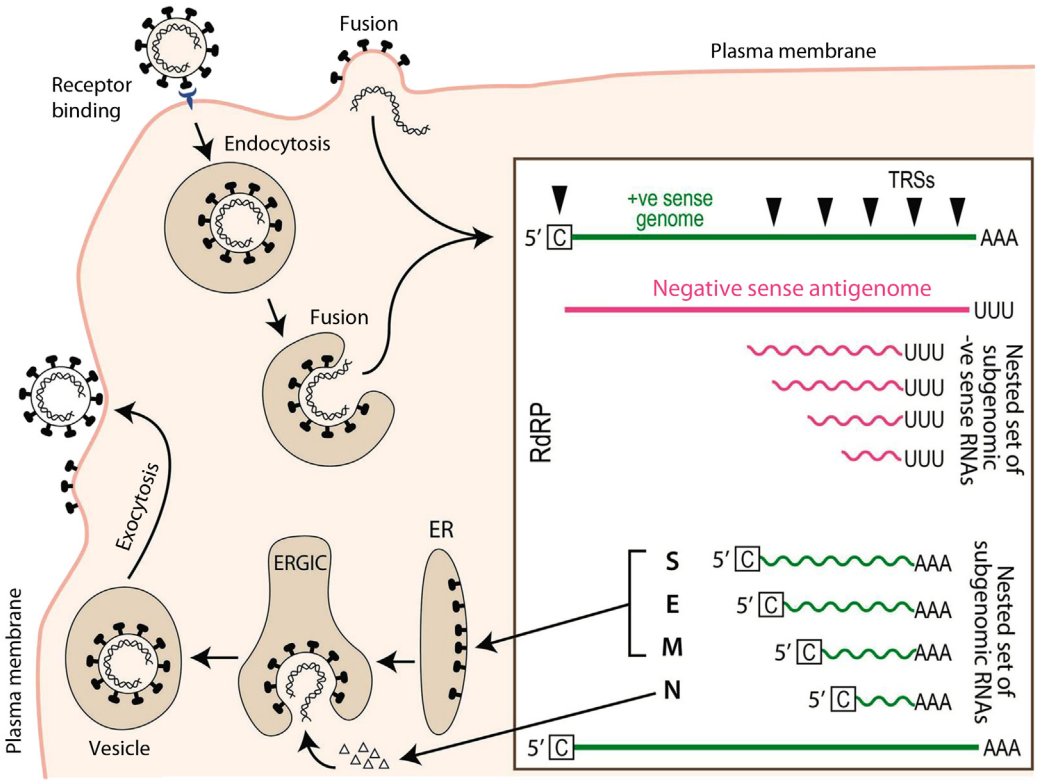

FIGURE 24.4 Coronavirus replication. ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment; RdRP, RNA-dependent RNA polymerase complex; TRS, transcriptional regulatory sequence. *Courtesy of G. Whittaker and R. Collins, Cornell University.*

within the network of rearranged membranes to form the active replicase–transcriptase complex, comprising the RNA polymerase (nsp12) and accessory proteins, including a 3′–5′ exonuclease that imparts some degree of "proof reading" function during replication, an unusual feature for RNA viruses. Such proof reading activity is thought to be important in maintaining the integrity of such large RNA genomes and in avoiding accumulation of excessive numbers of mutations associated with RNA polymerase infidelity.

The viral polymerase is used to synthesize full-length negative-sense (complementary) RNA by copying the genome starting at the 3′ end. The antigenome is then copied back into full-length positive-sense genomic RNA. The generation of full-length genomic RNA is done utilizing the replicase activity of the viral RNA dependent RNA polymerase. In addition, the RNA dependent RNA polymerase can also synthesize a nested set of RNAs with different sizes that are generated by a discontinuous synthesis of negative-sense RNAs. This is done using the transcriptase activity of the RNA dependent RNA polymerase. In this case, the RNA dependent RNA polymerasesynthesizes negative-sense RNA by starting to copy at the 3′ end of the genome, it then recognizes internal regulatory sequences, the transcriptional regulatory sequences (TRSs) found upstream of each open reading frame, where it pauses and translocates to the 5′ end of the genome, guided by sequence complementarity. The RNA dependent RNA polymerase then extends the nascent negative-sense RNA by copying the leader sequence found at the genome's 5′ end. These negative-sense template RNAs, sharing both 5′ and 3′ ends, are copied into positive-sense subgenomic mRNAs which then allow expression of viral genes downstream of the replicase. The template switching employed during transcription is at the heart of the RNA recombination that is a hallmark of coronavirus replication.

In addition to the accumulation of point mutations as a result of polymerase errors (infidelity) during transcription (genetic drift), genetic recombination occurs at high frequency between the genomes of different but related coronaviruses during coinfection situations. Recombination between coronaviruses is a direct result of the discontinuous transcription strategy employed by the viral polymerase, and the presence of transcriptional regulatory sequences in the viral genome. Such recombination is likely to 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, such as host range, tissue tropism, and virulence.

