

31

Coronaviruses

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Overview

The family *Coronaviridae* within the order *Nidovirales* consists of two subfamilies: (1) *Coronavirinae* comprising the genera *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus* and (2) *Torovirinae* comprising the genera *Torovirus* and *Bafinivirus* and an unassigned genus.

Five swine coronaviruses (CoVs) have been identified: transmissible gastroenteritis virus (TGEV) first described in 1946; porcine respiratory coronavirus (PRCV), a spike (S) gene deletion mutant of TGEV isolated in 1984; porcine epidemic diarrhea virus (PEDV) isolated in 1977; porcine hemagglutinating encephalomyelitis virus (pHEV) isolated in 1962; and porcine deltacoronavirus (PDCoV) detected in 2012. In addition, a TGEV/PEDV recombinant virus (TGEV backbone but with PEDV spike gene) has been identified in swine in Europe (Akimkin et al. 2016; Belsham et al. 2016; Boniotti et al. 2016), and a bat-HKU2-like alphacoronavirus has been identified in swine in China (Gong et al. 2017; Pan et al. 2017; Zhou et al. 2018). In pigs, CoVs and toroviruses (ToVs) affect a variety of organs, including the gastrointestinal and respiratory tracts, the peripheral and central nervous systems (CNS), and the mammary glands. Most ToVs and PRCV induce mainly subclinical infections in pigs, whereas TGEV, PEDV, PDCoV, and pHEV infections can result in fatal enteric or nervous diseases.

Swine CoVs comprise three distinct genera – *Alphacoronavirus*, *Betacoronavirus*, and *Deltacoronavirus* (Figure 31.1) – and share replication strategies common to *Coronaviridae*. TGEV and PRCV belong to the *Alphacoronavirus 1* species that also contains closely related CoVs of domestic cats and dogs. PEDV and two human CoVs (229E and NL63) are separate species in the same genus *Alphacoronavirus*. The newly identified bat-HKU2-like swine enteric alphacoronavirus also belongs to the genus *Alphacoronavirus*, but its taxonomic name has not been defined. pHEV and PDCoV are genetically

distinct, and they belong to the *Betacoronavirus* and *Deltacoronavirus* genera, respectively. Together with bovine, human OC43, equine, and canine respiratory CoVs, pHEV is a member of the *Betacoronavirus 1* species. PDCoV is most closely related to other mammalian deltacoronaviruses from Asian leopard cats and Chinese ferret badgers (Ma et al. 2015). For each swine CoV, only a single serotype is recognized.

CoVs are enveloped and pleomorphic, with an overall diameter of 60–160 nm as viewed by negative staining electron microscopy (EM) (Figure 31.2). Most have a single layer of club-shaped spikes (S protein) 12–25 nm in length, but pHEV and some other betacoronaviruses have a second shorter layer of surface spikes, the hemagglutinin-esterase (HE) protein.

Genomic organization and gene expression: TGEV prototype

Swine CoVs contain one large, polyadenylated, single-stranded, genomic RNA (25–30 kb) of positive-sense polarity. The genome organization, replication strategy, and expression of viral proteins are similar to those of other human and animal CoVs (Enjuanes and Van der Zeijst 1995; Gonzalez et al. 2003; Laude et al. 1993). The complete genomes of the Purdue and Miller strains of TGEV are 28,546–28,580 nucleotide (nt) long and share 96% overall identity (Penzes et al. 2001; Zhang et al. 2007). Most CoVs have buoyant densities in sucrose of 1.18–1.20 g/mL. The phospholipids and glycolipids incorporated into the virus envelope are derived from the host cell, and thus, the envelope composition is host cell dependent (Enjuanes and Van der Zeijst 1995).

Most CoVs contain four structural proteins: a large surface glycoprotein (spike or S protein visible as the corona; Figure 31.2), a small membrane protein (E), an integral membrane glycoprotein (M), and a nucleocapsid protein (N). However, pHEV also contains an HE protein (de Groot et al. 2008).

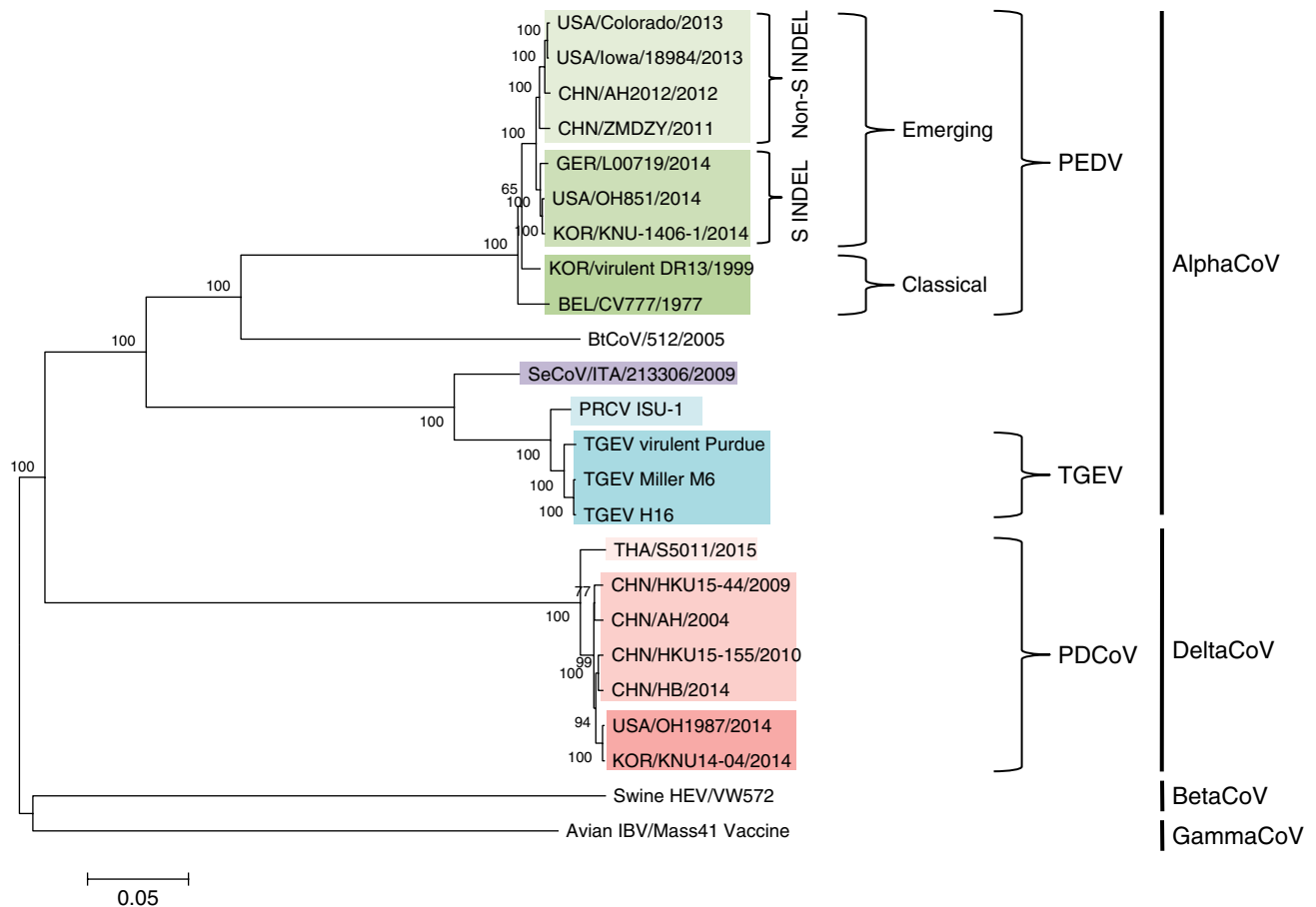


Figure 31.1 Phylogenetic tree of coronaviruses. The complete genomes of TGEV, PEDV, and PDCoV strains, closely related PRCV and a bat alphacoronavirus, a porcine HEV strain, and an avian IBV strain were selected. Multiple sequence alignments were performed using ClusterW, and a neighbor-joining phylogenetic tree supported with a bootstrap test of 1000 replicates was constructed using MEGA 6.0 software. The number on each branch indicates the bootstrap value. The scale represents the nucleotide substitutions per site. Color-shaded areas denote different swine virus clusters.

The N protein (47kDa) interacts with viral RNA to form a helical ribonucleoprotein complex. This structure, in association with M protein, forms an internal icosahedral core in TGEV. The 29–36kDa M glycoprotein is embedded in the viral envelope by 3–4 membrane-spanning regions. In TGEV, the hydrophilic N-terminus with a single accessible glycosylation site is responsible for interferon (IFN) induction (Charley and Laude 1988). Epitopes on protruding N- and C-terminal ends of the M protein of TGEV bind complement-dependent neutralizing monoclonal antibodies (MAbs) (Laude et al. 1992; Woods et al. 1988).

The TGEV S glycoprotein (220kDa) occurs as trimer complexes (Delmas and Laude 1990) and functions in virus neutralization (complement independent), virus-cell attachment, membrane fusion, and hemagglutination. The large deletion in the S gene of PRCV results in a smaller S protein (170–190kDa) (Figure 31.3). During fusion of TGEV with host cell membranes, two highly conserved heptad repeat regions (HR1 and HR2) of the S

protein undergo conformational changes important for fusion (Ma et al. 2005). Entry of TGEV into the cell is likely associated with cholesterol-rich membrane microdomains (Ren et al. 2008), since exogenous cholesterol rescued virus infectivity.

Epitope mapping of the S glycoprotein of TGEV revealed four antigenic sites (A, B, C, D) (Figure 31.3). Site A–B, the conserved immunodominant epitope, is recognized strongly by neutralizing MAbs (Correa et al. 1990; Delmas and Laude 1990; Simkins et al. 1992, 1993), although other sites (D, C) can also induce virus-neutralizing (VN) antibodies (Delmas and Laude 1990). The S protein mutations in attenuated TGEV strains or the natural TGEV deletion mutant PRCV include a serine/alanine mutation at amino acid (aa) 585 position associated with induction of VN antibodies, as well as receptor (aminopeptidase N) binding (Zhang et al. 2007).

Porcine aminopeptidase N (pAPN) has been identified as the TGEV cell receptor (Delmas et al. 1992). The receptor-binding and major neutralizing site (site A) on the S

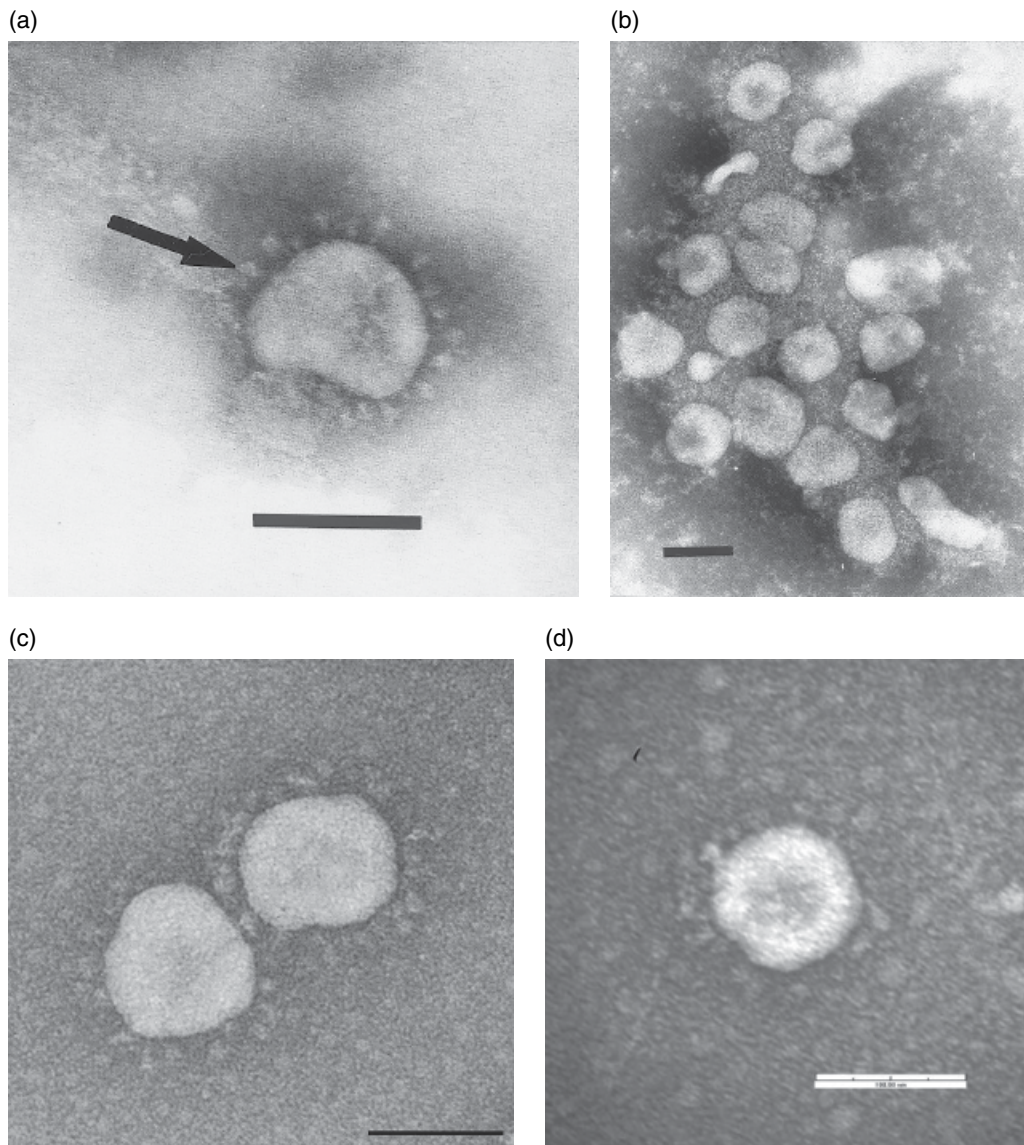


Figure 31.2 Electron micrographs. (a) A TGEV particle showing typical coronavirus morphology. Arrow points to the virus peplomers or spikes. Bar = 100 nm. (b) Typical virus–antibody aggregates observed by immunoelectron microscopy of TGEV and gnotobiotic pig anti-TGEV serum. Bar = 100 nm. (c) Two particles of emerging non-S INDEL PEDV PC22A strain, bar = 100 nm. *Source:* Oka et al. (2014). Reproduced with permission of Elsevier. (d) A PDCoV particle, bar = 100 nm. *Source:* Jung et al. (2015b).

protein of TGEV are located within the same domain (Figure 31.3) (Godet et al. 1994). TGEV binding to sialic acid residues on glycoproteins of target cells was proposed to initiate infection of intestinal enterocytes (Schwegmann-Wessels et al. 2002). Treatment of TGEV with sialidase enhanced hemagglutinating activity (Noda et al. 1987; Schultze et al. 1996). The hemagglutinating activity resides in the N-terminal region of the TGEV S protein, a region that is missing from the PRCV S protein; thus, determination of hemagglutinating activity (Schultze et al. 1996) could potentially differentiate PRCV and TGEV strains.

TGEV and PEDV as well as PDCoV also encode 1–2 accessory proteins encoded by open reading frame (ORF) 3 (TGEV and PEDV), ORF6 (PDCoV), and ORF7

(TGEV and PDCoV). The overall genome organization is 5'UTR-ORF1ab, S, ORF3, E, M, ORF6, N, ORF7-3'UTR.

Contrasts and comparisons

Seven CoVs are related antigenically or by their genomic sequences (Enjuanes and Van der Zeijst 1995): TGEV, PRCV, canine coronavirus (CCoV), feline infectious peritonitis virus (FIPV), feline enteric coronavirus (FECoV), PEDV, and human CoV 229E. CoVs within the species *Alphacoronavirus 1* (TGEV, PRCV, CCoV, FIPV, FECoV) are antigenically related, based on cross-reactivity in VN and immunofluorescence (IF) tests and with MAbs to the S, N, or M proteins, and all share the antigenic subsite

Ac on the S protein (Enjuanes and Van der Zeijst 1995). As members of the same species, these viruses likely represent host range mutants of an ancestral virus strain (de Groot et al. 2008). TGEV and CCoV could be distinguished *in vitro* by two-way cross-neutralization tests and other biological differences (Reynolds et al. 1980), i.e. both TGEV and CCoV grow in canine kidney cells and a feline cell line, whereas TGEV, but neither CCoV nor FIPV, grows in swine cells.

For the S glycoprotein that confers host range specificity, the 300 aa residues at the N-terminus are the most variable. In this domain, CCoV and FIPV are more similar to each other than to TGEV (Wesseling et al. 1994). Differentiation of the TGEV-related CoVs is possible using specific MAbs to the S glycoprotein of TGEV that recognize TGEV but not PRCV, FIPV, or CCoV strains (Callebaut et al. 1989; Laude et al. 1993; Sanchez et al. 1990; Simkins et al. 1992, 1993).

Interestingly, outbreaks of fatal acute gastroenteritis associated with TGEV-related CCoV type II (CCoV-II) were reported in European dogs (Decaro et al. 2009; Erles and Brownlie 2009). The close genetic relatedness (>96% aa identity) in the key replicase domains suggested that the recently emerged CCoV-II strains are host range variants of TGEV that infect dogs (de Groot et al. 2008). Based on the S protein, the CCoV-IIb strains (TGEV-like) (Decaro et al. 2010) may represent novel recombinant viruses of mixed (TGEV/CCoV) origin.

Several researchers reported that the severe acute respiratory syndrome (SARS) CoV cross-reacts with antibodies to *Alphacoronavirus 1* species (TGEV, PRCV, CCoV, FeCoV) through the N protein (Ksiazek et al. 2003; Sun and Meng 2004) and that this cross-reactivity mapped to the N-terminal region (Vlasova et al. 2007). This discovery led to use of SARS CoV-specific N peptide fragments in serologic assays to detect SARS CoV antibodies in animal sera (Vlasova et al. 2007). One-way

cross-reactivity with the N protein has also been reported for PEDV, FIPV, CCoV, TGEV, and a putative mink CoV (Have et al. 1992; Zhou et al. 2010). Although no cross-reactivity between PEDV and TGEV-related CoVs was initially reported (Enjuanes and Van der Zeijst, 1995), one-way cross-reactivity between TGEV Miller and several PEDV strains (classical CV777, emerging non-S INDEL and S INDEL US strains) was recently confirmed (Lin et al. 2015b).

