10 PORCINE NIDOVIRUSES

10.1 Porcine Coronaviruses

Karol Sestak and Linda J. Saif

SUMMARY

The family Coronaviridae belongs to the order Nidovirales. Coronaviruses are pathogens associated with infections of veterinary importance causing a spectrum of clinical syndromes that vary depending on the host. Due to their large, inherently error-prone RNA genome, coronaviruses are well adapted to changing environmental selective pressures. The dynamic, quasi-species character of this virus family was recognized two decades ago with the emergence of porcine respiratory coronavirus (PRCV), a deletion mutant of transmissible gastroenteritis virus (TGEV). It was later shown that the loss of only two amino acids from the TGEV major surface attachment protein might result in a change from gastrointestinal to respiratory tropism. In addition to TGEV and PRCV, two other antigenically distinct coronaviruses have been isolated from pigs: hemagglutinating encephalitis virus (HEV) and porcine epidemic diarrhea virus (PEDV). Due to their emerging/reemerging nature and impact on swine production, this chapter deals only with TGEV, PRCV, and PEDV. Historical and current aspects related to epidemiology, pathogenesis, diagnosis, prevention, and control of these three porcine coronavirus species are discussed.

INTRODUCTION

The Coronaviridae family consists of two genera of RNA viruses that infect vertebrates including humans, domestic animals, and birds (Horzinek 1999). Coronavirus infections are usually associated with respiratory, gastrointestinal, cardiovascular, and neurological diseases (Lai 1990). The best-known representatives of this virus family include avian infectious bronchitis virus (IBV), mouse hepatitis virus (MHV), porcine transmissible gastroenteritis virus (TGEV), bovine coronavirus (BCV), human coronavirus (HCV), feline infectious peritonitis virus (FIPV), canine coronavirus (CCV), turkey coronavirus (TCV), and several other virus species of veterinary importance (Siddell et al. 1983; Lai 1990). From pigs, four antigenically distinct coronaviruses have been isolated: transmissible gastroenteritis virus (TGEV), hemagglutinating encephalomyelitis virus (HEV), porcine epidemic diarrhea virus (PEDV), and porcine respiratory coronavirus (PRCV) (Pensaert 1989).

Historically, the first report of clinical disease in pigs caused by coronaviruses dates to 1946 (Doyle and Hutchings 1946) and TGE, which occurs throughout the world. According to serological surveys conducted in North America and Europe, a high seroprevalence (36% to 100%) to TGEV exists among swine [US Department of Agriculture (USDA) 1997; Pensaert et al. 1993]. Accurate TGEV seroprevalence assessments are complicated by the fact that antibodies to a natural deletion mutant of TGEV, PRCV, are indistinguishable from TGEV antibodies by routine serological diagnostic assays. According to the 1995 and the 1990 National Swine Surveys (United States), approximately 6% of swine operations reported problems with TGEV (USDA 1992, 1997). Diagnostic studies indicated that among the piglets that died from diarrhea before reaching the age of 1 month, 8% were positive for TGEV (Moon and Bunn 1993). A more recent serological survey showed that all of the 22 "medium to large" size swine herds studied in Iowa were positive for TGEV or PRCV antibodies, with 16 herds being specifically positive for TGEV antibodies (Wesley et al. 1997).

The emergence of PRCV from 1984 onward coincided with the disappearance of TGEV in Europe (McGoldrick et al. 1999). Based on several TGEV-PRCV cross-protection studies, it was suggested that repeated subclinical PRCV infections increased the level of immunoglobulin A (IgA) antibodies cross-reactive to TGEV in milk of lactating sows (Sestak et al. 1996). The TGEV infection in piglets born to such sows was characterized by reduced severity of clinical disease. In these situations, PRCV acts as a naturally modified-live vaccine to TGEV and induces active immunity in pregnant sows that is passively transferred to suckling piglets (Sestak et al. 1996).

Since the mid-1980s, a previously unrecognized porcine coronavirus spreading rapidly through Europe was identified (DeBouck et al. 1982). Epidemic spread, enteropathogenicity, and ability to cause diarrhea in swine of all ages were reported (Pensaert 1999). The agent was found to be antigenically distinct from TGEV, HEV, and other animal coronaviruses (Pensaert et al.

1981). The name porcine epidemic diarrhea virus (PEDV) was adopted. At present, PEDV has been identified in most swine-producing countries, except the Americas (Pensaert 1999).

EPIDEMIOLOGY

Transmissible Gastroenteritis Virus

Transmissible gastroenteritis can occur in three different forms, depending on the herd's health status (Bohl 1989). When the virus spreads within a fully susceptible herd with no previous history of TGEV, it is referred to as epizootic TGE, characterized by up to 100% mortality among newborn pigs, marked diarrhea and dehydration in weaned pigs, and inappetence, vomiting, and diarrhea in adult animals. Partial or total agalactia of lactating sows is common (Lanza et al. 1995). Epizootic TGE ends within several weeks. In herds where TGE is on the decline but the continuous introduction of susceptible animals occurs, infection becomes more chronic and is referred to as endemic or enzootic TGE (Bohl 1989). Mortality among endemic herds usually does not exceed 20%; however, the decline of colostral and milk antibodies contributes to the onset of diarrhea. A modification of endemic TGE is known as intermittent endemic TGE, where virus is introduced into a herd where only adult animals (sows) have been previously exposed and therefore can provide some passive immunity to their pigs (Bohl 1989). Because of better TGEV stability when kept cold and protected from the sunlight, TGE tends to be a seasonal infection with mainly a winter occurrence (Haelterman 1973). Transmission of virus by means of mechanical vectors or occasional hosts (dog, cat, fox, or starlings) can take place (Bohl 1989). In feces of young pigs, TGEV can be shed for up to 2 weeks and in the nasal secretions for 10 to 11 days (Kemeny et al. 1975).

Since its first description in an Indiana swine herd in 1946 by Doyle and Hutchings, TGE has been reported in all countries with an intensive pork industry (Bohl 1989; Doyle and Hutchings 1946; Saif and Wesley 1999). The economic losses caused previously by TGEV were significant, as reported from France, the United States, Czechoslovakia, England, and the Netherlands (Bohl 1989; Saif and Wesley 1999). In the United States, TGE remains a problem. TGEV was found in about half of the swine herds tested in 1987 and 1988 (Hill 1989; Polson et al. 1993) and was also responsible for 26% of all the cases of neonatal diarrhea reported to the Illinois Department of Agricultural Animal Diagnostic Laboratory (Hoefling 1989). Major economic losses to the swine industry occur from epizootic TGEV outbreaks that can cause 100% mortality among neonatal pigs (Saif and Wesley 1999), as well as from growth retardation and increased susceptibility to other infectious diseases in older TGEV-infected pigs (Hoefling 1989). In 1987 and 1988, it was estimated that the pork industry in Iowa alone lost $10 million as a result of TGEV infection (Hill 1989). A survey in 1990 conducted by the National Animal Health Monitoring System reported that 36% of swine herds in the United States were positive for antibodies to TGEV (Wesley et al. 1997). In 1995, 16 of 22 swine herds examined in Iowa were seropositive for TGEV (Wesley et al. 1997). The current economic impact of TGEV infections, since the occurrence of PRCV in the United States, has not been examined.