Among members of the subfamily *Torovirinae*, transcription and replication apparently are similar to those of coronaviruses, except that there are no common 5′ leader sequences on the mRNAs of viruses in the genus *Torovirus*. As occurs during replication of coronaviruses, 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. Viruses in the genus *Bafinivirus* use the same transcriptional strategy as the coronaviruses and produce their replicase polyproteins from the virus genome and the three structural proteins from a nested set of 3′-coterminal subgenomic mRNAs, each having a common 5′ leader sequence identical to that of the virus genome.

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 the cisternae of the endoplasmic reticulum and other pre-Golgi membranes. As a result, virions bud into the lumen of the endoplasmic reticulum–Golgi and not from the plasma membrane. Assembled virions are transported in Golgi-derived vesicles to the plasma membrane, where they are released by exocytosis (Fig. 24.5). After their release, many of the mature enveloped virions remain adherent to the outside of the cell. The spike

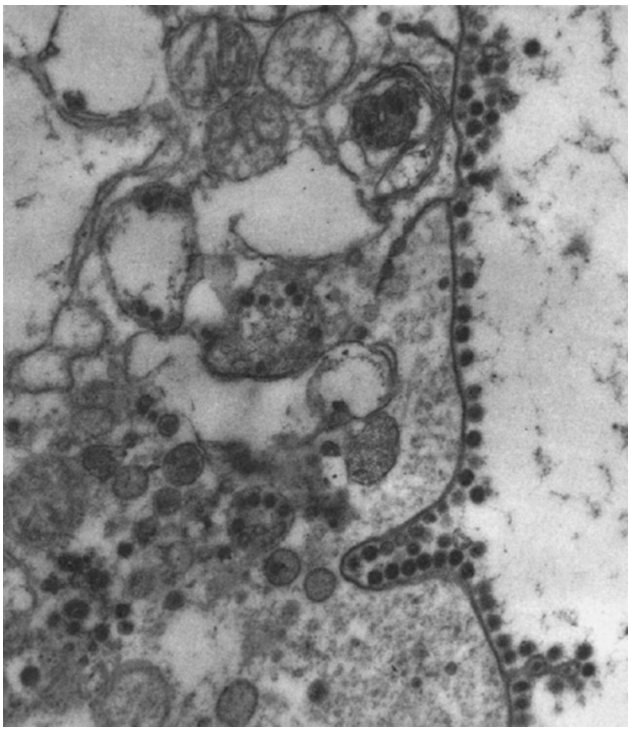

FIGURE 24.5 Mouse hepatitis virus infection in the duodenum of a 1-week-old mouse. Virions are transported to the plasma membrane from their site of formation in the endoplasmic reticulum in vesicles and are released by exocytosis. After their release, many virions remain adherent to the outside of the cell. Thin-section electron microscopy. Magnification: 30,000×.

proteins are coassociated with M at the endoplasmic reticulum−Golgi interface, but are also expressed on the cell surface where they can trigger extensive cell−cell fusion, resulting in syncytia formation.

MEMBERS OF THE SUBFAMILY *CORONAVIRINAE*

The subdivision of viruses included in the subfamily *Coronavirinae* into genera (*Alpha-, Beta-, Delta-*, and *Gammacoronaviruses*) is based largely on comparative genome sequence analyses, rather than the biological properties of individual viruses. Thus, these viruses will be grouped according to the animal species they infect, rather than their taxonomic assignment; specifically, coronaviruses of birds (infectious bronchitis, turkey coronavirus), cats, dogs and ferrets (feline enteric coronavirus, feline infectious peritonitis virus, canine coronavirus, canine respiratory coronavirus, ferret coronavirus), cattle and horses (bovine coronavirus, equine coronavirus), laboratory animals (mouse hepatitis virus, rat coronavirus (rat sialodacroadenitis coronavirus), guinea pig and rabbit coronaviruses), swine (transmissible gastroenteritis, porcine epidemic diarrhea, porcine respiratory corona virus, porcine hemagglutinating encephalomyelitis virus, porcine deltacoronavirus), and the zoonotic coronavirus infections (SARS and MERS coronaviruses).

CORONAVIRUSES OF BIRDS

INFECTIOUS BRONCHITIS VIRUS

Infectious bronchitis was the term coined in 1931 to describe the principal clinical-pathological feature of a transmissible respiratory disease of chickens 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 genus *Gammacoronavirus*; there are many genotypes and serotypes as a consequence of mutations in its large genome.

Clinical Features and Epidemiology

The clinical presentation of infectious bronchitis depends on the 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, poor ventilation 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 virus serotypes, with the first variant strains emerging in the 1940s and new variants continue to emerge today. 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, with additional boosts by live-attenuated and/or inactivated vaccines. The current trend in “free-range” and backyard poultry production is likely to lead to a resurgence of infectious bronchitis.

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, but this is dependent on the properties of individual virus strains. The intestinal tract is another site of primary infection, but damage usually is minimal.

