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Porcine respiratory coronavirus: molecular features and virus–host interactions

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Summary — Since 1984, a previously unrecognized respiratory coronavirus, causing a mostly unapparent infection, has rapidly and massively spread within the swine population in Europe, and few years later, a virus with similar characteristics has been identified in the USA. The agent, designated PRCV, appears to be derived from the porcine enteric coronavirus TGEV. The aim of the present article is to review comprehensively the state of the knowledge about this new virus and its infection. The review includes the following topics: epizootiology, molecular characterization and antigenic features of PRCV, pathogenesis and clinical aspects, immunity and laboratory diagnosis. The authors' views concerning the impact of the emergence of PRCV on both coronavirus research and swine production are presented in the conclusion.

porcine respiratory coronavirus / transmissible gastroenteritis virus variant / molecular characterization / epizootiology / pathogenesis / immunity / diagnosis

Résumé — **Coronavirus respiratoire porcin: aspects moléculaires et interactions virus-hôte.** À partir de 1984, un virus respiratoire inconnu jusqu'alors, responsable d'une infection essentiellement inapparente, a rapidement et massivement diffusé au sein de la population porcine européenne; quelques années plus tard, un virus présentant des caractéristiques similaires a été identifié aux USA. Cet agent, désigné PRCV, apparaît être dérivé du coronavirus entéropathogène porcin TGEV. L'objectif de cette revue est de faire le point de façon critique sur l'ensemble des connaissances accumulées sur ce nouveau virus et sur l'infection qu'il engendre. Epizootologie, caractérisation moléculaire et antigénique du PRCV, pathogénèse et aspects cliniques, immunité et diagnostic en laboratoire sont les principaux volets développés. En conclusion, les auteurs donnent leur point de vue sur les conséquences qu'a eu l'émergence du PRCV au plan des recherches en coronavirologie et sur la production porcine. (92 références)

coronavirus respiratoire porcin / variant du virus de la gastro-entérite transmissible / caractérisation moléculaire / épizootologie / pathogénie / immunité / diagnostic

* Correspondence and reprints

INTRODUCTION

The porcine respiratory coronavirus (PRCV) is a variant of transmissible gastroenteritis virus (TGEV). It appears to have emerged naturally in Europe during the early eighties (Pensaert *et al*, 1986). In more recent years, a similar virus has been detected in the USA (Wesley *et al*, 1990a).

In 1983–1984, a serological survey was carried out in Belgium in slaughterhouse sows to determine the prevalence of TGEV among the swine population (Pensaert *et al*, 1986). Surprisingly enough, it was found that 68% of the sows had neutralizing antibodies to TGEV. This was in sharp contrast to the results of previous surveys, which had been carried out during the seventies and early eighties and at which time the percentage of seropositive sows varied between 12–24%. In France, similar findings were reported during a study in 1985 when 73% of the farms had TGE antibodies in a region in Brittany (Jestin *et al*, 1987b).

TGE is a porcine viral disease which is well known all over the world. The virus was first identified in 1946 (Doyle and Hutchings, 1946) and, for decades, the disease has been known as a severe diarrhea in swine of all ages, with a high (nearly 100%) mortality rate in neonatal pigs. No epidemic of diarrhea resembling TGE had occurred in Belgium during the months prior to the 1983–1984 serological survey and to the finding of the abnormally high incidence of anti-TGEV antibodies. Moreover, in tracing back the positive sows to their farms of origin, it appeared that a high prevalence of antibodies was present in the sow population in these farms and that no history of an epidemic of diarrhea typical of TGE had occurred. Attempts to isolate TGE virus from the feces of pigs of different ages on such farms failed. Longitudinal serological studies of

groups of pigs on closed breeding–fattening farms showed that 2 situations were encountered. In some farms, sows were seropositive for TGEV and fattening pigs became negative after losing their maternal antibodies between 12 and 18 wk of age. On other farms, maternal anti-TGEV antibodies first declined but the antibody titer increased again around the age of 6–9 wk indicating the persistence of TGEV or a TGEV-like infection. On these farms, sentinel pigs were placed together with farm-born pigs at the age of 4 wk and nasal swabs and fecal samples were collected twice weekly. The sentinel pigs showed seroconversion to TGEV without any clinical sign and, subsequently, a virus was isolated from the nasal swabs which had been stored at -70°C . It was cultivable in cell cultures and examination with the electron microscope revealed that it was a coronavirus. The virus was neutralized by antiserum against TGEV but did not cause any disease upon inoculation of neonatal pigs. It was first called TGEV-like mutant because of its resemblance to TGEV and was later named PRCV because it clearly had a tropism for the respiratory tract (and not for the enteric tract). In recent years, PRCV has been compared to TGEV from the point of view of the structural and biological characteristics. These studies have shown that few genetic differences in PRCV have brought about large alterations in the virus–cell and virus–animal interactions. PRCV has, therefore, appeared to be a good model for coronavirologists to study how the modification of genes encoding structural and non-structural proteins may modulate the development of the disease in the animal. Since the emergence of PRCV, the clinical and economic importance of TGE has markedly lowered in Europe.

The present article is meant to review the state of knowledge of PRCV and its infection.

EPIZOOTIOLOGY

PRCV is widespread in Europe (Brown and Cartwright, 1986; Pensaert *et al*, 1986; Jestin *et al*, 1987; Madec *et al*, 1987; Henningsen *et al*, 1988; Yu *et al*, 1989; Lanza *et al*, 1990). A non-diarrheic TGEV-like virus with quite similar characteristics has also been found in the USA (Wesley *et al*, 1990a), Asia (Pensaert M, unpublished results) and Eastern European countries (Deriabine, personal communication).

In Europe, PRCV spread rapidly in all swine raising countries during the second half of the eighties. This spread to and within countries was so rapid and appeared to be so unavoidable that an aerogenic route of dissemination could not be overlooked. Rapid virus spread also occurred in countries with high hygienic standards or a highly developed SPF-farm system. Denmark, a country which has always been free of TGEV, was a good example to show that the aerogenic spread of PRCV had to be the main route of dissemination. The infection was first observed in Jutland along the German border and it progressed towards the inside of the country on farms where no introduction or sale of animals had occurred and with which no other direct or indirect links could be established (Henningsen *et al*, 1988).

Since that time, experimental inoculation studies have shown that aerolized virus initiates the infection very easily in the upper and deeper respiratory pathways and that high quantities of viruses are produced in the lungs and excreted in nasal fluids. Animals excrete the virus in oronasal secretions for 8–13 d after inoculation and long term virus carriers have not been observed. There are no indications that the fecal-oral transmission plays a role in the epizootiology of the natural infection and persons, footwear or lorries are unimportant in spreading the virus. Of course, introduction of infected pigs serves as a source of infection.

A sero-epizootiological study was carried out in the Belgian swine population in 1989–1990 (Pensaert *et al*, 1992). PRCV-induced neutralizing antibodies were found in 90.6% of the 160 sera from sows at slaughter. Fattening swine were followed on 33 closed breeding–fattening farms. The virus persisted on 22 farms, 11 of which were situated in a high farm density region (all the farms located in an area of 4 km²) and 11 of which were situated in a relatively low farm density region (1–4 farms per 12 km²). The growing pig population on the remaining 11 closed breeding–fattening farms became temporarily free of PRCV in spring and summer. All these farms became reinfected during the autumn. The latter pattern has been observed repeatedly in Belgium over several successive years and in other countries such as France (Jestin *et al*, 1987b; Laval *et al*, 1990). These observations indicate that waves of infection easily occur during the foggy and rainy season. The infection wave is not accompanied by manifest clinical disease signs.

Virus persistence, on a farm basis, occurs by infection of successive litters of pigs. Maternal antibodies in these pigs show a normal decline until the age of 5–8 wk and then the antibody titer rises again. This evolution points to a switch from passive to active immunity. In one farm, it was shown, by virus isolation from tonsillar swabs, that the infection occurs around the age of 5 wk. Maternal antibodies apparently postpone but do not prevent the infection of the respiratory tract.

In the study earlier mentioned, antibodies induced by TGEV as detected by a differential ELISA (Callebaut *et al*, 1989) were found in 7.6 % of the 130 sow sera and in sera from fattening pigs on 5 of the 33 farms. This shows that TGEV is still present in the swine population despite the high prevalence of PRCV.

It is clear that seasonal circumstances, swine density and distances between farms influence the epizootiology. In the above study, there was no correlation between the herd size and the temporary disappearance of PRCV on farms. Since farm density and distance vary greatly from country to country and from region to region, the epizootiological pattern of PRCV may be different in different countries.