In vivo biological differences in pathogenicity for neonatal pigs are evident among TGEV, CCoV, and FIPV strains. Whereas virulent FIPV caused diarrhea and intestinal lesions similar to those of virulent TGEV, CCoV caused no clinical signs and only slight villous atrophy. CCoV shed by acutely infected dogs, infected baby pigs, and induced serum VN antibodies to CCoV and TGEV (Woods and Wesley 1992). However, baby pigs and pregnant gilts infected with FIPV did not produce TGEV VN antibodies, but did develop some immunity to TGEV challenge.

PRCV strains have been characterized and sequenced (Britton et al. 1991; Costantini et al. 2004; Kim et al. 2000b; Rasschaert et al. 1990; Vaughn et al. 1995; Zhang et al. 2007). Two striking features characterize the PRCV genome: (1) a large deletion (621–681 nt) near the N-terminus of the S gene producing a smaller S protein (Figure 31.3) and (2) a variable region with deletions that compromise ORF3 downstream of the S gene. These genetic changes may account for the altered tissue tropism of PRCV (Ballesteros et al. 1997; Sanchez et al. 1999). An overall nucleotide and aa sequence identity of 96–98% between TGEV and PRCV suggests that PRCV evolved from TGEV (Zhang et al. 2007) and that this occurred on a number of independent occasions.

Disease outbreaks caused by swine CoVs are endemic or variable in swine-producing countries. Nevertheless, the diseases induced by these CoVs have resisted eradication

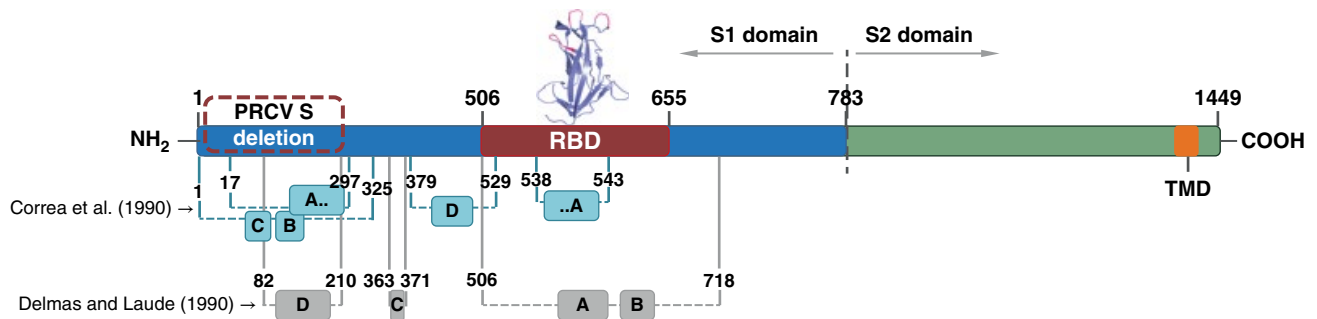


Figure 31.3 Schematic diagram of TGEV S protein. RBD, receptor-binding domain (shown in red); TMD, transmembrane domain (shown in orange). According to Correa et al.'s (1990) classification (light blue boxes), site A is discontinuous and located around positions 17 and 297 and 538 and 543, site B is located between amino acids 1 and 325, and site D is located between 379 and 529. The relative order of sites C and B and part of site A is not determined. According to Delmas and Laude's (1990) classification (light gray boxes), S protein antigenic sites D, C, and A/B are located between amino acids 82 and 210, between 363 and 371, and between 506 and 718, respectively. Dotted red box indicates a 227 aa deletion in the PRCV S protein. *Source:* Adapted from Correa et al. (1990) and Delmas and Laude (1990).

efforts, and the viruses may continue to evolve in domestic pigs, wild boars, and other potential reservoir (bats) or secondary hosts, such as carnivores, via interspecies transmission.

Transmissible gastroenteritis virus and porcine respiratory coronavirus

Relevance

TGE is a highly contagious enteric viral disease of swine characterized by vomiting, severe diarrhea, and high mortality (often 100%) in piglets less than 2 weeks of age. TGE was first described by Doyle and Hutchings (1946) in the United States and subsequently reported worldwide. Although swine of all ages are susceptible to TGEV or PRCV infection, the mortality in TGEV and/or PRCV seropositive herds and in swine over 5 weeks of age is generally low.

The appearance and widespread prevalence of PRCV, a naturally occurring deletion mutant of TGEV, lessened the clinical impact of TGE (Brown and Cartwright 1986; Laude et al. 1993; Pensaert et al. 1986, 1993; Pensaert 1989; Jaeger et al. 2002). However, sporadic outbreaks of severe diarrhea in piglets caused by TGEV in TGEV/PRCV seronegative herds are still reported in North America, Europe, and Asia. Currently, TGEV and PEDV co-circulate in Asia, Europe, and the United States, and recently, pathogenic recombinant TGEV/PEDV variants (swine enteric coronavirus [SeCoV]) were identified and characterized in Europe (Akimkin et al. 2016; Belsham et al. 2016; Boniotti et al. 2016). SeCoV that contains PEDV S gene on a TGEV backbone reportedly causes disease clinically indistinguishable from that caused by TGEV and PEDV. This epidemiological situation requires frequent monitoring and development of reliable tools for differential diagnosis (Kim et al. 2001; Masuda et al. 2016).

Etiology

TGEV antigen can be demonstrated by IF staining in the cytoplasm of infected cells 4–5 hours post infection (Pensaert et al. 1970). Maturation of virus occurs in the cytoplasm by budding through the endoplasmic reticulum, and viral particles (65–90 nm in diameter) are observed within cytoplasmic vacuoles (Figure 31.4a) (Pensaert et al. 1970; Thake 1968). Virus may line host cell membranes after exit from infected cells (Figure 31.4b). A similar intracellular replication scenario has been described for PEDV (Figure 31.4c). TGEV glycoproteins are also evident on the surface of infected ST cells (Laviada et al. 1990).

TGEV is stable when stored frozen, but labile at room temperature or higher. Infectious virus persisted in liquid manure slurry for more than 8 weeks at 5 °C (41 °F), 2 weeks at 20 °C (68 °F), and 24 hours at 35 °C (95 °F) (Haas et al. 1995). In recent studies that used TGEV as a surrogate for SARS CoV (Casanova et al. 2009), it remained infectious in water and sewage for several days at 25 °C (77 °F) and for several weeks at 4 °C (39 °F).

TGEV is highly photosensitive. Fecal material containing 1×10^5 pig infectious doses (PID) was inactivated within 6 hours when exposed to sunlight or to ultraviolet light (Cartwright et al. 1965; Haelterman 1962). TGEV is inactivated by exposure to 0.03% formalin, 1% Lysovet (phenol and aldehyde), 0.01% beta-propiolactone, 1 mM binary ethylenimine, sodium hypochlorite, NaOH, iodines, quaternary ammonium compounds, ether, and chloroform (Brown 1981; VanCott et al. 1993). TGEV field strains are trypsin resistant, relatively stable in pig bile, and stable at pH3 (Laude et al. 1981), allowing virus to survive in the stomach and small intestine. However, properties of attenuated and field strains of TGEV vary.

Public health

Pigs are the main species naturally susceptible to TGEV and PRCV. No infection of humans has been reported.

Epidemiology

On a herd basis, two epidemiologic forms of TGE are recognized: epidemic and endemic. Infections with the TGEV deletion mutant PRCV present a different pattern, greatly complicating seroprevalence studies of the epidemiology of TGEV (Pensaert 1989).

Epidemic versus endemic TGE

Epidemic TGE occurs when most of the animals in a herd are TGEV/PRCV seronegative and susceptible. After introduction, the disease spreads rapidly to swine of all ages, especially during winter. Inappetence, vomiting, or diarrhea occurs in most animals. Suckling pigs show marked clinical signs and rapidly dehydrate. Mortality is very high in pigs under 2–3 weeks of age but decreases in older pigs. Lactating sows often develop anorexia and agalactia, with reduced milk production, which further contributes to piglet mortality.

Endemic TGE refers to the persistence of the virus and disease in a herd perpetuated by the continual or frequent influx of susceptible swine. Endemic TGE is a common sequel to a primary outbreak and occurs in seropositive herds that have frequent farrowings (Stepanek et al. 1979), herd additions, or commingling of susceptible pigs. In endemically infected herds, TGEV

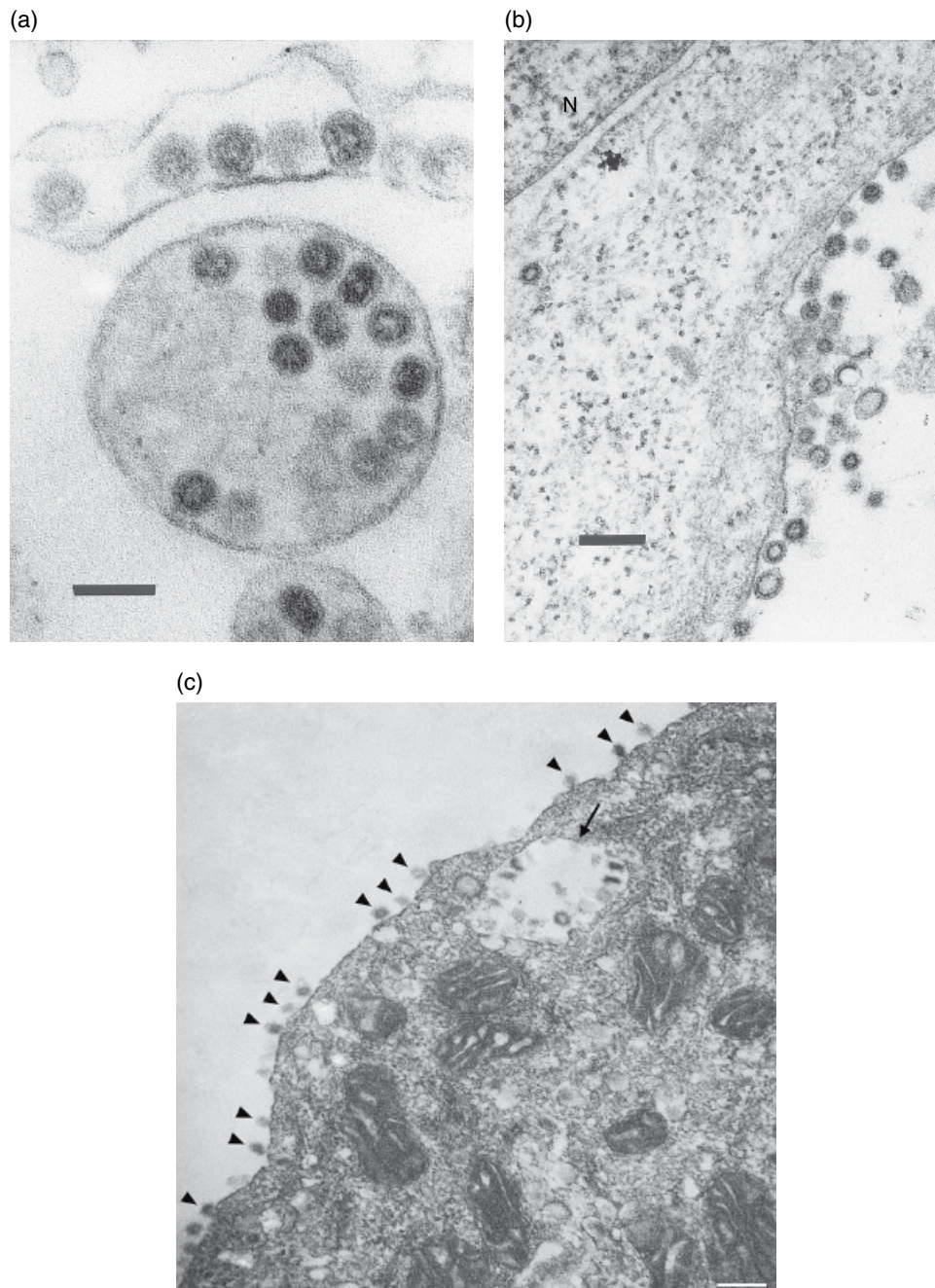


Figure 31.4 Electron micrographs. (a) TGEV in vesicles of the endoplasmic reticulum of a pig kidney cell (36 hours post infection). Bar = 100 nm. (b) TGEV lining the cell membrane of a pig kidney cell (36 hours post infection). N = nucleus; bar = 200 nm. (c) A PEDV-infected Vero cell. PEDV particles (arrow heads) on the cell surface and inside a vesicle (arrow) of the infected Vero cell. Scale bar = 200 nm. For (c), Source: Oka et al. (2014). Reproduced with permission of Elsevier.

spreads slowly among adult swine (Pritchard 1987). Sows are frequently immune and asymptomatic and will transfer a variable degree of passive lactogenic immunity to their progeny. In these herds, mild TGEV diarrhea occurs, and mortality is usually under 10–20% in pigs from approximately 6 days of age until approximately 2 weeks post weaning. The age-related effects are influenced by the management system and the degree of passive immunity from the sow.

Endemic TGE in suckling or recently weaned pigs can be difficult to diagnose and must be differentiated from other types of endemic diarrheal pathogens common in young pigs, such as PEDV, PDCoV, rotavirus, and *Escherichia coli*. Endemic TGE persists in the herd as long as susceptible or partially immune swine are exposed to TGEV. It is unclear whether the source of virus is from reactivation of virus shedding in carrier swine or reintroduction of virus into the herd from an external source.

Porcine respiratory coronavirus

PRCV is a TGEV variant that infects the respiratory tract with limited or no shedding in feces (Pensaert 1989). However, PRCV-infected pigs produce antibodies that neutralize TGEV. The first isolation of PRCV was in Belgium in 1984 (Pensaert et al. 1986). In 1989, PRCV was detected in two herds in the United States with no history of TGEV vaccination or clinical disease (Hill et al. 1990; Wesley et al. 1990).

Swine population density, distance between farms, and season influence PRCV epidemiology (Have 1990; Pensaert 1989). PRCV infects pigs of all ages by contact or airborne transmission. PRCV infections are often subclinical. The risk of PRCV spread increases in areas of high swine density, where the virus can travel several kilometers. The virus has spread rapidly and extensively in pigs in Europe (Brown and Cartwright 1986; Have 1990; Laude et al. 1993; van Nieuwstadt et al. 1989) and became endemic even in TGEV-free countries (Laude et al. 1993; Pensaert 1989; Pensaert et al. 1993). A limited serological survey in 1995 in the United States suggested that many asymptomatic herds in Iowa were seropositive for PRCV (Wesley et al. 1997).

PRCV circulates in the herd, infecting pigs before the age of 10–15 weeks after passively acquired maternal antibodies have declined. Introduction of pigs into fattening units and commingling of PRCV-negative and PRCV-positive pigs from diverse sources result in seroconversion to PRCV in pigs shortly after introduction into most units.

Susceptible pigs experimentally infected with PRCV shed virus from nasal secretions for less than 2 weeks (Onno et al. 1989; VanCott et al. 1993; Wesley et al. 1990). There is no evidence for the fecal–oral transmission of PRCV. PRCV persists in closed breeding farms by regularly infecting newly weaned pigs, even in the presence of maternal antibodies (Pensaert et al. 1993). PRCV can persist in the herd throughout the year, or it can disappear in summer and reappear in the nursery and fattening units in winter. Coincident with the widespread dissemination of PRCV, the seroprevalence of TGEV in Europe has decreased, to a low prevalence (Brown and Paton 1991; Pensaert et al. 1993).

Transmission and reservoirs

An epidemiological feature of TGE is its seasonal appearance during winter. Haelterman (1962) suggested that this is because the virus is stable when frozen and more labile when exposed to heat or to sunlight. This would allow virus transmission between herds in winter on fomites or animals. He proposed at least three possible reservoirs for TGEV between seasonal epidemics: (1) pig farms in which the virus spreads subclinically, (2) hosts other than swine, and (3) carrier pigs. There is evidence for the existence of TGEV in non-porcine hosts. Cats, dogs, and foxes have been suggested as possible carriers

of TGEV from one herd to another, since they can shed virus in their feces for variable periods (Haelterman 1962; McClurkin et al. 1970) and virus excreted by dogs was infectious for pigs (Haelterman 1962; Reynolds and Garwes 1979).

The concentration of starlings (*Sturnus vulgaris*) in winter in feeding areas of swine may foster mechanical spread of TGEV among farms. Pilchard (1965) reported that TGEV was detected in the droppings of starlings for up to 32 hours after feeding TGEV. Houseflies (*Musca domestica*) have also been proposed as possible mechanical vectors for TGEV. TGEV antigen was detected in flies within a swine herd, and experimentally inoculated flies excreted TGEV for 3 days (Gough and Jorgenson 1983). According to surveys conducted in Central Europe, antibodies against TGEV are also present in approximately 30% of the feral pig population (Sedlak et al. 2008).

The third possibility relating to TGE transmission is the duration of TGEV shedding and the role of the carrier pig. Nasal shedding of PRCV in experimentally infected pigs occurs through 10 days post infection (DPI) (Onno et al. 1989; Wesley et al. 1990). However, how long pigs clinically recovered from TGEV and PRCV infection in the field remain infectious is unknown. One report indicated chronic and/or persistent TGEV fecal shedding for up to 18 months, suggesting a possible role for the long-term carrier hog in transmitting TGEV (Woods and Wesley 1998). Although TGEV has been detected in the intestinal and respiratory tracts for periods of up to 104 DPI (Underdahl et al. 1975), it is unknown whether infectious virus is shed or transmitted. Addition of sentinel pigs to a herd at 3, 4, and 5 months after a previous TGE outbreak resulted in no infections in the introduced pigs, as determined by serologic tests (Derbyshire et al. 1969).

Pathogenesis

Intestinal and extraintestinal replication of TGEV

Jejunal enterocytes undergo massive necrosis within 12–24 hours after infection, resulting in marked reduction in enzymatic activity (alkaline phosphatase, lactase, etc.) in the small intestine. This disrupts digestion and cellular transport of nutrients and electrolytes (including sodium), thereby causing an accumulation of liquid in the intestinal lumen and acute malabsorptive diarrhea (Moon 1978) that leads to severe and fatal dehydration in piglets (Butler et al. 1974) and loss of extravascular protein. Dehydration is also related to metabolic acidosis coupled with abnormal cardiac function due to hyperkalemia.

