Transboundary and Emerging Diseases

RAPID COMMUNICATION

Characterization of a Novel Chimeric Swine Enteric Coronavirus from Diseased Pigs in Central Eastern Europe in 2016

G. J. Belsham[1], T. B. Rasmussen[1], P. Normann[1], P. Vaclavek[2], B. Strandbygaard[1] and A. Bøtner[1]

[1] DTU National Veterinary Institute, Technical University of Denmark, Lindholm, Kalvehave, Denmark
[2] Department of Virology, State Veterinary Institute Jihlava, Jihlava, Czech Republic

Keywords:
recombination; porcine epidemic diarrhoea virus; transmissible gastroenteritis virus

Correspondence:
A. Bøtner. DTU National Veterinary Institute, Technical University of Denmark, Lindholm, Kalvehave 4771, Denmark. Tel.: +45 3588 7858; Fax: +45 3588 7901; E-mail: aneb@vet.dtu.dk

Received for publication June 29, 2016

doi:10.1111/tbed.12579

Summary

During a severe outbreak of diarrhoea and vomiting in a pig herd in Central Eastern Europe, faecal samples were tested positive for porcine epidemic diarrhoea virus (PEDV) and negative for transmissible gastroenteritis virus (TGEV) using a commercial RT-qPCR assay that can detect both of these coronaviruses. However, further analyses, using other TGEV- and PEDV-specific RT-qPCR assays, provided results inconsistent with infection by either of these viruses. Sequencing of an amplicon (ca. 1.6 kb), generated by an RT-PCR specific for the PEDV S-gene, indicated a very close similarity (ca. 99% identity) to recently described chimeric viruses termed swine enteric coronaviruses (SeCoVs). These viruses (with an RNA genome of ca. 28 kb) were first identified in Italy in samples from 2009 but have not been detected there since 2012. A closely related virus was detected in archived samples in Germany from 2012, but has not been detected subsequently. Building on the initial sequence data, further amplicons were generated and over 9 kb of sequence corresponding to the 3′-terminus of the new SeCoV genome was determined. Sequence comparisons showed that the three known SeCoVs are ≥98% identical across this region and contain the S-gene and 3a sequences from PEDV within a backbone of TGEV, but the viruses are clearly distinct from each other. It is demonstrated, for the first time, that pigs from within the SeCoV-infected herd seroconverted against PEDV but tested negative in a TGEV-specific ELISA that detects antibodies against the S protein. These results indicate that SeCoV is continuing to circulate in Europe and suggest it can cause a disease that is very similar to PED. Specific detection of the chimeric SeCoVs either requires development of a new diagnostic RT-qPCR assay or the combined use of assays targeting the PEDV S-gene and another part of the TGEV genome.

Introduction

Coronaviruses have a large, positive-sense, RNA genome of about 28 kb. Full-length and multiple subgenomic mRNAs are expressed within infected cells, and these encode the structural and non-structural proteins of the virus (see Brian and Baric, 2005). The virus particles include the spike (S) protein, the envelope (E) protein, the membrane (M) protein and the nucleocapsid (N) protein. The spike protein is exposed on the virus surface and gives the virus particles their characteristic morphology, and it is also important for inducing neutralizing antibodies (Song and Park, 2012).

Several different porcine coronaviruses have been characterized including transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhoea virus (PEDV). These viruses each replicate within the intestine and cause clinical disease, including diarrhoea and vomiting, that can lead to severe dehydration in young piglets and consequently high levels of mortality (Stevenson et al., 2013).

Infections by PEDV were first recognized in Europe in the 1970s, and then, the virus spread widely within Europe and to Asia (Jung and Saif, 2015; Lee, 2015). The incidence of the disease declined in Europe in the late 1980s and 1990s but persisted within Asia. The occurrence of disease attributed to TGEV infection within Europe also declined; this was coincident with the spread of another porcine coronavirus, porcine respiratory coronavirus (PRCV), which is closely related to TGEV (but does not cause apparent disease) and acted like a vaccine against it (Saif et al., 2012).