Porcine Respiratory Coronavirus

Since the 1980s, the significance of TGEV has diminished in Europe with the appearance of the TGEV mutant, PRCV (Laude et al. 1993). Possibly, one factor that contributed to the emergence of this porcine coronavirus with respiratory tract tropism was the intensification of pig production during the late 1970s (Pensaert 1989). In contrast to TGEV, PRCV does not cause mortality among pigs and infections are usually subclinical. Some strains were described that produce mild respiratory symptoms (Paul et al. 1997). Aerogenic virus spread was described, and seroconversion could not be distinguished from TGEV-induced seroconversion without the use of monoclonal antibodies (Pensaert et al. 1986; Simkins et al. 1993). Moreover, PRCV also became endemic in countries like Denmark and England where the incidence of TGEV was very low or absent (Brown and Cartwright 1986). In endemic areas, newborn pigs receive PRCV antibodies via colostrum and milk. This passive protection lasts 3 to 4 weeks and is gradually replaced with active immune response. Experimental passive-immunity studies suggested that multiple PRCV reinfections in endemic areas could contribute to the decline in TGEV outbreaks that have been observed; thus, PRCV could act as a naturally modified-live vaccine (Lanza et al. 1995; Sestak et al. 1996). In young and adult pigs, PRCV is excreted nasally for 10 to 11 days. Similarly to TGEV, PRCV exhibits an autumn-winter incidence (Pensaert 1989). Reinfections of pigs with PRCV were reported in France and Belgium, with an increased autumn incidence for several successive years (Jestin et al. 1987; Laval et al. 1991). Serological studies of the prevalence of PRCV infection among fattening pigs (Belgium) suggested that more than 50% of animals were seropositive (Pensaert 1989). In Iowa swine herds, it was suggested that the recent increases in TGEV/PRCV seroprevalence was most likely due to subclinical PRCV infections (Wesley et al. 1997). High seroprevalence to PRCV (61%) was recently reported from South Korea, suggesting an extensive distribution of this virus throughout the Korean swine population (Chae et al. 2000).

Porcine Epidemic Diarrhea Virus

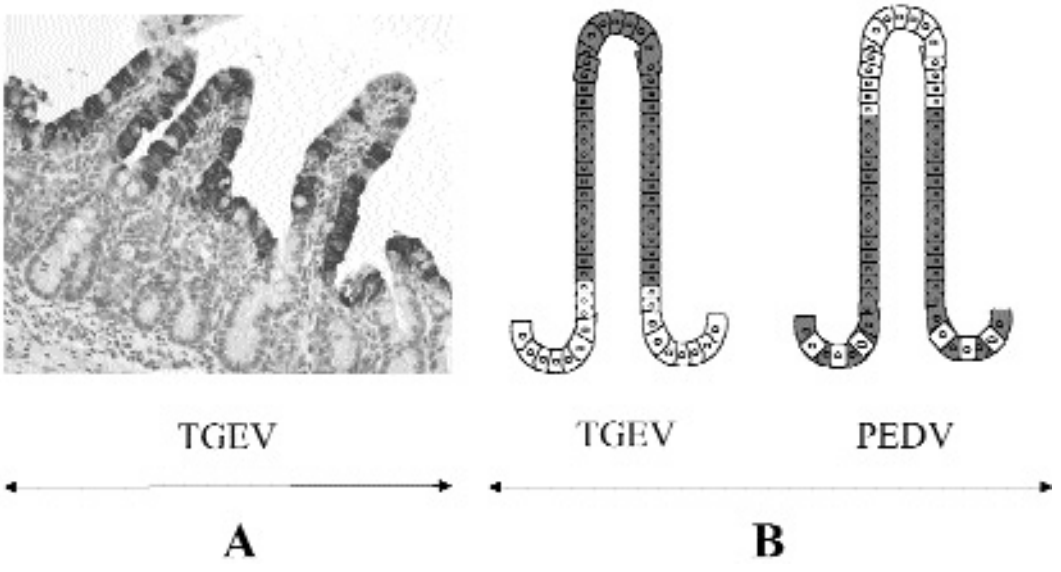
Although PEDV has been isolated in most swine-raising countries in Europe and Asia (Kweon et al. 1993; Mostl

et al. 1990), no virus isolation has yet been reported from the Americas. Changing patterns of PEDV epidemiology have been observed in Europe, where PED is no longer epidemic but endemic and sometimes persistent (Pensaert 1999). This persistence is characterized by the presence of virus-specific antibodies and was preceded by the stage of acute epizootics during the 1980s (DeBouck et al. 1982). An epidemic epidemiological pattern has been observed during recent years in Asia, where massive and severe PED outbreaks clinically resembling TGE previously were associated with large economic losses (Hwang et al. 1994; Sueyoshi et al. 1995). Similarly to TGEV, PEDV transmission is maintained via feces or other virus-carrying fomites by the oral route of ingestion. In contrast to TGEV, PEDV appears to persist in swine farms, but mechanisms of this persistence have not been fully elucidated.