The severe villous atrophy in the jejunum (Figure 31.5a and b) and to a lesser extent in the ileum of TGEV-infected pigs is often absent in the proximal duodenum (Hooper and Haelterman 1966a). Villous atrophy is more

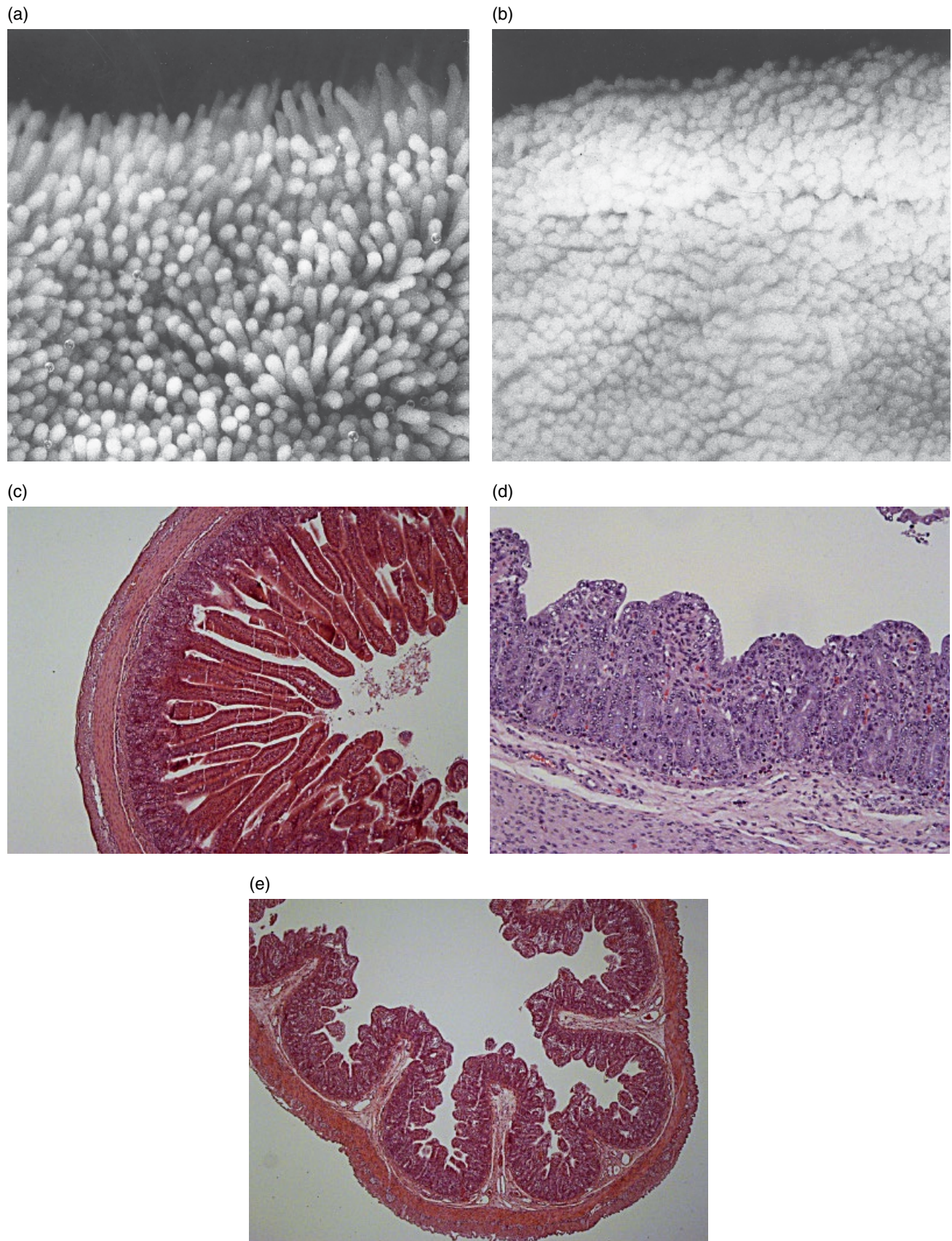


Figure 31.5 Villi of the jejunum from a normal pig (a) and from a TGEV-infected pig (b), as viewed through a dissecting microscope (approximately $\times 10$). Hematoxylin and eosin (H&E)-stained jejunum of a normal gnotobiotic pig (17 days of age), showing normal villi ($\times 80$) (c) (Source: Jung et al. 2015b); of a PEDV-infected gnotobiotic pig (26 days of age) at 46 hours post inoculation (at onset of clinical signs), showing acute diffuse, severe atrophic jejunitis ($\times 200$) (d) (Source: Jung et al. 2014); and of a PDCoV-infected gnotobiotic pig (17 days of age) at 3 days post inoculation ($\times 40$) (e) (Source: Jung et al. 2015b).

severe in newborn pigs than in 3-week-old pigs (Moon 1978), suggesting higher susceptibility of neonates to TGEV infection. A similar degree and distribution of small intestinal villous atrophy is also evident for PEDV (Figure 31.5c and d) and PDCoV (Figure 31.5c and e).

Mechanisms to account for age-dependent susceptibility to clinical disease include the slower replacement in newborn pigs of infected villous epithelial cells by migration of cells from crypts (Moon 1978). These newly replaced villous enterocytes are reportedly resistant to TGEV infection, possibly due to induction of innate immunity and intestinal IFN (Abou-Youssef and Ristic 1972) or the inability of the regenerating cells to support virus growth.

The exposure dose of infectious virus plays a major role in age-dependent susceptibility. The infectious dose of TGEV needed to infect a 6-month-old market hog was 10^4 times greater than that needed to infect a 2-day-old piglet (Witte and Walther 1976). Moreover, the severity of clinical signs due to TGEV increased when pigs were injected with a synthetic corticosteroid, dexamethasone (Shimizu and Shimizu 1979), similar to dexamethasone-aggravated lung pathology in PRCV infection (Jung et al. 2007; Zhang et al. 2008), indicating the possible effect of stress on TGEV/PRCV disease severity. In addition, TGEV in combination with other enteric pathogens, such as *E. coli* or porcine rotavirus, caused more severe enteritis than either infection alone (Underdahl et al. 1972). Likewise, PRCV respiratory infection and lung lesions were exacerbated by preexisting porcine reproductive and respiratory syndrome virus (PRRSV) infection (Jung et al. 2009; van Reeth et al. 1996).

Extraintestinal sites for TGEV replication include lungs (alveolar macrophages) and mammary tissues (Kemeny et al. 1975). Oronasal infection of pigs with TGEV caused pneumonia (Underdahl et al. 1975). Cell culture-attenuated but not virulent TGEV replicated in cultures of alveolar macrophages *in vitro*, suggesting a possible role for these cells in lung infection (Laude et al. 1984). Moreover, TGEV was detected in nasal secretions of infected piglets (VanCott et al. 1993) and lactating sows exposed to infected piglets (Kemeny et al. 1975). Cell-cultured strains of TGEV generally showed reduced virulence in pigs, with less replication in the gut and higher levels of replication in the upper respiratory tract compared with virulent TGEV (Frederick et al. 1976; VanCott et al. 1993).

TGEV replicated in mammary tissues of lactating sows (Saif and Bohl 1983) and infected sows shed virus in milk (Kemeny and Woods 1977). The clinical or epidemiological significance of mammary gland infection with TGEV under field conditions is unclear, but agalactia is often seen in TGEV-infected sows and TGEV spreads rapidly among pigs.

Replication of PRCV in the respiratory tract

PRCV has a tropism for the respiratory tract. It replicates to high titers in porcine lungs (1×10^7 – 10^8 TCID₅₀) in type 1 and 2 pneumocytes and infects epithelial cells of the nares, trachea, bronchi, bronchioles, alveoli, and, occasionally, alveolar macrophages (Atanasova et al. 2008; Jung et al. 2007, 2009; O'Toole et al. 1989; Pensaert et al. 1986). PRCV induces necrosis of infected cells, increasing innate immune responses at the infection sites, including high levels of IFN- α and nitric oxide in lungs (Jung et al. 2009, 2010). Innate cytokines inhibit initial viral replication and modulate Th1/Th2 responses with the latter enhancing B-cell responses, leading to secretion of VN antibodies.

Virus shedding in nasal secretions lasted for 4–6 days after experimental PRCV infection. The severity of PRCV-induced pneumonia and viral replication in lung peaked at 8–10 DPI, coinciding with increased numbers of T and B cells and frequency of lymphocytic inflammation. Thereafter, pulmonary lesions and clinical signs resolved concurrently with increased VN antibody titers (Atanasova et al. 2008; Jung et al. 2009).

Depending on the experimental conditions and the virus strains used, PRCV may be detected in blood, tracheobronchial lymph nodes, and occasionally the small intestines of infected pigs. However, virus in infected enterocytes does not spread to adjacent cells (Cox et al. 1990a,b), and fecal shedding is low or undetectable. The limited intestinal replication of PRCV may be related to the deletion in the S gene. When fecal and nasal isolates of PRCV from the same pigs were compared genetically, only point mutations, but not additional deletions, were noted in the S gene (Costantini et al. 2004).

Clinical signs

Epidemic TGE

Typical clinical signs of TGE in seronegative piglets are vomiting and profuse watery, yellowish diarrhea, with rapid loss of weight, dehydration, and high morbidity and mortality in pigs under 2 weeks of age. The severity of clinical signs, duration of disease, and mortality are inversely related to the age of the pig. Most pigs under 7 days of age will die in 2–7 days after onset of clinical signs. Most suckling pigs over 3 weeks of age will survive, but may remain stunted. Clinical signs of TGE in finishing swine and in sows include inappetence, transient diarrhea, and vomiting.

The incubation period is short, usually 18 hours to 3 days. Infection generally spreads rapidly through the entire group, and most swine are affected in 2–3 days, but this is more likely to occur in winter than summer (Haelterman 1962).

Endemic TGE

Endemic TGE occurs in large herds that farrow frequently and in TGEV or PRCV seropositive herds. Clinical signs are usually less severe than those in seronegative pigs of the same age. Mortality is low, especially if pigs are kept warm. The clinical signs in suckling pigs can resemble rotavirus, PEDV, or PDCoV diarrhea (Bohl et al. 1978; Pensaert and de Bouck 1978; Wang et al. 2014a). In some herds, endemic TGE is manifested primarily in weaned pigs and may be confused with PEDV (Madson et al. 2014), *E. coli*, coccidia, or rotavirus infections (Pritchard 1987).

Porcine respiratory coronavirus

Experimentally, PRCV infection of pigs is mostly subclinical with self-limiting respiratory infection. The early antiviral effects of innate immune responses to PRCV infection, followed by cell-mediated and antibody responses, likely effectively control the infection (Atanasova et al. 2008; Jung et al. 2007, 2009, 2010; Zhang et al. 2008). Clinical signs include (1) respiratory signs (e.g. coughing, abdominal breathing, dyspnea), (2) depression and/or anorexia, and (3) slightly decreased growth rates (Lanza et al. 1992; van Reeth et al. 1996; Wesley and Woods 1996).

The severity and frequency of clinical signs are influenced by the presence of other bacterial or viral pathogens in the herd. For example, coinfection with PRRSV can alter the severity of either PRCV or PRRSV infections. Inoculation with PRRSV followed by PRCV resulted in prolonged fever with respiratory disease, reduced weight gain, and prolonged severe pneumonia (Jung et al. 2009; van Reeth et al. 1996). Ongoing or pre-existing PRRSV infection significantly suppressed innate immune responses (reduced IFN- α levels in lung and blood natural killer [NK] cell cytotoxicity) during early PRCV infection, which may exacerbate PRCV pneumonia (Jung et al. 2009).

TGEV lesions

TGE gross lesions are confined to the gastrointestinal tract. The stomach is distended with curdled milk and may have petechial hemorrhages (Hooper and Haelterman 1966b). The small intestine is distended with yellow fluid and curdled, undigested milk. The wall is thin and transparent, due to villous atrophy. A major lesion of TGE is markedly shortened villi of the jejunum and ileum (Figure 31.5a and b), similar to PEDV and PDCoV lesions (Figure 31.5c–e) (Debouck et al. 1981; Jung et al. 2015b), but usually more severe and extensive than that seen in rotavirus diarrhea (Bohl et al. 1978). Infections with some strains of *E. coli* and coccidia may produce similar lesions (Hornich et al. 1977). Transmission EM of TGEV-infected villous enterocytes

has revealed alterations in the microvilli, mitochondria, endoplasmic reticulum, and other cytoplasmic components. Virus particles, primarily in cytoplasmic vacuoles, were observed in villous enterocytes and in M cells, lymphocytes, and macrophages in the dome regions of Peyer's patches (Chu et al. 1982; Thake 1968).

Pathologic findings and the extent of villous atrophy are highly variable in pigs from endemically infected herds (Pritchard 1987). Moxley and Olson (1989) showed that the level of passive immunity in TGEV-infected pigs influenced both the degree of villous atrophy and its segmental distribution. Villous atrophy was minimal in pigs nursing sows previously infected with virulent TGEV, compared with pigs nursing seronegative sows or sows given live attenuated vaccines. In partially protected pigs, villous atrophy was primarily in the ileum and not the jejunum. Similar observations were noted in pigs from herds with endemic TGE.

PRCV lesions

PRCV primarily causes upper and lower respiratory tract disease. The PRCV-induced lesions are generally limited to the lungs and commonly observed as consolidation of the lung and bronchointerstitial pneumonia, with frequent peribronchiolar and perivascular lymphohistiocytic cuffing (Atanasova et al. 2008; Cox et al. 1990a; Halbur et al. 1993; Jabrane et al. 1994; Jung et al. 2007, 2009). PRCV-induced bronchointerstitial pneumonia is characterized by (1) thickening of the alveolar septa by infiltration of inflammatory leucocytes, principally macrophages and lymphocytes; (2) type 2 pneumocyte hypertrophy and hyperplasia; (3) accumulation of necrotic cells and inflammatory leucocytes in alveolar and bronchiolar lumina due to airway epithelial necrosis; and (4) peribronchiolar or perivascular lymphohistiocytic inflammation. Within 10 days of PRCV infection, the virus simultaneously induces inflammatory (cell necrotizing) and proliferative (alveolar septal thickening) chronic-active bronchointerstitial pneumonia (Jung et al. 2007, 2009).

Diagnosis

The collection and preservation of appropriate clinical specimens is necessary for reliable diagnosis. Because clinical signs and atrophic enteritis caused by TGEV are frequently observed in other enteric infections (rotavirus, PEDV, PDCoV, and coccidia), laboratory diagnosis of TGE must be accomplished by one or more of the following procedures: detection of viral antigen or nucleic acids in feces or lesions, virus isolation from specimens, or detection of TGEV antibodies.

Diagnosis of PRCV requires similar procedures, but with a focus on respiratory specimens. Evaluation of

clinical signs, histologic lesions, and tissue distribution of viral antigen may provide a presumptive diagnosis. PRCV does not cause diarrhea or villous atrophy and replicates almost exclusively in respiratory tissues (Pensaert 1989). Thus, PRCV is suspected if there is antigen in lung tissues, seroconversion to TGEV/PRCV, and no signs of enteric disease.

Detection of viral antigens or nucleic acids

Detection of TGEV antigen in small intestinal enterocytes is commonly used to diagnose TGE. Either IF (Pensaert et al. 1970) or immunohistochemical (IHC) (Shoup et al. 1996) techniques using MAb against the highly conserved N protein of TGEV may be used in frozen or formalin-fixed tissues (Figure 31.6a), but they

require pigs in the early stage of infection. A similar viral antigen distribution is seen in the small intestine of PEDV- (Figure 31.6b) and PDCoV-infected pigs (Figure 31.6c). An exception for PEDV and PDCoV is the occasional detection of viral antigens in the crypt epithelial cells and the colon.

An enzyme-linked immunosorbent assay (ELISA) using MAb or polyclonal antibodies to TGEV is used to detect TGEV antigens in cell culture, feces, and intestinal contents (Lanza et al. 1995; Sestak et al. 1996, 1999a; van Nieuwstadt et al. 1988) or PRCV antigen in cell culture, nasal swabs, or lung homogenates (Lanza et al. 1995).

RT-PCR or real-time RT-PCR is currently used for diagnosis of TGEV and differentiation of TGEV, PRCV, PDCoV, and PEDV (Costantini et al. 2004; Kim et al.