Infectivity 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 pathologic finding is mucosal thickening within the upper and lower respiratory tract, 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 occur in some cases. 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 occur 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.

Diagnosis

Tracheal swabs or fresh samples of trachea are most useful for virus detection or isolation. 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 chicken eggs are inoculated via the allantoic sac route. Infectious bronchitis virus does not typically infect cells in culture, although primary chick kidney cells can propagate the virus. 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 serologic methods and nucleic acid analyses such as 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 bronchitis 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.

Live-attenuated virus vaccines are widely used to protect meat chickens. These vaccine viruses are derived by serial passage in embryonated chicken 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, live-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. Avian coronaviruses related to infectious bronchitis virus have also been identified in many wild bird species, but these are typically found in the gastrointestinal tract.

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 pigment 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, as a gammacoronavirus. 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, and turkey coronavirus likely represents a recombinant coronavirus containing a spike gene of unknown origin. Whether the origin of turkey coronavirus reflects altered enteric tropism or adaptation of an infectious bronchitis-like virus to the turkey, or whether infectious bronchitis virus is in itself a variant of an ancestral enteric avian coronavirus, is also unclear. Recently, bovine coronavirus was shown experimentally to infect turkey poults, but natural cases have not been described.

Turkey coronavirus can also 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 OF BIRDS AND BATS

Warm-blooded flying vertebrates likely serve as the definitive hosts that harbor the coronavirus gene pool, with alpha- and betacoronaviruses having their origin in bats, and gamma- and delta-coronaviruses having their origin in birds. A wide variety of coronaviruses have been identified in geese, guinea fowl, swans, gulls, shorebirds, vulture, sparrow-hawk, hawk, woodpecker, fruit crow, great kiskadee, ruddy turnstone, pigeons, ducks, parrots, and other species of birds. Similarly, genetically divergent species of coronaviruses have been identified in a wide variety of species of bats, implying that they, like birds, may be the source of future epidemics of human and/or animal disease.

CORONAVIRUSES OF CATS, DOGS, AND FERRETS

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 all feline enteric coronaviruses are classified as alphacoronaviruses (Table 24.1), two distinct serotypes of the virus have been identified, both being able to cause feline infectious peritonitis. The majority of circulating feline coronaviruses are designated as serotype I. The serotype II feline enteric coronaviruses appear to be relatively rare, and represent recombinants that include portions of the genome of canine coronavirus, presumably arising from coinfection situations of feline and canine coronaviruses. Serotype II feline infectious peritonitis viruses grow well in cell culture and utilize amino-peptidase-N (APN) as a receptor. In contrast, serotype I viruses are very difficult to culture, and appear to use a distinct and currently unidentified receptor. However, both virus types can cause the two clinical 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 and lethal disease of domestic and wild members of the family *Felidae*. Disease typically occurs in young or very old cats, or in the context of immune suppression. 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 noneffusive 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 shedding of feline enteric coronavirus. 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 coexist in the kitten, but the infection is modulated by an efficient cellular immune response that keeps virus replication in 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 variants 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 strains infecting fewer macrophages and producing lower virus titers than virulent strains. Avirulent strains 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 ultimately to cause feline infectious peritonitis. The most consistently occurring mutations appear to be within the cleavage-activation and fusion domains of spike, and within the 3C accessory gene. 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, 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, and 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 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 (Fig. 24.6). 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

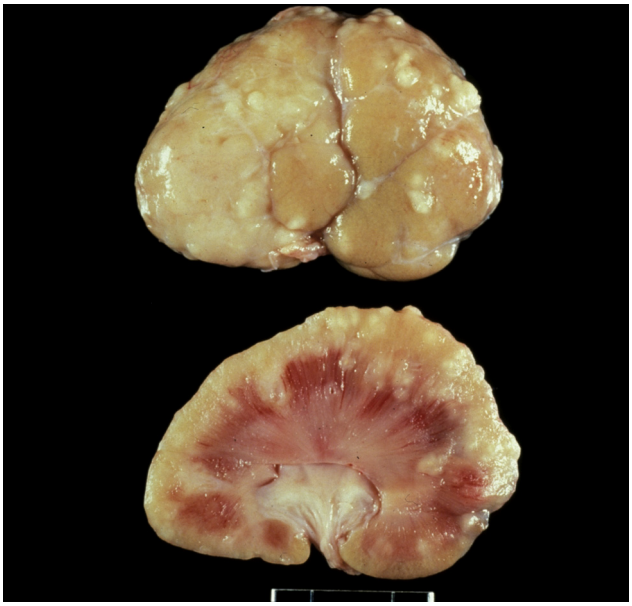

FIGURE 24.6 Feline infectious peritonitis. Granulomas (white nodules) disseminated throughout the kidney of an affected cat. *Courtesy of N.J. Maclachlan, University of California.*

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 and both forms may be present in a single cat.