In France, a longitudinal serological study was carried out in 10 fattening units and it was observed that seroconversion occurred during the fattening period in 8 (Laval *et al*, 1991). A study on the prevalence of PRCV infections in pigs shortly after entering intensive fattening units was recently carried out in Belgium (Van Reeth *et al*, unpublished results). In these farms, an all in – all out system is the rule. Pigs originate from numerous breeding herds and are introduced for fattening at the age of 9–12 wk. During the autumn of 1991, 10 groups of these pigs on 9 different farms were serologically examined 1 wk after entry and 3 wk later. Approximately 50% of the pigs had PRCV-neutralizing antibody titres of < 4 at the time of arrival. An infection with PRCV was observed in all 10 groups. A similar study which was performed in February–March 1992 involved 7 groups of pigs on 7 farms. PRCV-seroconversion was observed in 6 out of the 7 groups. These results show that the virus is introduced in practically every group with some animals and that an infection occurs in those pigs which have not yet been infected on the breeding farm of origin. In all these groups, the seroconversion was due to infection with PRCV and not with TGEV, as was shown by a differentiating ELISA (Callebaut *et al*, 1989).

CAUSAL AGENT

The demonstration that a previously unrecognized respiratory coronavirus, closely

related to TGEV, had emerged and spread massively within the European swine population gave rise to a number of purely conjectural hypotheses about the nature of this agent. The most alarming was that PRCV might represent a TGEV — possibly vaccine — strain of which the tropism for the respiratory tract was exalted as a result of a systematic aerogenic immunization (Jestin *et al* 1987a) or was a consequence of a laboratory manipulation. Other speculative views involved a spontaneous genome recombination between TGEV and either a heterologous coronavirus such as the feline infectious peritonitis virus FIPV or the canine coronavirus CCV (both of which are able to infect the porcine species to some extent), or a swine-specific, serologically unrelated virus, such as the porcine epidemic diarrhea virus PEDV or the hemagglutinating encephalitis virus HEV (Sanchez *et al*, 1990). The possibility that a preexisting coronavirus specific for a wild animal crossed accidentally the species barrier was also set forward.

The problem of the phylogenic origin of PRCV has been largely elucidated through the information provided by analysis of its genome. The most plausible scenario is that this agent originates from TGEV itself and that this occurred without incorporation of exogenous genetic material. At the same time, the studies raised stimulating questions about the genetic basis of the attenuation of PRCV, or in alternative terms, of the enterotropism of TGEV.

Molecular characterization

Coronavirus genome organization and expression

The genome of coronaviruses consists of one single-stranded mRNA molecule of positive polarity. With some 3×10^4 nucleotides (nt), coronaviruses have the largest

genome among RNA viruses, yet they do not encode a particularly high number of proteins. The 5' first 20 kilobases (kb) region is comprised of 2 large open reading frames (ORFs) from which a polyprotein with polymerase motifs is translated. Thus, nearly two-thirds of the coding capacity is devoted to the RNA transcription and replication machinery, which appears to be strikingly intricately in coronaviruses.

Most of the genes are clustered in the 3'-terminal third. They are expressed through the synthesis of 5–7 mRNAs of subgenomic size which are 3' coterminal, thus forming a so-called nested set structure. These mRNAs are thought to be produced following a mechanism of discontinuous, non-processive, leader-primed transcription. The leader is a short (50–90 nt) sequence which is homologous to the extreme 5' of the genome plus sense RNA. A hexameric conserved sequence (CUAAAC in several coronaviruses including TGEV), located upstream from each transcription unit, is assumed to provide a signal to the leader-polymerase complex for the specific transcription initiation of each mRNA species (review: Lai, 1990). Recently, the possibility that the different mRNAs could be amplified independently

has been questioned. This model derives further complexity from the high rate of recombination observed during coronavirus replication.

Coronavirus proteins are translated from the mRNA 5' part which is absent from the immediately smaller mRNA species. The translationally active part generally contains a single ORF; however, a few mRNAs are functionally bicistronic (eg mRNA 3 of TGEV) or tricistronic.

Comparison of PRCV and TGEV genomes

The genomic sequence of these 2 viruses has not been completed, contrary to the avian infectious bronchitis virus IBV and murine hepatitis virus MHV genomes. Published TGEV nucleotide data are yet limited to the 3' third region downstream the polymerase locus, *ie* ≈ 8.5 kb. This region contains 7 large ORFs (fig 1), that are expressed through 6 (in one case, 7) subgenomic mRNA species, numbered 2 to 7 from 5' to 3'.

The 3 ORFs named S, M and N encode the major structural proteins from which all coronaviruses are built (review: Spaan *et*

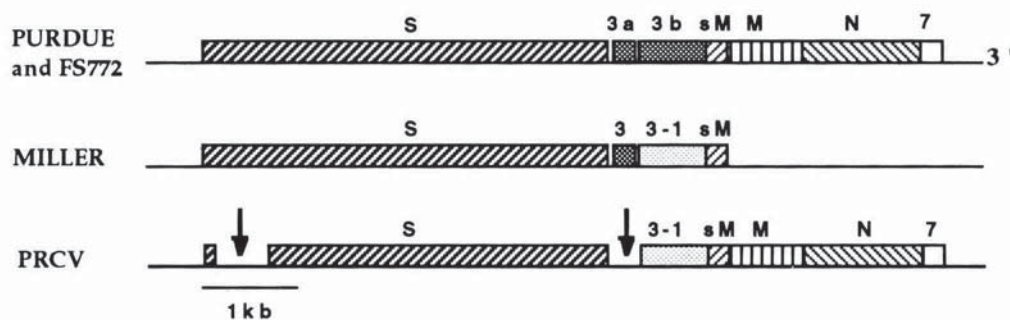


Fig 1. Compared genome organization of TGEV and PRCV viruses. An alignment of the genome 3' region of 3 TGEV strains and of PRCV is shown. The arrows denote the major changes found in PRCV genome: S protein gene truncated and open reading frame 3a non-functional (see text for details).

al, 1988). The protein S (former name E₂) is a large (220 kDa), membrane-anchored glycoprotein which, by trimerization (Delmas and Laude, 1990), forms the characteristic, petal-shaped spikes protruding from the virion envelope. The globular and the elongated domains roughly correspond to the amino- and carboxy-half parts of the polypeptide chain, respectively. S is the sole virion protein able to induce highly neutralizing antibodies and is considered as being the attachment protein. M (formerly E₁) is a 29–36 kDa-glycoprotein mostly embedded in the virion envelope. N is a 47 kDa-phosphoprotein associated with the genomic RNA to form the nucleocapsid (review: Laude *et al*, 1990). Recently, a 10 kDa polypeptide encoded by mRNA 4 has been proposed to be an additional virion-associated, integral protein, designated sM (Godet *et al*, 1992). The 3 other ORFs, numbered 3a, 3b and 7, code for assumedly non-structural polypeptides, the function of which is presently unknown.

The global organization of PRCV genome is identical to that of TGEV, as deduced from sequence data covering the whole region downstream from the S gene 5' end (Rasschaert *et al*, 1990; Britton *et al*, 1991; Page *et al*, 1991). The only striking difference consists of a number of deletions, which essentially affect two regions of PRCV genome: the S gene and the ORF3a (fig 1). No additional ORF is present between the polymerase locus and the S gene. Hence, PRCV and TGEV genomes differ by deletions and point mutations, with no sequence unique to PRCV being identified.

The 2 viruses exhibit an overall 3% nucleotide and amino acid divergence, not significantly different from that observed among 2 TGEV strains. The observed amino acid changes are not clustered but interspersed within the different genes. Pairwise alignment of the translated ORFs also revealed a slightly higher homology of

PRCV with the wild TGEV strains (Miller and FS772) than with the Purdue 115 strain, which has been isolated some 20 yr earlier (Rasschaert *et al*, 1990). The leader sequence of 2 PRCV isolates are identical to that of FS772 strain in length (91 nt) and composition, whereas that of Purdue strain differs at 2 positions (Page *et al*, 1990; Rasschaert and Laude, unpublished data). Based on the comparison of the S gene 5' half part of 6 Eur PRCV isolates and 5 TGEV strains, an evolutionary tree has been proposed in which the 2 viruses diverged recently from a common ancestor (Sanchez *et al*, 1992).

Another important finding revealed by the sequence data is that the deletions identified in 2 USA PRCV isolates (Wesley *et al*, 1991; Jackwood *et al*, 1992) differ from that of Eur PRCV isolates. This is quite unexpected in view of the fact that the latter have nearly identical sequences. Nevertheless, as detailed below, the genomes of Eur and USA PRCVs show the same alterations: i) S gene encoding a polypeptide with a large truncation at its N-terminus; ii) ORF3a converted to a pseudogene.