New strains of PEDV appeared in Asia from 2010 onwards, and in 2013, for the first time, PEDV infections occurred in the USA and spread to other countries in North, Central and Southern America. About 7 million piglets died as a result of the PEDV infections in the USA in a single year (Jung and Saif, 2015). Infections by two distinct genogroups of PEDV (termed 1b and 2b, based on the S-gene sequences) have occurred in the USA, while the initial outbreaks of disease in Europe were caused by genogroup 1a (the CV777 strain is the prototype). It seems that the genogroup 1b viruses, also referred to as 'INDEL' strains, are less virulent than the genogroup 2b viruses (Wang et al., 2014), but the relationship between virus genotype and clinical disease is still not fully understood and probably dependent on a number of factors (EFSA, 2014). Recently, new cases of PEDV infection have occurred in multiple European countries including Germany, Italy, France, Portugal, Austria and Slovenia (see Grasland et al., 2015; Hanke et al., 2015; Mesquita et al., 2015; Steinrigl et al., 2015; Boniotti et al., 2016; Toplak et al., 2016;). These new cases have all been caused by viruses within genogroup 1b. It should be noted that no vaccination against PEDV is used within Europe and thus no vaccine strains of PEDV are in circulation.

The re-emergence of PEDV infections in Europe (since 2014) has encouraged the search for these viruses in archived faecal and intestinal samples collected previously from pigs. From such analyses, Boniotti et al. (2016) identified a novel chimeric (recombinant) porcine coronavirus that was termed a swine enteric coronavirus (SeCoV), which circulated in Italy from 2009 until 2012 and then apparently disappeared. Most of the genome of this virus is derived from TGEV, but the S-gene, encoding the spike protein, is derived from PEDV. Similarly, in Germany, stored faecal samples from herds showing typical symptoms of PEDV infection were also analysed and a sample from 2012 was shown to contain coronavirus RNA with a genomic sequence that is 99.5% identical to the chimeric SeCoV described from Italy (Akimkin et al., 2016). The recombination junction on the 3′-side of the S-gene appears to be slightly different in these two virus strains, but the upstream junction is identical. No isolation of the SeCoV has been reported, so far, and no serological analysis has been performed on the infected herds in Italy and Germany as the SeCoV has been detected retrospectively. As both the S protein and the N protein are immunogenic, then it is clear that serological assays for the presence of antibodies to PEDV and TGEV could give apparently discordant results if an infection by SeCoV is present. We describe here the characterization of a new chimeric SeCoV identified in samples from clinically diseased pigs in Central Eastern Europe and have, for the first time, performed a serological analysis of a SeCoV-infected herd.

Material and Methods

Detection of viral RNA by RT-qPCR and sequence determination

The presence of viral RNA in samples was determined by RT-qPCR. Briefly, RNA was extracted from faecal samples using a ZR soil/faecal RNA MicroPrep kit (Zymo Research, Irvine, CA, USA). Samples were initially analysed using a multiplexed PEDV/TGEV virotype RT-qPCR assay (Qiagen, Hilden, Germany) that separately detects the S-gene of PEDV and TGEV. Subsequently, two samples (42845 and 42848), that scored positive in the PEDV assay, were tested using individual assays specific for the PEDV N-gene (as described by Lohse et al., 2016), the TGEV/PRCV N-gene and the TGEV S-gene (Kim et al., 2007) using a one-step RT-PCR kit (Qiagen). The assays were run for 40 cycles.

In conventional RT-PCRs (initially targeting the S-gene and then adjacent sequences), primers (see Table 1) were used to produce amplicons that were gel-purified and sequenced with a BigDye Terminator v. 3.1 Cycle Sequencing Kit and an ABI PRISM 3500 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences from overlapping amplicons were assembled using Seq Man Pro and analysed using Geneious® 6.1.7 and MUSCLE (using http://www.ebi.ac.uk/Tools). Alignments generated were edited manually.

Detection of anti-coronavirus antibodies in field sera

An 'in-house' blocking ELISA, using PEDV-infected cell harvest as antigen, as described previously (Lohse et al., 2016), and a commercial PEDV N protein-specific indirect ELISA (Biovet, Quebec, Canada) were used to detect anti-PEDV antibodies in pig sera. Antibodies to TGEV and PRCV were detected using a commercial blocking ELISA (Svanovir TGEV/PRCV-Ab assay; Svanova, Uppsala, Sweden).