Diagnosis

Serology utilizing either indirect immunofluorescence or ELISA assays 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. Diagnostic RT-PCR tests are available that can be used in feces or tissue/exudate samples, and can confirm the presence of feline coronavirus. Recent advances in understanding the mutations in the virus genome that correlate with macrophage infection may allow specific identification of feline infectious peritonitis virus. RT-PCR analysis of blood samples remains challenging as virus levels are often low, and viral variants may be present in blood without progression to feline infectious peritonitis. Immunohistochemistry is typically used to obtain definitive confirmation of coronavirus infection of macrophages within the lesions in tissues and biopsy samples of 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, based on a serotype II 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. In addition, experimental challenge of vaccinated cats has resulted in "early death" due to feline infectious peritonitis in some cases.

A broad spectrum coronavirus protease inhibitor drug has recently shown considerable therapeutic efficacy for treatment of cats with feline infectious peritonitis, a finding that suggests the disease might in the future be treated with antiviral drugs.

CANINE CORONAVIRUS

A canine coronavirus that usually produces only a mild gastroenteritis in infected dogs was originally identified in 1971. More recently, strains of this enteric canine coronavirus have been identified with different properties, including pantropic strains of the enteric 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. As with feline coronaviruses, there are two distinct serotypes of the enteric canine coronavirus (I and II), with equivalent biological properties: serotype I canine coronaviruses grow poorly in culture and have an ill-defined receptor, and serotype II canine coronaviruses grow readily in culture and use the APN receptor. Within the serotype II viruses, variant canine coronaviruses have been identified where the N-terminal domain of the spike protein is highly homologous to either transmissible gastroenteritis virus of swine or to serotype I feline/canine coronaviruses. These variant viruses would be expected to have major antigenic differences as compared to prototype serotype II canine coronaviruses.

Enteric canine coronavirus infection is common in dogs worldwide, and putative instances of coronavirus

enteritis have also been recorded in wild dogs. Similar or identical alphacoronaviruses have been identified in foxes, raccoon dogs (*Nyctereutes procyonoides*), and cats. The intestinal disease caused by canine coronavirus is similar to that caused by enteric coronaviruses in other species (see porcine transmissible gastroenteritis virus), with destruction of mature enterocytes lining the intestinal villi causing maldigestion, malabsorption, and subsequent diarrhea. Historically, severe cases of coronavirus infection have been associated with coinfection with canine parvovirus, but deaths due to canine coronavirus have increased recently in the absence of known coinfection, especially in high-density housing situations. 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, although these tests may not distinguish the different forms of canine coronavirus. 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 also been described as the putative cause of severe systemic disease in dogs that is characterized by pyrexia, anorexia, depression, vomiting, diarrhea, leukopenia, and neurologic signs of ataxia and seizures. Despite these reported systemic clinical signs, there is limited evidence for viremia in coronavirus-infected dogs. Furthermore, there are no indications that canine coronavirus can become tropic to macrophages and spread systemically, as in cats, despite many similarities between the canine and feline coronaviruses.

CANINE RESPIRATORY CORONAVIRUS

In 2003, a novel coronavirus was associated with canine infectious respiratory disease, so-called "kennel cough." The virus is genetically distinct from the enteric canine coronavirus; enteric canine coronavirus is classified as an alphacoronavirus whereas canine respiratory coronavirus is a betacoronavirus that is genetically similar to bovine coronavirus and the human "common cold" coronavirus OC43. Unlike the enteric canine coronavirus, canine respiratory coronavirus possesses a hemagglutinin-esterase (HE) gene.

The occurrence of canine infectious respiratory disease among dogs that enter kennels has been strongly associated with their subsequent seroconversion to canine respiratory coronavirus; however, respiratory disease in dogs is clearly multifactorial and the potential consequence of infection with a variety of infectious agents. Canine respiratory coronavirus is apparently spread rapidly by aerosol amongst susceptible dogs in kennels, sometimes leading to moderate or even severe disease characterized by respiratory distress and pneumonia, inappetence and even death. Disease is more common during the autumn/fall and winter months. Experimentally infected dogs also develop respiratory disease, including nasal discharge, sneezing and coughing. Virus is readily detected by RT-PCR in the oropharynx, tonsils, and respiratory tract of acutely affected dogs, and rarely in the gastrointestinal tract and feces. Virus-mediated injury to the ciliated respiratory epithelium is likely responsible for respiratory disease, and predisposes to bacterial infection of the lungs.

Diagnosis of canine respiratory coronavirus infection is accomplished using either RT-PCR or virus isolation procedures, although the latter is technically challenging and only done in specialized laboratories. Serologic detection of prior canine respiratory coronavirus infection in dogs can be accomplished by ELISA. Currently formulated canine vaccines do not include canine respiratory coronavirus, and those to canine enteric coronavirus are not cross-protective. Treatment of affected dogs is not specific and is currently reliant on supportive care and antimicrobial therapy to prevent bacterial infection. Infections also can be controlled in high-density environments by quarantine and by reducing overcrowding.

