Chapter 4
Porcine Coronaviruses

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Abstract Transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhoea virus (PEDV), and porcine deltacoronavirus (PDCoV) are enteropathogenic coronaviruses (CoVs) of swine. TGEV appearance in 1946 preceded identification of PEDV (1971) and PDCoV (2009) that are considered as emerging CoVs. A spike deletion mutant of TGEV associated with respiratory tract infection in piglets appeared in 1984 in pigs in Belgium and was designated porcine respiratory coronavirus (PRCV). PRCV is considered non-pathogenic because the infection is very mild or subclinical. Since PRCV emergence and rapid spread, most pigs have become immune to both PRCV and TGEV, which has significantly reduced the clinical and economic importance of TGEV. In contrast, PDCoV and PEDV are currently expanding their geographic distribution, and there are reports on the circulation of TGEV-PEDV recombinants that cause a disease clinically indistinguishable from that associated with the parent viruses. TGEV, PEDV and PDCoV cause acute gastroenteritis in pigs (most severe in neonatal piglets) and matches in their clinical signs and pathogenesis. Necrosis of the infected intestinal epithelial cells causes villous atrophy and malabsorptive diarrhoea. Profuse diarrhoea frequently combined with vomiting results in dehydration, which can lead to the death of piglets. Strong immune responses following natural infection protect against subsequent homologous challenge; however, these viruses display no cross-protection. Adoption of advanced biosecurity measures and effective vaccines control and prevent the occurrence of diseases due to these porcine-associated CoVs. Recombination and reversion to virulence are the risks associated with generally highly effective attenuated vaccines necessitating further research on alternative vaccines to ensure their safe application in the field.
**Keywords** Porcine coronaviruses · Transmissible gastroenteritis virus · Porcine epidemic diarrhoea virus · Porcine deltacoronavirus · Enteropathogens · Porcine respiratory coronavirus · Pathology · Detection · Vaccine

### 4.1 Prologue

All known porcine coronaviruses (CoVs) belong to the genera *Alphacoronavirus*, *Betacoronavirus* and *Deltacoronavirus* of the subfamily * Coronavirinae*, in the family *Coronaviridae*, of the order *Nidovirales* (de Groot et al. 2008) [https://data.ictvonline.org/taxonomy-search.asp?msl_id=30 (Fig. 4.1)]. Affections of gastrointestinal, respiratory, peripheral and central nervous systems are usually visualised. Five swine CoVs are recognised: (1) the transmissible gastroenteritis virus (TGEV), first defined in 1946; (2) the porcine respiratory coronavirus (PRCV), a mutant of TGEV, isolated in 1984; (3) the porcine epidemic diarrhoea virus (PEDV), isolated in 1977; (4) the PHEV (porcine haemagglutinating encephalomyelitis virus) isolated in 1962; and (5) the PDCoV (porcine deltacoronavirus) described in 2012. The first two, TGEV and PRCV, belong to the *Alphacoronavirus* species together with closely associated CoVs of cats and dogs, and PEDV and human CoVs (229E and NL63) form distinct species in the *Alphacoronavirus* genus. PHEV and PDCoV belong to the *Beta- (Betacoronavirus* 1 species) and *Deltacoronavirus* genera, respectively. PDCoV is closely related to the deltacoronaviruses from Asian leopard cats and Chinese ferret badgers (Ma et al. 2005). While PRCV induces primarily subclinical infections in pigs, enteropathogenic swine alphacoronaviruses (TGEV, PEDV, SeCoV, porcine enteric alphacoronavirus) and PDCoV are allied with a severe enteric disease of variable severity depending on the animal age and immune status. One serotype is recognised for each swine CoV species.

TGEV and PEDV have been reportedly co-circulating in Eurasia and the USA. Recently in Europe, a pathogenic recombinant TGEV/PEDV variant (swine enteric coronavirus, SeCoV) was recognised and described (Akimkin et al. 2016; Belsham et al. 2016; Boniotti et al. 2016). SeCoV that contains PEDV S protein on a TGEV backbone apparently leads to disease clinically indistinct from the TGEV- and PEDV-produced ones (Table 4.1). Additionally, a novel bat-HKU2-like porcine coronavirus [porcine enteric alphacoronavirus (PEAV), GDS04 strain] associated with severe diarrheal disease in suckling piglets was identified in Southern China in 2017 (Gong et al. 2017) (Table 4.1). However, its prevalence and adaptation status to the swine host are unknown.

Currently, PHEV, the only porcine betacoronavirus, has a worldwide prevalence (Li et al. 2016). In neonatal piglets devoid of maternal antibodies (generally in those purchased from infection-free herds), PHEV causes either encephalomyelitis or a condition characterised by vomiting and wasting. Generally, maternal immunity
**Fig. 4.1** Phylogenetic tree of porcine coronaviruses of the *Alpha-, Beta- and Deltacoronavirus* genera. Closed circles indicate potential ancestral non-porcine coronaviruses. Bootstrapping with 1000 replicates was used to determine the reliability of each node.
protects piglets which have taken colostrum for up to 15 weeks, while in pigs older than 3–4 weeks and adult swine the infection is mostly subclinical. Therefore, it is seldom considered to be of economic importance. However, a recent report of an uncommon respiratory (influenza-like) presentation and increasing prevalence of PHEV in adult exhibition swine in the USA (Lorbach et al. 2017) may indicate a potential tropism shift that could lead to a substantial change in its epidemiology. To clear this complex epidemiological position, continuing monitoring and development of state-of-the-art rapid and reliable tools and techniques are needed to confirm and provide a clear differential diagnosis (Kim et al. 2001; Masuda et al. 2016).

CoVs are enveloped and pleomorphic, 60–160 nm in diameter. Swine CoV shaves single-stranded, polyadenylated, large genomic RNA (25–30 kDa) of positive-sense polarity that is infectious. The genome profile, replication strategies as well as protein expression match to other human and animal CoVs (Enjuanes and Van der Zeijst 1995; Gonzalez et al. 2003; Laude et al. 1993). Most porcine CoVs have four basic structural proteins: a large surface glycoprotein (S, spike protein that forms a monolayer of club-shaped spikes defined as the corona); a small membrane protein (E); an integral membrane glycoprotein (M); and a nucleocapsid protein (N). However, PHEV contains a haemagglutinin-esterase (HE) protein that forms a second shorter layer of surface spikes (de Groot et al. 2008). TGEV, PEDV and PDCoV also transcribe 1–2 accessory proteins encoded by open reading frame (ORF)3 (TGEV and PEDV), ORF6 (PDCoV), and ORF7 (TGEV and PDCoV). The complete genome organisation is 5’UTR-ORF1ab, S, ORF3, E, M, ORF6, N and ORF7-3’UTR.