Results and Discussion

Very recently, in late 2015, outbreaks of PED have been described in Slovenia (Toplak et al., 2016). In February 2016, a large pig herd (30–130 kg pigs), also within Central

Table 1. Primers used for the production and sequencing of amplicons by RT-PCR from SeCoV/CEE/42845/2016

Forward primer	Reverse primer	Primer Sequence (5′-3′)	Location of 5′ nt[a]	Amplicon size (bp)
14-PPN 32		CCAAAAGTATGGTCTCGAGGATTATGG	19077	1020
	14-P PN 33	CCCATCTATTGCTTTGTCACAG	20093	
14-PPN 12		CATCATCATCAGAAGGGTTTCTGATTGG	20045	1220
	14-PPN1	GCACGCTCAGCAGCAGCTCC	21269	
13-DPN 5		GTCTTTTGGCCATTCCTAAGA	21190	1600
	13-DPN 2	CTGGGTGAGTAATTGTTTACAACG	22794	
13-DPN 3		GGCCAAGTCAAGATTGCACC	22650	1060
	14-PPN21	CCATAACGCTGAGATTGCGATTTGACGC	23707	
14-PPN 20		CCCTCGCTAAGTATACTGAGGTTCAGGC	23644	1430
	14-PPN18	CGGTGCAGCTCTGCCATGTACAAACATG	25073	
14-PPN 17		TATTGATGGCGTTGTTACAACAACTGTC	24962	840
	14-PPN 16	ATCAATATTATAGACCAGCTGAAGTTCC	25806	
14-PPN 15		CATGTCGCAATAGCACAGCGTCTGATTG	25731	800
	14-PPN 14	ACATAAGTTCCAAAATTTTGCACCTTGC	26527	
14-PPN 13		CCGTGGTCGGAAGAGTAATAATATACC	26515	750
	TGE F2	ACATTCAGCCAGTTGTGGGTAA	27265	
TGE F1		GCAGGTAAAGGTGATGTGACAA	27167	940
	VT27	TTTTTTTTTTTTTTTTTTTTTTTTTTTV	28110	

[a]nt locations based on sequence of SeCoV/Italy/213306/2009 (Boniotti et al., 2016).

Eastern Europe (CEE), experienced severe clinical disease in all age groups, including diarrhoea and vomiting with poor growth performance and about 10% mortality; hence, the occurrence of PEDV infection was suspected. Indeed, initial testing of the faecal samples from the diseased pigs using a commercial PEDV/TGEV RT-qPCR assay (targeting the S-genes) identified two samples (42845 ad 42848) as PEDV positive but TGEV negative (see Table 2). As part of a process to characterize further the presumed PEDV, the extracted RNA from these two samples was then analysed using a collection of other RT-qPCR assays (see Table 2). Unexpectedly, using an assay specific for the N-gene of PEDV (that recognizes genogroups 1 and 2, as described by Lohse et al., 2016), the samples were scored as negative for PEDV. In contrast, in another assay that detects both TGEV and PRCV (targeted at the N-gene, Kim et al., 2007), the samples scored positive but they scored negative in a TGEV-specific assay (targeted at the TGEV S-gene, Kim et al., 2007). Thus, the results (Table 2) were not consistent with the presence of either PEDV or TGEV and therefore did not provide an explanation for the clinical disease.

Table 2. Detection of coronavirus RNA by RT-qPCR assays

Sample	PEDV RT-qPCR (N-gene)	PRCV/TGEV RT-qPCR (N-gene)	TGEV RT-qPCR (S-gene)	Qiagen RT-qPCR PEDV (S-gene)	Qiagen RT-qPCR TGEV (S-gene)
42845	No Ct	13.9	No Ct	15.5	No Ct
42848	No Ct	25.2	No Ct	26.9	No Ct
PEDV	27.7[a]	–	–	29.1[c]	–
PEDV US	31.4[b]	–	–	–	–
TGEV	–	32.9	26.9	–	27.0[c]
PRCV	–	26.3	No Ct	–	–

–, Not tested.
[a]PEDV (Br1/87) Genogroup 1a.
[b]PEDV (US) Genogroup 2a.
[c]Internal controls provided with assay kit.