PATHOGENESIS

Transmissible Gastroenteritis Virus

The gateway for TGEV infection is the oral cavity, although the virus can be inhaled as well (Aynaud et al. 1991). After the virus is swallowed, it survives the low pH of the stomach and resists the proteolytic environment of the duodenum. In the small intestine, it infects the villous epithelial cells (Bohl 1989). Peplomer-shaped protrusions, i.e., the viral spike (S) glycoproteins bind to aminopeptidase N, a TGEV receptor expressed on the intestinal brush border (Delmas et al. 1993). The most prominent factor accounting for TGE pathology and diarrhea is destruction of villous epithelium. A typical pattern is detection of TGEV in villous, but not crypt, epithelium (Figure 10.1.1). Only the enzymatically mature villous cells (absent in crypt epithelium) are infected. As a consequence of virus infection, discernible morphological changes in the intestinal epithelium were described, such as reduction and blunting of the villi (Saif and Wesley 1999). After 1 to 3 days of infection, the undifferentiated cells from the crypts start to migrate upward to replace the destroyed villous epithelium (Wege 1995). The time necessary for villous replacement depends on the age of animals (up to 10 days in the case of suckling pigs and 2 to 4 days in the case of weaned pigs) (Moon 1971). The reduction in enzymatic activity of the villous epithelium accounts for alterations in digestion, cellular transport, hydrolysis of lactose from milk, and subsequent development of a malabsorptive syndrome (Frederick et al. 1976). In contrast to the normal osmotic force in a healthy intestine, in TGEV-infected intestines, undigested lactose and the Na^+ accumulate in the gut lumen, which contributes to the withdrawal of body fluids and accounts for metabolic acidosis, diarrhea, and dehydration (Saif and Wesley 1999). TGEV infection of the respiratory tract has been described (Underdahl et al. 1975), and these virus strains (attenuated P115) were also found to replicate in lavaged alveolar macrophages (Laude et al. 1984). The TGE gross lesions involve the accumulation of undigested milk in the stomach and small intestine, thinner intestinal walls due to the villous atrophy and, in some cases, pneumonic lesions (Bohl 1989; Saif and Wesley 1999).



10.1.1. *Epithelial cell (small intestine) tropism of enteropathogenic porcine coronaviruses.* ***A:*** *Detection of transmissible gastroenteritis virus (TGEV) antigens by immunohistochemistry. Courtesy of Dr. J. Hayes.* ***B:*** *Usual distribution of TGEV/porcine epidemic diarrhea virus (PEDV) antigens.* Shaded cells *are likely virus targets.*

Porcine Respiratory Coronavirus

The loss of PRCV enteropathogenicity is explained by alterations in the spike (S) glycoprotein, which mediates attachment and thus plays a critical role during the early stages of cell infection (Ballesteros et al. 1997; Pensaert 1989). Aminopeptidase N, an enzyme expressed by the villous enterocytes of the small intestine, is known to be the major receptor for TGEV (Delmas et al. 1993). PRCV also uses aminopeptidase N as a cell receptor in the respiratory tract (Delmas et al. 1993). Two theories for the emergence of PRCV as a deletion mutant are (1) PRCV is a particular TGEV strain originally having a respiratory tropism, and (2) PRCV gained its ability to replicate in the respiratory tract because of the S-gene and possibly 3a-gene alterations (Pensaert 1989). Although some findings suggested that the 3a gene might be responsible for loss of PRCV enteric tropism (Paul et al. 1997), experiments with amino acid changes at the N terminus of TGEV S proteins suggested that the TGEV S gene is a determinant of enteric tropism (Ballesteros et al. 1997; Sanchez et al. 1999). However, it was still speculated that 3a-gene deletions could be a condition or prerequisite for the occurrence of the S-gene deletion. Recent characterization of British porcine coronavirus isolates suggests that virulent, enterotropic TGEV can have a large deletion in its 3a gene without any impact on S-gene completeness and virus tropism (McGoldrick et al. 1999). It was suggested that the severity of PRCV infections differs with the age of inoculated animals (Cox et al. 1990a). When animals younger than 5 weeks were inoculated by the nasal route, PRCV infected both the respiratory and intestinal tracts. However, because of a substantially lower extent of multiplication and infection of nonepithelial cells, the gut is not considered a target organ for PRCV (Saif and Wesley 1999). PRCV can be isolated from nasal mucosa, tonsils, trachea, and lungs and, with lower virus titers, also from the gastrointestinal tract (Cox et al. 1990b; O'Toole et al. 1989). Maximum antigen expression was demonstrated at postinoculation day 3 in epithelial cells of the pulmonary and bronchiolar alveoli (Cox et al. 1990b).

Investigation of the TGEV- and PRCV-shedding duration showed that PRCV-nasal shedding persisted (adult swine) until postinoculation day 10, whereas TGEV-fecal shedding persisted (suckling and weaned pigs) until postinoculation day 14, with TGEV-nasal shedding up to postinoculation day 11 (Laude et al. 1993; Saif and Wesley 1999). PRCV infections usually remain subclinical, although some investigators reported mild clinical signs of respiratory tract infections, such as sneezing, cough, dyspnea, and short-lasting fever (Cox et al. 1990a; Pensaert et al. 1986; Vannier 1990). Gross lesions have been described after experimental infection of gnotobiotic pigs and consist of catarrhal lobular bronchopneumonia, interstitial pneumonia with infiltration of macrophages, plasma cells, and lymphoblasts (Cox et al. 1990b; Van Nieuwstadt and Pol 1989). Both PRCV and TGEV induce interferon-α secretion (Charley and Laude 1988; Van Reeth and Nauwynck 2000). It was suggested that dual infection of pigs with porcine reproductive and respiratory syndrome virus (PRRSV) and PRCV could result in more severe disease and growth retardation than only single PRRSV infection (Van Reeth et al. 1996).

Porcine Epidemic Diarrhea Virus

The severity of clinical PED depends on the immune status of the affected herd. In cases where PEDV is introduced into a nonimmune, fully susceptible population, clinical symptoms may resemble TGE, and mortality in neonatal piglets can reach about 80% (Pensaert 1999). This acute PED is characterized by watery diarrhea and dehydration in young piglets. In fattening pigs, an association between PED rate and stress was observed (Pensaert 1999). Subclinical, persistent PEDV infections are typical for populations with previous PED history that also possess virus-specific immunity. The mechanism of viral replication and consequent villous degeneration is similar to that described for TGE (Pospischil et al. 1981). The affected villous epithelial cells (Figure 10.1.1) can be seen in the small intestine and colon as early as 12 to 18 hours and as late as 5 days after inoculation (Pensaert 1999). When introduced into a seronegative herd, clinical and pathological signs associated with PEDV tend to be similar to those for TGEV, but less severe, except that the diarrhea may persist 2 to 3 weeks (Pensaert 1999).