PRCV S gene

The position and length of the deletions identified in the S gene 5' region of European and USA isolates are shown in figure 2. The first 4 amino acids are conserved and the next 224 amino acids are deleted in the mature S polypeptide encoded by the Eur isolates, whereas the deletion in the USA isolates involves 227 residues from position 7 to 233 (Rasschaert *et al*, 1990; Britton *et al*, 1991; Wesley *et al*, 1991). As a consequence, the S protein of Eur and USA PRCV isolates are predicted to be 1209 and 1206 amino acids long, respectively, instead of 1431 or 1433 for the TGEV strains (Rasschaert and Laude, 1987; Britton and Page, 1990).

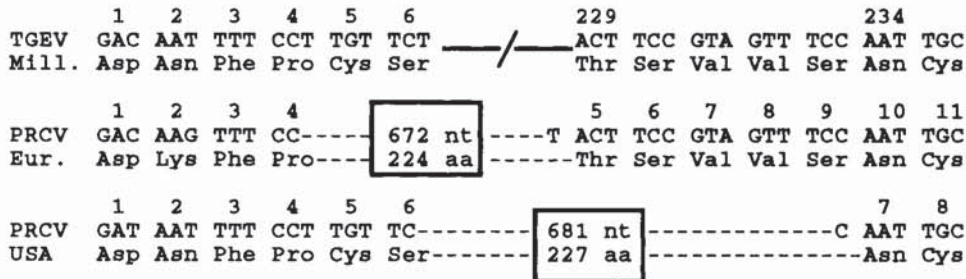


Fig 2. Deletions observed in the spike protein gene of PRCV. An alignment of part of the nucleotide and deduced amino acid sequences of the S gene of the TGEV strain Miller and of the European and USA PRCV isolates is shown. The alignment starts from the first codon in the mature protein (*ie* signal sequence omitted). The size of the observed deletions is boxed. The codons 7 to 228 of TGEV S gene are not shown.

The relative mass (M_r) of the monomeric S protein synthesised in PRCV-infected cells has been shown to be reduced in the expected proportion, *ie* 170 kDa instead of 220 kDa, while the M and N proteins have a similar M_r (Rasschaert *et al*, 1990). As a consequence, some antigenic sites are no longer expressed by PRCV (see below). Whether such a large truncation alters any biological function of the protein is so far unknown.

PRCV ORF3a pseudogene

The ORF3a region of the Eur PRCV genome contains 3 major deletions (Rasschaert *et al*, 1990; Page *et al*, 1991): a 13 nt deletion which covers part of the consensus sequence; a 22 nt deletion which suppresses a non-coding stretch, the initiation AUG codon and 2 ORF3a codons; a 36 nt deletion internal to the ORF3a coding sequence (fig 3). In contrast, a unique deletion 5 nt long and located within the ORF3a coding sequence is observed in the USA isolate (Wesley *et al*, 1991). However, transcripts of the relevant size were

not detected in USA PRCV-infected cells, consistent with the conversion of the CUA AAC sequence in CUAAA U, which should be not (or poorly) functional for transcription.

PRCV ORF3-1

In the Purdue-115 and FS772 TGEV strains, ORF3b is assumed to be expressed from the functionally bicistronic mRNA3 since no functional conserved sequence is found downstream of ORFs 3a (Rasschaert *et al*, 1987; Britton *et al*, 1990). In contrast, the Miller strain, which has a CUA AAC motif between ORF3a and 3b, produces an additional mRNA species (3.7 kb; Wesley *et al*, 1989), designated mRNA3-1 in accordance with the rules proposed by the ICTV coronavirus subgroup (Cavanagh *et al*, 1990).

In PRCV, the ORF3b sequence is expressed through the synthesis of a specific mRNA species: in both Eur and USA isolates a CUA AAC sequence is present upstream (instead of CUAAA U in Purdue and FS772 TGEV strains), so that transcription

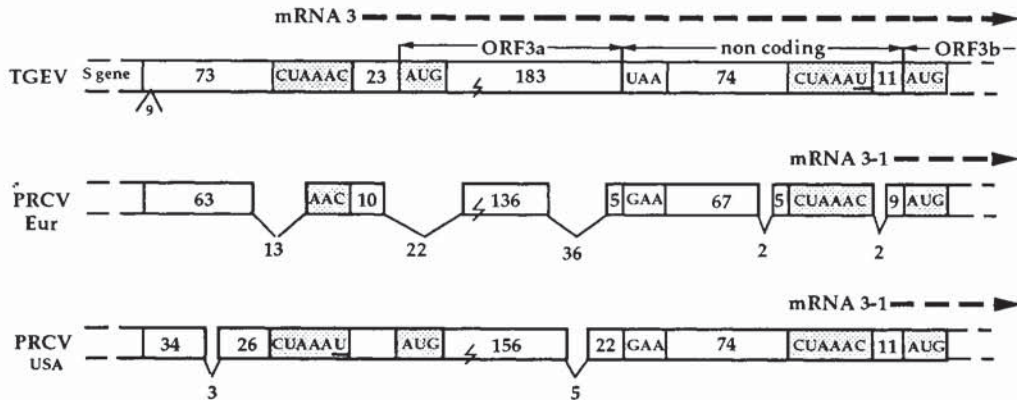


Fig 3. Compared structure of TGEV and PRCV genomes in the ORF3a/ORF3b region. The indicated numbers relate to nucleotide stretches. The position and size of the deletions are shown beneath the frames. Nucleotide substitutions in the CUA AAC sequence assumed to alter the initiation of transcription are underlined. The diagram was drawn according to sequence data of the SF772 TGEV strain, the European RM-4 and the USA Ind89 PRCV isolates (see text for details).

can be initiated at this site (Rasschaert *et al*, 1990; Britton *et al*, 1991; Wesley *et al*, 1991) (fig 3). The corresponding PRCV transcript is thus homologous to the extra mRNA species (mRNA3-1) produced by the Miller strain. Whether the synthesis efficiency of the ORF3b product is modified compared to that from mRNA3a-b has not been examined.

How related are Eur and USA PRCV viruses ?

As mentioned above, the alterations observed in USA and Eur isolates, though being identically targeted in PRCV genome, are different: i) the S gene truncation differs in length and position; ii) 3 deletions are present in Eur PRCV ORF3a gene instead of 1 short deletion for USA PRCV; in the latter, the hexameric sequence is mutated, not deleted. This raises the question of the phylogenetic relationship of the 2 variants. Theoretically, they

could have derived from a common ancestor having the ORF3a gene altered by mutation of the hexameric sequence or short frameshift deletion in the coding sequence. Subsequently, additional and possibly different modifications may have occurred. It is to note that the 36 nt deletion in Eur ORF3a overlaps the 5 nt deletion in USA ORF3a (fig 3). However, such a mechanism does not apply simply to the S gene, since the respective deletions overlap only partially. Moreover, the nonoverlapping sequences, *ie* codons 5, 6 in USA PRCV and 5 to 9 in Eur PRCV are endogenous TGEV sequences (fig 2). It can be inferred from these findings that the 2 pneumotropic variants arose independently, a conclusion further strengthened by the fact that they do not cocirculate but spread — up to now — in geographically distinct swine populations. One important implication of this notion is the possibility of a functional link between the ORF3a and S gene alterations (one acting in such a way that it compensates a defect induced by the other?).

How the deletions were generated into the PRCV genome is less clear. A unique phenomenon associated with coronaviruses is the extremely high-frequency RNA recombination that can be evidenced during a single round of infection. Recombination is thought to occur through a copy-choice mechanism, reflecting the tendency of the polymerase to pause then to jump to another RNA template (review: Lai, 1990). In addition, defective RNAs, composed of several discontinuous parts of the parental genomic RNA are frequently observed in infected cells. Therefore, deletions may have been introduced into the PRCV genome by a very same mechanism, implying the binding of pausing RNA intermediates to a site downstream of the original pausing sites, thus deleting various internal portions of the genome. Inspection of TGEV sequence did not reveal any repeated sequences flanking the different cross-over sites, as frequently observed for intra- or intergenomic homologous recombination; 2 identical heptanucleotide sequences were found in the vicinity of the segment unique to TGEV S gene, but not right each side of the putative cross-over sites (Rasschaert *et al*, 1990). In conclusion, PRCV derived from TGEV by successive deletions which likely occurred through a mechanism analogous to illegitimate recombination. As an important consequence, the appearance of a revertant PRCV is highly improbable.