FERRET CORONAVIRUS

Ferrets are commonly infected with an enteric alphacoronavirus that is similar to the viruses that occur in mink, but distinct from the related viruses of pigs, cats, and dogs. In addition to widespread, but generally benign gastrointestinal infection, ferret coronaviruses can cause the more serious epizootic catarrhal enteritis, or "green slime" disease, as well as a systemic disease with many similarities to feline infectious peritonitis. In this case, characteristic effusion can occur in ferrets, but most reported cases appear to be of the "dry" form of the disease. While specific viruses termed ferret systemic coronavirus have been reported, their relationship to ferret enteric coronaviruses remains unclear.

CORONAVIRUSES OF CATTLE AND HORSES

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, betacoronaviruses such as bovine and SARS coronaviruses (Table 24.1) can infect other animal species, including wildlife. Bovine coronavirus is closely related to the human coronavirus OC43 that causes the common cold; indeed, OC43 has been proposed to represent prior zoonotic transmission of bovine coronavirus. Bovine coronavirus has also been shown 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 (*Cerous unicolor*), waterbuck (*Kobus ellipsiprymnus*), giraffe (*Giraffa camelopardalis*), and white-tailed deer (*Odocoileus virgineanus*). 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. The host cell receptor for bovine coronavirus is sialic acid, which reflects the wide tropism of this virus and explains the presence of a HE gene in the virus.

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. Coinfections with other enteric pathogens such as rotavirus, torovirus, cryptosporidia, and enterotoxigenic or enteropathogenic *E. 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 enteric 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 coronaviruses 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 also 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 comingled, 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 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 maldigestion and malabsorption, 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. Cell culture isolation became a viable option when it was discovered that the virus could be grown when trypsin was added to the medium—virus replication is recognized by hemadsorption or cytopathogenic effects, and the presence of coronavirus is 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. The use of RT-PCR for detection of bovine coronavirus has significantly increased the detection of this agent, particularly in respiratory samples, and has also substantially increased the recognized period of virus shedding by infected animals. Postmortem diagnosis is performed on acute fresh or fixed respiratory or intestinal tissues using hyperimmune antisera or monoclonal antibodies for immunofluorescence 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 IgG_1 antibodies in colostrum and milk. In ruminants, IgG_1 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 adjuvanted inactivated bovine coronavirus vaccines effectively boosts IgG_1 antibody titers 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. The serum antibody titer to bovine coronavirus may be a marker for respiratory protection, as coronavirus-specific antibody titers and isotype (IgG_1, IgG_2, IgA) were correlated with protection of calves and feedlot cattle against subsequent occurrence of respiratory disease, pneumonia, or coronavirus shedding. However, it can be difficult to distinguish whether serum antibodies are correlates of protection, or whether they merely reflect prior enteric or respiratory coronavirus infection.

Intranasal vaccination using live-attenuated enteric coronavirus vaccine has been proposed to reduce the risk of bovine respiratory disease complex (so-called "shipping fever") in cattle entering feedlots.

EQUINE CORONAVIRUS

Equine coronavirus infections have been historically associated with sporadic, relatively mild cases of diarrhea in horses, with severe disease being rare and occurring typically in foals. The virus was first discovered associated with outbreaks of enteric diseases in foals in the United States in 2000, and later among adult horses with

enteric disease in Japan in 2011. More recently, this virus has been associated with a self-limiting enteric disease syndrome among horses in boarding and breeding facilities and racetracks in North America, Europe, and Japan. Affected horses exhibit anorexia, lethargy, and fever. The causative virus is classified along with bovine coronavirus as a group A betacoronavirus. Thus, these two viruses likely share common features in their epidemiology and pathogenesis. Equine coronavirus can be detected by RT-PCR amplification of the N-gene, however, the virus is commonly present in the gastrointestinal tract of horses, including apparently normal horses.

CORONAVIRUSES OF LABORATORY ANIMALS

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. The enteric coronaviruses are at one end of the spectrum, as these viruses 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 involves the 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 tissues, liver, and the 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, lethal intestinal virus of infant mice was considered to be distinct from mouse hepatitis virus.

Mouse hepatitis virus in the most widely investigated coronavirus and there are numerous laboratory strains of 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 coronaviral 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. The 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 runting 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-gamma-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 reinfecting 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 a 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 the virus receptor, CEACAM-1. 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 hematopoiesis. Central nervous system disease can arise directly through olfactory neural pathways (nasoencephalitis) 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-gamma may develop chronic polyserositis, which features prominent syncytia 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. The different strains of the virus are all highly cross-reactive serologically, so antigen is typically prepared from polytropic strains of virus propagated in cell culture. Acute (active) infections can be diagnosed at necropsy, and virus detected by RT-PCR or isolation in cell culture

(especially for polytropic strains of the virus). There is little practical utility to virus strain identification by sequence analysis.

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. 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 "rederived" 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 reestablished 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).