In a conventional RT-PCR, using primers that are specific for the PEDV S-gene (see Table 1), an amplicon was produced (ca. 1600 bp) from sample 42845 (which had the lowest Ct for PEDV in the S-gene-specific assay) and then sequenced. The sequence generated was found (using BLAST) to be most closely related to the S-gene from the recently described SeCoV (SeCoV/Italy/213306/2009, Acc. No. KR061459, see Boniotti et al., 2016). Using additional primers (Table 1), designed from known porcine coronavirus sequences, in further RT-PCRs, some nine, overlapping, amplicons were produced. Using Sanger sequencing, a total of ca. 9050 nt of contiguous sequence, upstream from the poly(A) tail, was determined (GenBank, Acc. No. KX689261). Using BLAST, the consensus sequence of this 'contig' proved to be very closely related (99% identical, across 9016 nt) to the equivalent portion of the sequence of the chimeric SeCoV from Italy (Boniotti et al., 2016) and 98% identical (across 8957 nt) to the closely related, but distinct, virus from Germany (Akimkin et al., 2016; Acc. No. LT545990). Thus, on this basis, a third SeCoV has been identified, from CEE, and is termed here the SeCoV/CEE/42845/2016 (but abbreviated to SeCoV/CEE/16 subsequently). For comparison, this SeCoV/CEE/16 sequence was 92% identical (across the

4234 nt including the S-gene and putative 3a coding sequence) to the corresponding region of the prototypic CV777 strain of PEDV (genogroup 1a, Acc. No. AF353511) and 91% identical in the same region to genogroup 1b PED viruses (e.g. OH851, Acc. No. KJ399978 that is 99.5% identical to recent European strains of PEDV, see Hanke et al., 2015). This partial SeCoV/CEE/16 sequence contains a region that is 96% identical (across some 3551nt) to the TGEV TS strain (Acc. No. DQ201447), but this match is to the region downstream of the S and 3a sequences but includes the M- and N-genes (see Fig. 1a). The level of sequence identity within the S-gene to PEDV is clearly high (≥91%), but the sequences are significantly diverged from both the genogroup 1a and genogroup 1b sequences known to have been in Europe, and thus, the actual parental PEDV responsible for donating the S-gene portion of the SeCoVs is not yet apparent. Furthermore, there are numerous sequence differences between the three different SeCoVs (Fig. 1b). The sequence data (see Figures S1 and S2) showed that the recombination junctions in the SeCoV/CEE/16 sequence match those reported for the SeCoV/Italy/213306/2009 by Boniotti et al. (2016). The recombination event has resulted in the presence of the complete S-gene and a short putative 3a coding sequence mainly derived from the PEDV sequence within a TGEV backbone (it should be noted, however, that PEDV does not encode a separate 3a protein but does have a functional ORF 3). In all three SeCoV sequences known, the 5′-recombination junction, upstream of the S-gene, appears to be the same [note the high level of sequence identity between TGEV