DIAGNOSIS

Laboratory diagnosis of PRCV, TGEV, and PEDV infections usually involves one or more of the following: detection of virus, its genome, antigen components, or antibody response. PRCV antigen can be detected by a direct immunofluores (or immunoperoxidase) antibody test on formalin- or paraffin-fixed lung sections (Pospisil et al. 1969). An indirect immunofluorescence test has been used for the detection of virus antigen in nasal smears (Onno et al. 1989). Electron microscopy can be used to examine the cells of bronchiolar and alveolar tissues, including macrophages, for the presence of coronavirus particles (Cox et al. 1990b). Detection of TGEV/PRCV/PEDV-specific nucleic acid was performed by the use of dot-blot hybridization, reverse-transcription (RT) polymerase chain reaction (PCR), or RT-nested PCR (Benfield et al. 1991; Britton et al. 1993; Jackwood et al. 1993; Kim et al. 2000; Kubota et al. 1999; Kwon et al. 1998; Paton et al. 1997; Wesley et al. 1991; Woods 1997). A simple and reliable method to confirm TGEV infection is to detect TGEV antigens or virus contained within small intestinal fluids by enzyme-linked immunosorbent assay (ELISA) (Lanza et al. 1995). TGEV antigens can also be detected by immunofluorescence or immunoperoxidase techniques within virus-infected cells (Shoup et

al. 1996). However, this must be done during an early stage of infection (1 to 2 days after inoculation) since the infected enterocytes are rapidly destroyed and released from the villi (Pensaert et al. 1981). Clarified, diluted intestinal contents can be subjected to immunoelectron microscopy or ELISA (Horzinek et al. 1982; Saif and Wesley 1999).

During the mid-1990s, with the emergence of PRCV, the necessity for a new test arose, primarily because of export requirements for TGEV-seronegative animals. To meet this requirement, monoclonal antibodies and oligonucleotide probes specific for TGEV/PRCV were prepared, and differential ELISAs and RT-PCR assays were developed (Callebaut et al. 1989; Garwes et al. 1988; Have 1990; Kim et al. 2000; Sestak et al. 1999b; Simkins et al. 1993). These tests are used to detect and differentiate between TGEV- and PRCV-induced antibodies or viral RNA extracted directly from feces or nasal secretions of infected pigs.

In contrast, PEDV does not cross-react with TGEV/PRCV and exhibits a distinct pathogenesis in the intestinal tract. Because of its common host and cell tropism with TGEV, PEDV has to be considered when diagnosis is based solely on electron microscopy of fecal specimens (Kusanagi et al. 1992). PEDV can be confirmed by direct immunofluorescence or immunohistochemistry of the small intestine from piglets with acute diarrhea within 3 days after onset (Pensaert 1999). ELISA can be used for detection of PEDV antigens (Carvajal et al. 1995) or antibodies (De Arriba et al. 1995). Primers specific for the PEDV nucleocapsid (N) protein gene have been used for detection of virus in intestinal contents by RT-nested PCR (Kubota et al. 1999). Shedding of PEDV in feces of experimental pigs was detected between 3 and 11 days after inoculation (Pensaert 1999).

PREVENTION AND CONTROL

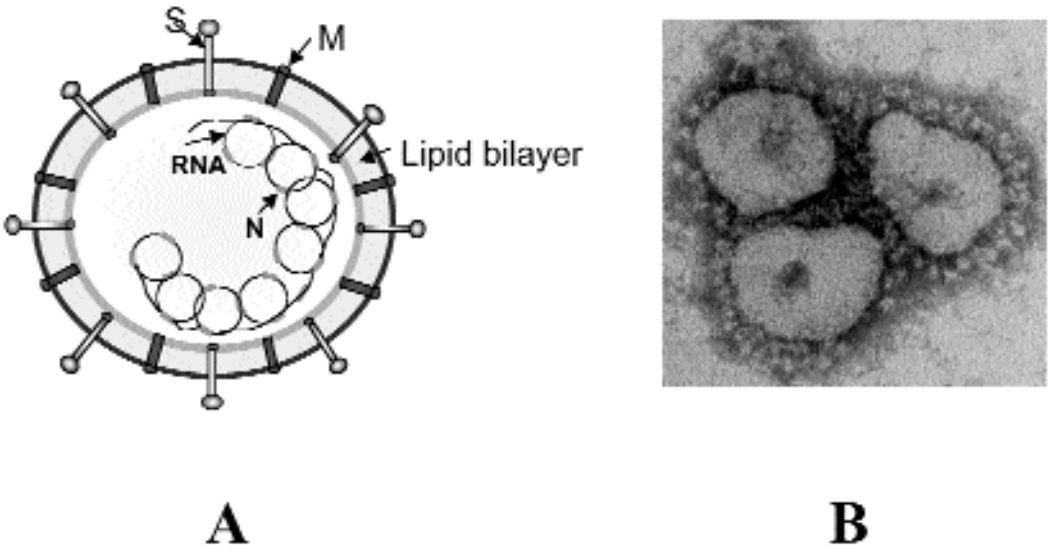
Application of general preventive measures such as "all-in all-out" herd turnover and the "black-and-white" system of sanitation helps to prevent infection with porcine coronaviruses (Bohl 1989; Stepanek et al. 1974). An efficient and historically the oldest immunization method to prevent TGE or PED is based on feeding the infectious gut materials from diarrheic piglets to pregnant sows and gilts approximately 3 weeks prepartum (Bohl 1989). Although effective active immunity of a sow and, subsequently, also passive immunity of suckling piglets can be induced by this method, it can also lead to uncontrollable perpetuation of other intestinal pathogens. The necessity to better characterize the potential vaccine dose and virus or antigen source led to the development of commercial vaccines.

Current commercial TGEV vaccines consist of attenuated or killed virus that does not induce sufficient immune responses in the gut, resulting in irregular levels of active and passive immunity. Commercial vaccines have been available since 1966 (Welter 1986). These vaccines were inactivated or modified-live virus, and were applied intramuscularly (IM) to sows before farrowing; they contributed mostly to systemic immunity (IgG) and to moderate or no reductions in the rate of piglet mortality (Bohl 1989). Several US companies reported the testing of attenuated live vaccines for IM administration (Welter 1986). It was found that passive immunity induced by a federally licensed, attenuated live vaccine for oral and IM use was overwhelmed after TGEV challenge exposure of suckling piglets (Moxley and Olson 1989). The immunity induced by these attenuated live vaccines functioned by means of stimulation of gut-associated lymphoid tissue, with secretory IgA production and prompting the gut-mammary homing pathway. An attenuated live vaccine is still one of the currently available commercial TGE vaccines licensed by Veterinary Biologics (USDA).

An important requirement for an oral TGEV vaccine is that it possess minimal pathogenicity for piglets while retaining the ability to deliver immunogenic antigens to gut-associated lymphoid tissue (Saif and Wesley 1999). The incomplete protection against TGEV induced by oral vaccines currently available is the result of their inability to infect the villous enterocytes of the small intestine. As a consequence, there is very low stimulation of intestinal IgA B-cell precursors (Saif and Wesley 1999; Sestak et al. 1999a).