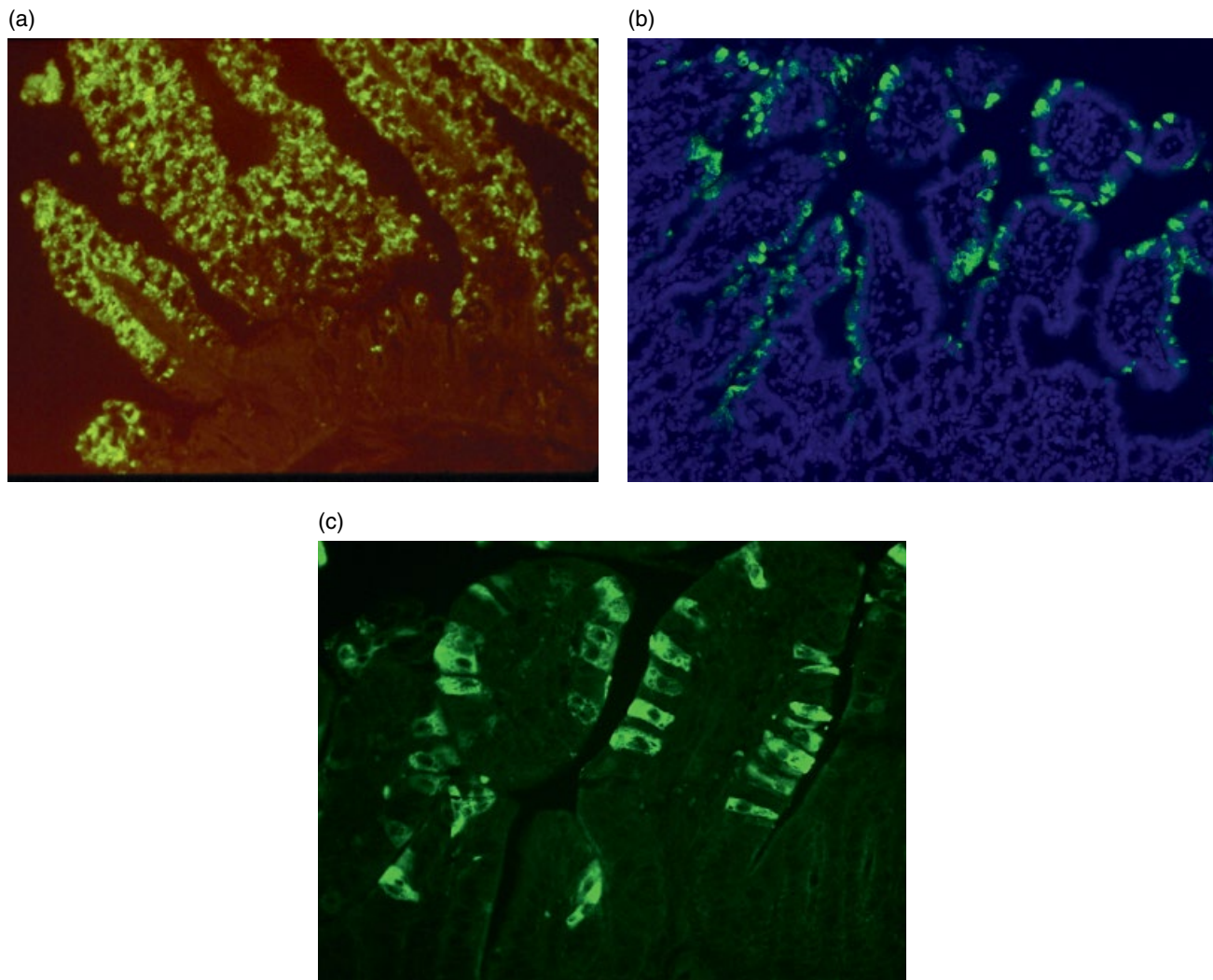


Figure 31.6 Immunofluorescent staining (green) of (a) TGEV antigens in almost 100% of the ileal enterocytes lining the villi of a TGEV-infected piglet. Note absence of TGEV antigens in the crypt epithelial cells. (b) PEDV antigens in the enterocytes of the jejunum of a piglet at 67 hours post inoculation with the emerging non-S INDEL PEDV strain PC21A (37–41 hours after onset of clinical signs), indicating that the epithelial cells lining atrophied villi are positive for PEDV ($\times 200$). *Source:* Jung et al. 2014. (c) PDCoV antigens in the jejunum of a gnotobiotic pig at 3 days post inoculation with PDCoV strain OH-FD22, showing similar localization of PDCoV antigens in the cytoplasm of villous epithelial cells ($\times 400$).

2000a, 2001, 2007; Masuda et al. 2016; Ogawa et al. 2009). PRCV/TGEV differentiation is accomplished using PCR primers targeting the S gene deletion region in PRCV strains. Multiplex RT-PCR and real-time RT-PCR assays have been developed for the simultaneous detection of major porcine viruses associated with diarrhea including rotavirus, TGEV, PDCoV, and PEDV (Masuda et al. 2016; Ogawa et al. 2009). These assays permit detection of up to nine viruses in a sample. Moreover, multiplex microarray hybridization was employed for the rapid differential diagnosis of eight CoVs including TGEV (Chen et al. 2005).

Electron microscopy (EM)

TGEV can be demonstrated in the intestinal contents and feces of infected pigs by negative contrast transmission EM (Figure 31.2a). Immune electron microscopy (IEM) has advantages over conventional EM in being more sensitive for detecting TGEV and distinguishing it from PEDV, PDCoV, and enveloped membranous debris, as well as concurrently detecting the presence of other enteric viruses (Figure 31.2b) (Saif et al. 1977).

Virus isolation

Primary and secondary pig kidney (PK) cells (Bohl and Kumagai 1965) or cell lines (Laude et al. 1981), porcine thyroid cells (Witte 1971), and the McClurkin swine testicle (ST) cell line (McClurkin and Norman 1966) are recommended for the isolation of TGEV from feces or gut contents of infected pigs. Distinct cytopathic effects (CPE) may be negligible upon primary isolation of field strains, requiring additional passages. The CPE consists of enlarged, rounded cells with a balloon-like appearance (Bohl and Kumagai 1965). For detecting viral CPE or plaques, the sensitivity of ST cells can be further enhanced by adding pancreatin or trypsin to cell culture media (Bohl 1979) and using older cells.

Pig kidney and ST cells are preferred for isolating PRCV from nasal swab fluids or lung tissue homogenates. PRCV- and TGEV-induced CPE are similar, with syncytia frequently observed as also reported for PEDV and SARS CoV grown in Vero cells (Hofmann and Wyler 1988; Ksiazek et al. 2003). Identification of cell culture virus can be done by VN, IF staining, or IEM using specific TGEV antiserum or differential MAbs (Garwes et al. 1988) and RT-PCR using virus-specific primers (Enjuanes and Van der Zeijst 1995; Kim et al. 2000a; Laude et al. 1993).

Serology

TGEV antibodies can be detected by several serologic tests. However, TGEV serology is complicated by the fact that both TGEV and PRCV induce VN antibodies that are qualitatively and quantitatively similar (Pensaert 1989). A blocking ELISA test can differentiate between

these antibodies based on using MAbs to TGEV antigenic sites that are absent on the PRCV S protein (Bernard et al. 1989; Callebaut et al. 1989; Delmas and Laude 1990; Garwes et al. 1988; Sanchez et al. 1990; Sestak et al. 1999b; Simkins et al. 1992, 1993). Blocking ELISAs should only be applied on a herd basis because some pigs with low TGEV or PRCV antibody titers may not be detected (Callebaut et al. 1989; Sestak et al. 1999b; Simkins et al. 1993) and the accuracy of commercial ELISAs for differentiating US strains of PRCV and TGEV is low (Sestak et al. 1999b). ELISA tests were used to differentiate not only between TGEV and PRCV antibodies but also between TGEV and TGEV-like CCoV or classical CCoV-II antibodies (Elia et al. 2010; Lopez et al. 2009).

A rise in antibody titer between acute and convalescent serum samples provides retrospective evidence for TGEV or PRCV infection. To determine the presence of endemic TGE or PRCV, serum samples from 2- to 6-month-old swine (often free of passively acquired antibodies at this age) can be tested for antibodies (Derbyshire et al. 1969). The VN test using cell culture-adapted viruses has been the most widely used (Bohl 1979; Bohl and Kumagai 1965). VN antibodies to TGEV are detectable in serum by 7–8 DPI and persist for at least 18 months. Little is known regarding the persistence of VN antibodies to PRCV within a herd. Antibody ELISA tests (Bernard et al. 1989; Berthon et al. 1990; Callebaut et al. 1989; Garwes et al. 1988; Sestak et al. 1999a, b; van Nieuwstadt et al. 1989) have been reported, but they require concentrated purified virus or S or N protein for coating ELISA plates.

Immunity

Active immunity to TGEV

The duration of active immunity in swine after oral infection with virulent TGEV has not been well characterized. Intestinal infection of breeding-age swine results in detectable serum antibodies that persist for at least 6 months and possibly several years (Stepanek et al. 1979). Although serum antibodies provide serologic evidence of TGEV or PRCV infection, they afford little indication of the degree of active immunity to TGEV. Swine that have recovered from TGE are immune to subsequent short-term challenge, presumably due to local immunity within the intestinal mucosa (Brim et al. 1995; Saif et al. 1994; VanCott et al. 1993, 1994). The age and immune status of the animal at initial infection and the severity of the challenge influence the completeness and duration of active immunity.

The mechanism of active immunity in the gut relates to stimulation of the secretory IgA (sIgA) immune system with production of sIgA antibodies by intestinal plasma cells (Saif et al. 1994; VanCott et al. 1993, 1994). IgA TGEV antibodies and antibody-secreting cells (ASCs)

have been detected in the intestine and serum of pigs after oral, but not parenteral inoculation with TGEV (Kodama et al. 1980; Saif et al. 1994; VanCott et al. 1993, 1994). Kodama et al. (1980) proposed that detection of IgA antibody in the serum, presumably intestinally derived, might serve as an indicator of active immunity to TGE. Enzyme-linked immunospot (ELISPOT) assay was used to investigate the kinetics of IgA and IgG TGEV antibody production by the pig's systemic and local gut-associated lymphoid tissues (GALT). High numbers of IgA ASCs were induced in GALT only by virulent TGEV. In contrast, live attenuated (vaccine) TGEV or PRCV strains induced significantly fewer IgA ASCs (Berthon et al. 1990; Saif et al. 1994; VanCott et al. 1993, 1994). Besides local antibody-mediated immunity, cell-mediated immunity (CMI) may also be important in active immunity against TGEV infections. However, only indirect evidence exists concerning the role of CMI in resistance to TGEV infection. CMI was demonstrated with lymphocytes obtained from GALT of swine orally infected with virulent TGEV (Brim et al. 1995; Frederick et al. 1976; Shimizu and Shimizu 1979), whereas swine parenterally or oronasally inoculated with attenuated TGEV or PRCV developed CMI mainly in systemic sites. Lymphoproliferative responses to TGEV persisted within GALT, but not systemic lymphocytes, for at least 110 days after oral infection of 6-month-old swine (Shimizu and Shimizu 1979), but for only about 14–21 days after infection of younger (7- to 11-day-old) pigs (Brim et al. 1995). CD4 T helper cells are involved in lymphoproliferative responses to TGEV (Anton et al. 1995). Potent production of antiviral IFN- α by plasmacytoid dendritic cells (DCs) derived from TGEV-infected swine was observed upon stimulation of these cells *in vitro* with TGEV antigens (Calzada-Nova et al. 2010).

A correlation between lymphoproliferative responses and lactogenic immunity to TGEV was described in sows vaccinated with attenuated or recombinant TGEV vaccines (Park et al. 1998). Although T-cell epitopes were identified by lymphoproliferation studies for each of the three major proteins of TGEV, a dominant functional T helper epitope was defined on the N protein (N321) (Anton et al. 1995). The N321 peptide-induced T cells collaborated in the *in vitro* synthesis of TGEV VN antibodies specific for the S protein. Maximal responses were induced by native S protein combined with recombinant N protein. Such findings have important implications for design of CoV subunit or other recombinant CoV vaccines.

Because lymphocyte cytotoxicity was absent in newborn piglets and decreased in parturient sows, it was proposed that a lack of NK cell activity against TGEV-infected cells might correlate with the increased susceptibility of newborn piglets and parturient sows to TGEV infection (Cepica and Derbyshire 1984). Thus, CMI or

innate immunity may play a role in either recovery from TGEV infection or resistance to reinfection via the rapid elimination of TGEV-infected epithelial cells. Some TGEV strains can also downregulate host immune responses. A virulent (SHXB) but not attenuated (STC3) TGEV strain impaired the ability of porcine intestinal DCs or monocyte-derived DCs to recognize antigen, migrate, and induce T-cell proliferation *in vivo* and *in vitro* (Zhao et al. 2014).

PRCV-induced active immunity to TGEV

The dramatic decline in epidemic outbreaks of TGE in Europe following the widespread dissemination of PRCV prompted researchers to examine if respiratory PRCV infection could induce protective intestinal immunity against TGEV. The consensus from several studies was that prior infection of nursing or weaned pigs with PRCV provided partial immunity against TGEV challenge, as evidenced by a reduced duration and level of virus shedding and diarrhea in most pigs studied (Brim et al. 1995; Cox et al. 1993; VanCott et al. 1994; Wesley and Woods 1996).

This partial immunity presumably is related to the rapid increase in TGEV VN antibodies (Cox et al. 1993; Wesley and Woods 1996) and numbers of IgG and IgA ASCs in the intestines of PRCV-exposed pigs after TGEV challenge (Saif et al. 1994; VanCott et al. 1994). The altered tissue tropism of PRCV was also linked to a shift in antibody responses; that is, in TGEV-infected pigs, more IgA ASCs were found in gut, whereas PRCV predominantly induced IgG ASCs in the lung (VanCott et al. 1994). Migration of PRCV IgG and IgA ASCs from the bronchus-associated lymphoid tissues (BALT) to the gut of the PRCV-exposed pigs after TGEV challenge might explain the rapid anamnestic response and the partial protection induced (VanCott et al. 1994). However, neonatal pigs required at least 6–8 days after PRCV exposure to develop partial immunity to TGEV challenge (Wesley and Woods 1996).

Passive immunity to TGEV

Passive lactogenic immunity is critical to provide newborn piglets with immediate protection against TGEV infection. Circulating passive antibodies, acquired after absorption of colostral immunoglobulin (primarily IgG), protect the neonate against systemic but generally not intestinal infection (Hooper and Haelterman 1966a; Saif and Sestak 2006). Mechanisms of passive immunity to TGEV infections have been reviewed (Chattha et al. 2015; Saif and Bohl 1979; Saif and Jackwood 1990; Saif and Sestak 2006). Swine recovered from TGE transmit passive immunity to their suckling pigs by the frequent ingestion of colostrum or milk (lactogenic immunity) that contains TGEV VN antibodies (Hooper and Haelterman 1966a). Such antibodies in the lumen of the

intestine neutralize the ingested TGEV and protect the susceptible small intestinal enterocytes. This is accomplished naturally when piglets suckle immune sows frequently or by continuous feeding of antiserum to piglets. During the first week of lactation, IgA becomes dominant in milk and IgG decreases.

TGEV IgA antibodies in milk are stable in the gut and provide the most effective protection, but IgG antibodies are also protective if high titers are maintained in milk after vaccination (Bohl and Saif 1975) or by artificial feeding of colostrum IgG antibodies (Stone et al. 1977). TGEV IgG antibodies are produced in the sow's milk after parenteral or systemic immunization, whereas TGEV IgA antibodies occur in milk after intestinal infection. It is postulated that IgA immunocytes migrate to the mammary gland after antigenic stimulation in the gut where they localize and secrete IgA antibodies into colostrum and milk that play a key role in passive intestinal immunity of suckling pigs (Bohl and Saif 1975; Saif and Bohl 1979; Saif and Jackwood 1990; Saif and Sestak 2006). The "gut–mammary" immunologic axis, first proposed in relation to TGEV infections in swine (Bohl et al. 1972; Saif et al. 1972), provided the initial concept for a common mucosal immune system. This concept continues to be important in the design of maternal vaccines that are capable of providing effective lactogenic immunity against enteric pathogens.

PRCV-induced passive immunity to TGEV

The incidence and severity of TGE in countries with PRCV has declined since PRCV has become widespread (Schwegmann-Wessels and Herrler 2006). This suggests that prior exposure of swine to PRCV imparts partial immunity to TGEV (Laude et al. 1993; Pensaert 1989).

Prior natural exposure of sows to PRCV induced a variable degree of passive protection (44–53% mortality) against experimental TGEV challenge of suckling pigs (Bernard et al. 1989; Paton and Brown 1990). Variable protection in the field during TGE outbreaks was also noted among litters of PRCV-exposed sows (Pensaert 1989; Sanchez et al. 1990). Similar variable levels of protection (30–67% mortality) were reported after TGEV challenge of piglets suckling sows that had been experimentally infected or reinfected with PRCV during pregnancy (De Diego et al. 1992; Lanza et al. 1995; Sanchez et al. 1990; Sestak et al. 1996; Wesley and Woods 1993). In the latter two studies, litter mortality was lowest (range = 0–27%), and IgA and IgG milk antibody titers were highest in sows multiply exposed to PRCV during two subsequent pregnancies. These experimental findings agreed with field reports that naturally PRCV-exposed sows reinfected with PRCV during pregnancy secreted PRCV IgA antibodies in milk and provided a high degree of protection (0–12.5% mortality) to TGEV challenge (Sanchez et al. 1990). Besides PRCV IgA

antibodies in milk, a hallmark of protection in these and other studies (Wesley and Woods 1993) was induction of active immunity to TGEV in the sow preventing clinical disease or agalactia.

Besides quantitative differences in the levels of IgA antibodies induced in milk of sows after exposure to TGEV or PRCV, researchers have investigated potential differences in virus epitopes recognized by the milk IgA antibodies (De Diego et al. 1992, 1994). In TGEV-infected sows, antigenic subsite A (Aa, Ab, Ac), followed by antigenic subsite D, was the best inducer of IgA antibodies, while after PRCV infection, antigenic site D and subsite Ab were immunodominant (Figure 31.3). Thus, only IgA recognizing at least antigenic sites A and D conferred protection *in vivo*, whereas any immunoglobulin isotype reactive to one antigenic site neutralized virus *in vitro*.

Prevention and control

Treatment

No antiviral drugs have been developed for treatment of TGE. After the discovery of SARS CoV, studies were conducted with various surrogate viruses, including TGEV, to develop anti-CoV agents. Ortego et al. (2007) used TGEV deletion mutants to show that absence of the E protein blocks virus trafficking in the endoplasmic reticulum and prevents virus maturation. RNA interference (RNAi) targeting the viral RNA polymerase was studied *in vitro* as a strategy to prevent TGEV infection (Zhou et al. 2007). Although protective *in vitro*, the results of analogous *in vivo* experiments were less convincing (Zhou et al. 2010).

Studies suggest that IFN may activate NK cells in newborn pigs, contributing to resistance to challenge with TGEV (Lesnick and Derbyshire 1988; Loewen and Derbyshire 1988). In addition, during a field outbreak of TGE, 1- to 12-day-old piglets treated orally for 4 days with 1–20 IU of human IFN- α had significantly greater survival rates than placebo-treated piglets (Cummins et al. 1995).

The only available treatment for TGE is to alleviate starvation, dehydration, and acidosis. Parenteral treatment with fluids, electrolytes, and nutrients are effective in treating young pigs, but not practical under farm conditions. Oral therapy with balanced electrolyte or glucose solutions is contraindicated in young pigs (Moon 1978). The following measures are suggested: provide a warm (above 32°C [90°F]), draft-free, dry environment and provide water or nutrient solutions freely to TGEV-infected pigs. Such measures reduced mortality in pigs that were infected at more than 3–4 days of age. Antibacterial therapy is beneficial in 2- to 5-week-old pigs if there is concurrent infection with bacterial pathogens. Cross-fostering of infected or susceptible litters

onto TGE-immune sows was useful in some field outbreaks (Stepanek et al. 1979).