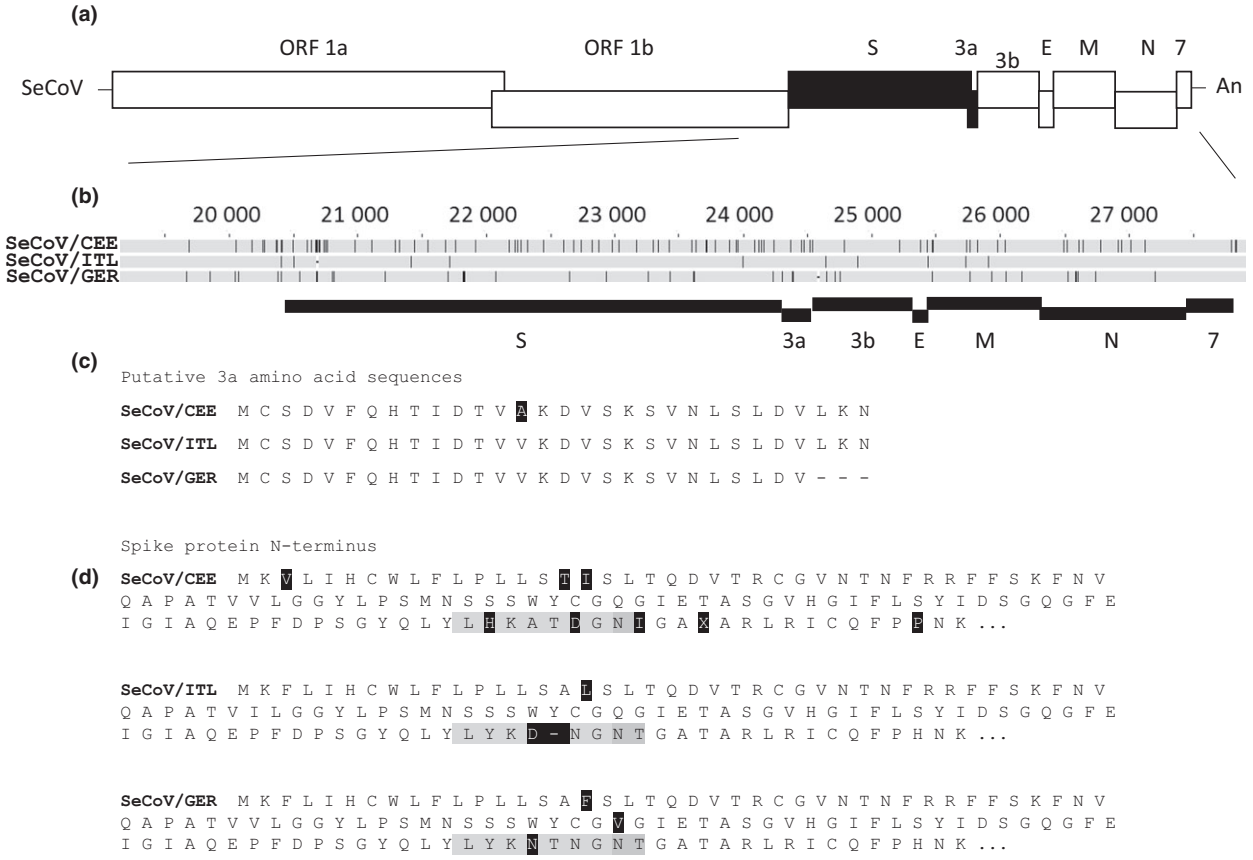

Fig. 1. Genome organization of SeCoV and sequence differences between strains. Panel (a). Representation of the genome organization of SeCoVs. The complete polyadenylated genome sequences of the SeCoV/ITL and SeCoV/GER are known (Akimkin et al., 2016; Boniotti et al., 2016) and about 9 kb from the 3′-terminus of the SeCoV/CEE genome has been determined. This region includes the coding regions for the S (spike) protein from PEDV (indicated in black) and the TGEV E (envelope), M (membrane) and N (nucleoprotein) ORFs indicated by open rectangles. Panel (b). Sequence differences within this 9 kb fragment between the three SeCoVs are indicated by black lines. The three sequences are >98% identical throughout this region. Panel (c). A short putative 3a protein coding region is present in each SeCoV, but the ORF is truncated by 9 nt in the SeCoV/GER sequence; this results in the predicted loss of the C-terminal 3 amino acids. Panel (d). A deletion of 3 nt within the spike protein coding region of the SeCoV/ITL modifies the N-terminal region of that protein. Individual amino acid differences between the SeCoV proteins are highlighted (in panels c and d) in black, while a short variable region of the S protein (panel d) that lacks a single amino acid in the SeCoV/ITL sequence is highlighted in grey.