With current vaccines being either too attenuated or applied at a dosage that is too low, protection is inconsistent (Saif and Jackwood 1990; Shoup et al. 1997; Van Cott et al. 1993; Saif 1996) and the search for more reliable vaccines continues. For the protection of suckling piglets, research continues to focus on the principle of colostral and lacteal intake of secretory IgA antibodies after immunization of sows with attenuated live vaccines (Park et al. 1998; Saif 1996; Sestak et al. 1996).

During this decade, emphasis has been on the construction of TGEV protein subunit vaccines. Among the three major structural proteins of TGEV (Figure 10.1.2), the S protein contains immunodominant epitopes that are recognized by virus-neutralizing antibodies (Delmas et al. 1986; Jimenez et al. 1986). Some of these epitopes were shown to be continuous domains (Delmas et al. 1990; Gebauer et al. 1991; Posthumus et al. 1990). Therefore, the objective of some studies was to design antigenic synthetic peptides derived from the S protein (Posthumus et al. 1991). It was found that the N protein and not S protein contains T-helper cell epitopes (Anton et al. 1995). A synthetic 15-mer peptide epitope derived from the N protein was shown to cooperate with the S protein for in vitro induction of TGEV-specific antibody (Anton et al. 1996).



10.1.2. *Transmissible gastroenteritis virus (TGEV), porcine respiratory coronavirus (PRCV), and porcine epidemic diarrhea virus (PEDV) exhibit typical coronavirus morphology.* ***A:*** *Virions are pleomorphic, 60 to 200 nm, with club-shaped sparse spikes (S protein). In addition to S protein, membrane (M) and helical nuleocapsid (N) proteins are major structural components of the virus particle. The internal core contains the N protein and continuous mRNAs that are produced in host cells (TGEV and PEDV, small intestine villous epithelium; and PRCV, respiratory tract epithelium).* ***B:*** *In the electron micrograph, TGEV particles are indistinguishable from PRCV or PEDV particles.*

To express the TGEV S, membrane (M), or N proteins, several prokaryotic and eukaryotic systems such as *Escherichia coli*, *Salmonella*, adenovirus, vaccinia virus, baculovirus, and plants were used (Britton et al. 1987; Chen and Schifferli 2001; Enjuanes et al. 1992; Godet et al. 1991; Gomez et al. 1998, 2000; Park et al. 1998; Pulford and Britton 1991; Shoup et al. 1997; Smerdou et al. 1996a,b; Torres et al. 1995, 1996; Tuboly et al. 1994, 2000). In some studies, protective antibodies were induced in inoculated animals, correlating with partial protection (Torres et al. 1995). In other studies, induction of protective antibodies was not reported (Gomez et al. 1998, 2000; Smerdou et al. 1996a,b; Tuboly et al. 2000), or they were detected as IgG virus-neutralizing antibodies (Park et al. 1998; Shoup et al. 1997). In the first attempts with prokaryotic expression systems, TGEV immunogens did not induce any neutralizing antibodies (Saif and Wesley 1999). Human adenovirus vectors were reported to undergo an abortive replication in the porcine gut and lose the TGEV (S) inserts (Torres et al. 1996). The baculovirus-expressed S protein induced virus-neutralizing antibodies to TGEV, as detected in the serum of rats and pigs (Shoup et al. 1997; Tuboly et al. 1995). However, the protective capability of these systemic antibodies was insufficient (Godet et al. 1991; Shoup et al. 1997; Tuboly et al. 1995). Similarly, when baculovirus-expressed S protein with incomplete Freund's adjuvant was administered intramammary and IM to TGEV-seronegative, pregnant sows, only IgG antibodies to TGEV were detected in sows' colostrum and milk (Shoup et al. 1997). Moreover, there was no significant impact on morbidity or mortality after TGEV challenge exposure of litters from these sows (Shoup et al. 1997).

In studies using baculovirus-expressed TGEV structural proteins (S, N, and M) coadministered intraperitoneally with *E. coli* mutant thermolabile toxin (LT-R192G), immune responses associated with IgA antibodies to TGEV resulted in reduced TGEV shedding in the feces of challenged pigs (Sestak et al. 1999a). These results suggested that vaccines based on the three major TGEV proteins (S, N, and M) could stimulate both mucosal and systemic immune responses. Since the pathology of TGEV remains localized in the intestine, an effective vaccine should primarily elicit an intestinal immune response that can be targeted by oronasal immunizations with adequate doses and forms of attenuated vaccines (Saif and Jackwood 1990; Van Cott et al. 1993). TGEV vaccines might be improved further by the use of supplementary carrier systems such as immunostimulating complexes, biodegradable microspheres, or recombinant *Salmonella* expression and delivery vectors.

REFERENCES

Anton IM, Suñé C, Meloen RH, et al. 1995. A transmissible gastroenteritis coronavirus nucleoprotein epitope elicits T helper cells that collaborate in the in vitro antibody synthesis to the three major structural viral proteins. Virology 212:746–751.

Anton IM, González S, Bullido MJ, et al. 1996. Cooperation between transmissible gastroenteritis coronavirus (TGEV) structural proteins in the in vitro induction of virus-specific antibodies. Virus Res 46:111–124.

Aynaud JM, Bernard S, Bottreau E, et al. 1991. Induction of lactogenic immunity to transmissible gastroenteritis virus of swine using an attenuated coronavirus mutant able to survive in the physicochemical environment of the digestive tract. Vet Microbiol 26:227–239.

Ballesteros ML, Sanchez CM, Enjuanes L. 1997. Two amino acid changes at the N-terminus of transmissible gastroenteritis coronavirus spike protein result in the loss of enteric tropism. Virology 227:378–388.

Benfield DA, Jackwood DJ, Bae I, et al. 1991. Detection of transmissible gastroenteritis virus using cDNA probes. Arch Virol 116:91–106.

Bohl EH. 1989. Transmissible gastroenteritis virus. *In:* Virus Infections of Porcines. Pensaert MB (ed). Elsevier Science, Amsterdam, pp 139–153.

Britton P, Garwes DJ, Page K, Walmsley J. 1987. Expression of porcine transmissible gastroenteritis virus genes in *E. coli* as beta-galactosidase chimaeric proteins. Adv Exp Med Biol 218:55–64.

Britton P, Kottier S, Chen CM, et al. 1993. The use of PCR genome mapping for the characterization of TGEV strains. Adv Exp Med Biol 342:29–34.

Brown I, Cartwright S. 1986. New porcine coronavirus? Vet Rec 119:282–283.

Callebaut P, Pensaert MB, Hooyberghs J. 1989. A competitive inhibition ELISA for the differentiation of serum antibodies from pigs infected with transmissible gastroenteritis virus (TGEV) or with the TGEV-related porcine respiratory coronavirus. Vet Microbiol 20:9–19.