An overall nt and amino acid sequence similarity of 96–98% among TGEV and PRCV proposes that PRCV evolved from TGEV. Two characteristic features of the PRCV genome that may account for its altered tissue tropism include a large

| CoV | Symptoms                  | Clinical disease onset | Lesions          | Morbidity | Mortality | Most affected age group |
|-----|---------------------------|------------------------|------------------|-----------|-----------|------------------------|
| TGEV | Diarrhoea, vomiting, dehydration | 24 h                   | Jejunum, ileum | 100%      | Up to 100% | Neonatal piglets up to 3 weeks of age |
| PEDV | Diarrhoea, vomiting, dehydration | 24–36 h                | Jejunum, ileum | 100%      | Up to 100% | Neonatal piglets up to 3 weeks of age |
| SeCoV | Diarrhoea, vomiting, dehydration | 24–36 h                | Jejunum, ileum | 100%      | Up to 100% | Neonatal piglets up to 3 weeks of age |
| PEAV | Diarrhoea, dehydration    | 3–4 days               | ?                | 100%      | ?         | Neonatal piglets up to 3 weeks of age |
| PDCoV | Diarrhoea, vomiting, dehydration | 1–3 days               | Jejunum, ileum, colon | Up to 100% | 40–80%     | Neonatal piglets up to 3 weeks of age |

Table 4.1 Comparative pathogenesis of porcine enteric CoVs
omission (621–681 nt) in the N-termini of the S gene resulting in a reduced S protein size and variable sequence changes in the ORF3 (Ballesteros et al. 1997; Sanchez et al. 1999).

While there is no evidence of the existence of different PEDV serotypes (Lin et al. 2015a), genetically, PEDV strains are classified into two groups: (1) classical (isolates from Eurasia that are genetically similar to the prototype CV777 strain) and (2) emerging PEDV strains (Lin et al. 2016; Vlasova et al. 2014). All classical PEDV strains contain inserts and omissions in the spike gene (S INDEL) that are not present in the majority of the highly virulent emerging PEDV strains (Vlasova et al. 2014). Thus, these highly virulent strains that originally emerged in China in 2010 and transmitted to the USA, Europe and other Asian parts are referred to as emerging non-S INDEL PEDV strains. Recombinants between these two major groups of PEDV contain a set of deletions-insertions in their spike gene identical to those of the classical strains. They are called S INDEL strains and circulate in Asia, Europe and the USA. Additionally, a few reports described other uncommon PEDV variants that bear large deletions (194–216 aa) in the N-terminal domain (NTD) of the S protein and designated as S1 NTD-del type of PEDV (Diep et al. 2017; Oka et al. 2014; Suzuki et al. 2015). Unlike the altered tissue tropism from enteric TGEV to respiratory PRCV, these (S INDEL and S1 NTF have been reported-del) PEDV strains have kept their enteric predilection, but with lower virulence (Suzuki et al. 2016; Hou et al. 2017).

Swine enteric CoVs (TGEV, PEDV and PDCoV) are highly contagious and are associated with severe disease forms such as diarrhoea and vomition, and increased mortality in young ones (often 100%). They can cause sporadic outbreaks (endemic) or large-scale epidemics in swine-producing countries (Fig. 4.2). No specific treatments are available for any of the swine enteric CoVs that so far have resisted eradication
efforts in different geographic regions. In this chapter, we have reviewed the diseases due to CoVs that continue evolving in domestic and wild swine, as well as another possible reservoir (avian or bat species) or secondary hosts including carnivores, or via the interspecies spread, recombination and generation of deletion escape variants. We also review PRCV that has lost its enteric tropism but is capable of inducing protective immune responses against TGEV that altered its global epidemiology.

4.2 Pathogenesis and Clinical Signs

4.2.1 TGEV

Extensive necrosis of mature enterocytes of jejunum and ileum within 24 h after infection results in reduced enzymatic activity (alkaline phosphatase, lactase, etc.), disrupted digestion, and cellular electrolyte (including sodium) balance. These changes primarily lead to the deposition of fluid in the intestinal lumen, acute malabsorptive diarrhoea (Moon 1978). The loss of extravascular protein and copious dehydration in piglets can be fatal (Butler et al. 1974). The latter can also lead to metabolic acidosis and hyperkalaemia, causing abnormal cardiac function.

TGE gross lesions are limited to the gastrointestinal tract. The distension of the stomach and the small intestine are seen to be filled up with curdled milk and sometimes petechial haemorrhages are visualised (Hooper and Haelterman 1966a). The small intestinal wall is thin and transparent. The villous atrophy in the jejunum and lesser in the ileum regions are the major TGE lesions and are more pronounced in neonatal pigs than in ≥3-week-old piglets (Moon 1978; Hooper and Haelterman 1966b). The increased severity of TGEV infection results in higher mortality (often 100%) in piglets less than 2 weeks of age that decreases in older pigs (Table 4.1). Although swine of any age is susceptible to TGEV, the mortality in TGEV seropositive groups and swine more than 5 weeks of age is usually low. Mechanisms that represent age-dependent susceptibility to clinical ailment comprise the slower substitution of tainted villous epithelial cells with newly differentiated enterocytes migrating from crypts in newborn pigs (Moon 1978). These lesions are similar to PEDV/PDCoV (Debouck et al. 1981; Jung et al. 2015a) lesions, but more severe than those caused by rotavirus (RV) (Bohl et al. 1978). Pathologic observations and degree of villous atrophy are exceptionally variable in pigs from endemic herds (Pritchard 1987).

Lungs (alveolar macrophages) and mammary gland tissues are recognised extra-intestinal sites for TGEV replication (Kemeny et al. 1975). Hitherto report shows pneumonia due to oronasal infection of pigs with TGEV (Underdahl et al. 1975), and the clinical significance of mammary gland infection is imprecise. However, agalactia is frequently observed in TGEV-affected sows, and TGEV spreads quickly among the population.
4.2.2 PRCV

PRCV replicates efficiently in porcine type 1 and 2 pneumocytes and is seen in epithelial cells of the nares, trachea, bronchi and bronchioles, and alveoli, and on occasion in alveolar macrophages (Atanasova et al. 2008; Pensaert et al. 1986; O’Toole et al. 1989). It can be noticed in blood and tracheobronchial lymph nodes. After experimental infection, nasal PRCV shedding usually lasts for 4–6 days. Pulmonary lesions and clinical signs subside consequently with an increase in the virus-neutralising (VN) antibody titres (Atanasova et al. 2008). Although PRCV is sometimes found in enterocytes, it does not spread efficiently to adjacent epithelial cells (Cox et al. 1990), and faecal shedding is low or undetectable.

PRCV predominantly causes upper and lower respiratory tract disease. The lesions appear to include lung and bronchointerstitial pneumonia, with regular peribronchiolar and perivascular lymphohistiocytic handcuffing (Atanasova et al. 2008; Cox et al. 1990; Halbur et al. 1993; Jung et al. 2007). The PRCV-induced bronchointerstitial pneumonia results in (1) thickened alveolar septa due to macrophage and lymphocyte infiltration, (2) hypertrophy and hyperplasia of type 2 pneumocytes, (3) aggregation of cell debris and inflammatory leukocytes in alveolar and bronchiolar lumina because of airway epithelial necrosis and (4) peribronchiolar or perivascular lymphohistiocytic inflammation.

4.2.3 PEDV

Clinical signs are evident between 22 and 36 h postinfection and match with the peak of viral replication (Table 4.1). The clinical presentation (watery malabsorptive diarrhoea, vomiting, depression and anorexia) and pathological lesions of PEDV are clinically indistinguishable from those of TGEV (Debouck et al. 1981; Coussement et al. 1982).

