

Update on porcine epidemic diarrhea

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Porcine epidemic diarrhea virus (PEDV), a member of the Coronaviridae, is antigenically distinguishable from the other porcine coronaviruses, transmissible gastroenteritis virus (TGEV), and hemagglutinating encephalomyelitis virus (HEV).^{1,2,3} Diarrhea caused by PEDV is clinically difficult to distinguish from diarrhea caused by infection with TGEV.^{4,5} Transmissible gastroenteritis (TGE)-like outbreaks of acute diarrhea were first reported in feeder and grower pigs in England in 1971, but TGEV and other known enteropathogenic agents were ruled out as the cause.⁶ During these outbreaks, suckling pigs (less than 4 to 5 weeks of age) did not become sick. In 1976, other European countries reported similar disease outbreaks in pigs of all ages, including suckling pigs.^{4,7,8} The disease was named “epidemic viral diarrhea” (EVD) and the designates EVD type 1 (EVD1) and EVD type 2 (EVD2) were used to differentiate between the earlier outbreaks and those affecting pigs of all ages.^{7,8} In 1978, a coronavirus-like agent was demonstrated as the causative agent of EVD2 and probably the cause of EVD1 outbreaks, and the name “porcine epidemic diarrhea” (“PED”) was proposed.^{7,8} The reason for the different clinical presentations of EVD1 and EVD2 remains unknown. Since then, the disease has been recognized in a number of European countries, and more recently in China, Korea, and Japan.^{5,9–13} In recent years, acute outbreaks have become rare in regions where the virus is widespread. Presently in Europe, diarrhea due to PEDV

is most often seen in feeder and grower pigs and in young breeding animals, whereas suckling piglets are rarely affected. In Asia, however, severe epizootics with high mortality still occur in swine of all ages, and these outbreaks cannot be differentiated clinically from acute TGE.^{5,12,13}

Characteristics of PEDV

The morphologic, physicochemical, and biological features of PED virus particles are characteristic for the coronavirus family.^{2,8,11} Porcine epidemic diarrhea viral particles are pleomorphic and range in diameter from 90 to 190 nm (Figure 1).^{2,14–16} They have an electron-dense core with central halo and club-shaped projections of approximately 20 nm length radiating from the core.^{2,14–16} The virus is ether- and chloroform-sensitive and does not hemagglutinate erythrocytes from a wide variety of species.^{11,17}

Attempts to grow PEDV in cell culture proved difficult, and it took more than 10 years for the virus to be propagated in Vero cells.^{17,18} Viral growth depends on the presence of trypsin in the cell culture medium. Vero cell-adapted PEDV has been successfully propagated in other cell types.^{12,19} Only one serotype of PEDV has been identified. Isolates from Europe, Korea, and China are serologically identical to the prototype CV777 strain.^{4,12,17}

The sequence of the entire genome of strain CV777 has only recently been determined and was found to be 28,033 nucleotides (nt) in length (excluding the poly A-tail).^{20,21} Analysis of the nucleotide

sequences revealed a small open reading frame (ORF) located near the 5' end (nt 99 to 137), and two large, slightly overlapping ORFs, ORF1a (nt 297 to 12650) and ORF1b (nt 12605 to 20641).^{20,22,23} The ORF1a and ORF1b sequences overlap at a potential ribosomal frame shift site. The amino acid sequence analysis suggests the presence of several functional motifs within the putative ORF1 protein. There are major genomic differences between cell culture-adapted (ca-PEDV) and wild type virus (wt-PEDV).^{20–23}

Comparative amino acid sequence alignments revealed that PEDV is most closely related to human coronavirus (HCoV) 229E and transmissible gastroenteritis virus (TGEV) and is more distantly related to murine hepatitis virus and infectious bronchitis virus.^{20,22,24} Antigenic determinants common to PEDV and feline infectious peritonitis virus are located on the nucleocapsid protein.²⁵ Identification of the membrane glycoprotein supports classification as a group I coronavirus.^{2,11,24,26}

Geographic distribution

Porcine epidemic diarrhea was first reported in the UK in 1971, and the virus was identified during episodes of epizootic diarrhea in pigs in Belgium and in the UK.^{7,8} The disease has subsequently been reported in Canada, Hungary, Germany, China, Korea, and Japan.^{5,11–13,16} However, outbreaks of severe PED are now rare in Europe. Very likely, this is due to the enzootic character of the virus and the presence of lactogenic immunity, which protects suckling pigs. More recently, severe outbreaks of PED with high mortality have been reported in Spain, Japan, and Korea.^{12,27,28} The Asian epizootics are so severe that they cannot be differentiated clinically from acute TGEV outbreaks, and they incur heavy economic losses. Until now, PEDV has not been detected in the United States or South America. Seroprevalence of PEDV has been confirmed in most major swine-producing countries worldwide, but no recent