Carvajal A, Lanza I, Diego R, et al. 1995. Evaluation of a blocking ELISA using monoclonal antibodies for the detection of porcine epidemic diarrhea virus and its antibodies. J Vet Diagn Invest 7:60–64.

Chae C, Kim O, Min K, et al. 2000. Seroprevalence of porcine respiratory coronavirus in selected Korean pigs. Prev Vet Med 46:293–296.

Charley B, Laude H. 1988. Induction of alpha interferon by transmissible gastroenteritis coronavirus: Role of transmembrane glycoprotein E1. J Virol 62:8–11.

Chen H, Schifferli DM. 2001. Enhanced immune responses to viral epitopes by combining macrophage-inducible expression with multimeric display on a *Salmonella* vector. Vaccine 19:3009–3018.

Cox E, Hooyberghs J, Pensaert MB. 1990a. Sites of replication of a porcine respiratory coronavirus related to transmissible gastroenteritis virus. Res Vet Sci 48: 165–169.

Cox E, Pensaert MB, Hooyberghs J, Van Deun K. 1990b. Sites of replication of a porcine respiratory coronavirus in 5-week-old pigs with or without maternal antibodies. Adv Exp Med Biol 276:429–433.

De Arriba ML, Carvajal A, Lanza I, et al. 1995. Development of an ELISA for the detection of antibody isotypes against porcine epidemic diarrhoea virus (PEDV) in sow's milk. *In:* Proceedings of the Third Congress of the European Society of Veterinary Virology, pp 222–225.

DeBouck P, Callebaut P, Pensaert M. 1982. Prevalence of the porcine epidemic diarrhea (PED) virus in the pig population of different countries. Proc Int Pig Vet Soc Congr 7:45.

Delmas B, Gelfi J, Laude H. 1986. Antigenic structure of transmissible gastroenteritis virus. II. Domains of the peplomer glycoprotein. J Gen Virol 67:1405–1418.

Delmas B, Rasschaert D, Godet M, et al. 1990. Four major antigenic sites of the coronavirus transmissible gastroenteritis virus are located on the amino-terminal half of spike glycoprotein S. J Gen Virol 71:1313–1323.

Delmas B, Gelfi J, Sjostrom H, et al. 1993. Further characterization of aminopeptidase-N as a receptor for coronaviruses. Adv Exp Med Biol 342:293–298.

Doyle LP, Hutchings LM. 1946. A transmissible gastroenteritis in pigs. J Am Vet Med Assoc 108:257–259.

Enjuanes L, Sune C, Gebauer F, et al. 1992. Antigen selection and presentation to protect against transmissible gastroenteritis coronavirus. Vet Microbiol 33:249–262.

Frederick GT, Bohl EH, Cross RF. 1976. Pathogenicity of an attenuated strain of transmissible gastroenteritis virus for newborn pigs. Am J Vet Res 37:165–169.

Garwes DJ, Steward F, Cartwright SF, Brown I. 1988. Differentiation of porcine coronavirus from transmissible gastroenteritis virus. Vet Rec 122:86–87.

Gebauer F, Posthumus WP, Correa I, et al. 1991. Residues involved in the antigenic sites of transmissible gastro-enteritis coronavirus S glycoprotein. Virology 183:225–238.

Godet M, Rasschaert D, Laude H. 1991. Processing and antigenicity of entire and anchor-free spike glycoprotein S of coronavirus TGEV expressed by recombinant baculovirus. Virology 185:732–740.

Gomez N, Carrillo C, Salinas J, et al. 1998. Expression of immunogenic glycoprotein S polypeptides from transmissible gastroenteritis coronavirus in transgenic plants. Virology 249:352–358.

Gomez N, Wigdorovitz A, Castanon S, et al. 2000. Oral immunogenicity of the plant derived spike protein from swine-transmissible gastroenteritis coronavirus. Arch Virol 145:1725–1732.

Haelterman EO. 1973. On the control of transmissible gastroenteritis of swine. Proc Annu Meet US Anim Health Assoc 77:345–349.

Have P. 1990. Infection with a new porcine respiratory coronavirus in Denmark: Serologic differentiation from

transmissible gastroenteritis virus using monoclonal antibodies. Adv Exp Med Biol 276:435–439.

Hill HT. 1989. Preventing epizootic TGE from becoming enzootic TGE. Vet Med 84:432–436.

Hoefling D. 1989. Tracking the culprits behind diarrhea in neonatal pigs. Vet Med 84:427.

Horzinek MC. 1999. Molecular evolution of corona- and toroviruses. Adv Exp Med Biol 473:61–72.

Horzinek MC, Lutz H, Pedersen NC. 1982. Antigenic relationships among homologous structural polypeptides of porcine, feline, and canine coronaviruses. Infect Immun 37:1148–1155.

Hwang EK, Kim JH, Jean YH, et al. 1994. Current occurrence of porcine epidemic diarrhoea in Korea. RDA J Agric Sci 36:587–596.

Jackwood DJ, Bae I, Jackwood RJ, Saif LJ. 1993. Transmissible gastroenteritis virus and porcine respiratory coronavirus: Molecular characterization of the S gene using cDNA probes and nucleotide sequence analysis. Adv Exp Med Biol 342:43–48.

Jestin A, Leforban Y, Vannier P, et al. 1987. Un nouveau coronavirus porcin: Etudes sero-epidemiologiques retrospectives cans les elevages de Bretagne. Recl Med Vet Ec Alfort 163:567–571.

Jimenez G, Correa I, Melgosa MP, et al. 1986. Critical epitopes in transmissible gastroenteritis virus neutralization. J Virol 60:131–139.

Kemeny LJ, Wiltsey VL, Riley JL. 1975. Upper respiratory infection of lactating sows with transmissible gastroenteritis virus following contact exposure to infected piglets. Cornell Vet 65:352–362.

Kim L, Hayes J, Lewis P, et al. 2000. Molecular characterization and pathogenesis of transmissible gastroenteritis coronavirus (TGEV) and porcine respiratory coronavirus (PRCV) field isolates co-circulating in a swine herd. Arch Virol 145:1133–1147.

Kubota S, Sasaki O, Amimoto K, et al. 1999. Detection of porcine epidemic diarrhea virus using polymerase chain reaction and comparison of the nucleocapsid protein genes among strains of the virus. J Vet Med Sci 61:827–830.

Kusanagi K, Kuwahara H, Katoh T, et al. 1992. Isolation and serial propagation of porcine epidemic diarrhea virus in cell cultures and partial characterization of the isolate. J Vet Med Sci 54:313–318.