RAT CORONAVIRUS (RAT SIALODACRYOADENITIS CORONAVIRUS)

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 can occur in suckling rats, complicated by failure to nurse as a result of destruction of olfactory epithelium. Clinical features in older rats include nasal and ocular discharge, cervical swelling, photophobia, keratitis, and dyspnea. Lacrimal secretions surrounding the eyes are tinted with porphyrin pigment derived from the affected retro-orbital Harderian glands. Lesions consist of necrotizing rhinitis, necrosis of salivary 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 infections. Immunodeficient rats are uncommon, but chronic wasting syndrome may occur in athymic and severe combined immunodeficient rats, which succumb to progressive pneumonia.

Although rats are immune to reinfection with the homologous strain, they can be reinfected 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.

GUINEA PIG AND RABBIT CORONAVIRUSES

In juvenile European (*Orcytolagus*) 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 subclinically, 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 experimentally maintained by intratesticular inoculation of laboratory rabbits. 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.

CORONAVIRUSES OF SWINE

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 nonimmune 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 maldigestion 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 (Fig. 24.7). The destruction of enterocytes lining the villi leads to maldigestion because of the loss of critical digestive enzymes such as lactase and other disaccharidases, normally present in the microvillus brush border of villus enterocytes, that are responsible for digestion of milk. Thus destruction of villus enterocytes results in both malabsorption and maldigestion. 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 pertubations 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 (Fig. 24.8).

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 procedures—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

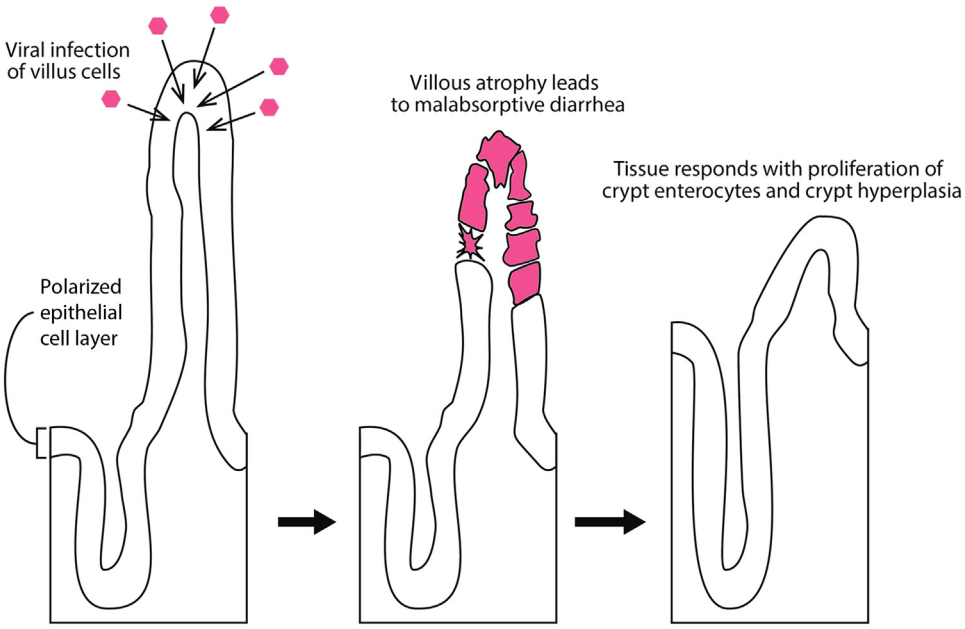

FIGURE 24.7 Pathogenesis of transmissible gastroenteritis. Schematic diagram showing viral infection and destruction of enterocytes lining small intestinal villi, leading to malabsorption diarrhea. *Courtesy of L. Saif, The Ohio State University, adapted by R. Collins, Cornell University.*

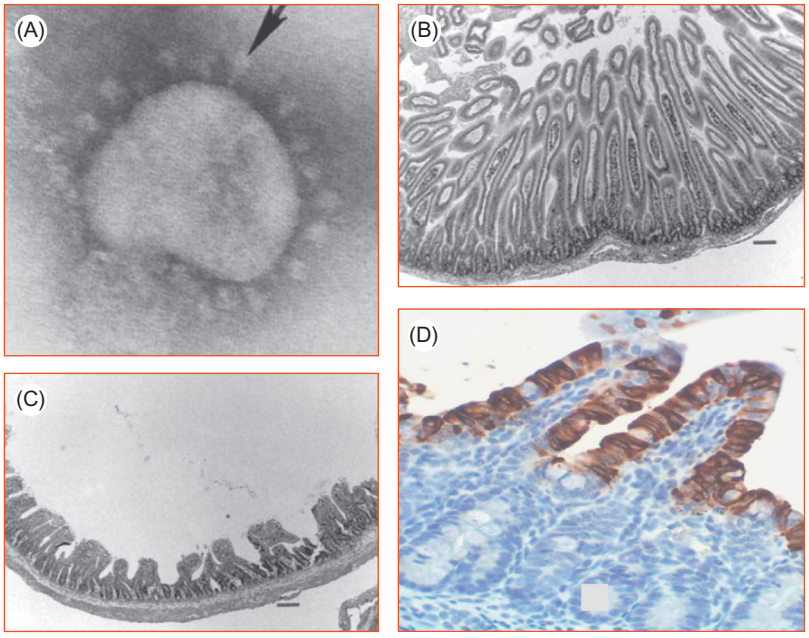

FIGURE 24.8 Pathogenesis of transmissible gastroenteritis. (A) Electron micrograph of causative virus, with prominent envelop spikes (arrow). Histologic appearance of small intestine of (B) normal piglet and (C) piglet with transmissible gastroenteritis. (D) Immunohistochemical staining showing selective viral infection of enterocytes lining the intestinal villi. *Courtesy of L. Saif, The Ohio State University.*