Genetic basis of the PRCV phenotype

In this section, the possible significance of the major modifications – *ie* deletions – identified in the sequenced part of PRCV genome with respect to its altered tropism will be discussed. For the sake of clarity, 2 aspects will be considered separately, which does not imply that they are actually disconnected.

Loss of enteropathogenicity of PRCV

Like many spike glycoproteins, the coronavirus S protein plays a crucial role in both the attachment of virions to the target cells and the fusion between the viral and cellular membranes. In several reports, the altered neurovirulence of murine hepatitis virus (MHV) variants has been related to the presence of a deletion or even of a point mutation in the amino-half, globular part of the S protein, thus indicating that the latter bears major virulence determinant(s) (Fazakerly *et al*, 1992 and references therein). Also, several TGEV neutralization escape mutants encoding a S protein with a single substitution or a short deletion within its N-terminal region were found to exhibit a markedly reduced enteropathogenicity for the newborn piglet (Bernard and Laude, to be published). In this regard, the truncation of PRCV S protein appears to be highly relevant.

Aminopeptidase N (APN), an ectoenzyme abundantly expressed at the brush border membrane of the small intestinal villi, is known to act as a major receptor for TGEV (Delmas *et al*, 1992a). The first hypothesis coming naturally to mind is that the modified tropism of PRCV could be due to an impaired interaction between attachment protein and cellular receptor. However, recently reported observations have indicated that PRCV may also use APN for gaining entry into cells (Delmas *et al*, 1992b). First, BHK cells, which are refractory to PRCV, became susceptible once transfected with the cDNA encoding porcine APN. Second, anti-APN monoclonal antibodies were able to block efficiently the multiplication of PRCV in cell culture. Yet these *in vitro* findings may reflect the *in vivo* situation incompletely. It can be speculated for instance that the N-terminal domain contributes in some way to the stability of the S protein. Then, exposure to the physicochemical environment of the diges-

tive tract could result in a decreased attachment or fusogenic capacity of PRCV S protein. This should not be an all or none phenomenon, however, since both isolation and replication of PRCV in the neonate small intestine have been described (see *Pathogenesis* section).

In any case, the possibility that PRCV restriction may take place after penetration into the enterocyte should not be disregarded. Examining the susceptibility to TGEV of clones of cell lines derived from various tissues or species and stably expressing porcine APN, led to an intriguing observation. All the transfected clones underwent cell lysis upon infection at an appropriate multiplicity and synthesized the viral structural polypeptides, yet only part of them yielded infectious particles (Delmas *et al*, 1992b). This suggests that the capacity to bind the receptor is a crucial but not unique determinant of the viral tropism.

These observations lead focusing the attention towards the second alteration of PRCV genome, *ie* the conversion of ORF3a to a pseudogene. ORF3a encodes a 71 to 72 amino acid-long non-structural polypeptide, which has been detected in TGEV infected-cells (Godet, 1992). A homologous gene is present only in the enterotropic canine CCV and feline FECV/FIPV coronaviruses. Two cell-adapted TGEV strains, SP and Nouzilly SG188 exhibit, like PRCV, a small plaque phenotype in cell culture and a markedly reduced growth in the intestine. Strikingly enough, a deletion involving part of the ORF3a and part of the downstream ORF3b was identified in both strains, which apparently encode a S protein of normal size (Wesley *et al*, 1990b; Britton *et al*, 1992). In addition, mRNA 3 of the high-passage, avirulent Miller-60 virus, could not be detected by Northern blot analysis (Wesley, unpublished data). Even though the attenuation of these viruses cannot be related unequivocally to the ORF3a defect, these findings point to a possible involvement of ORF3a in the expression of TGEV enteropathogenicity. If confirmed, this would mean that the ORF3a product, which is dispensable for virus replication in cell culture, plays a crucial role in modulating the virulence for the animal.

Respiratory tropism of PRCV

Two kinds of cells have been shown to support PRCV replication in the respiratory tract: the epithelial cells, that are likely to express APN at their membrane surface (Ito *et al*, 1980) and the alveolar macrophages; interestingly enough, APN and CD13 – a marker of the monocyte-macrophage lineage – are the same molecule in the human species (see Delmas *et al*, 1992). It is thus conceivable that PRCV uses APN as a receptor for gaining entry into the pulmonary cells, although the use of an alternative molecule cannot be formally excluded. The recent finding that HCV 229E, a human respiratory coronavirus, also uses APN as a receptor (Yeager *et al*, 1992) brings some support to this view.

Whether PRCV possesses an increased tropism for the respiratory tract compared to TGEV may be a more controversial issue. Indeed, replication of TGEV in the respiratory tract has been reported by a number of authors (Harada *et al*, 1969; Underdahl *et al*, 1974, 1975; Kemeny *et al*, 1975, 1977, 1978; Furuuchi *et al*, 1979; Sprino *et al*, 1982; La Bonnardière and Laude, 1983 and unpublished data; Wesley *et al*, 1990a; Cubero *et al*, 1992). Also, the alveolar macrophages have been shown to support the replication of cell-adapted and virulent strains *in vitro* or *in vivo* (Laude *et al*, 1984). Attention should be drawn, however, to the nature of the strain assayed as well as to the technique employed for assessing virus multiplication

(eg histoimmunodetection *versus* infectivity titration), when interpreting the published data. A tentative conclusion from the available information is that many cell-adapted strains (including a pathogenic strain having no more than 10 passages in culture, such as D52) replicate in the lungs to substantial titers. This might be no longer true with pig-passaged wild strains of TGEV. Growth of the Miller and Purdue wild strains was found to occur only at a limited extent (Wesley *et al*, 1990a; Pensaert *et al*, unpublished results); there might be exceptions, however, such as the Nebraska isolate, which has been reported to replicate to a similar efficiency in the digestive and respiratory tracts (Underdahl *et al*, 1974). Additional studies comparing the multiplication of PRCV and TGEV in pulmonary tissues may be necessary to answer that question conclusively. In any case, it is worth to keep in mind that, even though airborne infection by TGEV has been reported to occur, the efficiency of spreading by aerogenic way does not appear to be comparable to that of Eur PRCV (the limited diffusion of USA PRCV is likely to be related to differences in the European and US industries rather than to a distinct phenotype).

Finally, one remains faced to 2 main hypotheses. Firstly, PRCV originates from a particular TGEV strain having originally a respiratory tropism. Secondly, the ability of PRCV to replicate in pulmonary cells to a high level relies on the alteration of S and/or ORF3a genes. That a virus could acquire such a capacity by the introduction of deletions in its genome is puzzling and, to our knowledge, unprecedented. One possibility that could be explored in this respect is whether the introduced genomic change(s) may result in a delayed or decreased cytopathic effect, thus allowing the virus multiplication to take place for several virus cycles, which is unlikely to occur in TGEV-infected enterocytes.

Antigenic features

The fact that PRCV and TGEV share many antigenic determinants and cannot be differentiated by conventional serological techniques was acknowledged at the very beginning of the investigations. The 2 agents exhibit a complete 2-way cross-neutralization when using polyclonal sera. At the level of individual polypeptides, immunoblotting reveals a reciprocal cross-reactivity of the S, M and N antigens (Callebaut *et al*, 1988). The identification of distinctive determinants could only be achieved through the use of panels of monoclonal antibodies (MAbs) previously raised against TGEV (Jimenez *et al*, 1986; Laude *et al*, 1986; Garwes *et al*, 1987).

S antigen

Earlier studies on the antigenic structure of Purdue strain S protein have defined 4, possibly 5 major antigenic sites, all located in the amino-half of the polypeptide chain (Delmas *et al*, 1986; Correa *et al*, 1988, 1990; Delmas *et al*, 1990; Gebauer *et al*, 1991). From the analysis of the PRCV-binding activity of anti-TGEV S MAbs (Callebaut *et al*, 1988; Laude *et al*, 1988; Sanchez *et al*, 1990), 3 main conclusions can be drawn (fig 4).

Firstly, the MAbs defining the partially overlapping sites A and B are fully reactive towards PRCV. Both these sites are comprised of epitopes which are highly conserved among TGEV strains and induce strongly neutralizing antibodies. The site A has been recently reported to be immunodominant in both TGEV- and PRCV-immune sows (De Diego *et al*, 1992).