Morbidity is nearly 100% in piglets and variable in sows. Neonates below 1 week of age often die due to severe dehydration, and mortality touches 50–100%, whereas mortality is low in older pigs and they recover within a week. In sows, severity of diarrhoea is constant and frequently shows only depression and anorexia. Similarly, fattening pigs may develop watery faeces and can become anorexic and depressed within a week. As with TGEV, slower enterocyte turnover and immature innate immune system may add to the more extreme clinical signs, higher mortality and slower recuperation in PEDV-tainted piglets in contrast to weaned pigs (Jung et al. 2015a; Moon et al. 1975; Annamalai et al. 2015).

Each outbreak generally lasts for ~3–4 weeks; however, it might be longer on large breeding farms with multiple, isolated units and variable levels of lactogenic immunity in gilts/sows. PEDV-exposed pregnant sows can provide sufficient lactogenic immunity to protect their piglets, and PED outbreaks stop. After the passage of acute outbreak, diarrhoea may persist and is recurrent in weaned pigs, resembling endemic TGE form (Martelli et al. 2008).
The severity of lesions and the virus replication levels in naturally and experimentally infected suckling piglets vary for classical PEDV, emerging non-S INDEL and S INDEL PEDV strains (Jung et al. 2015a; Coussement et al. 1982; Kim and Chae 2003; Pospischil et al. 1981; Sueyoshi et al. 1995; Lin et al. 2015b; Madson et al. 2014). Lesions remain localised to the small intestine that is swollen and filled up with watery, yellowish liquid. Microscopic examination shows syncytia, vacuolation and shedding of small intestinal enterocytes primarily on the proximal villi. Similar to TGEV, PEDV infection results in degeneration of enterocytes that reduces the villous height:crypt depth (VH: CD) ratios and the enzymatic activity. Although PEDV antigens were detected in colonic epithelial cells, no associated histopathologic changes have been observed (Debouck et al. 1981).

Viral RNA has been confirmed in the serum, and different tissues (including lung, spleen, liver and muscle) of pigs euthanised during PEDV infection (Suzuki et al. 2016; Jung et al. 2014, 2015a; Lohse et al. 2017; Chen et al. 2016a; Park and Shin 2014) with high RNA titres in the serum of $7–8 \log_{10}$ GE/mL coinciding with peak RNA titres in faeces ($11–12 \log_{10}$ GE/mL) (Jung et al. 2015a). Additionally, PEDV RNA is identified in 40.8% (20/49) of sow milk samples during the epidemics caused by emerging PEDV strains (Sun et al. 2012).

4.2.4 PDCoV

The clinical signs are observed within 1–3 days after PDCoV infection in suckling and older pigs. Although clinical symptoms are similar (Table 4.1), they are less pronounced compared to PEDV and TGEV infections (Chen et al. 2015; Hu et al. 2016; Jung et al. 2015b; Ma et al. 2015). They include acute, watery diarrhoea due to malabsorption induced by the massive loss of absorptive enterocytes. Additional signs may include vomiting, dehydration, weight loss, lethargy and death. Vacuolation of the infected colonic epithelial cells may inhibit water and electrolyte reabsorption. The seronegative pigs are susceptible to PDCoV infection at any age, with high morbidity that can reach 100% in piglets. Evaluation of filed cases in the USA, China and Thailand in 2014 shows that PDCoV infection is associated with up to 40–80% mortality among suckling pigs (Anon 2014). The infection on breeding establishments remains self-limiting and stops when pregnant sows develop lactogenic immunity adequate to secure their offspring.

Gross lesions include thinned and transparent intestinal walls (jejunum to the colon) with a collection of a lot of yellow liquid with gas. Often stomach is found bloated with curdled milk.

PDCoV replicates in the epithelial cell of the large and small intestine. Lesions look like those seen in TGEV and PEDV infections but are mild (Chen et al. 2015; Hu et al. 2016; Jung et al. 2015b; Ma et al. 2015). Histological findings are intense, multifocal to diffuse, mild to extreme atrophic enteritis of jejunum and ileum, at some point joined by mild vacuolation of caecal and colonic epithelial cells (Jung et al. 2015b). Amid acute infection, PDCoV antigens are available in the villous
epithelium of the mid-jejunum to the ileum and a lesser degree, in the duodenum, and caecum/colon (Jung et al. 2016a). PDCoV antigens may also be noticed in immune cells of the intestinal lamina propria, Peyer’s patches and mesenteric lymph nodes (Hu et al. 2016). Inflammatory cell (macrophage, lymphocyte and neutrophil) infiltration can be observed in the lamina propria. Acute necrosis of PDCoV-infected enterocytes (Jung et al. 2016a) results in marked villous atrophy in jejunum and ileum, but not duodenum or large intestine, which coincides with fewer PDCoV antigen-positive duodenal, caecal or colonic epithelial cells (Chen et al. 2015; Jung et al. 2015b). Acute-phase viremia with low PDCoV RNA titres in serum is observed (Chen et al. 2015; Hu et al. 2016). After recovery of pigs from clinical disease, huge amount of PDCoV antigens are found in the gut lymphatic tissues (Hu et al. 2016). Additionally, low or moderate quantities of PDCoV RNA, but not antigens, are detected in multiple organs, feasibly as of viremia (Chen et al. 2015; Ma et al. 2015; Jung et al. 2016b). Decreased levels of PDCoV shedding (compared with PEDV and TGEV) in the faeces may be indicative of its incomplete adaptation to pigs and can contribute to its slower spread among swine herds and the lower mortality of nursing pigs (Jung et al. 2015b).

4.3 Incidence and Prevalence of the Disease

4.3.1 TGEV

TGEV was first detected in the USA in 1946 from outbreaks of acute diarrhoea with high mortality in piglets (Doyle and Hutchings 1946). Since then the disease has been reported in several pig-rearing countries practicing intensive pig farming system, including Europe, Asia (Japan, Korea, Malaysia and Taiwan), the Americas (North, Central and South) and Africa (Zaïre, Ghana). Despite the widespread application of vaccines, TGEV infections were a prime reason for enteric disease and mortality in piglets in the USA and globally in the 1960s–1980s. The presence and extensive prevalence of PRCV, a deletion mutant of TGEV, narrowed the clinical impact of TGE (Laude et al. 1993; Pensaert et al. 1986, 1993; Brown and Cartwright 1986; Pensaert 1989; Yaeger et al. 2002). Currently, sporadic outbreaks of profuse diarrhoea in piglets due to TGEV in TGEV/PRCV seronegative herds are yet to be confirmed in North America, Europe and Asia. However, careful differentiation between TGEV and emerging TGEV/ PEDV recombinants may be needed.

Two epidemiologic forms of TGE are apparent: epidemic and endemic. Epidemic TGE noticed transcendentally in seronegative flocks. After entry, the illness transmits quickly to swine of any age, particularly in winters, with inappetence, vomition or diarrhoea in affected animals. Suckling pigs exhibit prominent clinical signs and get quickly dehydrated. Lactating sows usually show anorexia and agalactia, with reduced milk production, which further adds to piglet mortality.
Endemic TGE indicates the persistence of the virus and disease in a group perpetuated by the continuous influx of susceptible swine. It is a classic sequel to a primary outbreak and occurs in seropositive animals that regularly have farrowing (Stepanek et al. 1979), additions of the herd or mixing of susceptible pig population. In endemic groups, TGEV spreads slowly among grown-up pigs (Pritchard 1987). Sows are most of the time resistant and asymptomatic and will transfer a variable level of passive lactogenic immunity to their offspring. In these groups, mild TGEV diarrhoea is seen with mortality under 10–20% in pigs from ~6 days of age until ~2 weeks post-weaning.