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serologic surveys or diagnostic studies have been published in Europe or North America.^{10,29}

Transmission

Modes of transmission of PEDV are similar to those of TGEV, but PEDV tends to persist more easily on infected premises. Fecal-oral transmission is probably the main or only route of infection.^{30,31} Most commonly, the introduction of infected pigs into susceptible farms causes outbreaks of PED within 4 to 5 days.⁴ Virus may also be introduced through contaminated equipment and other fomites or personnel. After a disease outbreak, PEDV may disappear, or it may become enzootic on farms where there are sufficient litters of pigs to allow the virus to be maintained through

Figure 1: Intracytoplasmic vacuole in a porcine small intestinal enterocyte, containing pleomorphic porcine epidemic diarrhea virus particles of 90 to 190 nm in diameter ($\times 20,000$).

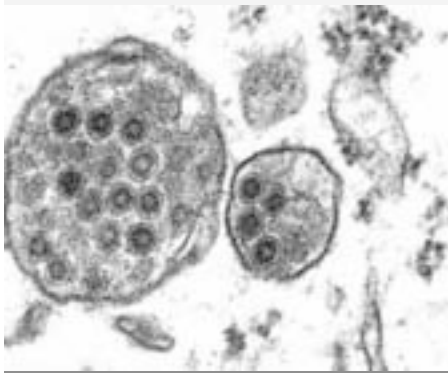
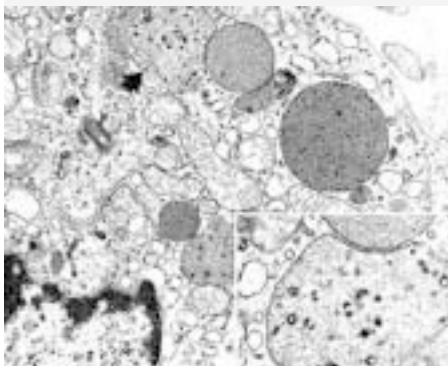


Figure 2: Replication of porcine epidemic diarrhea virus in a cytoplasmic vacuole (arrow) of a porcine small intestinal enterocyte ($\times 5000$). Insert: higher magnification of the cytoplasmic vacuole containing pleomorphic virus particles of 90 to 190 nm in diameter ($\times 10,000$).



infection of consecutive litters that have lost their lactogenic immunity at weaning.

Zoonotic potential and public health implications

Infection of human beings and other species with PEDV has not been reported.

Clinical presentation

The severity of clinical disease caused by PEDV is highly variable, depending largely on the immunological or enzootic status of the pig farm. Nevertheless, the main and often only clinical sign is watery diarrhea.^{4,7-9,16,30-32} Diarrheic feces are watery and flocculent, and have a characteristic fetid smell owing to steatorrhea.^{9,16,30-32} Affected animals commonly vomit. In susceptible breeding herds, pigs of all ages may be affected, with morbidity approaching 100%. Watery diarrhea, and dehydration and metabolic acidosis causing mortality of 50 to 80%, are the principal features of PED in suckling pigs, whereas infections of feeder and grower pigs are characterized by diarrhea, anorexia, depression, and a high morbidity but low mortality (1 to 3%).^{9,11,16,30-32} In enzootic situations, PEDV may cause persistent diarrhea in recently weaned pigs. Clinical presentation may be very similar to TGE, but there is often a slower spread of disease and lower mortality in baby piglets, and more severe disease than with TGEV if infection occurs toward the end of the growing period. Mortality in grower pigs may reach 1 to 3%, and pigs often appear to have abdominal pain and may die acutely during the early stages of diarrhea or even prior to its onset.^{4,8,9,11,16,30-32} Recovery occurs after approximately 7 to 10 days.

Pathogenesis

Experimental oral inoculation of caesarian-derived, colostrum-deprived pigs with the CV777 isolate of PEDV causes diarrhea 36 hours post inoculation (PI). Like TGEV, PEDV replicates in the cytoplasm of villous enterocytes throughout the small intestine (Figure 2), causing epithelial cell degeneration with subsequent villus shortening.^{14,16} However, the effects of TGE are more intense and dramatic.¹¹ Enterocytes along the entire length of villi are susceptible to infection with PEDV, and infected enterocytes have been observed as early as 12 to 18 hours PI, with a maximum reached between 24 and 36 hours PI. The clinical course in gnotobiotic pigs

experimentally infected with ca-PEDV was much milder than that in pigs infected with wt-PEDV. The ca-PEDV was notably less virulent, viral replication was slower, and histopathological changes were less evident.

Porcine epidemic diarrhea virus has also been detected in epithelial cells of the colon in conventional grower pigs with both experimental and naturally occurring PED.^{11,16} The significance of colonic infection and its role in the development of more severe disease in older pigs is unknown. Also, the cause of sudden death accompanied by acute back muscle necrosis that has been described in finishing and adult pigs with PED is unknown. Replication of PEDV in pigs has not been detected in cells outside the intestinal tract.