Kweon CH, Kwon BJ, Jung TS, et al. 1993. Isolation of porcine epidemic diarrhea virus (PEDV) in Korea. Korean J Vet Res 33:249–254.

Kwon HM, Saif LJ, Jackwood DJ. 1998. Field isolates of transmissible gastroenteritis virus differ at the molecular level from the Miller and Purdue virulent and attenuated strains and from porcine respiratory coronaviruses. J Vet Med Sci 60:589–597.

Lai MMC. 1990. Coronavirus: Organization, replication and expression of genome. Annu Rev Microbiol 44: 303–333.

Lanza I, Shoup DI, Saif LJ. 1995. Lactogenic immunity and milk antibody isotypes to transmissible gastroenteritis virus in sows exposed to porcine respiratory coronavirus during pregnancy. Am J Vet Res 56:739–748.

Laude H, Charley B, Gelfi J. 1984. Replication of transmissible gastroenteritis coronavirus (TGEV) in swine alveolar macrophages. J Gen Virol 65:327–332.

Laude H, Van-Reeth K, Pensaert MB. 1993. Porcine respiratory coronavirus: Molecular features and virus-host interactions. Vet Res 24:125–150.

Laval A, Le Foll P, Gestin G, Reynaud G. 1991. Grippes et coronavirus respiratoire porcin: Etude serologique dans dix elevages bretons. Recl Med Vet Ec Alfort 167:521–528.

McGoldrick A, Lowings JP, Paton DJ. 1999. Characterization of a recent virulent transmissible gastroenteritis virus from Britain with a deleted ORF 3a. Arch Virol 144:763–770.

Moon HW. 1971. Epithelial cell migration in the alimentary mucosa of the suckling pig. Proc Soc Exp Biol Med 137:151–154.

Moon HW, Bunn TO. 1993. Vaccines for preventing enterotoxigenic *Escherichia coli* infections in farm animals. Vaccine 11:200–213.

Mostl K, Horvath E, Burki F. 1990. Erhebungen uber porcine Coronaviren in Osterreich. II. Porcine epidemic diarrhea virus (PEDV) der Schweine. Wien Tierarztl Monatsschr 77:10–18.

Moxley RA, Olson LD. 1989. Clinical evaluation of transmissible gastroenteritis virus vaccines and vaccination procedures for inducing lactogenic immunity in sows. Am J Vet Res 50:111–118.

Onno M, Jestin A, Cariolet R, Vannier P. 1989. Rapid diagnosis of TGEV-like coronavirus in fattened pigs by indirect immunofluorescence labeling in nasal cells. Zentralbl Veterinarmed [B] 36:629–634.

O'Toole D, Brown I, Bridges A, Cartwright SF. 1989. Pathogenicity of experimental infection with "pneumotropic" porcine coronavirus. Res Vet Sci 47:23–29.

Park S, Sestak K, Hodgins DC, et al. 1998. Immune response of sows vaccinated with attenuated transmissible gastroenteritis virus (TGEV) and recombinant TGEV spike protein vaccines and protection of their suckling pigs against virulent TGEV challenge exposure. Am J Vet Res 59:1002–1008.

Paton D, Ibata G, Sands J, McGoldrick A. 1997. Detection of transmissible gastroenteritis virus by RT-PCR and differentiation from porcine respiratory coronavirus. J Virol Methods 66:303–309.

Paul PS, Vaughn EM, Halbur PG. 1997. Pathogenicity and sequence analysis studies suggest potential role of gene 3 in virulence of swine enteric and respiratory coronaviruses. Adv Exp Med Biol 412:317–321.

Pensaert M. 1999. Porcine epidemic diarrhea. *In:* Straw BE, D'Allaire S, Mengeling WL, Taylor DJ (eds). Diseases of Swine (8th edition). Iowa State University Press, Ames, pp 179–185.

Pensaert M, Cox E, Van Deun K, Callebaut P. 1993. A seroepidemiological study of porcine respiratory coronavirus in Belgian swine. Vet Q 15:16–20.

Pensaert MB. 1989. Coronaviridae. *In:* Virus Infections of Porcines. Pensaert MB (ed). Elsevier Science, Amsterdam, pp 139–185.

Pensaert MB, DeBouck P, Reynolds DJ. 1981. An immunoelectron microscopic and immunofluorescent study on the antigenic relationship between the coronavirus-like agent, CV 777, and several coronaviruses. Arch Virol 68:45–52.

Pensaert MB, Callebaut P, Vergote J. 1986. Isolation of a porcine respiratory, non-enteric coronavirus related to transmissible gastroenteritis. Vet Q 8:257–261.

Polson D, Marsh W, Morrison R, Dial G. 1993. A methodology for evaluating the financial consequences of a disease outbreak of transmissible gastroenteritis and pseudorabies virus. Prev Vet Med 16:61–63.

Pospischil A, Hess RG, Bachmann PA. 1981. Light microscopy and ultrahistology of intestinal changes in pigs infected with enzootic diarrhea virus (EVD): Comparison with transmissible gastroenteritis (TGE) virus and porcine rotavirus infections. Zentralbl Veterinarmed [B] 28:564–577.

Pospisil Z, Mesaros E, Stepanek J. 1969. Immunofluorescent detection of transmissible gastroenteritis virus in the intestines of piglets. Zentralbl Veterinarmed [B] 16:840–846.

Posthumus WP, Lenstra JA, Schaaper WM, et al. 1990. Analysis and simulation of a neutralizing epitope of transmissible gastroenteritis virus. J Virol 64: 3304–3309.

Posthumus WP, Lenstra JA, Van Nieuwstadt AP, et al. 1991. Immunogenicity of peptides simulating a neutralizing epitope of transmissible gastroenteritis virus. Virology 182:371–375.

Pulford DJ, Britton P. 1991. Intracellular processing of the porcine coronavirus transmissible gastroenteritis virus spike protein expressed by recombinant vaccinia virus. Virology 182:765–773.

Saif LJ. 1996. Mucosal immunity: An overview and studies of enteric and respiratory coronavirus infections in a swine model of enteric disease. Vet Immunol Immunopathol 54:163–169.

Saif LJ, Jackwood DJ. 1990. Enteric virus vaccines: Theoretical considerations, current status, and future approaches. *In:* Saif LJ, Theil KW (eds). Viral Diarrhea of Man and Animals. CRC, Boca Raton, FL, pp 313–329.

Saif LJ, Wesley RD. 1999. Transmissible gastroenteritis and porcine respiratory coronavirus. *In:* Straw BE, D' Allaire S, Mengeling WL, Taylor DJ (eds). Diseases of Swine (8th edition). Iowa State University Press, Ames, pp 292–325.