antisera, usually using an ELISA. Serology using paired serum samples and either serum neutralization or ELISA 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 EPIDEMIC DIARRHEA VIRUS

Porcine epidemic diarrhea is a disease of piglets that was first described in the 1970s in Europe, and subsequently spread throughout Asia, where it remains a significant problem. The virus was introduced into the United States in 2013 as a point-source infection where it proved to be highly transmissible and spread rapidly across the country, causing high mortality in piglets. Porcine epidemic diarrhea virus has now been reported widely across North America, Europe and Asia. The disease is clinically similar to transmissible gastroenteritis and the two infections likely share a similar or identical pathogenesis, but porcine epidemic diarrhea is caused by a distinct alphacoronavirus with distinct serological properties. While porcine epidemic diarrhea and transmissible gastroenteritis viruses may share a common receptor (APN), they have distinct growth properties in cell culture. The closest known relatives of porcine epidemic diarrhea virus are found in bats and humans (HCoV-NL63).

The main clinical sign in young pigs is watery diarrhea, sometimes preceded by vomiting. Mortality can be very high (up to 100%) in piglets. The virus also can cause diarrhea in growing and fattening pigs. Infection of adult swine is frequently subclinical, although diarrhea occurs sometimes. A diagnosis may be confirmed by the isolation of virus in primary porcine cell culture or more typically Vero (African green monkey kidney) cells with added trypsin. Immunofluorescence or ELISA tests for porcine epidemic diarrhea virus antigens can be carried out in intestine or feces, respectively, and diagnosis may also be by RT-PCR assay to detect viral RNA, or by the demonstration of virus-specific antibodies in convalescent swine. Inactivated and live-attenuated vaccines are available in some countries for vaccination of pregnant sows to provide passive antibodies to nursing piglets.

PORCINE RESPIRATORY CORONAVIRUS

The respiratory variant of transmissible gastroenteritis virus, porcine respiratory coronavirus, was discovered in 1986 when seroconversion was detected in swineherds in countries (eg, Denmark) known to be free of transmissible gastroenteritis; the virus causing this disease pattern is a spike protein (*S 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. Coinfection 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 swineherds 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 subclinically infected 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 nonimmune sows. The disease is characterized by repeated vomiting after feeding, depression, progressive emaciation, and death. In contrast to transmissible gastroenteritis, diarrhea is not common in vomiting and wasting disease. Infection also can lead to neurologic signs similar to those of porcine polioencephalomyelitis (caused by a picornavirus); specifically, affected piglets may show a dog-sitting posture, paddling movements, opisthotonos, paralysis or convulsions.

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 DELTACORONAVIRUS

Novel coronaviruses, which have been classified as deltacoronaviruses, have recently been identified from cases of enteric disease of pigs in the United States. These viruses were closely related to deltacoronaviruses identified previously in pigs in China. The clinical signs were similar to those associated with porcine epidemic diarrhea virus infection, including watery diarrhea in sows and death in piglets. However, the death rate in piglets was lower than that typically observed with porcine epidemic diarrhea virus infection. Little information is available on porcine deltacoronavirus beyond its genotypic classification.

ZOONOTIC CORONAVIRUSES

SARS CORONAVIRUS

In 2002, a new coronavirus emerged in China, associated with a 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 so-called *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 type I pneumocytes and macrophages. The epidemic was contained by strict quarantine and sanitation strategies, 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 (*Betacoronavirus* group B). 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 overrepresented 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 (*Rhinolophus sinicus*) with a remarkable genetic spectrum of SARS-like coronaviruses has now been established.

Changes in three genes were identified during the adaption of SARS coronavirus to humans, including the *S* gene, as related to adaptation to the human cell receptor (ACE2) and in the accessory proteins encoded by open reading frames 3a and 8, which are of uncertain biologic significance. In 2004, SARS reemerged in China and, as determined from sequence analyses, the reemerged 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 potential "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, 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.