Pathology

Gross and microscopic lesions are similar to those described for TGE. Stomachs are typically empty due to vomiting, and lacteals are devoid of chyle due to malabsorption. Small intestines are fluid-filled and appear thin-walled due to severe mucosal atrophy (Figures 3 and 4).^{14,16,28} Intestinal contents are flocculent. In addition to lesions similar to those of TGE, acute necrosis of back muscle has been reported.

Microscopically, marked cytoplasmic vacuolation and exfoliation of enterocytes with subsequent considerable shortening and fusion of villi is noted, but is less severe than in TGE (Figures 3, 4, and 5).^{9,16,32} No microscopic lesions have been observed in the colon. Interestingly, ultrastructural studies revealed intracytoplasmic viral particles and cellular changes in epithelial cells of the small intestine and the colon.^{14,16,32} Ultrastructural changes were initially characterized by loss of cell organelles, microvilli, and the terminal web, and protrusion of parts of the cytoplasm into the intestinal lumen.¹⁶ Later, cells became flattened, the tight junctions were lost, and cells were released into the gut lumen.

Diagnosis

Porcine epidemic diarrhea cannot be diagnosed on the basis of clinical findings alone, and acute outbreaks of PED cannot be clinically differentiated from TGE regardless of the age of pigs infected. Laboratory methods are necessary for an etiologic diagnosis. The most commonly used diagnostic methods are the direct

Figure 3: Cross section of small intestine ($\times 20$) from a pig infected with porcine epidemic diarrhea virus. Note villous shortening.

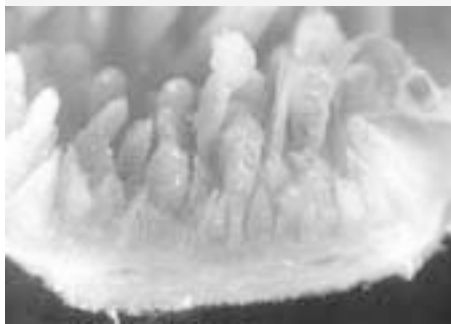
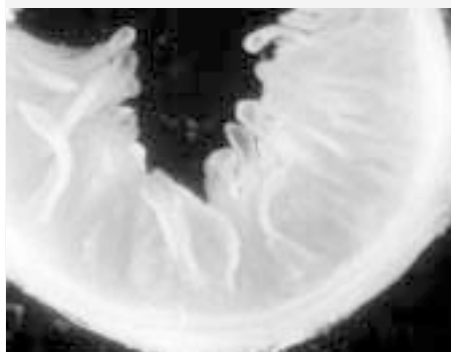


Figure 4: Cross section of small intestine ($\times 10$) from a control pig (non-infected with porcine epidemic diarrhea virus).



immunofluorescence test (IFT) for detection of viral antigen, and enzyme-linked immunosorbant assays (ELISA) to demonstrate PEDV antigen in feces or antibodies in serum.^{11,27,31,33} The virus can also be detected by immunohistochemistry (IHC),^{30,34} polymerase chain reaction (PCR),^{8,24,35–41} indirect fluorescent antibody test (IFA),⁴² in situ hybridization,⁴³ and electron microscopy (EM).^{10,16}

Fluorescent antibody tests and IHC (Figure 6) on sections of small intestine from pigs with acute diarrhea are the most sensitive, rapid, and reliable methods. They should be used preferably within 3 days of onset of diarrhea. Electron microscopy is widely used to detect viral particles in fecal material of pigs with diarrhea.^{9,28,30} Identification of coronaviruses in feces may be difficult because virus particles are not easy to detect if the spikes are lost or are not clearly visible. Electron microscopy is not a sensitive technique, and TGEV cannot be differentiated from PEDV. In contrast, the ELISA has a much higher sen-

sitivity than EM and also allows differentiation between TGEV and PEDV.^{27,31,33} Using ELISA tests, PEDV antigen can be detected in rectal swabs of experimentally inoculated pigs until 11 days PI.²⁷ The ELISA antigen test, using monoclonal or polyclonal antibodies, may be used to detect PEDV in endemically infected herds and cases of persistent diarrhea in breeding farms where the amount of virus is too small to be detected by any other methods.^{27,31,33} More recently, published reports show that reverse transcriptase PCR may be used in a similar manner to differentiate TGE from PEDV and is highly sensitive.^{35–41} Cell culture cannot be used for routine diagnosis.

Antibodies against PEDV can be detected in sera from swine with naturally occurring or experimentally induced PED. Antibodies are detectable by blocking ELISA 7 days PI and by IFA testing between 10 and 13 days PI.²⁷ Paired serum samples should be examined, and the convalescent sample should be collected 2 to 3 weeks after the onset of diarrhea.^{17,27,29} Antibodies generally persist for more than a year in the serum of infected pigs.