Secondly, the MAbs which discriminate between TGEV and PRCV generally exhibit no significant neutralizing activity. By differentiating MAbs, it is meant those which recognize epitopes expressed by all or

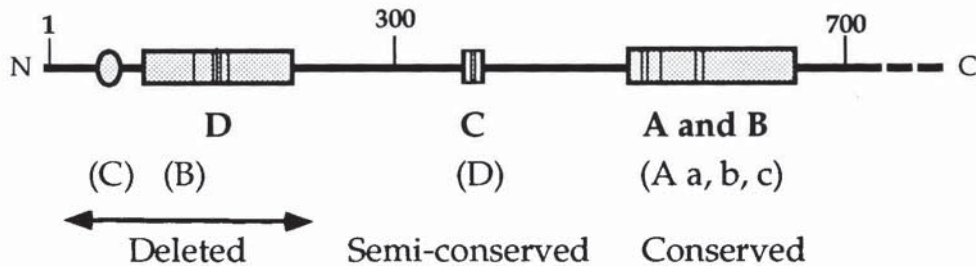


Fig 4. Expression by PRCV S protein of the antigenic sites identified on the TGEV S protein. The upper diagram shows the location of the major antigenic sites on the amino-half part of the TGEV S protein sequence (Paris group's designation in bold, Madrid group's designation within brackets). The position of residues involved in epitope formation is indicated by dashes within the boxes. The bottom line gives the status of PRCV S protein with respect to the expression of the different sites. The figure was constructed according to data cited in the text.

most TGEV strains, but not by any of the PRCV isolates tested so far. Such epitopes are grouped into 2 distinct antigenic sites: i) the site D (probably overlapping the site designated B by the Madrid group); ii) a site not expressed on the native virions (C Madrid), which is less conserved than the former. Both of these sites have been mapped within the region of the polypeptide chain predicted to be removed in the PRCV S molecule, thus providing a straightforward explanation for the lack of binding of the relevant MAbs. Few non-neutralizing anti-S MAbs, defining epitopes unrelated to the major sites, have also been found to be differentiating. Two site D MAbs exhibit interesting peculiarities (Laude *et al*, 1988): unexpectedly, the MAb 78.17 cross-reacts with PRCV, which may suggest that the cognate epitope is assembled, one component being located outside of the truncated domain; the MAb 40.1 is the sole differentiating MAb showing a high neutralizing activity; strikingly, it is by use of this MAb that escape TGEV mutants attenuated for the young piglet were selected.

Thirdly, the PRCV isolates originating from Belgium, Denmark, France, Great

Britain and The Netherlands display nearly identical reactivity patterns. This result, consistent with the sequence data, confirms the unicity of Eur PRCV. However, some antigenic variation can be evidenced by using MAbs defining the site C (D Madrid), which shows some variability among TGEV strains as well. The antigenic pattern of USA PRCV has not been studied in detail, but the reactivity of the Ind89 strain towards anti-TGEV MAbs representative of the sites A, B, C, and D is similar to that of Eur PRCV (Wesley *et al*, 1990a).

Information regarding PRCV S-specific determinants is comparatively scarce. Seven PRCV MAbs, selected on the basis of their lack of reactivity towards the Purdue TGEV strain, were all directed against the S protein. Three of them, all of which were neutralizing, did not react with any of the 5 TGEV strains tested, whereas the other, non-neutralizing MAbs recognised common epitopes (Deriabine and Laude, unpublished data). This is in contrast with the fact that nearly all differentiating anti-TGEV MAbs were not neutralizing. Whether the neutralizing PRCV MAbs define a site distinct from those previously identified on the S molecule remains to be investigated.

N and M antigens

Anti-N and anti-M MAbs raised against TGEV are not differentiating antibodies since they recognize epitopes which are either conserved or inconsistently expressed on both the viruses (Laude *et al*, 1988; Sanchez *et al*, 1990). Anti-N MAbs defining the antigenic site A, located within the amino-half part of the polypeptide chain, exhibit a good cross-reactivity, whereas those defining the site B, located within the carboxy half part, show an altered reactivity toward most of the PRCV isolates tested (Alonso *et al*, 1992). These studies also revealed some antigenic heterogeneity of PRCV N protein, not observed with TGEV, which may be the trait of a young virus, not fully adapted to its ecological niche (Sanchez *et al*, 1990).

Concerning the M antigen, MAbs directed against epitopes previously mapped within the short (some 30 residues) N-terminal region protruding at the outer face of the virion envelope, were found not to react with any of the 3 PRCV isolates tested, whereas MAbs assumed to recognize the C-terminal, internal region of the M protein were cross-reactive (Laude *et al*, 1988). These findings correlate well with the sequence data: the 64 carboxy-last amino acids of 2 PRCV isolates and of TGEV strains Purdue and FS772 are identical, whereas 4 differences are noted within the first 30 N-terminal residues, a divergence comparable to that of the 2 TGEV strains (Laude *et al*, 1987; Rasschaert *et al*, 1990; Britton *et al*, 1991). Moreover, 2 of these changes involve amino acids shown to be crucial for epitope expression (Laude *et al*, 1992). In essence, it would appear that the M protein epitopes conserved between TGEV and PRCV are internal. Finally, despite some amino acid changes in the N-terminal domain of M protein, PRCV is able to induce an α -interferon synthesis in naive swine lympho-

cytes similar to TGEV (Charley and Laude, 1988 and unpublished data).

In conclusion, several epitopes absent on PRCV have been identified by use of anti-TGEV S monoclonal antibodies. Such dissimilarities have been exploited for developing an ELISA test for the differential detection of antibodies against each virus (see *Diagnosis* section).

INFECTION

Pathogenesis

The pathogenesis of experimentally induced PRCV infections has been found to differ depending on the age at infection.

Pigs < 1 wk old, either hysterectomy-derived and colostrum-deprived (HDCC) or conventional but devoid of antibodies against PRCV, have been inoculated by aerosol (Cox *et al*, 1990a) or oronasally (O'Toole *et al*, 1989; Pensaert *et al*, 1986). They were euthanized at different time intervals after inoculation and examined for virus. PRCV was isolated from the nasal mucosa, tonsils, trachea and lungs. Virus was also recovered from the stomach and the small intestine and its contents (O'Toole *et al*, 1989; Cox *et al*, 1990a). In the aerosol infected group, a number of other tissues including plasma, mesenteric lymph nodes and colon were consistently positive, while virus was sometimes isolated from other lymph nodes, spleen, liver and thymus. Yet, the respiratory tract is the main target organ and virus titers in the apical lung lobe are as high as $10^{8.3}$ TCID₅₀/g tissue. These titers are significantly higher than those obtained in any other organ. Using immunofluorescence (IF), the virus has been shown to infect the epithelial cells of nasal mucosa, tonsils, trachea and lung tissue and the alveolar macrophages.

In the lungs, fluorescence was detected mainly in the epithelial cells of alveoli (fig 5), but also in bronchioli and bronchi. A maximum of infected cells is seen at 3 d post inoculation (PI) in the bronchi and between 3 and 5 d PI in alveoli and bronchioli (Cox *et al*, 1990a). Though viremia can occur as early as 2 d PI, as evidenced by virus isolation from plasma, no virus was seen by IF in parenchymal organs and lymph nodes. Viral antigen was demonstrated in a few epithelial cells in the small intestine of neonatal pigs (Cox *et al*, 1990a). The intestinal infection begins in the ileum and gradually moves upwards to the duodenum. Four to 5 d PI, all small intestinal segments are infected (Cox *et al*, 1990a) and a maximum of 4 fluorescing cells are present per ileal section. The infected cells were identified as villous enterocytes by electron microscopy

(Pospischil *et al*, 1990) and immunocytochemistry (O'Toole *et al*, 1989). Virus isolation from the stomach suggests that virus may reach the intestine after being ingested, but a spread from the respiratory tract to the gut *via* viremia cannot be ruled out. The way of virus spread from the caudal to the cranial small intestine is unclear. In piglets inoculated with PRCV directly into the lumen of the intestinal tract, more than 10^3 TCID₅₀ of virus were needed to start the intestinal infection (Cox *et al*, 1990c). These findings, together with the very limited extent of intestinal replication, indicate that the susceptibility of the small intestine to infection with PRCV is rather low. Small intestinal enterocytes are very susceptible cells to TGEV, allowing a highly productive virus replication, but the gut is not a target organ for PRCV.