Management

Biosecurity

Swine in the incubative or viral shedding stage of the disease or possibly carriers can transmit TGEV. To introduce swine into a herd, precautions are needed to assure that swine originate from herds free of TGE, are serologically negative, and/or have been placed in isolation on the farm for 2–4 weeks before being added to the herd. After a TGE outbreak, at least 4 weeks should elapse from the last sign of disease before introducing such animals into a “clean” herd. Feces from TGEV-infected swine can be carried on boots, shoes, clothing, truck beds, feeds, and so on and can be a source of infection to other herds, requiring strict disinfection regimes, especially in winter.

After onset of TGE and endemic TGEV

When TGE occurs on a farm and pregnant animals have not yet been exposed, two procedures may minimize losses of newborn pigs: (1) If the sows are due to farrow in at least 2 weeks, use feedback methods to orally expose them to virulent autogenous virus, such as a slurry of minced intestines of acutely infected pigs, so that they will be immune at farrowing. (2) If the sows will farrow in under 2 weeks, attempt to provide facilities and management procedures to avoid exposure to TGEV until at least 3 weeks post farrowing.

Some success has been achieved in elimination of TGEV from epidemically infected closed breeder herds without depopulation by the following procedures (Harris et al. 1987): (1) bring in breeding stock replacements for the next 4–6 months; (2) in the face of an outbreak, feedback TGEV acutely infected minced piglet intestines simultaneously to all pigs in the herd (including replacement stock) to eliminate susceptible hosts, shorten the time the disease progresses through the herd, and ensure more uniform exposure levels in all pigs; (3) maintain strict all-in/all-out production in farrowing and nursery units; and (4) add sentinel seronegative pigs about 2 months after clinical signs of TGE disappear and monitor these pigs for seroconversion to TGEV. Potential hazards associated with feedback control of TGE include possible spread of other pathogens to pregnant sows and throughout the herd.

Other approaches to control or terminate endemic TGE include the following. First, pregnant seropositive sows can be vaccinated intramuscularly late in gestation or shortly after farrowing with live attenuated TGEV vaccine to boost immunity, increase milk antibody levels, and maintain longer passive immunity in suckling pigs (Saif and Sestak 2006; Stepanek et al. 1979). Although this procedure may only delay onset of TGE in exposed

pigs, the delay itself can reduce mortality. Second, break the cycle of infection by eliminating reservoirs of susceptible pigs in a unit: prevent the continual influx of susceptible animals into the herd temporarily (alter farrowing schedule as possible), utilize other facilities, and create smaller farrowing and nursing units to achieve an all-in/all-out system.

Immunoprophylaxis

Vaccines and vaccinations

There are several licensed TGEV vaccines. All contain inactivated or live attenuated TGEV and are approved for use in pregnant or neonatal swine. These vaccines and their efficacy have been reviewed (Saif and Sestak 2006) but will be briefly summarized.

Many variables complicate the evaluation of both experimental and commercial TGEV vaccines, resulting in conflicting data. These include the challenge dose and strain of TGEV, the age of the pig at challenge, environmental conditions (especially temperature), the health status and milking efficiency of the vaccinated sow, and the immune status (for TGEV or PRCV antibodies) of the dam at vaccination. If previously infected sows were unknowingly used in vaccine challenge studies, this could account for discrepant results seen in immune responses and piglet protection. This possibility can only be eliminated by using sensitive tests (such as VN) to measure TGEV/PRCV antibodies and by knowing the herd history of test animals since occurrence of PRCV in herds further complicates TGEV vaccine studies.

TGE vaccination of the seronegative pregnant dam

A variety of viral vaccines (virulent, attenuated, inactivated, and subunit) and routes of administration (oral, intranasal, intramuscular, subcutaneous, and intramammary) (Bohl and Saif 1975; Moxley and Olson 1989; Saif and Bohl 1979; Saif and Jackwood 1990; Saif and Sestak 2006) have been tested for induction of lactogenic immunity. Only oral administration of live virulent virus to pregnant sows consistently stimulated high levels of protective immunity for the sow and persisting TGEV IgA antibodies in milk that passively protected piglets.

The generally poor results for oral or intranasal vaccination of sows using attenuated TGEV strains (Moxley and Olson 1989; Saif and Bohl 1979; Saif and Sestak 2006) may be attributed to the limited replication of most attenuated strains in the sow's intestine (Frederick et al. 1976). This results in little antigenic stimulation of intestinal IgA immunocytes and correspondingly little IgA antibody secretion in milk. Thus, the dilemma is how to develop commercial TGEV vaccines that are capable of stimulating IgA in the gut of sows, but sufficiently attenuated so as not to produce disease in newborn pigs.

Parenteral TGEV vaccines induced even lower or inconsistent protection rates in TGEV/PRCV seronegative swine. They have two major disadvantages: (1) Vaccinated swine develop little or no gut immunity and often get sick when exposed to TGEV, depriving their suckling pigs of milk. (2) The low titer IgG and no IgA TGEV antibodies in milk of vaccinated sows fail to provide optimal passive protection to suckling pigs. Currently available parenterally administered TGEV vaccines may be more effective in boosting immunity in pregnant swine previously infected with TGEV or PRCV than in initiating immunity in seronegative pregnant swine. These vaccines may be especially useful in herds in which endemic TGE is a problem (Stepanek et al. 1979).

TGE vaccination of neonatal or weaned pigs

Active immunization of suckling or feeder pigs could be important for control of endemic infections, especially in newly weaned pigs, in which TGEV infections result in increased mortality. Live attenuated and inactivated TGEV vaccines have been licensed in the United States for oral or intraperitoneal administration, respectively, shortly after birth. However, the presence of maternal antibodies in vaccinated pigs decreased or completely suppressed (Furuuchi et al. 1978; Hess et al. 1982; Lanza et al. 1995; Sestak et al. 1996) active antibody production following oral administration of attenuated TGEV vaccines. Other approaches using recombinant TGEV proteins (reviewed in next section) have been used in attempts to actively immunize young pigs against TGEV.

Recombinant vaccine approaches

Among the major structural proteins of TGEV, the S protein contains immunodominant epitopes recognized by VN antibodies. Epitopes for continuous domains (Delmas and Laude 1990) were incorporated into synthetic peptides derived from the S protein (Posthumus et al. 1991). However, a peptide containing the major T helper cell epitope derived from the N protein has been reported to cooperate with the S protein for *in vitro* induction of TGEV antibody (Anton et al. 1996).

To express the TGEV S (or S epitopes), M, or N proteins, several prokaryotic and eukaryotic systems such as *E. coli*, *Salmonella*, adenovirus, vaccinia virus, pox virus, baculovirus, DNA vectors, and plants were used (Enjuanes et al. 1992; Godet et al. 1991; Gomez et al. 2000; Meng et al. 2013; Park et al. 1998; Shoup et al. 1997; Smerdou et al. 1996; Torres et al. 1996; Tuboly et al. 2000; Yuan et al. 2015). In some studies (Torres et al. 1996), but not others (Gomez et al. 2000; Smerdou et al. 1996; Tuboly et al. 2000), protective antibodies were induced in inoculated animals correlating with partial protection (Park et al. 1998; Shoup et al. 1997). A novel approach to

passive immunization was suggested by feeding the recombinant immunoproteins capable of inducing TGEV VN antibodies to sows to confer passive immunity to piglets (Bestagno et al. 2007). The approach may be cost effective by expressing these proteins in plants (Monger et al. 2006).

Various levels of VN antibodies and protection were induced using eukaryotic vectors to express the TGEV S glycoprotein encoding the glycosylation-dependent antigenic determinants (sites A and B) with or without sites C and D (Figure 31.3). The baculovirus- or vaccinia virus-expressed S glycoprotein of TGEV induced low titers of VN antibodies in serum, colostrum, and milk, but low or no protection (Godet et al. 1991; Hu et al. 1985; Shoup et al. 1997; Tuboly et al. 1995). Only S glycoprotein constructs containing antigenic site A induced high VN antibody titers. Sites C and D induced only low titer VN antibodies, but interestingly, they primed pigs for secondary serum antibody responses after challenge (Shoup et al. 1997).

Similar findings were evident in studies using the same baculovirus-expressed S constructs administered IM to boost antibody responses in sows vaccinated orally with attenuated TGEV vaccines: the partial protection rates were comparable with IM boosting with attenuated TGEV vaccine (Park et al. 1998). Baculovirus-expressed TGEV structural proteins (S, N, and M) coadministered IP with *E. coli* mutant LT adjuvant induced TGEV IgA antibody responses associated with reduced TGEV shedding in challenged pigs (Sestak et al. 1999b).

Recent studies have used molecular approaches to develop vectored TGEV vaccines and test them in porcine and murine models. A human adenovirus engineered to express the TGEV or PRCV S proteins (Callebaut et al. 1996; Torres et al. 1996; Tuboly and Nagy 2001) elicited variable protection against TGEV mortality and little protection against TGEV or PRCV infection. An oral *Lactobacillus casei*-based vaccine expressing repetitive (20X and 40X) peptides of the antigenic D site of TGEV S protein induced humoral and T-cell-based immune responses and Th17 polarization in mice (Jiang et al. 2014, 2016). Additionally, an attenuated *Salmonella typhimurium* vector expressing TGEV N or M proteins and a recombinant *Bacillus subtilis* strain expressing TGEV S protein induced T-cell proliferation, antibody and cytokine responses in mice, and antibody responses in pigs, respectively (Mou et al. 2016; Qing et al. 2016; Zhang et al. 2016a). Yuan et al. (2015) expressed the A epitope of the S protein in swinepox virus and demonstrated that this vaccine administered to sows was immunogenic and protected piglets against clinical disease. DNA plasmids were generated for PEDV and TGEV for the development of DNA vaccines that were immunogenic in mice, but not tested in pigs (Meng et al. 2013).

An effective TGEV vaccine should primarily elicit an intestinal immune response (Saif and Jackwood 1990; Saif and Sestak 2006; VanCott et al. 1993). Further improvements of TGEV vaccines might be achieved by the use of mucosal adjuvants/delivery systems such as immunostimulating complexes (ISCOMs), vitamin A, probiotic bacteria, biodegradable microspheres, or infectious recombinant TGEV clones engineered to enhance TGEV immunogenicity and reduce pathogenicity (Chattha et al. 2015; Enjuanes et al. 2005). Studies of TGEV infectious cDNA minigenomes indicate that this approach also can be used for targeted delivery of immunogens derived from other pathogens to the intestine or respiratory tract.

Porcine epidemic diarrhea virus

Relevance

In 1971, acute outbreaks of diarrhea in feeder and finishing pigs were observed in England (Oldham 1972). The disease spread to other European countries, and the name “epidemic viral diarrhea” (EVD) was adopted. In 1976, similar outbreaks were observed, but in swine of all ages, including suckling pigs (Wood 1977) and, in 1978, a CoV-like agent was associated with the outbreaks in piglets (Chasey and Cartwright 1978; Pensaert and de Bouck 1978). Experimental inoculations with the Belgian isolate (CV777) revealed its enteropathogenicity for piglets and growing pigs (Debouck and Pensaert 1980), and the names “porcine epidemic diarrhea” (PED) and PED virus (PEDV) were adopted (Debouck et al. 1982). In the 1970s and 1980s, PEDV caused widespread epidemics in Europe, with severe losses in suckling pigs. Since then, PEDV has been associated more often with isolated outbreaks and recurrent diarrheic problems in weaned and feeder pigs. However, epidemics also occurred, as in Italy in 2005–2006.

In Asia, PEDV epidemics were first reported in 1982 and outbreaks continued through the 1990s and 2000s. The situation changed in 2010 when PED outbreaks in China caused by highly virulent PEDV strains resulted in the loss of >1 million piglets in 1 year (Sun et al. 2012). In 2013, PEDV outbreaks were reported in the United States, likewise with severe losses (Stevenson et al. 2013).

The initial PEDV strains are referred to as classical PEDV strains (Chen et al. 2013). The strains identified since 2010 are considered emerging PEDV strains. Variants of the emerging PEDV strains containing insertions and deletions in the S gene (“S INDEL” strains) were first detected in the United States (Lin et al. 2016; Wang et al. 2014b). By the end of 2016, the emerging PEDV strains, both non-S INDEL and S INDEL, had

spread throughout North and South America, Asia, and Europe.

The situation continues to evolve. For example, recombinant enteric CoVs between TGEV and PEDV have been detected in Italy (2009–2012), in Germany (2012), and in Eastern Europe (2016) (Akimkin et al. 2016; Belsham et al. 2016; Boniotti et al. 2016).

Etiology

Morphologic and physicochemical properties of PEDV (Figure 31.2c) are similar to those of other members of the family *Coronaviridae* (Figure 31.2). Based on genetic and antigenic criteria, PEDV is included in the genus *Alphacoronavirus* together with bat coronavirus (BtCoV)/512/2005, TGEV, PRCV, FECoV, FIPV, CCoV, and HCoV 229E. Based on phylogenetic analysis of the complete genomes, the global PEDV strains are divided into two major groups: the classical PEDV strains that first emerged in the 1970s in Europe and the PEDV strains appeared after 2010 (Lin et al. 2016) (Figure 31.1). The emerging PEDV strains are further divided into “non-S INDEL” (mainly highly virulent) and “S INDEL” subgroups (Figure 31.1) because the former and latter cause severe and mild PED, respectively, in the field and in experimental pig challenge studies (see “Pathogenesis”). The S INDEL strains contain insertions and deletions similar to the classical PEDV strains in the S1 subunit of the S protein (Vlasova et al. 2014). They likely resulted from multiple recombination events between the classical and emerging PEDV strains in Asia, perhaps related to widespread use of live classical PEDV vaccine strains in swine. Other minor PEDV variants have been reported, e.g. US TC-PC177, USA/OK10240-8/2017, and Japanese TTR-2 strains, bearing large deletions (194–200 aa) in the N-terminal domain (NTD) of the S protein (Oka et al. 2014; Suzuki et al. 2015; Zhang et al. 2018). The 197-aa deletion (residues 34–230) of PC177 strain occurred during Vero cell adaptation, whereas the 194-aa deletion (residues 23–216) of the TTR-2 strain and the 200-aa deletion (residues 31–230) of the OK10240-8 strain were detected in clinical swine samples in Japan and the United States, respectively. Unlike the altered tissue tropism seen for PRCV (enteric to respiratory), the two PEDV strains (PC177 and TTR-2) retained their enteric tropism, but with reduced virulence (Lin et al. 2016; Suzuki et al. 2016).

Although PEDV variants have different insertions or deletions in the S glycoprotein and variation in the S glycoprotein of PEDV may be related to pathogenesis and cross-neutralizing activity, there appears to be only one PEDV serotype (Choudhury et al. 2016; Lin et al. 2016). There is no cross-neutralization between PEDV and TGEV or between PEDV and PDCoV (Lin et al. 2015b; Ma et al. 2016). However, a low degree of cross-reactivity

was observed between PEDV and other animal alphacoronavirus antibodies. For example, a TGEV MAb recognized PEDV N protein (Lin et al. 2015b), TGEV Miller antiserum reacted with PEDV N protein (Gimenez-Lirola et al. 2017), TGEV and PRCV antisera reacted with PEDV M protein (Gimenez-Lirola et al. 2017), and mink alphacoronavirus antiserum reacted with PEDV M and N proteins (Have et al. 1992).

Vero (African green monkey kidney) cells support the growth of PEDV in culture medium supplemented with trypsin (Figure 31.4c). CPE consists of vacuolation and large multinucleated syncytia (Hofmann and Wyler 1988). PEDV also grows in various swine cell lines, including bladder and kidney cells (Shibata et al. 2000; Wang et al. 2016b), ST cells (Liu et al. 2015a), alveolar macrophage cell line 3D4 (Park and Shin 2014), and small intestinal epithelial cells (IECs) (Cao et al. 2015; Cong et al. 2015). As reviewed by Teeravechyan et al. (2016), PEDV can also replicate in bat lung cell line Tb1-Lu (Liu et al. 2015a), duck IEC line MK-DIEC (Khatri 2015), and human liver cell line HuH-7 (Wang et al. 2016b).

pAPN, the cell receptor used by TGEV, was initially considered to be the putative receptor of PEDV with some supporting evidence (Cong et al. 2015; Li et al. 2007; Nam and Lee 2010); however, some recent studies argue that pAPN may not be a functional receptor for PEDV (Li et al. 2017; Shirato et al. 2016).

Public health

PEDV is only infectious for swine and does not play a known role in public health.

Epidemiology

Classical PEDV regularly caused epidemics in Europe from 1971 until the late 1980s, but reports after 2000 are rare. An epidemic in Italy (2005–2006) affected 63 herds, but mortality was largely restricted to suckling piglets (Martelli et al. 2008). Until the emergence of new PED outbreaks in 2014, PEDV was not considered important, and therefore, the prevalence of classical PEDV in Europe is unknown. Except for an outbreak associated with the emerging non-S INDEL strain in Ukraine in 2014, subsequent outbreaks in France, Germany, Belgium, Slovenia, and the Netherlands were due to the emerging S INDEL strains (Lin et al. 2016).

In Asia, classical PED appeared in China in the late 1970s, causing serious losses in many provinces (Wang et al. 2016a; Xuan et al. 1984). PED was recognized in Japan in 1982 (Kuwahara et al. 1988; Sueyoshi et al. 1995; Takahashi et al. 1983) and Korea in 1993 (Chae et al. 2000; Hwang et al. 1994; Kweon et al. 1993), but is known to be present in India (Barman et al. 2003) and Thailand

(Puranaveja et al. 2009). In 2010, despite the widespread use of PEDV strain CV777 vaccines, severe PEDV outbreaks due to non-S INDEL strains occurred in China (Sun et al. 2012, 2016; Wang et al. 2016a). Later, emerging S INDEL strains were also detected in China (Wang et al. 2016a).