4.3.2 PRCV

PRCV infects the respiratory tract with limited or no shedding in faeces (Pensaert 1989). The first isolation of PRCV was from Belgium in 1984 (Pensaert et al. 1986) and 1989. PRCV was detected in the USA in the herds without a prior history of TGEV infection or vaccination (Hill et al. 1990; Wesley et al. 1990). Antibodies produced in PRCV-infected pigs neutralise the TGEV.

Since the first report, the virus has been introduced rapidly in Europe (Laude et al. 1993; Brown and Cartwright 1986; Have 1990; van Nieuwstadt et al. 1989) and attained endemic status worldwide, including entering TGEV-free countries (Laude et al. 1993; Pensaert 1989; Pensaert et al. 1993). A serological survey from the USA in 1995 demonstrated that clinically healthy pigs from different herds were found to be seropositive for PRCV (Wesley et al. 1997) in Iowa State.

4.3.3 PEDV

The classical PEDV strains were the cause of several epidemics with heavy mortality in Europe from 1971 until the late 1980s. However, after 2000, reports are very rare. In Italy, an epidemic involving 63 herds occurred in 2005 and 2006 where pigs of all ages were found affected, but mortality was mainly limited to suckling piglets (Martelli et al. 2008). Because of the low clinical importance of the disease, no surveillance studies were conducted until the emergence of new PEDV variants in Europe in 2014. Note that the historical prevalence of classical PEDV in the European swine population is unknown. An emerging non-S INDEL strain led to an outbreak in Ukraine in 2014; while outbreaks in European countries (Germany, Belgium, France, the Netherlands and Slovenia) were confirmed as of S INDEL strains (Lin et al. 2016).

Infections associated with classical PEDV strains were originally reported in China in the late 1970s. Since then, PED has spread among swine farms and became leading cause of viral diarrhoea, despite the use of vaccines (targeting the prototype PEDV strain CV777) (Wang et al. 2016a; Xuan et al. 1984). In Japan PEDV was
first detected in 1982 (Takahashi et al. 1983) and outbreaks continued during the 1990s with mortality between 30% and 100% in suckling pigs (Sueyoshi et al. 1995; Kuwahara et al. 1988). PEDV was first recognised in Korea in 1993 (Kweon et al. 1993) and became prevalent accounting for more than 50% of the enteric viral infections diagnosed in suckling piglets in the 1990s (Chae et al. 2000; Hwang et al. 1994). In India, 21.2% of 528 serum samples from pigs (2–6 months old) were confirmed positive for PEDV antibodies (Barman et al. 2003). In Thailand, between 2007 and 2008, PED occurred in eight provinces (Puranaveja et al. 2009) affecting all age-group pigs but mortality reached 100% in newborn piglets.

In October 2010, a massive series of PEDV outbreaks occurred in China, resulting in tremendous economic losses (Sun et al. 2012, 2016). These outbreaks were caused by the emerging extremely virulent non-S INDEL strains, and the mortality in neonatal piglets reached 50–100% (Wang et al. 2016b). Subsequently, emerging S INDEL strains were also identified in China (Wang et al. 2016b), demonstrating that classical and emerging (non-S INDEL and S-INDEL PEDV) strains are co-circulating in China.

In 2013, the emerging PEDV strains started spreading to other countries outside China. At the same period, outbreak due to highly virulent non-S INDEL PEDV strains started in US swine farms (Stevenson et al. 2013), which was followed by outbreaks associated with milder S-INDEL PEDV strains in January 2014 (Wang et al. 2014a). However, S INDEL strains were retrospectively found in pig samples collected as early as June 2013 (Vlasova et al. 2014). In 2013–2014, PEDV led to the loss of nearly 10% of the US swine stock (7 million pigs) and the associated profits. By 2018, PEDV had reportedly spread to 39 US states and Puerto Rico (www.aphis.usda.gov/animal-health/secd). In the USA, the last big outbreak of PEDV occurred in the spring of 2014, and the emerging non-S INDEL PEDV strains have reportedly spread to the neighbouring countries, including Canada and Mexico (Lin et al. 2016).

In 2013, the emerging non-S INDEL PEDV strains were identified in Japan (Masuda et al. 2015) and then reportedly spread to 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 strains were detected in Japanese (Suzuki et al. 2015) and Korean (Lee et al. 2014) farms in 2013 and 2014, respectively. To date, other PEDV variants that carry a large deletion in the NTD of the S protein were detected exclusively in pigs in Japan. Besides the avirulent TTR-2 strain (Suzuki et al. 2016), 15 novel field PEDV mutants with large deletions in the NTD of S gene ranging from 582 to 648 nt were detected from diarrhoeic pig faecal or intestinal samples collected between December 2013 and June 2015 (Diep et al. 2017). Interestingly, all of these samples contained at least two PEDV strains with distinct large genomic deletions, and the majority of these PEDV strains were confirmed to contain an intact S gene. These variants with large deletions in the S gene were found in both primary and recurring PED outbreaks. In summary, classical, emerging non-S INDEL and S-INDEL PEDV strains exist in Asia and Europe, but only emerging non-S INDEL and S-INDEL PEDV strains are currently confirmed to circulate in the Americas. No PEDV infections have been reported in the African and Australian continents so far.
4.3.4 PDCoV

The initial surveys in China and Hong Kong during 2007–2011 identified DCoVs in pigs and wild birds (Woo et al. 2012). Nonetheless, the DCoV presence was previously confirmed at Chinese live-animal markets in 2005–2006 in small mammals, including Chinese ferret badgers and Asian leopard cats (Dong et al. 2007). Because their helicase and S genes are closely related with PDCoV, interspecies transmission of DCoVs among Asian carnivores, porcine and avian species is suggested. The origin of PDCoV remains unclear, but considering its recent emergence, its adaptation to pigs may be incomplete.

In early 2014, the first outbreak of PDCoV-associated diarrhoea was documented in swine, in Ohio. Among intestinal or faecal samples obtained from diarrhoeic pigs from 5 Ohio farms, 92.9% were found to be positive for PDCoV by RT-PCR (Wang et al. 2014b). The PDCoV sequences shared high nucleotide similarity with the two Chinese prototype PDCoV strains, HKU15-44 and HKU15-155, identified in 2012. Other two genetically similar PDCoV strains, USA/IA/2014/8734 and SDCV/USA/Illinois121/2014, were detected in US states during the same time period (Li et al. 2014; Marthaler et al. 2014a). After this PDCoV has been confirmed in 19 US states (www.aphis.usda.gov/animal-health/secd) but is still less widespread than PEDV. PDCoV origin in US swine is unknown; however, serologic evidence suggests that it was circulating in US swine before its recognition in 2014 (Sinha et al. 2015; Thachil et al. 2015).