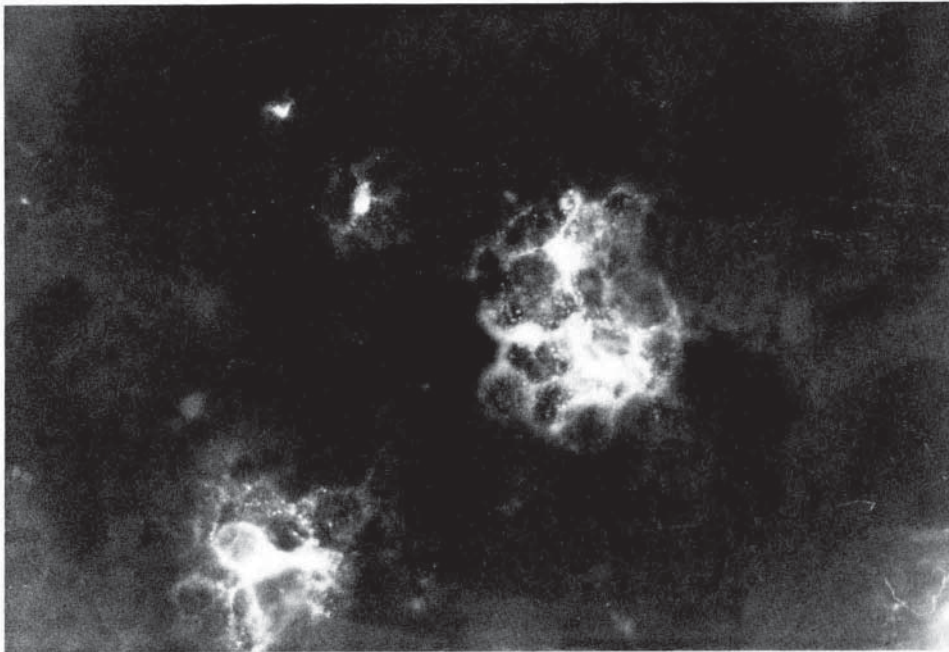


Fig 5. Immunofluorescence in the alveolar epithelium of a 5-week-old piglet upon experimental PRCV-infection.

In 5-wk-old HDCD pigs inoculated with PRCV by aerosol, virus replication was limited to the respiratory tract (Cox *et al*, 1990b). The pathogenesis of the respiratory infection was similar to that in neonatal piglets except for the absence of fluorescence in tonsils. However, the number of fluorescent cells in the lungs was lower in these pigs compared to the 1-wk-old piglets and fluorescence in bronchi was even rare. Viremia was not detected. Of main importance is that no intestinal replication was observed. Yet, infectious virus was ingested since PRCV was isolated at low titres from the cranial intestinal tract of some of the piglets. When 5-wk-old pigs with maternal neutralizing antibody titer of 48 were inoculated, virus production in the respiratory tract was not decreased compared to that in pigs without PRCV-neutralizing antibodies.

In adult swine, the pathogenesis of PRCV has not been studied in detail, but some data are available (Van Reeth *et al*, unpublished results). Conventional fattening swine (80–100 kg) without PRCV-seroneutralizing antibodies were infected by aerosol with 7 different Belgian PRCV field isolates. Virus titers in lung tissues between 4–6 d ranged from $10^{3.2}$ – $10^{7.2}$ TCID₅₀/g. No clear-cut differences appeared between the distinct isolates with regard to virus quantity and degree of fluorescence in the lungs. Fluorescence was restricted to a few alveolar epithelial cells, whereas bronchi and bronchioli were negative. The extent of virus replication was similar in apical, cardiac and diaphragmatic lung lobes. In pigs which were clinically followed, virus shedding in nasal secretions started 1 to 2 d PI and mean viral titres ranged from $10^{5.5}$ – $10^{6.5}$ TCID₅₀/g nasal secretions between 2 and 5 d PI. Thereafter, virus amounts gradually decreased and excretion continued until 8 d PI.

Clinical signs and pathology

It is still a subject of debate whether or not PRCV causes clinical disease signs. At the time of the first detection of PRCV in the Belgian swine population, the virus was considered to be non pathogenic. First isolations of PRCV were made from the respiratory tract of clinically normal piglets (Pensaert *et al*, 1986). Clinical disease is not a feature when a PRCV infection-wave occurs on closed breeding–fattening farms. In the longitudinal serological studies mentioned above, no disease was associated with seroconversion of fattening pigs on the closed farms.

However, some field reports have linked a PRCV infection with respiratory disease in growing, finishing and adult swine. A serological survey in pig herds with respiratory disease in Brittany (France), revealed seroconversion to PRCV and not to influenzaviruses or Aujeszky's disease virus in 2 out of 6 herds (Jestin *et al*, 1987b). Pigs became infected with PRCV either shortly after arrival on the fattening farm or towards the end of the fattening period. Anorexia and fever were the main clinical signs, coughing was seen on occasion. Similar observations were made by Laval *et al* (1991). Clinical manifestations have also been reported in lactating sows undergoing a PRCV infection (Ulbrich *et al*, 1991). They were characterized by fever and inappetence, followed by a barking cough a few days later. Such observations have not been made by the authors since experimental infections of sows in the field remained completely subclinical.

Whether field infections with PRCV result in respiratory disease or not will remain an open question. Multiple environmental factors such as climate, housing, presence of concomitant infections and many others will play a role in determining the appearance of disease signs. In the

study mentioned earlier (Van Reeth *et al*, unpublished results) newly arrived groups of feeder pigs were examined in industrial fattening units with respiratory disease in autumn and with minimal or negligible respiratory signs in spring. In both circumstances, infections with PRCV occurred together with H1N1-and/or H3N2-influenzavirus infections. Simultaneous experimental infections with H1N1 or H3N2-influenzavirus and PRCV did not appear to enhance the pathogenicity of these viruses (Lanza *et al*, 1992). However, in most recent experiments where 9-wk-old piglets were infected 2 or 3 d after an experimental PRCV infection, clinical signs and lung lesions were more severe in dual infected animals than in those infected with either of the respiratory agents alone (Van Reeth *et al*, unpublished results).

Clinical signs have been reported in some experimental inoculation studies and not in others. They vary considerably in severity and this may be influenced by the age of pigs and/or the inoculation technique. One-wk-old specific pathogen free (SPF) pigs inoculated either by aerosol (Cox *et al*, 1990a) or oronasally (O'Toole *et al*, 1989) failed to develop clinical signs. Five-wk-old piglets, either HD CD or conventional with or without PRCV maternal antibody titers, remained asymptomatic following aerosol inoculation (Cox *et al*, 1990b). Also, in another study, pigs at the age of 5 wk were clinically healthy after intranasal infection with PRCV (van Nieuwstadt and Pol, 1989). On the other hand, intratracheal inoculation of SPF pigs at 90 d of age resulted in mild clinical signs (Vannier, 1990). Dyspnea, polypnea, short lasting fever and prostration with temporary loss of performances were recorded in most but not in all the pigs. More severe disease signs have been described by Duret *et al* (1988) following intratracheal inoculation of SPF pigs at 25 kg. All the pigs experienced fever, sneezing and some dyspnea and

body temperatures did not return to normal until 2 wk PI. Nasal discharge and epistaxis were observed occasionally.

Recently, experimental infections of conventional fattening swine (80–100 kg) have been carried out in the authors' laboratory (Van Reeth *et al*, unpublished results). Each of 7 PRCV Belgian field isolates was inoculated by aerosol in 2 fattening pigs, free of PRCV-sero-neutralizing antibodies. Fever and inappetence generally developed 2 d PI. Mild respiratory signs such as nasal discharge and sneezing were recorded. Two of 13 pigs, each infected with a different isolate, did not show any sign of disease. Variation in virulence between the isolates tested was not detected. With one of these isolates, aerosol inoculation was performed on a larger group of 6 fattening swine. These 6 pigs showed symptoms suggesting an involvement of the deeper airways including coughing, laboured breathing and increased respiration.

Pathological changes on field cases have not been reported. A catarrhal lobular bronchopneumonia is most consistently found in experimentally inoculated pigs (Duret *et al*, 1988; O'Toole *et al*, 1989; van Nieuwstadt *et al*, 1989; Cox *et al*, 1990a; Vannier *et al*, 1990). Histological examination of the lungs of HD CD piglets revealed an interstitial pneumonia with some degeneration of the alveolar and bronchiolar epithelium. The lumen of bronchioli and alveoli is filled with macrophages and cellular debris. Regeneration starts at \approx 1 wk PI and is characterized by hyperplasia of bronchiolar epithelium, alveolitis and modest peribronchial cuffs of lymphoblasts, macrophages and plasma cells (O'Toole *et al*, 1989).

Immunity to PRCV and TGEV

Upon aerosol inoculation of pigs with PRCV, serum antibodies are detectable 1

wk later. The antibody titre increases until 3–4 wk after inoculation. Protection of the respiratory tract against reinfection is not long lasting since regular reinfections with PRCV occur in sows in the field (Callebaut *et al*, 1990). Also, experimental reinfection of 6 pigs was possible as soon as 6 wk after a primary infection using the intranasal or intrapulmonary inoculation technique (van Nieuwstadt *et al*, 1992). Virus neutralizing antibody titers in these pigs increased from 794 to 10 000. In the field, attempts were made at experimental reinfection of fattening swine with PRCV serum antibodies (VN titers 16–192). Upon challenge, virus replication did not occur in some of these pigs, as concluded from the absence of seroconversion. In other pigs though, neutralizing antibody titers up to 3 072 have been detected 3 wk after challenge (Van Deun *et al*, unpublished results).