Since 2013, the emerging non-S INDEL PEDV strains have been detected in other Asian countries/regions outside of China, including Japan (Masuda et al. 2015), South Korea (Kim et al. 2015), Vietnam (Vui et al. 2014), Thailand (Cheun-Arom et al. 2015), Taiwan (Lin et al. 2014), and the Philippines (Kim et al. 2016). The S INDEL PEDV was also detected in Japan in 2013 (Suzuki et al. 2015) and Korea in 2014 (Lee et al. 2014).

The first highly virulent PED outbreak caused by non-S INDEL PEDV occurred in swine farms in the United States in April 2013 (Stevenson et al. 2013), followed by the detection of milder PED outbreaks caused by the S INDEL PEDV in January 2014 (Wang et al. 2014b). From 2013 to 2014, PEDV killed approximately 7 million piglets in the United States. In January 2017, PEDV had spread to 39 US states and to Puerto Rico. PEDV has also spread to other countries (e.g. Canada and Mexico) in the Western Hemisphere (Lin et al. 2016). PEDV has not been reported in Africa or Australia.

Direct or indirect fecal–oral transmission is the main route of PEDV transmission. Contaminated equipment, feed and feed ingredients, transportation, or personnel may serve as vehicles for PEDV transmission (Dee et al. 2014, 2016; Schumacher et al. 2016). Evidence of PEDV aerosol transmission has been reported in some (Alonso et al. 2014), but not other studies (Niederwerder et al. 2016). In emerging non-S INDEL PEDV experimentally infected 4-week-old pigs, infectious virus excretion assessed by PEDV transmission to susceptible sentinel pigs lasted 14–16 days (Crawford et al. 2015). However, at 42 days post-initial oral exposure, some pigs still shed PEDV RNA in feces, illustrating discordance between prolonged detection of PEDV RNA in feces and the transmission of infectious PEDV to susceptible pigs.

After an outbreak on a breeding farm, PEDV can become endemic through a cycle of infection of consecutive litters as they lose lactogenic immunity at weaning. Although a study from South Korea showed a PEDV infection rate of 9.75% in wild boars (Lee et al. 2016a), their role in the maintenance and transmission of PEDV is unknown.

Pathogenesis

The pathogenesis of PED is related to the age of pigs at the time of infection, virus strain virulence, inoculation routes, and doses.

The PEDV pathogenesis was first studied in piglets (3 days of age) orally inoculated with the classical PEDV

CV777 isolate (Coussement et al. 1982; Debouck et al. 1981) (Table 31.1). Clinical signs were observed after 22–36 hours. Viral replication occurred mainly in the cytoplasm of villous epithelial cells throughout the small intestine as early as 12–18 hours post inoculation (PI), peaking at 24–36 hours. Infection resulted in degeneration of enterocytes, leading to a reduction in the villous height/crypt depth (VH/CD) ratios from the normal 7:1 to $\leq 4:1$. The pathogenic features of classical PEDV in the small intestine of piglets were very similar to those of TGEV, but somewhat less pronounced (Figure 31.5). PEDV replication was also observed in the colonic epithelium where slight cell degeneration was seen (Ducatelle et al. 1982). Occasionally, PEDV-positive crypt cells were also observed by IHC or IF staining, but the enterocyte regeneration capacity was preserved (Debouck et al. 1981; Sueyoshi et al. 1995). Shibata et al. (2000) showed that SPF pigs inoculated with field PEDV between the ages of 2 days and 12 weeks developed age-dependent resistance. That is, mortality was only observed in 2- to 7-day-old piglets. Pathogenic features of PEDV caused by classical PEDV strains described in Korea and Japan are very similar to those reported in Europe (Kim and Chae 2003; Sueyoshi et al. 1995).

Lohse et al. (2016) studied PEDV pathogenicity in 5-week-old pigs using classical PEDV strain (TC Br1/87, P3), an emerging S INDEL strain in Germany, and a non-S INDEL strain in the United States. Unfortunately, the S INDEL PEDV failed to infect pigs. Compared with the classical PEDV-infected pigs, the non-S INDEL PEDV-infected pigs had more severe clinical signs and histopathological changes, higher peak viral RNA shedding titers in feces, and longer detection of viral RNA in serum. These results suggested that the emerging non-S INDEL PEDV was more virulent than classical PEDV. However, concerns related to data interpretation include the following: (1) The Br1/87 inoculum was the Vero cell culture-adapted virus at passage 3, whereas the non-S INDEL was the wild-type virus from pigs and its infectious dose was not determined, so the disease outcomes may be due to different infectious doses. (2) The non-S INDEL inoculum contained a low amount of rotavirus by conventional RT-PCR. Therefore, coinfection with PEDV and rotavirus may influence disease severity. Generally, the pathogenesis and the age-dependent resistance of the emerging non-S INDEL PEDV strains were similar to those of the classical PEDV strains (Table 31.1) (Jung et al. 2014, 2015a; Madson et al. 2014; Niederwerder et al. 2016; Pensaert and Martelli 2016; Stevenson et al. 2013). Compared with the emerging non-S INDEL PEDV strains, S INDEL PEDV-infected piglets had lower mortality rates and less severe histopathological changes (milder villous atrophy) and less antigen in the small intestine (Table 31.1) (Chen et al. 2016a; Lin et al. 2015a). The pathogenicity of PEDV

TTR-2 and TC-PC177 strains that have a large deletion in the NTD of the S protein was milder compared with that of the emerging non-S INDEL PEDV strains (Lin et al. 2016; Suzuki et al. 2016).

The infectious doses of PEDV differ for different ages of pigs: 100- to 1000-fold less PEDV was needed to infect younger pigs compared with the dose required to infect 3-week-old pigs (Thomas et al. 2015). The infectious dose of an emerging non-S INDEL PEDV strain (PC22A) was as low as 0.1 plaque-forming unit (PFU)/pig in 4-day-old Cesarean-derived colostrum-deprived (CDCD) piglets (Liu et al. 2015b). Doses of 0.1 PFU/pig and 1–10,000 PFU/pig caused diarrhea in 40 and 100% piglets, respectively. Thomas et al. (2015) compared the infectious doses for another emerging non-S INDEL PEDV strain (USA/IN19338/2013) in 5-day-old and 3-week-old pigs: 0.056 and 0.56–5600 TCID₅₀/pig caused diarrhea in 25 and 100% of neonatal piglets, respectively, and at least 100-fold higher doses (56–5600 TCID₅₀/pig) caused diarrhea in 100% of 3-week-old pigs. However, the infectious dose for older pigs, such as finisher pigs, has not been determined, but is expected to be higher than that needed to infect weaned pigs, as was observed for TGEV (Witte and Walther 1976).

During the acute phase of PEDV infection, viral RNA was detected transiently in the serum of PEDV-infected suckling and weaned pigs (Chen et al. 2016a; Jung et al. 2014, 2015a; Lohse et al. 2016; Suzuki et al. 2016). Peak RNA titers in serum were low (7–8 log₁₀ GE/mL) compared with concurrent high peak RNA titers in feces (11–12 log₁₀ GE/mL) (Jung et al. 2015a). Whether detection of viral RNA in serum represents infectious virus and the role of viremia in PEDV pathogenesis is unknown. In general, PEDV RNA titers are about 4–6 log₁₀ higher than infectious titers (PFU or TCID₅₀) depending on different PEDV strains and/or the real-time RT-PCR assays (Jung et al. 2014; Song et al. 2016; Thomas et al. 2015).

Low levels of PEDV RNA were also detected in other tissues, such as the lung, liver, spleen, and muscle of pigs euthanized during acute PEDV infection (Chen et al. 2016a; Lohse et al. 2016; Park and Shin 2014). However, because the blood was not drained before collecting each tissue, the viral RNA was most likely from blood, except for the lungs, where PEDV antigens were detected by IHC (Park and Shin 2014). In the later study, the researchers found that a wild-type Korean PEDV non-S INDEL strain CNU-091222-01/2009 replicated in alveolar macrophages of infected pigs. Because no others have reported the detection of PEDV in the lungs, whether this is a unique characteristic of earlier emerging non-S INDEL strains (pre-2010) is unknown and needs to be investigated. In addition, PEDV RNA was detected from 40.8% (20/49) of sow milk samples during the emerging PEDV epidemics (Sun et al. 2012). TGEV replicated in the mammary glands of sows injected intramammarily

Table 31.1 Comparative pathogenesis of different clusters of PEDV in experimentally infected piglets (younger than 6 days of age).

PEDV strain	Inoculum/dose per pig	Pig type/age (day) at inoculation	Villous atrophy (VH:CD ratios)	Onset of clinical signs (hpi)	Vertical location of PEDV		Longitudinal distribution of PEDV		References
					Villous	Crypt	D, J, I	C	
Classical									
CV777	Fecal suspension/4 log ₁₀ PID	CDCD/2–3	Moderate to severe (1.5–4.2)	22–36	+++ (entire)	+	D, J, I (cont)	+	Coussement et al. (1982)
SNUVR971496	Cell culture (P3)/6.8 log ₁₀ TCID ₅₀	Colostrum-deprived/1	Severe (1.1–3.3)	12–36	+++ (entire)	–	D, J, I (cont)	–	Kim and Chae (2003)
Non-S INDEL									
IN19338	Cell culture (P7)/0.056–5600 TCID ₅₀	Conventional/5	Severe (1.2–1.7)	24	+++ (entire)	NR	D, J, I (cont)	NR	Thomas et al. (2015)
PC22A	Cell culture (P3)/1–4 log ₁₀ PFU	CDCD, conventional/3–4	Severe (0.8–2.3)	< 24	+++ (entire)	+	D, J, I (cont)	+	Liu et al. (2015b)
S INDEL									
Iowa106	Fecal suspension/10–12 log ₁₀ GE	Conventional/4	Moderate to severe (1.4–5.4)	24–72	++ (entire)	–	D (patchy), J, I (cont)	–	Lin et al. (2015)
IL20697	Cell culture/5 log ₁₀ TCID ₅₀	Conventional with milk replacer/5	Mild	NR	+ / ++	NR	D, J (NR), I (patchy)	+	Chen et al. (2016a)

Source: This table is adapted/updated from Saif (1989). Reproduced with permission of Taylor and Francis.

PID, pig infectious dose; TCID₅₀, 50% tissue culture infectious dose; PFU, plaque-forming unit; GE, genomic equivalent; CDCD, Cesarean-derived colostrum-deprived; SPF, specific pathogen free; VH: CD, villous height/crypt depth ratio; hpi, hours' post inoculation; D, duodenum; J, jejunum; I, ileum; cont, continuous; NR, not reported; –, +, ++, and +++ denotes none, less than 30%, 30–60%, and more than 60% of villous enterocytes that were positive for PEDV antigens, respectively.

with live TGEV during lactation (Saif and Bohl 1983). Whether PEDV replicates in the mammary glands of sows, whether the PEDV RNA represents infectious PEDV, or whether the PEDV RNA in milk is from the saliva of their infected nursing piglets is unclear. Although some PEDV strains may replicate at low levels outside of the intestine, it remains unclear whether it contributes to PEDV pathogenesis.

PEDV infection results in massive loss of enterocytes and the malfunction of infected enterocytes, leading to maldigestive and malabsorptive diarrhea (Coussement et al. 1982; Debouck et al. 1981; Jung et al. 2006). During acute PEDV infection, gut integrity was reduced, leading to loss of water into the intestinal lumen and high osmotic pressure (Annamalai et al. 2015). The following factors may contribute to the more severe clinical signs, higher mortality rates, and slower recovery in PEDV-infected neonatal piglets compared with weaned pigs:

- 1) Slower turnover of villous enterocytes in neonatal piglets (5–7 days) compared with 2–3 days in 3-week-old weaned pigs (Jung et al. 2015a; Moon et al. 1975).
- 2) Increased numbers of intestinal stem cells and proliferation of crypt cells occurred later (3 days post-PEDV) in neonatal piglets than in weaned pigs (1 day post-PEDV) (Jung et al. 2015a).
- 3) Deficiency in innate immunity in suckling pigs compared with weaned pigs (Annamalai et al. 2015) (see “Immunity”).

Clinical signs

PED shares most clinical features with TGE including watery diarrhea, vomiting, anorexia, and depression. On breeding farms, pigs of all ages become sick. Morbidity approaches 100% in piglets, but can vary in sows. Piglets up to 1 week of age may die from dehydration, and mortality ranges from 50 to 100%. Older pigs recover after about 1 week. In sows, diarrhea is variable, and they may only show depression and anorexia. In fattening pigs, all pigs in the unit may have watery feces within a week and often show severe anorexia and depression.

The disease on a breeding farm is self-limiting and stops when the pregnant sows develop lactogenic immunity to protect their offspring. The interval between onset and cessation of the disease is generally 3–4 weeks, but may be much longer in large breeding farms with multiple separated units. After the acute outbreak has passed, diarrhea may persist on the farm in weaned pigs and become recurrent (Martelli et al. 2008). PEDV may also be involved in a multi-etiological diarrhea syndrome in feeder pigs appearing 2–3 weeks after they enter the fattening units, particularly when the pigs originate from different sources and when new pigs are continuously added to the fattening unit (van Reeth and Pensaert 1994).

Lesions

Lesions have been described in experimentally and naturally infected suckling piglets for classical PEDV (Coussement et al. 1982; Kim and Chae 2003; Pospischil et al. 1981; Sueyoshi et al. 1995), emerging non-S INDEL (Chen et al. 2016a; Jung et al. 2015a; Madson et al. 2015; Stevenson et al. 2013), and S INDEL PEDV infections (Chen et al. 2016a; Lin et al. 2015a). Lesions are confined to the small intestine that is distended with watery, yellowish fluid. Microscopically, vacuolation, syncytia, and exfoliation of small intestinal enterocytes occur mainly on the proximal villi. The small intestinal villi are reduced in length (Figure 31.5d), and the enzymatic activity of the intestine is markedly decreased. This pathology is very similar to that observed in TGE and PDCoV (Figure 31.5). No histopathologic changes have been observed in the colon, although PEDV antigens were detected in vacuolated colonic epithelial cells (Chen et al. 2016a; Debouck et al. 1981; Jung et al. 2014).

Diagnosis

Diagnosis should be made based on both clinical signs and laboratory detection of viral RNA, viral antigens, or increased PEDV antibodies. For the detection of PEDV RNA, the most widely used laboratory diagnostic method is RT-PCR (Ishikawa et al. 1997; Kim et al. 2001; Kubota et al. 1999; Liu and Wang 2016) or real-time RT-PCR (Kim et al. 2007; Wang et al. 2014d; Zhang et al. 2016b). Loop-mediated isothermal amplification (LAMP) assays (Ren and Li 2011; Yu et al. 2015) have been developed for the detection of PEDV RNA but are used less in diagnostic laboratories. Newer technology such as the specific primer-independent metagenomic sequencing (next-generation sequencing) can be used to determine the nearly complete (lacking 5' and 3' ends) viral genome sequences from clinical specimens (Chen et al. 2014; Marthaler et al. 2013). *In situ* hybridization can be used to detect PEDV RNA in fixed tissues (Kim and Chae 2000; Stadler et al. 2015).

Diagnosis can be made by direct demonstration of PEDV and/or its antigens using IF or IHC tests on the small intestinal tissues of pigs euthanized acutely near the onset of diarrhea and prior to the desquamation of enterocytes (Debouck et al. 1981; Guscetti et al. 1998; Jung et al. 2014; Stevenson et al. 2013; Sueyoshi et al. 1995) (Figure 31.6b). PEDV particles can be demonstrated using direct EM or IEM of feces of pigs collected acutely after diarrhea onset. Virus particles are difficult to recognize when the virion spikes are lost or not clearly visible. Furthermore, IEM must be applied to differentiate PEDV from TGEV and PDCoV because the CoVs have identical morphology (Figure 31.2).

Isolation of field strains of PEDV from intestinal contents/homogenates or feces is done in Vero cells or in other cell types. Trypsin treatment and blind passages

may be needed before CPE appears, but early detection can be done by IF staining (Hofmann and Wyler 1988; Shibata et al. 2000). Successful isolation of PEDV in Vero cells is higher with intestinal contents/homogenates than with feces (Chen et al. 2014; Oka et al. 2014).

Antigen-capture ELISAs have been developed for detection of PEDV antigens in feces using polyclonal antibodies and MAbs (Callebaut et al. 1982; Carvajal et al. 1995), but they are not widely used.

Paired serum samples are required for serologic diagnosis of endemic PEDV. Recently, IgG and IgA antibodies to PEDV were detected in oral fluids, suggesting they may be suitable to monitor prior herd exposure to PEDV (Bjstrom-Kraft et al. 2016). PEDV antibodies have been demonstrated with indirect ELISAs using antigens consisting of cell-cultivated virus (Carvajal et al. 1995; Hofmann and Wyler 1990; Kweon et al. 1994; Thomas et al. 2015), or S and N viral proteins extracted from infected Vero cells (Knuchel et al. 1992; Oh et al. 2005), or expressed in bacteria or using mammalian expression systems (Chen et al. 2016b; Gerber et al. 2014; Gerber and Opriessnig 2015; Hou et al. 2007; Okda et al. 2015; Paudel et al. 2014; Wang et al. 2015). Blocking and competitive ELISAs have also been developed for the detection of PEDV antibodies using MAbs or polyclonal antibodies as competitive antibodies (Carvajal et al. 1995; Okda et al. 2015; van Nieuwstadt and Zetstra 1991). Serum IgG antibodies against the N proteins of PEDV can be detected by 9–14 DPI, with titers peaking around 21 DPI and then declining gradually (Okda et al. 2015). Recently, a fluorescent microsphere immunoassay (FMIA) was developed (Gimenez-Lirola et al. 2017; Okda et al. 2015), but it is not widely used due to the need for specific equipment. The VN test in Vero cells is critical to assess VN antibodies to PEDV (Oh et al. 2005; Okda et al. 2015; Paudel et al. 2014; Thomas et al. 2015). These serological assays have been widely used to monitor prior exposure to the virus and to evaluate the efficacy of vaccines.

PEDV infections must be differentiated from TGE, SeCoV, and PDCoV, which in the case of acute diarrhea in swine of all ages can only be done through laboratory testing. Since SeCoVs are recombinants between TGEV (backbone) and PEDV (mainly S protein), only assays targeting both TGEV (any genes except for S gene) and PEDV (S gene) fragments can identify those viruses. In neonatal colibacillosis or rotavirus diarrhea, adult animals are not affected, and sick pigs usually are born from gilts or young sows. Laboratory techniques must be used to differentiate PED from other causes of diarrhea in weaned or feeder pigs.