PDCoV has also been documented in Canada (March 2014), Korea (April 2014), mainland China (2015), Thailand (2015) and Vietnam and Lao PDR. A Korean study described that of 691 faecal samples from 59 farms collected from diarrhoeic pigs from 2014 to 2015, only 2 samples originating from a single farm were PDCoV RNA positive (Lee et al. 2016a). The two Korean PDCoV strains, SL2 and SL5, were genetically closely related to the US PDCoV strains but differed from the older Korean strain KNU14-04. Following the first PDCoV identification in Hong Kong in 2012 (Woo et al. 2012), it was confirmed in diarrhoeic pigs in mainland China (Dong et al. 2015; Song et al. 2015). PDCoV high prevalence (>30%) and frequent co-infections with PEDV (51%) were reported. All Chinese PDCoV strains shared high nt identities (≥98.9%) with the global PDCoV strains. The Thai PDCoV strains shared the highest nt identities (≥98.4%) with the Chinese strain CHN-AH-2004 (Janetanakit et al. 2016), forming, however, a cluster separate from Chinese and US strains (Zhang 2016). Strains genetically close to the Thai PDCoV lineage were found in Lao PDR, whereas strains of the US PDCoV lineage were detected in Vietnam (Saeng-Chuto et al. 2017).
4.4 Immunobiology

4.4.1 TGEV/PRCV

Infection of adult swine results in noticeable serum antibodies that could persist for 6 months to several years (Stepanek et al. 1979). Serum antibody presence confirms serological evidence for TGEV or PRCV infection; their association with protection against TGEV is not established. Swine that recover from TGEV infection show protection from subsequent short-term challenge, as of intestinal mucosal immunity (Brim et al. 1995; Saif et al. 1994; VanCott et al. 1993, 1994).

Protective immunity depends on the secretory IgA (sIgA) antibodies produced by intestinal plasma cells (Saif et al. 1994; VanCott et al. 1993, 1994). Intestinal and serum IgA TGEV antibodies and antibody-secreting cells (ASCs) have been detected in pigs after oral, but not after parenteral, administration with TGEV (Saif et al. 1994; VanCott et al. 1993, 1994; Kodama et al. 1980). Presence of IgA antibody (likely of intestinal origin) in the serum is considered to be an indicator of active immunity to TGE (Kodama et al. 1980; Saif 1999). Besides local antibody responses, cell-mediated immunity (CMI) is also important in protection against TGEV infections (Brim et al. 1995; Frederick et al. 1976; Shimizu and Shimizu 1979). Retinoic acid may enhance CMI after immunisation of piglets with an inactivated TGEV vaccine by increasing the trafficking of CD8+ T cells to lymph nodes and small intestine (Chen et al. 2016b). It was conceived that a low natural killer (NK) cell activity might associate with the higher susceptibility of piglets and parturient sows to TGEV infection (Cepica and Derbyshire 1984). A virulent (SHXB) however not attenuated (STC3) TGEV strain impeded the capacity of porcine intestinal DCs or monocyte-derived DCs to sample antigen, migrate and incite T-cell expansion in vivo and in vitro (Zhao et al. 2014), suggestive of the immune-suppressive potential of TGEV.

PRCV-inoculated pigs developed potent systemic and bronchus-associated but not gut-associated antibody, ASC and T-cell responses (VanCott et al. 1993; Brim et al. 1994). Other than quantitative differences in the count of IgA antibodies in the sow milk induced by TGEV or PRCV exposure, putative divergences in virus epitopes observed by the milk IgA antibodies were recommended (De Diego et al. 1992).

Subsequently to extensive decrease in epidemic outbreaks of TGE in Europe following the widespread distribution of PRCV, the research suggested that PRCV infections induce partial immunity against TGEV, as proven by a reduced length and duration of virus shedding and diarrhoea in most of the pigs examined (Brim et al. 1995; VanCott et al. 1994; Cox et al. 1993; Wesley and Woods 1996). This partial immunity is associated with rapid increases in TGEV-VN antibodies (Cox et al. 1993; Wesley and Woods 1996) and numbers of IgG and IgA ASCs in the intestines (Saif et al. 1994; VanCott et al. 1994). Movement of PRCV IgG and IgA ASCs from the bronchus-associated lymphoid tissues (BALT) to the gut of the PRCV-exposed pigs after TGEV challenge may clarify the quick anamnestic response and induces the partial protection (VanCott et al. 1994). However, neonatal pigs needed a week after PRCV encounter to develop partial immunity to subsequent TGEV challenge (Wesley and Woods 1996).
Circulating passive antibodies (primarily IgG), acquired with colostrum, protect the neonate against systemic, however not the intestinal, infection (Hooper and Haelterman 1966a; Saif and Sestak 2006). Between the first 7 days of lactation, IgA prevails in milk and IgG reduces. Researchers have reviewed the mechanisms of passive immunity to TGEV infection (Saif and Sestak 2006; Chattha et al. 2015; Saif and Jackwood 1990; Saif and Bohl 1979). Swine recovered from TGE spread passive immunity to their suckling pigs with colostrum or milk (lactogenic immunity) containing TGEV virus-neutralising antibodies (Hooper and Haelterman 1966a) that nullify the ingested TGEV in the intestinal lumen. This is accomplished naturally when piglets feed on immune mother regularly or experimentally by constant feeding of antiserum.

IgA TGEV antibodies in milk are stable in the gut and give the best protection, but IgG antibodies are likewise protective if high titres are restored in milk after parenteral or systemic vaccination (Bohl and Saif 1975) or by ingestion of colostral IgG antibodies (Stone et al. 1977). Following TGEV infection and antigenic stimulation in the gut, IgA immunocytes move to the mammary gland where they confine and produce IgA antibodies into colostrum and milk that play a major role in passive protection of suckling pigs (Saif and Sestak 2006; Saif and Jackwood 1990; Saif and Bohl 1979; Bohl and Saif 1975). This “gut-mammary” immunologic axis was first proposed concerning TGEV infections in swine (Bohl et al. 1972; Saif et al. 1972), providing the first concept for a common mucosal immune system.

4.4.2 PEDV

Even though all age pigs are susceptible to PED, highest mortality is seen in 1-week piglets and younger, and their survival is dependent on the transfer of maternal antibodies, especially VN and sIgA (via colostrum and milk from immunised or previously exposed sows). The components of lactogenic security depicted for TGEV infection apply to PEDV also (described previously in TGEV section) (Chattha et al. 2015; Langel et al. 2016). Pigs lose lactogenic protection at weaning becoming susceptible to PEDV infection. Humoral immune response to PEDV infection is quite the same as seen for TGEV (described in TGEV section) (Saif and Sestak 2006; Chattha et al. 2015). VN antibodies appear in the serum but do not play any significant role in protection against the clinical disease since protection basically relies on the presence of sIgA antibodies in the intestinal mucosa (Chattha et al. 2015; Langel et al. 2016). Immunity may not persist long, yet a fast anamnestic response upon re-exposure may decrease the severity of reoccurring disease or even prevent it.