Because of the high prevalence of PRCV, the majority of litters on a farm are born from immune sows and acquire serum antibodies through the colostrum. In the absence of an infection, these maternal antibodies decline with a mean half-life of 12.04 d and persist at the latest until the age of 16 wk (Pensaert *et al*, 1986). Declining maternally-derived antibody titres do not protect against a PRCV infection because PRCV virus titres and sites of replication in the respiratory tract were not different when 5-wk-old pigs with or without maternal PRCV-antibodies were experimentally inoculated (Cox *et al*, 1990b). Besides, infectious virus was excreted during 8–13 d by the maternally immune pigs, compared to 6–11 d by the seronegative pigs. In the field, passive immunity frequently turns to active immunity between the age of 5–10 wk.

The wide distribution of PRCV in the swine population has been accompanied by a marked reduction in the number of TGEV outbreaks. In the 3 diagnostic laboratories in Belgium (Laboratory of Veteri-

nary Virology, Faculty of Veterinary Medicine, Gent; Regional Veterinary Investigation Centre, Torhout and National Institute for Veterinary Research, Brussels), diagnosis of TGEV has been made 68 times during the year 1982–1983, 61 times in 1985–1986, but only 7 times in 1988–1989. From the beginning of 1992 till now (end of November 1992) only one case has been reported. Therefore, the question has arisen if PRCV and TGEV, whose structural antigens are nearly identical, can induce cross-protection *in vivo*. Protection of suckling piglets against an enteric TGEV infection is based on the uptake of specific (lactogenic) IgA antibodies in the milk of TGEV-immune mothers (Bohl *et al*, 1972). Sows immune following a natural PRCV infection do secrete TGEV-neutralizing antibodies in their milk. These antibodies though are not always of the IgA class (Callebaut *et al*, 1990). Experiments have been carried out in (field) sows with PRCV serum antibodies and thus previously infected with PRCV at an unknown time. When these sows were reinfected with PRCV oronasally during the last weeks of pregnancy or early in lactation, either induction or a rise in titer of lactogenic anti-TGEV IgA was obtained in all of them. By 24 wk after the experimental reinfection however, IgA antibody titers were significantly decreased or even absent (Van Deun *et al*, 1990 and unpublished results). Regular PRCV infection-waves on breeding farms, therefore, most probably favor and maintain the presence of anti-TGEV IgA in sow milk. The origin of IgA in sow-milk after a respiratory PRCV infection is unclear since there is no direct evidence of an immunological link between the respiratory tract and the mammary gland, as is known to exist between the gut and mammary gland (Bohl *et al*, 1972).

Yet, the main point is the efficiency with which PRCV-induced lactogenic IgA protects suckling piglets against an enteric

TGEV infection. In the aforementioned experiment (Van Deun *et al*, 1990 and unpublished results) naturally PRCV-infected sows, reinfected with PRCV during pregnancy (and secreting lactogenic TGEV-IgA) protected their offspring to a certain degree. Piglets of these sows developed diarrhea upon challenge with TGEV but the incubation period was delayed by 1–2 d and mortality rates were markedly lowered in 3 out of 6 litters (0–12.5%). Partial protection was also reported by Bernard *et al* (1989) in litters of sows which had been naturally infected with PRCV. Five out of 7 litters were protected against mortality after an experimental TGEV challenge, resulting in an overall mortality rate of 44%. Similar levels of protection were obtained upon TGEV challenge of piglets nursing sows which had been infected experimentally with PRCV at 84 and 104 d of pregnancy (De Diego *et al*, 1992). Generally, PRCV-induced lactogenic antibodies protect pigs against TGEV to a lesser degree than TGEV-induced antibodies. This difference is not due to IgA characteristics since the neutralizing capacity and/or the secretory component of both IgA's are the same (Callebaut *et al*, unpublished results). In experiments carried out by De Diego *et al* (1992) lactogenic protection in piglets suckling from TGEV-immune mothers was higher than in piglets from PRCV-immune sows and this corresponded with higher milk antibody titers. Cross-protection between PRCV and TGEV was not observed by Paton and Brown (1990), since litters from PRCV-immune sows were not protected against TGEV in their experiments. TGEV-antibodies in sowmilk have been demonstrated in this study but no characterization with regard to immunoglobulin class was done.

In PRCV-immune breeding herds, TGEV outbreaks are of a milder course which may be due to the presence of lactogenic IgA in sowmilk, but they are also of a shorter duration. Prior to the situation with PRCV being

enzootic, a 90–100% death loss of neonatal pigs during a period of 3 wk after the introduction of TGEV was a common finding. In 1987, 3 outbreaks of enteric TGEV were followed on PRCV-immune breeding farms and litters born 10, 11 and 14 d after the start of the outbreak remained healthy (Pensaert *et al*, 1987). This can be explained by a priming effect of a PRCV infection in the sows. When PRCV-primed sows subsequently become infected with TGEV, an anamnestic response will quickly develop. That way, a rapid increase of IgA antibodies in milk will occur and piglets born a few d after the start of the outbreak already receive a solid lactogenic immunity.

Another immunological aspect is the question of whether a respiratory PRCV infection can induce an intestinal mucosal immunity and protection against TGEV. Van Nieuwstadt *et al* (1989) could not demonstrate such protection in specific pathogen-free pigs. TGEV antigen excretion in the faeces was of the same duration in PRCV immune as in non-immune control pigs. Recently, however, it was demonstrated that conventional pigs which are inoculated by aerosol with PRCV at the age of 6 wk and in the absence of maternal PRCV antibodies, are partially protected when challenged with TGEV 4 wk later (Cox *et al*, 1992). Aerosol inoculation with PRCV appears to prime both the systemic and the intestinal immune system. Challenge of the PRCV-immune pigs with TGEV resulted in a rapid and drastic increase in TGEV neutralizing antibody titres—with titers ranging up to 1 280 2 wk later— and infectious TGEV was excreted in feces during 0–4 d, compared to 5–6 d when fully susceptible pigs are inoculated. The mechanism through which PRCV primes the intestinal immune system is not known at the present time, but the PRCV-TGEV tandem may be very suitable to study the common mucosal immune system in more detail.

LABORATORY DIAGNOSIS

A clinical diagnosis of PRCV is not possible. A PRCV infection can be diagnosed in the laboratory by isolating the virus, by demonstrating viral antigens in lung tissue or by detecting seroconversion.

PRCV can be cultivated in a variety of cell cultures. The virus was first isolated in primary pig kidney cells, but also grows in continuous cell cultures of swine kidney (PK15), swine testicle (ST) and a continuous cell line of cat fetuses (FCWF). Because of its high susceptibility, the ST swine testis cell line (McClurkin and Norman, 1966) has become most widely applied for virus isolation (Pensaert, 1989). Five d after seeding, fully sheeted swine testis cells are most sensitive. The cytopathic effect in ST cells is characterized by syncytium formation, followed by rounding up of cells. Formation of small syncytia may also be observed when wild TGEV strains are first isolated in ST cells. With PRCV, the entire monolayer may be destroyed by 2–3 d after inoculation. Identification can be performed by immunofluorescence, and differentiation between the 2 agents should be possible by the use of available TGEV- or PRCV-specific anti-S MAbs (see above: antigenic features). Lung tissue or nasal swabs are the specimens of choice for virus isolation.

The fluorescent antibody test on lung tissue of experimental animals is often performed. Cryostat sections are stained with an anti-TGEV conjugate and examined by fluorescence microscopy. This approach is also not PRCV-specific, since some TGEV strains have been reported to grow in the respiratory tract (Underdahl *et al*, 1974; Kemeny *et al*, 1975). Onno *et al* (1989) have described an indirect immunofluorescence test on smears of nasal cells. Alternatively, viral antigen can be detected by immunoperoxidase staining on frozen sec-

tions of lung tissue (O'Toole *et al*, 1989; van Nieuwstadt *et al*, 1989), but this technique is not applied for routine diagnosis. By electron microscopic examination, particles with a typical coronavirus morphology were detected in bronchiolar cells, in alveolar macrophages and free in the alveoli (O'Toole *et al*, 1989). Differentiation with TGEV is not possible with the electron microscope. Finally, the use of nucleic acid probes in dot-blot hybridisation has been recently proposed as a means for selective diagnosis (Jackwood *et al*, 1992).