Immunity

Evidence shows that PEDV has the ability to evade host IFN responses. Of 21 PEDV-encoded proteins, at least

11 proteins have been identified as IFN antagonists, which include both ORF1ab-encoded NS proteins (nsp1, nsp3, nsp5, nsp7, nsp14, nsp15, nsp16), structural proteins (E, M, N), and the accessory protein ORF3 (Ding et al. 2014; Wang et al. 2015; Zhang et al. 2016c). Identification of the virus-encoded IFN antagonists and understanding their mechanism of action may lead to novel therapeutic targets and more effective vaccines.

PEDV-infected suckling pigs had significantly lower NK cell frequencies, undetectable NK cell activity, and lower IFN- γ -producing CD3⁺CD4⁺CD8⁺ NK cells in blood and ileum compared with PEDV-infected weaned pigs (Annamalai et al. 2015). Deficiency in innate immune function of neonatal NK cells may contribute to the more severe PEDV infection in suckling pigs compared with weaned pigs as also reported for TGEV infections (Derbyshire et al. 1969).

Inflammatory responses play a significant role in the pathogenesis of enteric CoVs. Compared with suckling pigs, weaned pigs had a delayed proinflammatory cytokine induction that coincided with the delayed onset of infection, disease, and shedding of PEDV RNA in feces (Annamalai et al. 2015). Toll-like receptor 2 (TLR2), TLR3, and TLR9 may contribute to NF- κ B activation in response to PEDV infection in small IECs *in vitro* (Cao et al. 2015). The viral proteins E and N upregulated IL-8 expression by inducing endoplasmic reticulum stress and subsequent activation of the NF- κ B pathway (Xu et al. 2013a,b).

Humoral immune responses to PEDV infection are very similar to those described for TGEV [reviewed in TGEV section (Chattha et al. 2015; Saif and Sestak 2006)]. VN antibodies are detectable in the serum, but may not play an important role because protection against enteric disease is primarily dependent on the presence of sIgA antibodies in the intestinal mucosa (Chattha et al. 2015; Langel et al. 2016). Immunity may not be long lasting, but a rapid anamnestic response upon reexposure may prevent reoccurrence of disease.

Although PED occurs in pigs of all ages, piglets up to 1 week of age may experience high mortality and need to be protected by maternal antibodies, especially VN and sIgA, via colostrum and milk from immunized dams. The mechanisms of lactogenic protection described for TGEV infection apply to PED as well (reviewed in TGEV section [Chattha et al. 2015; Langel et al. 2016]). Lactogenic immunity is induced in sows by intestinal infection with PEDV, which then activates the gut–mammary–sIgA axis. Pigs lose lactogenic protection at weaning and soon become susceptible to PEDV infection. Cell-mediated immunity likely plays a role in viral clearance, but there is no experimental data on this topic. PEDV may persist on the farm in susceptible pigs as part of recurring weaning diarrhea after an acute outbreak.

Prevention and control

PEDV is highly contagious, and strict sanitation and biosecurity are required to prevent virus entrance. Do not commingle sources or groups of pigs; ensure facilities and transportation vehicles are thoroughly washed, disinfected, and dried before pigs enter; and do not share boots, clothing, or equipment between different ages of pigs.

Feedback (intentional exposure of sows to virus using feces or small intestines from acutely infected piglets) will stimulate lactogenic immunity in the sow herd, reduce clinical signs in piglets, and shorten clinical outbreaks. Feedback may also be used in the nursery, grower, or finisher pigs, but nose-to-nose contact and fecal–oral spread will quickly contaminate the entire facility. It should be recognized that other pathogens present in clinically affected animals can be transmitted via the feedback process.

In Europe, the disease has been of insufficient economic importance to develop vaccines. In China, various inactivated and/or attenuated bivalent (TGEV and PEDV) or trivalent (TGEV, PEDV, and rotavirus strain NX) vaccines based on the classical PEDV strains have been available as early as in 1999 (Ma et al. 1995; Sun et al. 2016; Wang et al. 2016a). However, classical PEDV vaccines were not efficacious in protecting pigs against the highly virulent non-S INDEL PEDV infection (Li et al. 2012; Sun et al. 2012; Wang et al. 2013) and newer vaccines (e.g. a bivalent inactivated PEDV and TGEV vaccine based on the emerging non-S INDEL PEDV strain AJ1102) have appeared in the market. In Japan, a commercial attenuated PEDV vaccine based on the classical PEDV 83P-5 strain has been in use since 1997 (Sato et al. 2011). In Korea, attenuated vaccines based on strains KPEDV-9 (Kweon et al. 1999) or DR13 (Song et al. 2007) were commercialized in 1999 and 2004, respectively. Reportedly, not all sows given the vaccines developed protective lactogenic immunity (Song et al. 2015a). Two commercial vaccines are available in the United States. The first vaccine (June 2014) was developed using a replication-deficient Venezuelan equine encephalitis virus packaging system to express the PEDV S protein (Crawford et al. 2016). The second vaccine (September 2014) is an inactivated whole virus vaccine based on an emerging non-S INDEL PEDV strain (Crawford et al. 2016).

Porcine deltacoronavirus

Relevance

In February 2014, acute outbreaks of diarrhea associated with PDCoV were observed in sows and their piglets on five Ohio farms in the United States (Wang et al. 2014a).

Previously, PDCoV had been reported in the feces of domestic pigs in China in 2012 (Woo et al. 2012), but the role of the virus as an enteric pathogen was unclear at that time. PDCoV has spread nationwide in the United States (Wang et al. 2014c) and caused deaths among suckling pigs (Anon 2014). Experimental studies verified that US PDCoV isolates are enteropathogenic in nursing pigs, as evident by acute, watery diarrhea and severe intestinal lesions (Figure 31.5c and e) (Chen et al. 2015; Jung et al. 2015b). However, the clinical impact and disease severity of PDCoV is less than that of epidemic PEDV and TGEV (Anon 2014).

Since the PDCoV outbreaks in the United States, it has also been identified on swine farms in Canada, Korea, China, Thailand, Vietnam, and Laos PDR, but in Canada and Korea, PDCoV failed to spread nationwide (Lee et al. 2016b; Marthaler et al. 2014b). Differential diagnosis of PDCoV, PEDV, and TGEV is critical to control CoV diarrheas in pig farms, especially in the regions where these CoVs have emerged or reemerged.

Etiology

PDCoV belongs to the genus *Deltacoronavirus* of the family *Coronaviridae*. Morphologic and physicochemical properties of PDCoV are similar to those of other members in the family *Coronaviridae* (Figure 31.2).

All global PDCoV strains overall share high nucleotide identities (Zhang 2016) (see “Epidemiology”). However, a comprehensive genetic analysis of global strains revealed that US/Korean PDCoV strains clustered together, Chinese strains clustered separately, and Thai strains formed another cluster (Zhang 2016) (Figure 31.1). Chinese PDCoV strains had multiple mutation or deletion sites in their S, NSP, or 3′ untranslated region (UTR) genes, whereas these mutations were not found in the genomes of US PDCoV strains (Wang et al. 2016c). Several investigators reported no cross-reactivity of PDCoV with antibodies to either PEDV or TGEV (Chen et al. 2015; Ma et al. 2015). However, another study reported antigenic cross-reactivity between US PDCoV and PEDV strains, possibly sharing at least one epitope on their N proteins (Ma et al. 2016).

LLC porcine kidney (LLC-PK) and ST cells supplemented with exogenous trypsin or pancreatin support the isolation and serial propagation of PDCoV in cell culture (Hu et al. 2015). The CPE consisted of enlarged and rounded cells and then cell shrinkage and detachment.

Recent studies have demonstrated that PDCoV employs pAPN as a major receptor for cellular entry, although it remains to be elucidated whether another receptor is involved in PDCoV infection (Li et al. 2018; Wang et al. 2018; Zhu et al. 2018).

Public health

There is no evidence that PDCoV is infectious for humans or plays a role in public health.

Epidemiology

The ancestral origin of PDCoV is unclear, but considering that PDCoV emerged recently, PDCoV may be incompletely adapted to pigs. Molecular surveillance in China and Hong Kong in 2007–2011 detected DCoVs only in pigs and wild birds (Woo et al. 2012). However, DCoVs were previously isolated from rectal swabs of small mammals, including Asian leopard cats and Chinese ferret badgers, at Chinese live animal markets in 2005–2006 (Dong et al. 2007). Their helicase and S genes were closely related to those of PDCoV. The data suggest the potential interspecies transmission of DCoVs between these wild small mammals, pigs, and birds. A recent study also revealed that PDCoV-inoculated gnotobiotic (Gn) calves exhibited an acute infection without disease or intestinal lesions, but with persisting fecal viral RNA shedding and seroconversion (Jung et al. 2017). Consequently, the potential ability of PDCoV and other DCoV isolates from birds or small mammals to infect different species should be investigated.

In February 2014, PDCoV was detected in US swine. Among 42 fecal or intestinal samples collected from diarrheic sows and piglets on five Ohio farms, 39 (92.9%) were positive for PDCoV by RT-PCR (Wang et al. 2014a). The PDCoV Ohio strain HKU15-OH1987 had a 99% nucleotide identity to the two prototype strains of PDCoV, HKU15-44 and HKU15-155, reported in Chinese pigs in 2012. During a similar period, genetically similar strains, USA/IA/2014/8734 and SDCV/USA/Illinois121/2014, were identified by other US diagnostic laboratories (Li et al. 2014; Marthaler et al. 2014a). Among PDCoV-positive premises, coinfection with PEDV is common (Zhang 2016). The origin of PDCoV in US swine is unknown, although there was serologic and virologic evidence suggesting its presence in the United States prior to its detection in February 2014 (Sinha et al. 2015; Thachil et al. 2015).

PDCoV has also been reported in Canada, Korea, mainland China, Thailand, Vietnam, and Lao PDR (Dong et al. 2015; Janetanakit et al. 2016; Lee et al. 2014; Lorsirigool et al. 2016; Marthaler et al. 2014a; Saeng-Chuto et al. 2017; Song et al. 2015b). The Korean PDCoV strains (KUN14-04, SL2, and SL5) had high nucleotide identities (98.7–99.2%) to US PDCoV strains (Lee et al. 2014, 2016b). In mainland China, coinfections with PDCoV and PEDV were common (Dong et al. 2015; Song et al. 2015b). Chinese PDCoV strains had $\geq 98.6\%$ nucleotide identities with each other and $\geq 97.1\%$ nucleotide identities with the global PDCoV strains (Zhang 2016).

The Thai PDCoV strains were highly similar to each other but formed a novel phylogenetic cluster separated from US and Chinese PDCoVs (Janetanakit et al. 2016; Saeng-Chuto et al. 2017). PDCoVs identified in Lao PDR were more closely related to Thai PDCoVs, whereas PDCoVs detected in Vietnam were more closely related to US PDCoVs (Saeng-Chuto et al. 2017).

Fecal–oral is the main PDCoV transmission route. Feces and/or vomitus and other contaminated fomites are major transmission sources of the virus. Based on experimental findings (Hu et al. 2016; Ma et al. 2015; Zhang 2016), diarrhea in infected piglets was observed for approximately 5–10 days, with persisting viral RNA shedding for up to 19–28 days in feces and for up to 42 days in oral fluids.

Pathogenesis

The pathogenesis of PDCoV has been studied in Gn or conventional piglets orally inoculated with US and/or Chinese PDCoV isolates at 5–21 days of age (Chen et al. 2015; Dong et al. 2016; Hu et al. 2016; Jung et al. 2015b; Ma et al. 2015). Clinical signs (diarrhea and/or vomiting) occurred at 1–3 DPI. Replication of PDCoV is confined to the small and large intestinal epithelia. PDCoV-infected enterocytes rapidly undergo acute necrosis (Jung et al. 2016a), leading to marked villous atrophy in the small intestine (Figure 31.5c and e), but not in the large intestine. During acute infection, PDCoV antigens are detected mainly in the villous epithelium of the atrophied mid-jejunum (Figure 31.6c) to ileum and, to a lesser extent, in duodenum, proximal jejunum, and cecum/colon. Occasionally, a few PDCoV antigens are detected in crypt epithelial cells of the jejunum and ileum (Jung et al. 2016a) and immune cells in the intestinal lamina propria, Peyer's patches, and mesenteric lymph nodes (Hu et al. 2016). Frequently, acute viremia with low PDCoV RNA titers in serum was observed (Chen et al. 2015; Hu et al. 2016; Ma et al. 2015). After pigs recovered from clinical disease, larger amounts of PDCoV antigens were detected in the gut lymphatic tissues (Hu et al. 2016). PDCoV antigens were not detected in other organs, including the respiratory tract of pigs (Jung et al. 2016b). However, by real-time RT-PCR, PDCoV RNA could be detected in low to moderate quantities in multiple organs, possibly due to viremia (Chen et al. 2015; Ma et al. 2015).

Clinical signs

Clinical signs of PDCoV infection in suckling and older pigs are similar, but milder, than those of PEDV and TGEV infections. In suckling piglets, PDCoV induces acute, watery diarrhea, frequently accompanied by vomiting, leading to dehydration, loss of body weight,

lethargy, and death. Experimentally, the onset of diarrhea coincided with, or was detected 1–2 days later than, the first detection of viral RNA in feces (Jung et al. 2015b; Ma et al. 2015).

Diarrhea is probably a consequence of malabsorption due to massive loss of absorptive enterocytes, resulting in decreased brush border membrane-bound digestive enzymes, similar to PEDV infection (Jung et al. 2006). Mild vacuolation observed in the infected colonic epithelial cells may interfere with the reabsorption of water and electrolytes (Jung et al. 2015b). Dehydration is also exacerbated by vomiting.

Seronegative pigs of all ages are susceptible to PDCoV infection. On seronegative farrowing farms, morbidity can reach up to 100% in piglets but can vary in sows. Based on field observations in US swine in 2014 (Anon 2014), PDCoV infection caused a number of deaths (up to a 40% mortality) among suckling pigs. Similarly, PDCoV diarrhea outbreaks in breeding farms in China and Thailand resulted in 64–80% mortality among suckling piglets. PDCoV infection is more severe and more likely to result in mortality in piglets as compared with older pigs. On many farms, morbidity and mortality may be affected by coinfections with other enteric viruses, such as PEDV and rotavirus (Marthaler et al. 2014b; Song et al. 2015b). The disease on breeding farms is self-limiting and stops when pregnant sows develop lactogenic immunity to protect their offspring.

PDCoV infection shares several clinical features with TGEV and PEDV infections, but the virus likely spreads more slowly among pigs, possibly due to its lower adaptation to pigs. Relative to PEDV infections, PDCoV-infected pigs shed less PDCoV RNA in the feces (Jung et al. 2015b), indicating lower replication of PDCoV in the intestine of pigs. This aspect of PDCoV infection may be a contributing factor to its lower mortality in nursing piglets, as compared with PEDV infections.

Lesions

Lesions have been described in suckling piglets experimentally and naturally infected with US, Chinese, or Thai PDCoV strains (Chen et al. 2015; Dong et al. 2016; Hu et al. 2016; Janetanakit et al. 2016; Jung et al. 2015b; Ma et al. 2015; Wang et al. 2016c). Lesions resemble those observed in TGEV and PEDV infections (Figure 31.5), but are usually less extensive.

Gross lesions are limited to the gastrointestinal tract and are characterized by thin and transparent intestinal walls (proximal jejunum to colon) with accumulation of large amounts of yellow fluid. The stomach is frequently filled with curdled milk. The transparency and fragility of affected intestines are milder, as compared with PEDV and TGEV infections. Histological lesions are characterized by acute, multifocal to diffuse, mild to severe

atrophic enteritis in the proximal jejunum to ileum (Figure 31.5c and e), occasionally accompanied by mild vacuolation of the superficial epithelial cells in the cecum and colon (Jung et al. 2015b). No villous atrophy or histologic lesions were evident in the duodenum, which coincided with few PDCoV antigen-positive duodenal epithelial cells (Chen et al. 2015; Jung et al. 2015b). During acute infection, vacuolated enterocytes or massive cell exfoliation was seen on the tips or the entire villi in the jejunum and ileum. Atrophied villi were frequently fused and covered with a degenerated or regenerated flattened epithelium. Infiltration of inflammatory cells, such as macrophages, lymphocytes, and neutrophils, was evident in the lamina propria. No lesions were seen in other organs.

Diagnosis

The diagnostic approaches described earlier for TGEV and PEDV also apply to PDCoV diagnosis. Laboratory techniques should be used to differentiate PDCoV infection from PEDV, TGEV, and rotavirus diarrhea in pigs. Definitive diagnosis of PDCoV infection includes detection of PDCoV RNA or antigens in the feces or intestinal tissues from diarrheic pigs. Diagnosis can be made by RT-PCR assays that target a conserved region of PDCoV M or N genes (Marthaler et al. 2014b; Wang et al. 2014a; Zhang et al. 2016b), IF or IHC using virus-specific MAbs or polyclonal antibodies (Chen et al. 2015; Jung et al. 2015b; Ma et al. 2015), and *in situ* hybridization (Jung et al. 2015b). A real-time duplex RT-PCR assay for detection of PDCoV and/or differentiation of the virus from PEDV in intestines and feces was developed (Zhang et al. 2016b).

Direct EM can be used to demonstrate PDCoV particles in feces collected from diarrheic pigs (Figure 31.2d), but IEM using hyperimmune or convalescent sera is essential to differentiate PDCoV from PEDV or TGEV (Jung et al. 2015b). Isolation of PDCoV from feces or intestinal tissues was attempted in LLC-PK or ST cells, but the success rate was low except for a few strains (OH-FD22) (Hu et al. 2015). Serologic diagnosis of PDCoV can be conducted by IFA, VN, and ELISA assays. Isotypes of PDCoV antibodies in serum and milk can be quantitated by ELISA using antigens consisting of cell culture-grown virus (Ma et al. 2016) or S1 and N viral proteins (Okda et al. 2016; Su et al. 2016; Thachil et al. 2015).