At least 11 proteins [ORF1ab-encoded NS proteins (nsp1, nsp3, nsp5, nsp7, nsp14–16), structural proteins (E, M, N) and accessory protein ORF3s] have been recognised as IFN antagonists allowing PEDV to evade host interferon (IFN) responses (Ding et al. 2014; Wang et al. 2015; Zhang et al. 2016a). Decreased innate immune responses (specifically frequencies and function of NK cells) (Annamalai et al. 2015) likely contribute to the increased severity of PEDV infection in suckling vs. older (weaned, finisher, adult) pigs, as observed for TGEV infections (Derbyshire et al. 1969).
4.4.3 PDCoV

The immune response to PDCoV infection in pigs is still unclear but presumed to be similar to that mentioned for TGEV and PEDV. Gnotobiotic pigs orally inoculated with the original or tissue-culture-developed PDCoV strain (OH-FD22) exhibited serum IgG, IgA and VN antibodies by 14 dpi that shows a peak at 24 dpi when the pigs had recouped from the clinical form and faecal virus shedding (Hu et al. 2016). Similar to TGEV and PEDV, the supply of maternal antibodies with colostrum and milk from immune sows, especially IgA and VN antibodies, should neutralise PDCoV in the gut protecting young piglets (Bohl et al. 1972; Saif et al. 1972).

4.5 Diagnosis

4.5.1 TGEV/PRCV

Since clinical signs and atrophic enteritis due to TGEV are also seen in other enteric infections (RV, PEDV, PDCoV and coccidia), lab-based diagnosis of TGE must be followed utilising the following tests: identification of viral antigen or nucleic acids in faeces, virus seclusion from samples or detection of TGEV antibodies. Similar methods are used for the diagnosis of PRCV, but with an emphasis on respiratory specimens (nasal swabs or lung homogenates). Evaluation of clinical signs, histologic lesions and viral antigen distribution in tissues might yield a presumptive diagnosis.

Probing of TGEV antigen in small intestinal enterocytes by immunofluorescence (IF) (Pensaert et al. 1970) or immunohistochemical (IHC) (Shoup et al. 1996) techniques using monoclonal antibodies (MAb) against the highly conserved TGEV N protein may be conducted for formalin-fixed or frozen tissues harvested in the early stage of infection.

A mono- or polyclonal antibody-based enzyme-linked immunosorbent assay (ELISA) is utilised to recognise TGEV antigens in cell cultures, intestinal contents and faeces (Lanza et al. 1995; Sestak et al. 1996, 1999a; van Nieuwstadt et al. 1988) or PRCV antigen in nasal swabs or lung homogenates (Lanza et al. 1995); however, the sensitivity of available ELISA tests is generally lower than that of RT-PCR assays. RT-PCR or real-time qPCR is now more commonly used for detection of TGEV and distinguishing TGEV, PRCV, PDCoV and PEDV (Kim et al. 2000, 2001; Masuda et al. 2016; Costantini et al. 2004; Ogawa et al. 2009). PRCV/TGEV distinction is usually performed through PCR assay based on the S gene deletion region in PRCV strains. Multiplex RT-PCR and real-time qPCR have been used for the concurrent identification of major porcine viruses related to diarrhoea including RV, TGEV, PDCoV and PEDV (Masuda et al. 2016; Ogawa et al. 2009). Also, multiplex microarray hybridisation assay was used for quick differential identification of eight CoVs, including TGEV (Chen et al. 2005).
Transmission electron microscopy (TEM) is used to show TGEV in the faecal or intestinal contents of infected pigs. Further, immune EM (IEM) possesses many advantages over TEM and is more sensitive and better in differentiating TGEV from PEDV and PDCoV (Saif et al. 1977).

Primary and secondary pig kidney cells (Bohl and Kumagai 1965) or cell lines (Laude et al. 1981), porcine thyroid cells (Witte 1971) and McClurkin swine testicle (ST) cell line (McClurkin and Norman 1966) are advocated for TGEV isolation from infected pig faeces or intestinal contents. Typical cytopathic effects (CPE) might be negligible upon primary isolation of field strains and require additional blind passages. The CPE observed comprises swollen, round cells with a balloon-like appearance (Bohl and Kumagai 1965). For observing viral CPE or plaques, the addition of pancreatin or trypsin to the ST cell culture media as well as using older cells can improve detection of viral CPE and plaques (Bohl 1979).

ST cells and pig kidney cells are best for isolation of PRCV from a nasal swab or lung tissue homogenates. PRCV- and TGEV-induced CPE are comparable with syncytia usually seen that is also observed in PEDV grown in Vero cells (Hofmann and Wyler 1988; Ksiazek et al. 2003). Detection of virus in the cell culture is confirmed by VN, IF or IEM using specific TGEV antiserum or differential monoclonal antibodies (Garwes et al. 1988) and virus-specific RT-PCR (Enjuanes and Van der Zeijst 1995; Laude et al. 1993; Kim et al. 2000).

TGEV antibodies can be identified by several serological tests. However, TGEV serology is complex, as both TGEV and PRCV prompt VN antibodies that are quantitatively and qualitatively similar (Pensaert 1989). A blocking ELISA that uses MAbs to TGEV differentiates easily from PRCV (Garwes et al. 1988; Bernard et al. 1989; Callebaut et al. 1989). However, blocking ELISA works better on a group basis since certain pigs with low antibody titres to TGEV or PRCV may not show positivity (Callebaut et al. 1989; Sestak et al. 1999b; Simkins et al. 1993). Further, the accuracy of commercial ELISAs for distinguishing US strains of PRCV and TGEV is low (Sestak et al. 1999a).

4.5.2 PEDV

As for TGEV, diagnosis of PEDV must be based on clinical signs and lab identification of viral RNA, viral antigens or increased PEDV-species antibodies. For the location of PEDV RNA, the most broadly utilised lab technique is conventional PCR (Kim et al. 2001; Ishikawa et al. 1997) or real-time RT-PCR (Kim et al. 2007), which is sensitive, specific and rapid for the detection of viral RNA in different clinical samples. Although loop-mediated isothermal amplification (LAMP) assays (Ren and Li 2011; Yu et al. 2015) are recognised more recently as highly sensitive in detecting PEDV RNA, they have still not reached diagnostic labs. In situ hybridisation can be utilised to recognise PEDV RNA in fixed tissues (Stadler et al. 2015).
Direct display of PEDV and additionally its antigens is done utilising IF or IHC tests on the small intestinal tissues of piglets euthanised close to the onset of diarrhoea and before the desquamation of enterocytes. The virus particles can be shown in direct EM or IEM on diarrhoeic pig faeces. However, IEM must be used to distinguish PEDV from other CoVs, viz. TGEV and PDCoV, as all CoVs possess indistinguishable morphology.

Successful isolation of PEDV in Vero cells is increased when using intestinal contents/homogenates compared to faeces (Oka et al. 2014; Chen et al. 2014). ELISA tests based on polyclonal antibodies and MAbs are available for detection of PEDV antigens in faeces (Callebaut et al. 1982; Carvajal et al. 1995).