and the SeCoV sequences upstream of the S protein initiation codon (Figure S1B) and the high sequence similarity between PEDV and SeCoV sequence downstream of this point (Figure S1A)]. In contrast, the sequences of the SeCoVs differ around the location of the 3′-recombination junction, downstream of the S-gene. The sequences of the SeCoV/ITL and the SeCoV/CEE are identical in this region. However, in the German strain (SeCoV/GER/L00930/2012), there is an absence of 10 nt relative to the SeCoV/ITL and SeCoV/CEE strains around this point. The lack of 9 nt truncates the coding region (only 90 nt in length) for a putative SeCoV 3a protein by three codons and, if translated, would result in the loss of the C-terminal amino acid residues, LKN (Fig. 1c). A single nt deletion is located downstream of the 3a termination codon (see Figure S2B). The putative SeCoV 3a sequence contains part of the PEDV ORF 3 (Figure S2A) but has the termination codon (and in the case of the SeCoV/ITL and SeCoV/CEE sequences, some coding sequence) derived from TGEV ORF 3a (see Figure S2B). The function of the coronavirus 3a protein is not well defined and is highly variable among different coronaviruses; thus, the significance of the differences in the ORF3a is not yet clear. Each of the three SeCoVs contains an open reading frame (only 81–90 nt) that could encode for a short 3a protein, whereas this sequence within PEDV (which is highly similar to that within the SeCoVs, see Figure S2A) is part of the larger ORF3. It is currently unknown whether this sequence within SeCoV is translated and detailed analysis of the expression of the various SeCoV subgenomic mRNAs and the encoded proteins will probably require the isolation of a SeCoV and characterization in cell culture. In TGEV, the 3a ORF is 219 nt in length and is extended at both termini compared to the 3a ORF in the SeCoVs (Figure S2B). The exact 3′-recombination junction cannot be determined, but it is clearly very close to the termination codon of the SeCoV 3a ORF. It is apparent that upstream of this point, there is a very close sequence similarity between the SeCoV sequences and PEDV (see Figure S2A), whereas downstream of this point, the SeCOVs are much closer to the TGEV (see Figure S2B). The TGEV 3b protein (the homologue of the PEDV ORF 3 protein) should be expressed by each of the SeCoVs.

A deletion of 3 nt (one codon) was observed in the S-gene of the Italian strain relative to both the German and CEE SeCoV strains (Fig. 1d). This resulted in the spike protein amino acid sequence including -LYKDNGNT- in the Italian sequence, while it is -LYKNTNGNT- in the German strain and -LHKATDGNI- in the CEE strain; this is a variable region within the S1 domain close to the N-terminus of the PEDV spike protein. In addition, there are many single nucleotide differences between these strains of SeCoV strain (see Fig. 1b) and thus these viruses are clearly distinct. It is currently unknown whether the different SeCoVs have been generated independently or whether they have been produced from a single recombination event and then diverged. It is noteworthy that the S-gene sequence in each SeCoV is very closely related (>98% identity) in each case but significantly different (only 91–92% identity) from the S-gene of known PEDVs. It should be noted that detection of the SeCoV RNA was achieved in six of 11 faecal/intestinal samples tested from this herd early on the outbreak.

Sera were collected in March 2016 from 39 pigs from the herd experiencing the outbreak of diarrhoea (Farm 1). The herd had been suspected of being infected with PEDV but was identified here as being infected with a chimeric SeCoV (see above). The sera were analysed for the presence of antibodies to PEDV, TGEV and PRCV. In total, 14 of these 39 sera were clearly positive (>40% blocking) for anti-PEDV antibodies in the 'in-house' ELISA while five gave inconclusive results (>35% but <40% blocking) and the rest were negative (Table 3). Furthermore, 33 of these sera were positive for antibodies to PRCV but none of these sera scored positive in the commercial TGEV- specific ELISA assays. From a second farm (Farm 2), within the same area but without clinical disease, 36 of the 40 sera tested were positive for anti-PRCV antibodies (plus two inconclusive results) but were each negative in the TGEV- and PEDV-specific assays. On Farm 3, also in the same area and without clinical disease, just two sera (from 40 tested) scored

Table 3. Serological status of pigs sampled in Central Eastern Europe in 2016

Farm	Sample collection	PED-like disease	Anti-PEDV prevalence	Anti-TGEV prevalence	Anti-PRCV prevalence
1	18/03/16	Yes[a]	36% (plus 13% incon[b])	0%	85%
1	12/04/16	Yes[a]	40% (plus 10% incon[b])[c]	N.D.	N.D.
2	18/03/16	No	0%	0%	90% (plus 5% incon[b])
3	18/03/16	No	0%	0%	5%

N.D., not determined.

[a]faecal samples tested positive for PEDV in TGEV/PEDV RT-qPCR assay.