A serological diagnosis can be made with the classical virus neutralization test (Voets *et al*, 1980) or with an ELISA test (Paton *et al*, 1991), using TGEV as an antigen. One of the major shortcomings of the test is that it cannot differentiate between PRCV- and TGEV- induced antibodies. If no problems of diarrhea have been encountered in a herd and if there is no evidence of enzootic TGEV, then TGEV-neutralizing antibodies are considered to be PRCV-induced. However, no guarantee can be given that TGEV is not involved. In the mid eighties, the wide prevalence of PRCV had created serious obstacles for export of pigs to TGE-free countries. A clear need for a differential test had therefore arisen, not only for export purposes but also for research. To meet this requirement, efforts have been channeled into developing a differential competitive inhibition ELISA (Garwes *et al*, 1988; Callebaut *et al*, 1989; Have, 1990). All these tests use a non-neutralizing MAb directed against an epitope on the S protein in TGEV with no counterpart in PRCV, in order to permit the detection of TGEV-specific antibodies without interference of PRCV antibodies. Two conditions are required for the test to be operational; first, the assay must be sensitive enough to detect the low levels of antibodies that may be present early in TGEV infections; second, the reactivity of the MAb(s) used must be extended to a panel

of TGEV strains circulating in the field. The principle of a test which has been used at a large scale and proved to be equivalent in sensitivity to neutralization (Callebaut *et al*, 1989) is schematized in figure 6. False positive results have not been reported but false negative results may occur (Callebaut *et al*, 1989; Have, 1990). It is recommended therefore, to test several serum samples from the same herd. The classical neutralization test no longer provides a method for the serological diagnosis of TGE, but is still essential. It is the combination of a negative result in the blocking ELISA and a positive result in the seroneutralization test that gives evidence of a PRCV infection. Consequently, sera will

be tested first in the seroneutralization test and, if positive, the differential ELISA may then be performed.

CONCLUSIONS

There is now strong evidence that PRCV is nothing else than a deletion variant of TGEV. Whether a peculiar context has favoured the emergence of PRCV remains an unanswered question. In particular, the fact that actually 2 different deletion mutants have emerged independently in 2 different continents (Europe and the USA), while TGEV apparently had undergone no recognizable changes since its original iso-

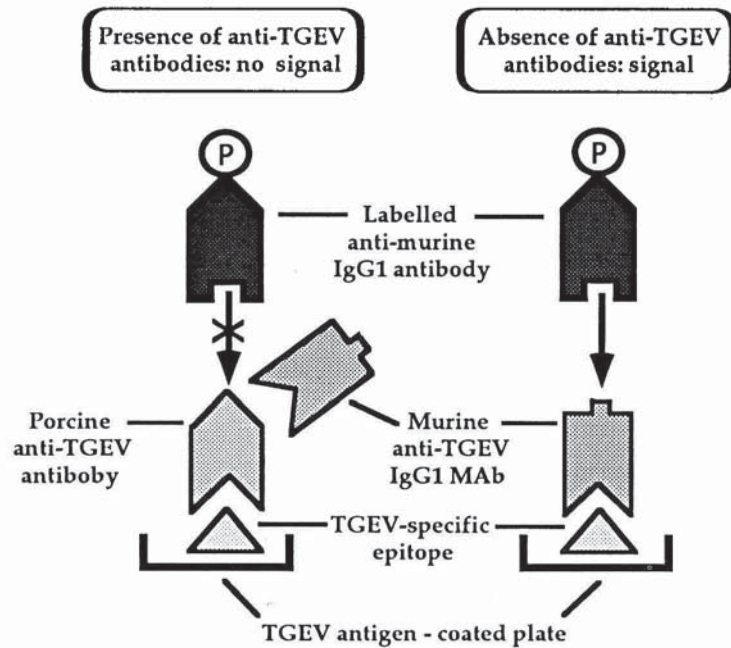


Fig 6. Differential competitive inhibition ELISA. The test is based on the use of a monoclonal antibody (MAb, IgG1 isotype) directed against a TGEV-specific epitope. Microtiter plates coated with Purdue virus as an antigen are incubated in the presence of the sample serum to test. TGEV-specific but not PRCV-specific antibodies can block the target epitope. In the absence of TGEV-specific antibodies, the target epitope remains available for interaction with the differentiating MAb. The latter is revealed by addition of peroxidase-labelled antibodies then of a chromogenic substrate.

lation in 1946, is intriguing. Also, PRCV provides the first example of a coronavirus for which spontaneous genomic deletions have resulted in a considerable epizootiological impact. This supports the view that not only recombination but also deletion events represent a driving force in coronavirus evolution.

The emergence of PRCV has brought about consequences which are clearly beneficial to researchers and to the pig producers. The virus has become very widespread in Europe since its first appearance in the mid-eighties and is now enzootically present. This enzootic status will, based on the presently available information, not change in the future. TGE has clearly become a disease of minor importance in Europe since the establishment of this enzootic PRCV situation. Widespread infections, possibly also repeated ones, with PRCV have created an immune status in the swine population so that infections with the enteropathogenic TGEV have acquired a mild or short lasting character. The much-feared acute outbreaks of TGE with the high pig mortalities have disappeared since PRCV has become enzootic and this has given a tremendous beneficial effect on the pig population.

Through the emergence of PRCV, researchers have been provided with a tool to obtain new insights into molecular pathogenesis and common mucosal immunity. PRCV replicates highly in the respiratory tract and has, contrary to TGEV, no enterotropism in clinical terms. One would expect that PRCV is no longer able to recognize receptors on enterocytes, but recent studies have shown that this may not be the case, suggesting that a later step of the virus cycle may be hampered. Even more interesting is that PRCV has acquired a highly pronounced tropism for the respiratory tract. From the molecular studies performed up to now, a much fo-

cused picture has emerged, and laboratory tools for a selective diagnosis of each kind of infection have been provided. Yet, much remains to uncover about the intimate mechanisms which led to such a remodeling of the original TGEV tropism. Two genes have been shown to be altered in PRCV genome, but whether the observed phenotypical changes have a mono- or multigenic origin remains an open question. However, several of the issues raised in this article should be amenable to solution, in particular by the engineering of TGEV-PRCV chimaeras. Thus, through this TGEV deletion mutant, molecular virologists can be provided with clearer insights into the function of different gene products of TGEV-PRCV, information which may be useful not only for molecular pathogenesis of the respective infections, but also for that of other coronaviruses of different animal species. From a more general point of view, the finding that the apparently enhanced contagiousity exhibited by PRCV relative to the parental virus has been gained virtually through a loss of genetic material is worth reflecting upon.

Common mucosal immune mechanisms exist but are often difficult to study with viruses because many viruses either have a tropism for several mucosae or do not have a counterpart-virus for reciprocal challenge and evaluation of protection at a distant mucosal site. PRCV has provided immunologists with an ideal tool. The protective effect of immune responses which were initiated by a PRCV infection in the respiratory tract can be tested in the respiratory tract itself through challenge with PRCV and can also be tested in the enteric tract by challenge with the closely related enterotropic TGEV and *vice versa*. This way, immune processes interacting between the respiratory tract and the enteric tract can be examined. Moreover, the mechanism by which a respiratory tract in-

fection with PRCV leads to IgA production in the mammary gland of sows can be studied. It has long been thought that lactogenic immunity based on IgA secretion in the sows milk could only be obtained if an infection had occurred in the enteric tract.

Not all the effects which accompanied the emergence of PRCV have been beneficial. The possible association of a PRCV infection with the appearance of respiratory disease is still under discussion. There is no doubt that a new infectious, potentially pathogenic, respiratory virus has been added to the long list of now existing respiratory infectious agents in swine. PRCV infections are widespread and pigs thus have a high chance to become infected concomitantly with other, also widespread, respiratory pathogens such as influenzaviruses, porcine respiratory and reproductive disease virus, Aujeszky's disease virus, and some bacteria. It can be reasonably accepted that PRCV in these combined infections adds to the appearance of disease particularly in pigs "at risk" *ie* when maternal immunity wanes or when transfer occurs from the breeding to the fattening divisions of farms. The real importance of PRCV as pathogen for the respiratory tract is still under study both in field and experimental circumstances but the economic impact is certainly much lower than that of the earlier typical TGE outbreaks.

In conclusion, the different features which have accompanied the emergence of PRCV have been explained in this review article. It is shown that, contrary to other newly emerged viruses in the recent history of porcine viral infections, the emergence of PRCV has undoubtedly brought more favorable than unfavorable effects to the pig population itself, to the pig producer and to researchers studying different aspects of viral diseases in swine.

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