Paired serum samples are requisite for serologic diagnosis of endemic PEDV. Recently, IgG and IgA antibodies to PEDV are observed in oral fluids, suggesting that they may be appropriate to monitor prior herd exposure to PEDV (Bjostrom-Kraft et al. 2016). PEDV antibodies have been demonstrated using indirect ELISAs based on the whole cell-culture-adapted virus antigens (Carvajal et al. 1995; Hofmann and Wyler 1990; Kweon et al. 1994; Thomas et al. 2015), S/N viral proteins extracted from infected Vero cells (Knuchel et al. 1992; Oh et al. 2005) or bacteria or mammalian expression systems (Wang et al. 2015; Gerber et al. 2014; Gerber and Opriessnig 2015; Okda et al. 2015; Paudel et al. 2014). Blocking and competitive ELISAs have additionally been used for the detection of PEDV antibodies utilising MAbs or polyclonal 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; they peak around 21 dpi and then decline gradually (Okda et al. 2015). The VN test in Vero cell culture is used to assess VN antibodies to PEDV (Thomas et al. 2015; Okda et al. 2015; Paudel et al. 2014). These serological assays have generally been employed to screen prior exposure to the virus and to assess the viability of vaccines.

Overall, use of a laboratory test is suggested which must differentiate PEDV infections from TGEV, SeCoV and PDCoV. Especially for SeCoV that is a recombinant of TGEV (backbone) and PEDV (S protein), a selective assay based on detecting TGEV (any gene aside from S gene) and PEDV (S gene) would be preferred.

### 4.5.3 PDCoV

Selective laboratory assays must be opted to distinguish PDCoV infection from related PEDV, TGEV and RV infections. The methodologies discussed for TGEV and PEDV apply to PDCoV diagnosis. The confirmatory finding of PDCoV infection incorporates detection of PDCoV RNA or antigens in the faeces or intestinal substance/tissues. Diagnosis can also be made utilising RT-PCR assays that target a conserved region of PDCoV M or N genes (Marthaler et al. 2014b; Wang et al. 2014c), 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 hybridisation (Jung et al. 2015b). A duplex real-time RT-PCR assay for detection of PDCoV and/or differentiation from PEDV in intestines and faeces was developed (Zhang et al. 2016b).
Direct EM can be used to display PDCoV viral particles in faeces from diarrhoeic pigs. However, IEM use must distinguish PDCoV from PEDV or TGEV (Jung et al. 2015a) using hyperimmune or convalescent sera. However isolation of PDCoV has been attempted with limited success in LLC-PK or ST cells, except in few strains (OH-FD22) (Hu et al. 2015). The other serologic assays in use for the diagnosis of PDCoV are IF, VN and ELISA. Serum and milk PDCoV antibodies of different isotypes have been quantified using ELISA based on cell culture virus antigen (Ma et al. 2016) or S1 and N viral proteins (Thachil et al. 2015; Okda et al. 2016; Su et al. 2016).

4.6 Transmission, Risk Factors and Stability

4.6.1 TGEV

TGEV is more stable in frozen conditions, but becomes fragile at room or higher temperatures. In an experimental study, the virus infectivity was maintained for more than 8 weeks at 5 °C, 2 weeks at 20 °C and 24 h at 35 °C in liquid manure slurry (Haas et al. 1995). Further, the virus is highly photosensitive and gets inactivated less than 6 h in the exposure of sunlight or ultraviolet light (Cartwright et al. 1965; Haelterman 1962). It also gets inactivated to 1% Lysovet (phenol and aldehyde), 0.03% formalin, 0.01% beta-propiolactone, 1 mM binary ethylenamine, sodium hypochlorite, NaOH, iodines, quaternary ammonium compounds, ether and chloroform (VanCott et al. 1993; Brown 1981). TGEV field strains are resistant to trypsin and relatively stable in pig bile, and at pH 3 (Laude et al. 1981), letting the virus survive in the stomach and small intestine.

In temperate climates, TGE is a seasonal disease with most outbreaks occurring in the winter months. It is assumed that this could be due to the virus stability in frozen form, and frailty to heat or sunlight (Haelterman 1962), permitting easy virus spread among herds in winter on fomites or animals. Three potential reservoirs for TGEV between seasonal epidemics proposed are (1) herds with enzootic TGE; (2) hosts other than swine and (3) carrier pigs. Dogs, cats and foxes could be the possible carriers, facilitating its spread on farms, as the shedding of the virus occurs for variable periods (Haelterman 1962; McClurkin et al. 1970) with the excreted virus (by dogs) remaining infectious for pigs (Haelterman 1962; Reynolds et al. 1980).

The increased concentration of wintering starlings (Sturnus vulgaris) in feedlots was suggested to contribute to the mechanical spread of TGEV between farms during the cold season. In a study, TGEV was seen in the droppings of starlings for as long as 32 h after feeding TGEV (Pilchard 1965). Likewise, housefly (Musca domestica) is also proposed as a mechanical vector for TGEV (Pilchard 1965). TGEV antigen is seen in flies on a swine herd and TGEV shedding up to 3 days from experimentally feeding flies (Gough and Jorgenson 1983). Notably, surveys done in Central Europe confirmed the presence of TGEV antibodies in nearly 30% of the feral pigs (Sedlak et al. 2008). Although TGEV shedding is detectable for up to 104 dpi (Underdahl et al. 1975), it is undefined yet whether infectious virus
particles are shed or transmitted at that time. Adding of sentinel pigs to a herd at 3, 4 and 5 months after a past TGE outbreak resulted in no new disease in the introduced pigs (Derbyshire et al. 1969).

### 4.6.2 PRCV

Swine population density, farm distances and seasons influence PRCV epidemiology (Pensaert 1989; Have 1990). Pigs get PRCV infection at any age through contact or airborne transmission. The risk of spreading PRCV increases in zones of high swine density, where the virus can journey several miles.

### 4.6.3 PEDV

As for other enteric viral infections, in PEDV also direct or indirect faecal-oral transmission is the main route of virus transmission. Acute outbreaks in non-immune farms often occur within 4–5 days after newly purchased pig arrival. The virus enters farms mostly via infected pigs, but also by contaminated feed, trucks, boots or other fomites. Farm workers may also act as a vehicle for virus transmission to naïve pigs (Dee et al. 2014, 2016; Schumacher et al. 2016). Evidence of PEDV aerosol transmission is reported in some (Alonso et al. 2014) but not other studies (Niederwerder et al. 2016). In four-week-old pigs infected with emerging non-S INDEL PEDV strain, infectious virus excretion lasted for 14–16 days (Crawford et al. 2015). Nevertheless, a few pigs shed PEDV RNA, at 42 days post-initial oral exposure, but non-infectious virus particles were seen in faeces.

Similar to TGEV, after initial outbreaks on the breeding farms, PEDV can become endemic if sufficient litters of pigs are produced and weaned, allowing maintaining the virus. Of note, a report from South Korea showed 9.75% PEDV infection rate in wild boars (Lee et al. 2016b), although their role in maintenance and transmission of PEDV is unclear.

### 4.6.4 PDCoV

The main method of PDCoV transmission is the faecal–oral route. Faeces, vomit and other contaminated fomites are the major sources of the virus. Experimentally induced PDCoV diarrhoea lasted for ~5–10 days, with faecal virus RNA shedding lasting for up to 19 days (Hu et al. 2016; Ma et al. 2015). Pigs generally continue shedding PDCoV RNA in the faeces after recovery from disease; therefore, another possible reservoir for PDCoV may be subclinically infected or convalescent carriers.
4.7 Prevention and Control

4.7.1 TGEV

Treatment of clinically affected newborn piglets is usually ineffective in field situations; however, electrolyte/glucose solution supplementation of piglets that are 1 week or older may reduce their mortality (Bohl 1981). Extra heat, deep bedding and antibiotic solutions (to treat secondary infections) generally can improve piglet health.