[b]incon is inconclusive (In the PEDV assay, >40% block is positive, <35% block is negative, and intermediate values are defined as inconclusive while in the TGEV/PRCV assays, >60% block is positive, <45% block is negative, and intermediate values are defined as inconclusive).

[c]one of 20 sera tested positive in the Biovet ELISA that detects anti-PEDV N antibodies.

positive in the PRCV assay and all 40 were negative in the assays for antibodies against TGEV and PEDV (see Table 3). Almost 1 month later, a further 20 sera were collected from Farm 1 and tested in the same assays. A similar prevalence of anti-PEDV antibodies (40% positive) was observed, as seen initially (Table 3). Using the Biovet ELISA (that detects antibodies to the PEDV N protein), just one sample of these 20 sera tested positive, and this is a similar frequency as the detection rate in this test from uninfected animals (Strandbygaard et al., in preparation). Analysis of faecal samples collected at this time showed three positive samples of 10 tested, judged by the combined results of TGEV/PRCV and PEDV RT-qPCRs (data not shown).

The three different SeCoVs described so far are distinct but each have the PEDV S-gene (encoding the spike protein) together with adjacent sequences potentially encoding a short 3a protein (<30 amino acids) recombined into a TGEV genome. The spike protein is important for the immunogenicity of PEDV (Song and Park, 2012). Many sera from the farm in CEE with SeCoV-infected pigs scored positive in tests for antibodies to PEDV (Table 3), although not with the Biovet assay as this only detects antibodies to the PEDV N protein. Furthermore, these sera were scored negative in assays for TGEV antibodies as these tests determine antibodies specifically against the TGEV spike protein. Many of the animals were also seropositive against PRCV, a widely spread respiratory virus, that does not induce diarrhoea, and indeed has acted like a vaccine against TGEV (Saif et al., 2012). As many pig samples scored positive for anti-PRCV antibodies in pigs from Farm 2 but all pigs scored negative for anti-PEDV antibodies, it is clear that the positive PEDV reaction in pigs from Farm 1 is not due to a cross-reaction to PRCV in the PEDV ELISA. Thus, it is apparent that the presence of antibodies in pigs against PRCV does not prevent SeCoV infection.

Conclusions

The results presented here clearly shows the presence of a chimeric SeCoV in pigs on a farm in Central Eastern Europe, during early 2016, where marked clinical signs suggestive of PEDV infection, including diarrhoea and vomiting, were apparent. This recombinant virus is closely related to, but distinct from, the chimeric SeCoV viruses previously identified in samples from Italy (within 2009–2012) and Germany (2012) by Boniotti et al. (2016) and Akimkin et al. (2016) respectively. It is demonstrated, for the first time, that pigs that were infected with the SeCoV/CEE/16 seroconverted against PEDV (when tested using an appropriate test) but they do not seroconvert against TGEV, as determined using a spike protein-specific assay, consistent with the chimeric structure of the recombinant SeCoV. Clearly, the specific target sequences used in diagnostic RT-qPCR assays determine the recognition of the chimeric SeCoV in such assays. It is necessary to use either a PEDV S-gene-specific assay in conjunction with an assay for some other region of the TGEV genome (e.g. the N-gene) to identify the SeCoV RNA (see Table 2) or to develop a new SeCoV RT-qPCR-specific assay. Currently, SeCoV has not yet been isolated and it will be necessary to achieve this to prove that this virus alone is sufficient to induce the clinical disease that has been observed in pigs.

Acknowledgements

We are grateful for the excellent technical assistance from Jane Borch, Carina Becke, Helle Rasmussen and Pernille Manley Hansen. The work was funded from internal resources of the DTU National Veterinary Institute.

References

Akimkin, V., M. Beer, S. Blome, D. Hanke, D. Höper, M. Jenckel, and A. Pohlmann, 2016: New chimeric porcine coronavirus in swine feces, Germany, 2012. *Emerg. Infect. Dis.* 22, 1314–1315.

Boniotti, M. B., A. Papetti, A. Lavazza, G. Alborali, E. Sozzi, C. Chiapponi, S. Faccini, P. Bonilauri, P. Cordioli, and D. Marthaler, 2016: Porcine epidemic diarrhea virus and discovery of a recombinant swine enteric coronavirus, Italy. *Emerg. Infect. Dis.* 22, 83–87.