Immunity

The immune responses of pigs to PDCoV infection are largely undefined, but they are likely similar to those described earlier for TGEV and PEDV. Hu et al. (2016) reported the development of PDCoV antibodies in serum of PDCoV-infected pigs (Hu et al. 2016). Gn pigs orally

inoculated with the original or tissue culture-grown PDCoV strain OH-FD22 had detectable serum IgG, IgA, and VN antibodies by 14 DPI that peaked at 24 DPI, when the pigs had recovered from clinical disease and fecal virus shedding. While PDCoV infection is epidemic, young piglets can be protected by transfer of maternal antibodies via colostrum and milk from immune dams, especially IgA and VN antibodies that neutralize PDCoV in the gut. Lactogenic immunity is expected to be strongly induced in sows by oral infection with PDCoV, which then activates the gut–mammary link, as described earlier for TGEV (Bohl et al. 1972; Saif et al. 1972).

Prevention and control

The prevention and control measures described earlier for TGEV and PEDV infections also apply to PDCoV infection. There are no treatments or vaccines to control PDCoV infection. Preventive or therapeutic antibiotic therapy can be implemented if there is concurrent infection with enteric bacterial pathogens. Symptomatic treatment of suckling pigs with diarrhea includes intraperitoneal administration of bicarbonate fluids and free access to water to alleviate acidosis and dehydration. If mortality is substantial among suckling piglets, feedback methods (intentional exposure of pregnant sows to virus-positive minced intestines from acutely infected piglets) will stimulate lactogenic immunity and reduce the high mortality if administered to sows at least 2 weeks pre-farrowing. During PDCoV epidemics, high-level biosecurity procedures to reduce PDCoV transmission via contaminated fomites are essential.

Hemagglutinating encephalomyelitis virus (vomiting and wasting disease)

Relevance

In 1962, Greig and coworkers isolated a viral pathogen from the brains of suckling pigs with encephalomyelitis in Canada. Designated hemagglutinating encephalomyelitis virus (HEV), the virus was later classified as a CoV (Greig et al. 1971). In 1969, an antigenically identical virus was isolated in England from suckling pigs showing anorexia, depression, vomiting, and stunting, but without signs of encephalomyelitis (Cartwright et al. 1969). The condition was called vomiting and wasting disease (VWD). Both forms of the disease were experimentally reproduced by Mengeling and Cutlip (1976) using isolates from the same farm. pHEV is widespread among swine, but the infection is generally subclinical, although some outbreaks may cause losses (Alsop 2006; Quiroga et al. 2008).

Etiology

pHEV belongs to the genus *Betacoronavirus* of the family *Coronaviridae* (Figure 31.1). The virus agglutinates erythrocytes of mice, rats, chickens, and several other animals. The natural host of pHEV is the pig. Although pHEV may show different clinical manifestations, there is only one serotype. Age-related susceptibility of the pigs, possible strain differences in virulence, and variation in pathogenesis may influence clinical signs. pHEV shows a strong tropism for neural tissues in pigs. Likewise, the virus displays neurotropism in mice and Wistar rats (Hirano et al. 2004; Yagami et al. 1993).

In vitro, only porcine cells are susceptible to pHEV. pHEV was first isolated in primary PK cells with CPE characterized by syncytia (Greig et al. 1962). pHEV was also shown by IF staining to propagate in other porcine cell cultures: adult thyroid gland, embryonic lung, and cell lines such as ST, PK-15, IBRS₂, SK, SK-K, and KSEK6 swine embryo kidney.

Public health

Pigs are the only species known to be susceptible to pHEV and pHEV has no public health significance.

Epidemiology

Serologic surveys (1960–1990) revealed that pHEV infection in swine occurs worldwide and is endemic in both breeding and fattening swine (Pensaert 2006). The presence of pHEV, as detected by isolation or serology, was reported in Europe, in the Western Hemisphere (United States, Canada, Argentina), in Asia (Japan, Taiwan), and in Australia.

pHEV is maintained in swine populations by infecting successive groups of pigs after replacement or weaning. The virus is excreted oronasally (Hirahara et al. 1989; Pensaert and Callebaut 1974) for 8–10 days. Transmission occurs via nasal secretions, via nose-to-nose contact, and aerogenically. Persistent virus carriers are not known to exist.

Generally, pigs will only develop disease when they become infected oronasally prior to 3–4 weeks of age and if originating from nonimmune mothers (Appel et al. 1965). Pigs with maternally derived pHEV antibodies that prevent the virus from reaching neural target tissues are clinically unaffected when exposed to pHEV (Appel et al. 1965). Pigs infected at later ages normally do not develop clinical disease. Since pHEV is endemic in most swine populations, most sows are immune and protect their offspring by maternal antibodies. Thus, clinical outbreaks are rare and usually occur in litters from nonimmune mothers, often first-parity sows. Three outbreaks are notable. In 2001, pHEV was isolated from

newborn and early weaned pigs with vomiting and posterior paralysis on a Canadian farm (Sasseville et al. 2001). Alsop (2006) described a clinically diagnosed outbreak of VWD in 2002 in a 650-sow genetic nucleus herd. Quiroga et al. (2008) described a VWD outbreak with motor disorders in Argentina in 2006. It occurred in a three-site herd with 6,000 sows where the breeder stock consisted of 55% gilts and first- or second-parity sows.

Pathogenesis

The type and severity of clinical signs vary and are related to age, possible differences in virus virulence (Mengeling and Cutlip 1976), and the course of viral pathogenesis.

The primary site of replication of pHEV in pigs is the respiratory tract (Andries and Pensaert 1980b; Hirahara et al. 1987; Mengeling et al. 1972). IF staining revealed that epithelial cells of nasal mucosa, tonsils, lungs, and some unidentified cells in the small intestine were infected. Primary replication may result in mild or sub-clinical signs.

Experimental studies in colostrum-deprived piglets inoculated oronasally with pHEV provided insight into pHEV pathogenesis (Andries and Pensaert 1980a). From the primary sites of replication, the virus spread via the peripheral nervous system to the CNS via different pathways. One pathway led from the nasal mucosa and tonsils to the trigeminal ganglion and the trigeminal sensory nucleus in the brain stem. A second pathway was along the vagal nerves via the vagal sensory ganglion to the vagal sensory nucleus in the brain stem. A third pathway led from the intestinal plexuses to the spinal cord, also after replication in local sensory ganglia. Viremia was of little or no importance in the pathogenesis of the disease (Andries and Pensaert 1980b).

In the CNS, the infection started in well-defined nuclei of the medulla oblongata, but progressed into the entire brain stem, the spinal cord, and sometimes also the cerebrum and cerebellum. IF staining in the brain was always restricted to the perikaryon and processes of neurons. Vomiting was induced by viral replication in the vagal sensory ganglion (ganglion distale vagi) or by impulses to the vomiting center produced by infected neurons at different sites (Andries 1982). To elucidate the pathogenesis of wasting, Andries (1982) suggested that virus-induced lesions in the intramural plexuses of the stomach may contribute to gastric stasis and delayed stomach emptying.

Clinical signs

Sneezing or coughing may be the first sign of infection because of primary pHEV replication in the upper respiratory tract. Body temperature can be elevated at

disease onset, but returns to normal in 1–2 days. The incubation period for the appearance of more specific signs is 4–7 days. Two main clinical manifestations associated with pHEV neurotropism are possible in pigs below 3–4 weeks of age: (1) typical VWD with frequent vomiting leading to death or subsequent wasting and (2) acute encephalomyelitis with motor disorders. However, signs of both clinical forms may occur in the same herd during an outbreak.

For VWD, clinical signs are repeated retching and vomiting. Pigs start suckling, but withdraw from the sow and vomit the milk. The persistent vomiting and decreased food intake results in constipation and a rapid decline of condition. Neonatally infected pigs become severely dehydrated after a few days, exhibit dyspnea and cyanosis, lapse into coma, and die. Older pigs lose their appetite and become emaciated. They continue to vomit, although less frequently than in the acute stage. Wasting, often with distension of the cranial abdomen, may appear. This “wasting” state persists for several weeks and may be post weaning, requiring euthanasia. During the acute stage of VWD outbreaks, some pigs may show neurologic signs, such as abnormal gait, dullness, tremors, and nystagmus.

At the herd or farrowing unit level, morbidity varies greatly and probably depends on the proportion of non-immune neonatal litters present at the time of infection. In litters without maternal protection, morbidity is litter dependent and may approach 100% when the infection occurs near birth. Morbidity decreases markedly with increasing age at infection. Mortality is variable, but may be 100% in neonatally infected litters.

In the Argentina outbreak (Quiroga et al. 2008), only suckling pigs were involved. Vomiting and wasting were the main signs, with slight motor disorders. Disease occurred in 27.6% of pigs <1 week old and declined to 1.6% in pigs 3 weeks of age. In this pHEV outbreak, an estimated 12.6% (3683) of the suckling pigs in the affected farrowing units died or were euthanized. After weaning, a mean of 29% (15–40%) of the pigs coming from affected farrowing units showed wasting.

Outbreaks of the motor encephalomyelitis disease in suckling pigs may start with sneezing, coughing, and vomiting 4–7 days after birth. Vomiting continues intermittently for 1–2 days, but is rarely severe. In some outbreaks, the first sign is acute depression and huddling. After 1–3 days, pigs exhibit various combinations of nervous disorders. Generalized muscle tremors and hyperesthesia are common. Pigs may have a jerky gait and walk backward, ending in a dog-sitting position. They become weak, are unable to rise, and paddle their limbs. Blindness, opisthotonus, and nystagmus may also occur. Finally, the animals become dyspneic and lie prostrate on their sides. In most cases, coma precedes death.

Morbidity and mortality in neonatal pigs is usually 100%, but older pigs show a mild transient illness in which posterior paralysis may be the most common sign. Outbreaks described in Taiwan (Chang et al. 1993) in 30- to 50-day-old pigs were characterized by fever, constipation, hyperesthesia, muscular tremor, progressive anterior paresis, posterior paresis, prostration, recumbency, and paddling movements with a morbidity of 4% and a mortality approaching 100%. The pigs died 4–5 days after the onset of clinical signs.

Lesions

The only gross lesions reported in pHEV infections are cachexia, stomach dilatation, and distension of the abdomen in some chronically affected pigs (Schlenstedt et al. 1969).

Microscopic lesions of epithelial degeneration and inflammatory cell infiltration are found in the tonsils and respiratory system of acutely diseased pigs (Cutlip and Mengeling 1972; Narita et al. 1989). A nonsuppurative encephalomyelitis was reported in 70–100% of pigs with nervous signs and in 20–60% of pigs showing VWD. The lesions are characterized by perivascular cuffing, gliosis, and neuronal degeneration (Alexander 1962; Chang et al. 1993; Richards and Savan 1960). They are most pronounced in the gray matter of the pons Varolii, medulla oblongata, and the dorsal horns of the upper spinal cord.

Microscopic changes in the stomach wall were found only in pigs showing VWD. Degeneration of the ganglia of the stomach wall and perivascular cuffing were present in 15–85% of diseased animals. The lesions were most pronounced in the pyloric gland area (Schlenstedt et al. 1969).

Diagnosis

Diagnosis can be made by virus isolation, IHC, or RT-PCR (Quiroga et al. 2008). Tonsils, brain stem, and lungs dissected aseptically from young acutely diseased piglets can be used for testing. It is difficult to isolate the virus from pigs that have been sick for more than 2–3 days. For virus isolation, suspensions are inoculated onto primary PK cells, secondary pig thyroid cells, or porcine cell lines. pHEV is detected by the presence of syncytia, by hemadsorption, or by hemagglutination. One or more blind passages may be needed since specimens often contain small amounts of infectious virus.

Antibodies to pHEV can be detected by VN or hemagglutination inhibition (HI) tests. The HI and VN tests were almost equally diagnostic in swine sera, but VN is more specific (Sasaki et al. 2003). Antibody titer results must be evaluated carefully because subclinical infections with pHEV are very common. Moreover, a significant rise in antibody titer can be detected only if acute

sera are taken near the onset of clinical signs. Pigs may develop antibody titers as early as 6–7 DPI, which often coincides with early disease, making an interpretation of paired serology more difficult.

Differential diagnosis must be made between pHEV encephalomyelitis, Teschen–Talfan disease, and pseudorabies (Aujeszky's) disease. In the latter infections, clinical signs of encephalomyelitis, including motor disorders, are more severe and may appear in piglets and older pigs. Aujeszky's disease in non-vaccinated animals also induces respiratory signs in older pigs and abortions in sows. All these viruses can be grown in PK cells and pig thyroid cells, but the type of CPE differs and only pHEV causes hemadsorption and hemagglutination. They can be further differentiated by virus-specific tests.

Immunity

After infection, pigs develop detectable protective circulating antibodies (HI, VN) to pHEV in 7–9 days. The duration of antibodies has not been determined. The duration of immunity is less important in pHEV because of the resistance to disease that develops with age. Neonatal pigs born to immune mothers are fully protected by maternally derived antibodies that persist until the age of 4–18 (mean 10.5) weeks (Paul and Mengeling 1984).

Prevention and control

On most breeding farms, pHEV infection persists endemically by pig-to-pig transmission and through subclinical respiratory infections. Gilts usually contract the virus before their first farrowing and then provide protection to their offspring via colostral antibodies. When sows are not immune at farrowing, (e.g. in newly populated farms, well-isolated gilts, or small farms in which the virus is not maintained), infection of pigs within the first weeks after birth results in clinical signs. Promoting virus circulation in the farm so that gilts are immune at farrowing prevents disease in piglets.

Once clinical signs are evident, the disease will run its course; spontaneous recoveries are rare. Litters born 2–3 weeks after the onset of disease are usually protected because nonimmune gestating sows should have become infected and immune by farrowing. Piglets born to nonimmune sows early in the outbreak can be passively protected by parenteral inoculation with specific immune serum shortly after birth. Hyperimmune serum is not commercially available, but pooled serum collected from older sows (at the slaughterhouse) should be filter sterilized and tested to confirm presence of pHEV antibodies. No vaccines against pHEV are currently available.

Porcine torovirus

Relevance

Torovirus (ToV) particles were initially detected by EM in the feces of a 3-week-old piglet with diarrhea in England. Subsequent studies revealed a high seroprevalence (81–100%) in adults or young nursing piglets and high detection rates (50–75%) among subclinically infected weaned pigs (Kroneman et al. 1998; Pignatelli et al. 2010). Latter reports from Europe, North America, and South Africa suggested that ToV was endemic. In contrast, in Korea, only 6.4% (19 of 295) of diarrheic feces from 3- to 45-day-old piglets were positive for porcine ToV (Shin et al. 2010). Of these, about 74% also contained other enteric pathogens. Consequently, the link between porcine ToV and enteric disease is unclear, and there are no reports confirming porcine ToV pathogenicity or gut lesions.

Etiology

Porcine ToV represents a species within the genus *Torovirus* of the subfamily *Torovirinae* in the family *Coronaviridae*. The genomic organization, replication strategy, and properties resemble other members in the family *Coronaviridae* (de Groot et al. 2008). Like some betacoronaviruses, porcine ToVs also possess an HE protein. Notable differences from CoVs include a smaller N protein (approximately 18.7 kD) and a tubular nucleocapsid, leading to differences in ToV particle morphology (spherical, elongated, or kidney shaped) (Kroneman et al. 1998). Multiple clusters of porcine ToVs have been identified based on gene sequence analysis (de Groot et al. 2008; Pignatelli et al. 2010; Shin et al. 2010).

Epidemiology and immunity

Based on serologic and shedding data from clinically normal pigs or sows in European herds, ToVs were endemic in 14 farms tested. High seroprevalence rates (81%) were detected in sows on 10 Dutch farms by testing for cross-reactive VN antibodies to equine ToV (Kroneman et al. 1998). Similarly, 100% of sows, nursing, and older pigs in three farms in Spain were seropositive for ToV antibodies using an ELISA based on porcine ToV N protein (Pignatelli et al. 2010). Longitudinal studies revealed fecal shedding (80%) (RT-PCR or real-time RT-PCR) post weaning at 4–14 days for 1–9 days (Kroneman et al. 1998) or at 4 and 8 weeks post weaning (50–75%) (Pignatelli et al. 2010). In both studies, maternal antibody titers were initially high in piglets, declined at weaning, and then increased post infection at 11 or 15 weeks of age.

Because most pigs become infected with ToVs post weaning, maternal antibodies apparently provide at least partial protection. However, the immune correlates of protection to porcine ToV infection are not known. In one of four farms, it was postulated that ToV infection of suckling piglets in the presence of maternal antibodies delayed development of active immune responses such that these pigs, but not pigs from the other farms, shed the same ToV strain pre- and post weaning (Pignatelli et al. 2010). Genetically diverse ToV strains were detected within herds in the latter study and in Korean farms. The porcine ToVs were associated with sporadic infections among diarrheic pigs from 65 Korean farms surveyed (6.2% of farms positive) (Shin et al. 2010). Based on phylogenetic analysis of the S and N genes, the Korean ToV strains formed distinct branches with clusters corresponding to the farm of origin.

Diagnosis

Methods to propagate porcine ToVs in cell culture have not been described. For serologic studies, a cell culture-adapted equine ToV has been used to assess cross-reactive VN antibodies in swine (Kroneman et al. 1998; Pignatelli et al. 2010). Recently an indirect ELISA using recombinant purified porcine ToV N protein as antigen was developed (Pignatelli et al. 2010). In most, but not all cases, there was a good correlation between ELISA and VN tests. Discrepancies observed could reflect use of heterologous equine ToV antigen in VN, compromising detection of low titer antibodies.

Porcine ToVs have been detected in feces using IEM to identify antibody-aggregated ToV particles and differentiate them from other fecal porcine CoVs (TGEV, PEDV, and PDCoV) (Kroneman et al. 1998). For detection of ToV-specific viral RNA, RT-PCR and real-time RT-PCR targeting conserved regions of the porcine ToV N gene or the 3' UTR of the genome have been described (Kroneman et al. 1998; Pignatelli et al. 2010; Shin et al. 2010).

Prevention and control

Based on the limited data available, the stress of transport, movement, and redistribution of pigs, even within multisite farms, could precipitate porcine ToV infection with similar or distinct co-circulating strains (Pignatelli et al. 2010). Thus, management practices applicable to control of other enteric CoV infections should be implemented for control of porcine ToVs.

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