Treatment and prevention

Specific therapeutic recommendations for PED are not available. Symptomatic treatment of diarrhea is recommended, including free access to water to diminish dehydration, and withholding of feed, particularly in growing swine.

Sanitary measures should be taken to prevent introduction of PEDV to the farm. Introduction of persistently infected pigs poses the highest risk, and disease can also be spread by human traffic between affected units. After diagnosis of PED, because of the slow spread of disease, the primary concern should be initiation of preventive measures to temporarily prevent virus entrance into farrowing units. Artificial exposure of pregnant sows to feces from PEDV-infected pigs stimulates lactogenic immunity and helps to postpone infection of these sows' piglets until they are older, resulting in fewer deaths.¹¹ If persistence of the virus is diagnosed in consecutive litters of weaned piglets after an outbreak has occurred, virus elimination can be attempted by removing pigs immediately after weaning to another site for at least 4 weeks.

Recently, the immunoprophylactic effects of chicken egg yolk immunoglobulin (IgY)

against PEDV were investigated in neonatal pigs.⁴² Administration of IgY was associated with reduced mortality and increased survival rate in piglets after challenge exposure to PEDV. This suggests that IgY against PEDV might be an alternative prophylactic measure similar to stimulated lactogenic immunity.

Porcine epidemic diarrhea virus is stable between pH 5.0 and 9.0 at 4°C and between pH 6.5 and 7.5 at 37°C.¹¹ Culture-adapted PEDV loses infectivity when heated to 60°C for 30 minutes, but is moderately stable at 50°C.

Porcine epidemic diarrhea virus is inactivated by most virucidal disinfectants, including cresol, sodium hydroxide (2%), formalin (1%), sodium carbonate (4% anhydrous or 10% crystalline, with 0.1% detergent), ionic and non-ionic detergents, strong iodophors (1%) in phosphoric acid, and lipid solvents such as chloroform. Examples of disinfectants effective against PEDV are shown in Table 1.

Figure 5: Section of small intestine from a pig infected with porcine epidemic diarrhea virus. Note villous shortening and vacuolation of enterocytes (H&E, $\times 200$).

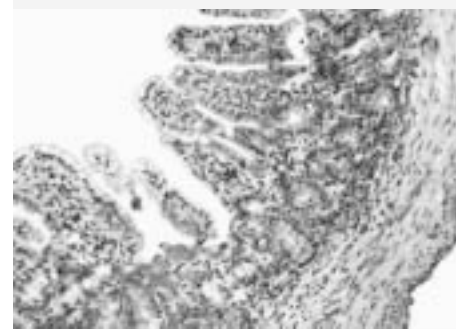


Figure 6: Immunohistochemical labeling of porcine epidemic diarrhea virus antigen (brown staining) within porcine small intestinal enterocytes ($\times 200$).

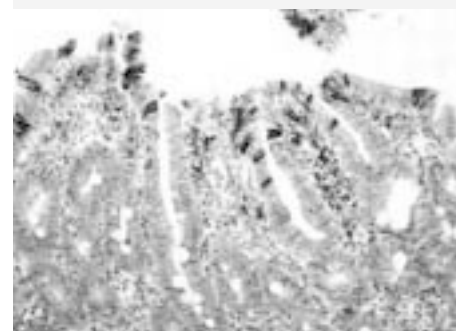


Table 1: Disinfectants effective against porcine epidemic diarrhea virus

Product	Manufacturer	Disinfectant class	Dilution rate	Precautions
Virkon S	Antec International, Arlington, Texas	Oxidizing agent (potassium peroxymonosulfate)	1:100	Contact with skin is corrosive: use of goggles and rubber gloves is recommended.
Clorox bleach	The Clorox Company Oakland, California	Oxidizing agent (sodium hypochlorite)	1:32	Disinfectant properties inactivated by organic material and diminished by alkaline materials (eg, lime) and moisture. Contact with skin is irritating.
1 Stroke Environ	Calgon Vestal Saint Louis, Missouri	Phenolic compound	1-2%	Disinfectant properties not inactivated by organic debris, enhanced by warm temperatures, diminished by cold temperatures. Contact with skin is corrosive: use of goggles and rubber gloves recommended.
Tek-Trol	Bio-Tek Industries, Inc Atlanta, Georgia	Phenolic compound	1-2%	Disinfectant properties not inactivated by organic debris, enhanced by warm temperatures, diminished by cold temperatures. Contact with skin is corrosive: use of goggles and rubber gloves recommended.

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Editor's note: This article is the fourth in a series dedicated to foreign animal diseases. Although US swine veterinarians are unlikely to see these diseases in practice, awareness is vital to early detection.

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