Enhanced biosecurity measures should be maintained to decrease a chance of introduction of infected animals, and contaminated vehicles from TGEV-affected farms to susceptible herds. TGEV infection can be spread not only with infected live animals but also with unprocessed tissues of slaughtered TGEV-infected animals (Forman 1991).

Many methods for immunising sows to induce lactogenic immunity and consequent protection of neonatal piglets have been attempted (Chattha et al. 2015; Saif and Jackwood 1990; Bohl and Saif 1975). Several viral vaccines (virulent, attenuated, inactivated and recombinant subunit) with different routes of administration (oral, intra-nasal, subcutaneous, intramuscular and intra-mammary) (Saif and Sestak 2006; Moxley and Olson 1989) have been evaluated in the past. To note, intramuscular, parenteral or intra-mammary administration of pregnant sows with live attenuated, inactivated or subunit vaccines did not offer complete protection but were found to be effective to reduce piglet mortality rates (Brim et al. 1994). Unlike natural intestinal infection with the virulent virus, attenuated viruses do not stimulate the gut-MG-sIgA axis sufficiently for the induction of immunity similar to that observed following this. There are two commercial vaccines based on a live-modified TGEV strain for combined oral-intramuscular administration produced by Merck Animal Health: PROSYSTEM® TGE/Rota and PROSYSTEM® TREC. These vaccines can effectively stimulate a response in previously exposed pigs, but do not protect the naïve population.

Herd immunity can be enhanced by exposing all the sows to virulent TGEV (using intestinal contents or gut tissues of affected pigs) to boost lactogenic (milk) immunity (Bohl and Saif 1975; Bohl et al. 1972). This practice is called feedback and results in the rapid development of immunity in pregnant sows (particularly in those due to farrow 2 weeks or more after the start of the outbreak) and reduces losses in newborn piglets. However, it may also result in dissemination of other pathogens (potentially present in TGEV-containing faeces/intestinal contents) to adjacent herds. On small-scale farms, herd immunity is accomplished, and TGEV infection is self-limiting. In contrast, in larger farms (≤200 sows) with a continuous farrowing system and continual influx of susceptible animals, TGEV infection frequently becomes endemic after the primary outbreak (Saif and Sestak 2006). Elimination of endemic TGE in a herd can be attempted using the feedback method. After this is done, no weaning of piglets should occur during the following 3–4 weeks so that there remains no susceptible host in the herd while TGEV is circulating on the farm.
4.7.2 **PEDV**

Due to the lack of PEDV-specific antivirals, the treatment is focused on alleviating the diarrhoeal disease. PEDV-infected pigs must get enough water to reduce dehydration, which exacerbates the severity of the disease. Temporary withholding of feed may benefit fattening pigs during the acute stage of the disease.

As in the case with TGEV, appropriate biosecurity measures should be applied to avoid the introduction of PED onto farms. Present epidemiological knowledge indicates that virus is spread between farms mainly through animal and human traffic, and contaminated feed (https://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/seed_final_report.pdf). Careful disposal of the dead stock is recommended.

In contrast to the present situation in Asia, in Europe, PEDV infection (with mostly mild S INDEL strains in circulation) is considered to be of marginal economic importance and therefore does not warrant the development of a vaccine (Lee 2015). However, severe classical PEDV outbreaks in Asia have necessitated the development of PEDV vaccines to prevent and control the infection. In China, the CV777-based inactivated and attenuated PEDV vaccines were approved in 1995 and 1998, respectively (Wang et al. 2016b). Soon after, attenuated vaccines based on classical PEDV strains KPEDV-9 and DR13 were commercialised in 1999 and 2004, respectively, in Korea (Kweon et al. 1999; Song et al. 2007). Since 1997 a commercial attenuated PEDV vaccine based on cell culture-adapted classical PEDV P-5V strain (Nisseiken Co. Ltd., Japan) is administered to sows in Japan (Sato et al. 2011). These vaccines based on classical PEDV strains appeared to satisfactorily control PED in Asia until the highly virulent non-S INDEL PEDV strains have emerged (Lee 2015). As demonstrated in the field, the classical PEDV vaccines have failed to protect pigs from severe diarrhoeal disease associated with the emerging highly virulent non-S INDEL PEDV strains (Lee 2015).

The deliberate exposure of pregnant sows (feedback method) to PEDV promotes the rapid development of lactogenic immunity and thus shortens the course and the severity of the disease on the farm (Chattha et al. 2015). However, as mentioned in the TGEV section, this method may contribute to the spread of other infectious agents throughout the farm. We have recently demonstrated that high dose of virulent PEDV administered to sows can substantially increase their piglet survival rate as compared to low-dose and mock-infected sows (Fig. 4.3) (Langel et al. 2016). This novel finding suggests that the current feedback-based control strategies can be improved by ensuring the uniform administration of high-dose PEDV to pregnant sows.

Since the outbreaks of 2013, the USA has conditionally licensed two PEDV vaccines targeting emerging non-S INDEL PEDV strains: alphavirus-based vaccine (Harris vaccines™, now Merck Animal Health) and an inactivated vaccine (Zoetis) (2014). The first vaccine was developed in June 2014 using a replication-deficient Venezuelan equine encephalitis (VEE) virus packaging system expressing the S protein of an emerging non-S INDEL PEDV strain (Crawford et al. 2016).
The second vaccine developed in September 2015 was an inactivated whole-virus (non-S INDEL PEDV) vaccine plus an adjuvant (Crawford et al. 2016). In October 2016, an inactivated vaccine based on non-S INDEL PEDV strain AJ1102 was licensed in China (Wang et al. 2016b). In South Korea, an inactivated vaccine candidate based on non-S INDEL strain KNU-141112 was demonstrated to be protective in sows and their suckling piglets (Baek et al. 2016). However, the efficacy of these vaccines/vaccine candidates in the field is not assessed. To date, reverse genetics platforms have been generated for both classical and emerging non-S INDEL PEDV strains using different approaches (Beall et al. 2016; Jengarn et al. 2015; Li et al. 2013) and can be used for the future rational design of safe and effective PEDV vaccines.

4.7.3 PDCoV

The disease-preventive measures adopted for TGEV and PEDV control and prevention can be useful for PDCoV infection too. In the absence of any suitable vaccines or antivirals to control PDCoV disease, reliable regime includes symptomatic action giving bicarbonate liquids and ad lib water to alleviate acidosis and dehydration in suckling pigs. Antibiotics administration may be beneficial in the case complicated by concurrent/secondary bacterial infection. In the event of heavy mortality, feedback techniques must be opted to stimulate lactogenic immunity and reduce mortality. Additionally, during PDCoV epidemics, a strict biosecurity plan must be implemented to lessen PDCoV spread via infected fomites. The systems all in/all out and thorough disinfection (using phenolic disinfectants, bleach, peroxides, aldehydes or iodophors) can break the disease cycle.
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