Brian, D. A., and R. S. Baric, 2005: Coronavirus genome structure and replication. *Curr. Top. Microbiol. Immunol.* 287, 1–30.

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2014: Scientific Opinion on porcine epidemic diarrhoea and emerging pig deltacoronavirus. *EFSA J.* 12, 3877.

Grasland, B., L. Bigault, C. Bernard, H. Quenault, O. Toulouse, C. Fablet, N. Rose, F. Touzain, and Y. Blanchard, 2015: Complete genome sequence of a porcine epidemic diarrhea S gene indel strain isolated in France in December 2014. *Genome Announc.* 3,e00535–15.

Hanke, D., M. Jenckel, A. Petrov, M. Ritzmann, J. Stadler, V. Akimkin, S. Blome, A. Pohlmann, H. Schirrmeier, M. Beer, and D. Höper, 2015: Comparison of porcine epidemic diarrhea viruses from Germany and the United States, 2014. *Emerg. Infect. Dis.* 21, 493–496.

Jung, K., and L. J. Saif, 2015: Porcine epidemic diarrhea virus infection: etiology, epidemiology, pathogenesis and immunoprophylaxis. *Vet. J.* 204, 134–143.

Kim, S. H., I. J. Kim, H. M. Pyo, D. S. Tark, J. Y. Song, and B. H. Hyun, 2007: Multiplex real-time RT-PCR for the simultaneous detection and quantification of transmissible gastroenteritis virus and porcine epidemic diarrhea virus. *J. Virol. Methods* 146, 172–177.

Lee, C., 2015: Porcine epidemic diarrhea virus: an emerging and re-emerging epizootic swine virus. *Virol. J.* 12, 193.

Lohse, L., J. S. Krog, B. Strandbygaard, T. B. Rasmussen, J. Kjær, G. J. Belsham, and A. Bøtner, 2016: Experimental infection of young pigs with an early European strain of porcine epidemic diarrhoea virus and a recent US strain. *Transbound. Emerg. Dis.* [Epub ahead of print] doi: 10.1111/tbed.12509

Mesquita, J. R., R. Hakze-van der Honing, A. Almeida, M. Lourenço, W. H. van der Poel, and M. S. Nascimento, 2015: Outbreak of porcine epidemic diarrhea virus in Portugal, 2015. *Transbound. Emerg. Dis.* 62, 586–588.

Saif, L. J., M. P. Pensaert, K. Sestak, S. G. Yeo, and K. Jung, 2012: Coronaviruses. In: Zimmerman, J. J., L. A. Karriker, A. Ramirez, K. J. Schwartz, and G. W. Stevenson (eds), Diseases of Swine (Tenth Ed.), pp. 501–524. Wiley-Blackwell, Iowa State University, IA, USA.

Song, D., and B. Park, 2012: Porcine epidemic diarrhoea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines. *Virus Genes* 44, 167–175.

Steinrigl, A., S. R. Fernández, F. Stoiber, J. Pikalo, T. Sattler, and F. Schmoll, 2015: First detection, clinical presentation and phylogenetic characterization of porcine epidemic diarrhea virus in Austria. *BMC Vet. Res.* 11, 310.

Stevenson, G. W., H. Hoang, K. J. Schwartz, E. R. Burrough, D. Sun, D. Madson, V. L. Cooper, A. Pillatzki, P. Gauger, B. J. Schmitt, L. G. Koster, M. L. Killian, and K. J. Yoon, 2013: Emergence of porcine epidemic diarrhea virus in the United States: clinical signs, lesions, and viral genomic sequences. *J. Vet. Diagn. Invest.* 25, 649–654.

Toplak, I., M. Ipavec, U. Kuhar, D. Kušar, B. Papić, S. Koren, and N. Toplak, 2016: Complete genome sequence of the porcine epidemic diarrhea virus strain SLO/JH-11/2015. *Genome Announc.* 4,e01725–15.

Wang, L., B. Byrum, and Y. Zhang, 2014: New variant of porcine epidemic diarrhea virus, United States, 2014. *Emerg. Infect. Dis.* 20, 917–919.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Localisation of the SeCoV 5′-recombination site.

Figure S2. Localisation of the SeCoV 3′ recombination site.