Sanchez CM, Izeta A, Sanchez-Morgado JM, et al. 1999. Targeted recombination demonstrates that the spike gene of transmissible gastroenteritis coronavirus is a determinant of its enteric tropism and virulence. J Virol 73:7607–7618.

Sestak K, Lanza I, Park SK, et al. 1996. Contribution of passive immunity to porcine respiratory coronavirus to protection against transmissible gastroenteritis virus challenge exposure in suckling pigs. Am J Vet Res 57:664–671.

Sestak K, Meister RK, Hayes JR, et al. 1999a. Active immunity and T-cell populations in pigs intraperitoneally inoculated with baculovirus-expressed transmissible gastroenteritis virus structural proteins. Vet Immunol Immunopathol 70:203–221.

Sestak K, Zhou Z, Shoup DI, Saif LJ. 1999b. Evaluation of the baculovirus-expressed S-glycoprotein of TGEV as antigen in a competition ELISA to differentiate PRCV from TGEV antibodies in pigs. J Vet Diagn Invest 11:205–214.

Shoup DI, Swayne DE, Jackwood DJ, Saif LJ. 1996. Immunohistochemistry of transmissible gastroenteritis virus antigens in fixed paraffin-embedded tissues. J Vet Diagn Invest 8:161–167.

Shoup DI, Jackwood DJ, Saif LJ. 1997. Active and passive immune response to transmissible gastroenteritis virus (TGEV) in swine inoculated with recombinant baculovirus-expressed TGEV spike glycoproteins vaccines. Am J Vet Res 58:242–250.

Siddell SG, Wege H, Ter Meulen V. 1983. The biology of coronaviruses. J Gen Virol 64:761–776.

Simkins RA, Weilnau PA, Van Cott JL, et al. 1993. Competition ELISA, using monoclonal antibodies to the transmissible gastroenteritis virus (TGEV) S protein, for serologic differentiation of pigs infected with TGEV or porcine respiratory coronavirus. Am J Vet Res 54:254–259.

Smerdou C, Anton IM, Plana J, et al. 1996a. A continuous epitope from transmissible gastroenteritis virus S protein fused to *E. coli* heat-labile toxin B subunit expressed by attenuated *Salmonella* induces serum and secretory immunity. Virus Res 41:1–9.

Smerdou C, Urniza A, Curtiss III R, Enjuanes L. 1996b. Characterization of transmissible gastroenteritis coronavirus S protein expression products in avirulent *S. typhimurium* delta cya delta crp: Persistence, stability and immune response in swine. Vet Microbiol 48: 87–100.

Stepanek J, Mensik J, Rozkosny V. 1974. Viral gastroenteritis of swine: Antibody production in sows in locations with different epizootological courses of the disease. Vet Med 19:625–636.

Sueyoshi M, Tsuda T, Yamazaki K, et al. 1995. An immunohistochemical investigation of porcine epidemic diarrhoea. J Comp Pathol 113:59–67.

Torres JM, Sanchez C, Sune C, et al. 1995. Induction of antibodies protecting against transmissible gastroenteritis coronavirus (TGEV) by recombinant adenovirus expressing TGEV spike protein. Virology 213:503–516.

Torres JM, Alonso C, Ortega A, et al. 1996. Tropism of human adenovirus type 5-based vectors in swine and their ability to protect against transmissible gastroenteritis coronavirus. J Virol 70:3770–3780.

Tuboly T, Nagy E, Dennis JR, Derbyshire JB. 1994. Immunogenicity of the S protein of transmissible gastroenteritis virus expressed in baculovirus. Arch Virol 137:55–67.

Tuboly T, Nagy E, Derbyshire JB. 1995. Passive protection of piglets by recombinant baculovirus induced transmissible gastroenteritis virus specific antibodies. Can J Vet Res 59:70–72.

Tuboly T, Yu W, Bailey A, Degrandis S, et al. 2000. Immunogenicity of porcine transmissible gastroenteritis virus spike protein expressed in plants. Vaccine 18:2023–2028.

Underdahl NR, Mebus CA, Torres-Medina A. 1975. Recovery of transmissible gastroenteritis virus from chronically infected experimental pigs. Am J Vet Res 36:1473–1476.

US Department of Agriculture (USDA)-Animal and Plant Health Inspection Service (APHIS)-Veterinary Services (VS). 1992. National Swine Survey: Morbidity/Mortality and Health Management of Swine in the United States. 555 South Howes, Fort Collins, CO 80521.

US Department of Agriculture (USDA)-Animal and Plant Health Inspection Service (APHIS)-Veterinary Services (VS). 1997. Swine '95 Study, Part III: 1990–1995 Changes in the U.S. Pork Industry. 555 South Howes, Fort Collins, CO 80521.

Van Cott JL, Brim TA, Simkins RA, Saif LJ. 1993. Isotype-specific antibody secreting cells to transmissible gastroenteritis virus and porcine respiratory coronavirus in gut- and bronchus-associated lymphoid tissues of suckling pigs. J Immunol 150:3990–4000.

Van Nieuwstadt AP, Pol JM. 1989. Isolation of a TGE virus-related respiratory coronavirus causing fatal pneumonia in pigs. Vet Rec 124:43–44.

Van Reeth K, Nauwynck H. 2000. Proinflammatory cytokines and viral respiratory disease in pigs. Vet Res 31:187–213.

Van Reeth K, Nauwynck H, Pensaert M. 1996. Dual infections of feeder pigs with porcine reproductive and respiratory syndrome virus followed by porcine respiratory coronavirus or swine influenza virus: A clinical and virological study. Vet Microbiol 48:325–335.

Vannier P. 1990. Disorders induced by the experimental infection of pigs with the porcine respiratory coronavirus (P.R.C.V.). Zentralbl Veterinarmed [B] 37:177–180.

Wege H. 1995. Immunopathological aspects of coronavirus infections. Springer Semin Immunopathol 17:133–148.

Welter CJ. 1986. Strategies for a successful coronavirus (TGE) vaccine for swine. Adv Exp Med Biol 218:551–557.

Wesley RD, Wesley IV, Woods RD. 1991. Differentiation between transmissible gastroenteritis virus and porcine respiratory coronavirus using cDNA probe. J Vet Diagn Invest 3:29–32.

Wesley RD, Woods RD, McKean JD, et al. 1997. Prevalence of coronavirus antibodies in Iowa swine. Can J Vet Res 61:305–308.

Woods RD. 1997. Development of PCR-based techniques to identify porcine transmissible gastroenteritis coronavirus isolates. Can J Vet Res 61:167–172.