MERS CORONAVIRUS

In May 2012, a new and fatal respiratory disease was recognized in a patient who died in Saudi Arabia and, soon thereafter, another patient in the United Kingdom who had recently traveled from the Middle East. A novel coronavirus was isolated from both patients. A similar virus emerged and spread in South Korea in 2015, with infections principally being associated with hospitals and healthcare workers. The virus is classified as a betacoronavirus in lineage C (Table 24.1). Notably, the MERS coronavirus is distinct from SARS coronavirus in several aspects: it uses a distinct receptor (DPP4) and has been classed as a "generalist" coronavirus, in that the virus is able to infect a broad range of cells in culture. Such a polytropic coronavirus is highly unusual and particularly alarming from an epidemiological standpoint as it represents an ideal candidate for zoonotic transfer from an animal reservoir. The MERS coronavirus appears to undergo only limited human−human transmission; it is most often transmitted in health care facilities, with serious disease typically occurring in patients already having significant underlying health conditions.

Viruses essentially identical to MERS coronavirus have now been found widely in camels, and the closest related viruses to MERS coronavirus are bat coronaviruses. A recent survey of dromedary camels from Oman showed high seroprevalence (100%) to MERS coronavirus, whereas only 15% of camels from Spain were seropositive. Other livestock (sheep, cows, goats, and other camelids) in the region were all seronegative. There is little evidence that camels infected with either MERS or MERS-like coronaviruses become clinically ill, although mild respiratory signs were present in some camels from which the viruses were isolated. However, infected camels shed large amounts of virus in their respiratory secretions, raising the question as to whether they are true virus reservoirs or intermediate hosts in the transmission of viruses to humans, possibly from an original bat reservoir. Aerosol transmission of MERS coronavirus from camels to other animals and possibly humans is suspected, along with virus transmission via unpasteurized camel's milk or in camel meat. In humans, the disease is a severe respiratory syndrome analogous to SARS, and a concern to veterinarians treating infected camels and for camel owners. Diagnosis of MERS coronavirus infection can be done using RT-PCR assay, and serological tests are available to detect prior exposure.

MEMBERS OF THE SUBFAMILY *TOROVIRINAE*

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 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 neonatal and postweaning diarrhea, but infection is apparently often subclinical. 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 39-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 nonimmune conventional calves; these animals develop watery diarrhea lasting for 4−5 days, with virus shedding for at least several days thereafter. Diarrhea is more severe in calves with a normal intestinal flora than in gnotobiotic calves. Histologic lesions include necrosis of enterocytes with subsequent villus 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.

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. Similarly, toroviruses can be detected in feces or intestinal contents of swine using RT-PCR or metagenomic analyses (next generation sequencing).

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, supportive 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.

GENUS *BAFINIVIRUS*

The first member of the genus *Bafinivirus* was isolated from a cyprinid, the white bream (*Blicca bjoerkna*), in Germany during a routine examination of healthy wild fish. Electron micrographs of virus propagated in a cyprinid cell line showed bacilliform virions 130–160 nm in length and 37–45 nm in diameter with prominent surface projections of 20–25 nm similar to the peplomers of coronaviruses. Genetic analysis of the white bream virus showed it to be most closely related to viruses in the genus *Torovirus*, but with sufficiently distinct features to justify establishment of a new genus, *Bafinivirus*, with white bream virus as the type species. A second bafinivirus was isolated from moribund juvenile fathead minnows (*Pimephales promelas*) farmed in the United States. Sick fish showed hemorrhages in the eyes and skin, and necrosis within the kidney, liver, and spleen. The virus produced a syncytial-type of cytopathic effect in cell lines and electron microscopy revealed virions with bacilliform morphology. Experimental infections produced up to 90% mortality among groups of fathead minnows, but not in several other commercially important freshwater fish species, including channel catfish, goldfish, golden shiners, and rainbow trout. Genetic analysis showed the fathead minnow nidovirus was most closely related to white bream virus with which it shared a similar gene order, genome size, and replication strategy. However, fathead minnow nidovirus has sufficient sequence divergence to be considered a second species of bafinivirus. Surveillance confirms that this virus is present in several locations in the United States and appears to be moving with the unregulated shipment of baitfish. The bafiniviruses characterized to date can be isolated by cultivation in cyprinid cell lines and identified by RT-PCR assay. No control strategies are available.

CURRENTLY UNCLASSIFIED NIDOVIRUSES

Currently unclassified nidoviruses recently have been detected in insects (mosquitoes) and animals, including cattle, turtles, and snakes. Severe respiratory disease of captive ball pythons (*Python regius*) has been described since the late 1990s. Sometimes fatal, the disease is characterized by a proliferative interstitial pneumonia often accompanied by pharyngitis, sinusitis, stomatitis, tracheitis, or bronchial epithelial hyperplasia. The apparent causative agent was not isolated in cell culture, but electron microscopic examination showed bacilliform virions in lung tissues of affected snakes. Metagenomic analyses of tissues from diseased snakes showed the presence of a novel nidovirus. Phylogenetic analyses confirm that the ball python nidovirus, while most closely related to bafiniviruses of fish and mammalian toroviruses, may be a member of a third genus in the subfamily *Torovirinae*. The routes of transmission for the virus are not known currently, but it appears to be widely present among populations of captive pythons, probably due to the frequent movement of animals in the pet trade. Infections can be detected by RT-PCR assay. No vaccines are available.