Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Partial sequence of the spike glycoprotein gene of transmissible gastroenteritis viruses isolated in Korea

Soo Joung Kim, Jeong Hee Han, Hyuk Moo Kwon*

Department of Veterinary Medicine, Kangwon National University, Chunchon 200-701, Republic of Korea

Received 22 November 2002; received in revised form 20 March 2003; accepted 20 March 2003

Abstract

The spike (S) glycoprotein of transmissible gastroenteritis virus (TGEV) is the predominant inducer of neutralizing antibodies and has been implicated in virulence and host cell tropism. In this study, the nucleotide and deduced amino acid sequences of the amino terminal half of the S glycoprotein gene of one Korean field TGEV strain (133) isolated in 1997 and three Korean field TGEV strains (KT2, KT3 and KT4) isolated in 2000 and HKT2 strain, KT2 passaged 104 times in ST cells, were determined. The amino terminal half of the S glycoprotein gene including antigenic sites A, B, C and D, were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR). Amplified PCR products were cloned, sequenced, and compared with published sequences for non-Korean TGEV strains. Korea TGEV field strains had 98.5–99.5% nucleotide sequence and 97.2–99.0% amino acid sequence similarity with each other. They had 96.5–99.0% nucleotide sequence similarity and 94.9–97.6% amino acid sequence similarity compared to non-Korean TGEV strains. Korean TGEV strains had several specific nucleotide and amino acid sequences which were not found in foreign TGEV or PRCV strains. HKT2 strain differed by 0.89% in nucleotide and 2.03% amino acid sequences compared to original KT2 strain although the regions forming four antigenic sites were not changed. By phylogenetic tree analysis, Korean field TGEV strains were branched into different groups from non-Korean TGEV or PRCV strains. Korean TGEV field strains KT2 and 133 were branched in separate groups that were differentiated from the other Korean TGEV strains. The Korean TGEV strains seemed to be evolved from a separate lineage of TGEV strain.

© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Transmissible gastroenteritis virus; Spike protein; Sequence; Serial passages; Korea

* Corresponding author. Tel.: +82-33-250-8652; fax: +82-33-244-2367.
E-mail address: kwonhm@kangwon.ac.kr (H.M. Kwon).

0378-1135/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0378-1135(03)00101-9
1. Introduction

Transmissible gastroenteritis virus (TGEV) is the etiological agent of transmissible gastroenteritis (TGE), which is a highly contagious enteric disease in piglets causing vomiting, severe yellowish diarrhea, weight loss, dehydration and high mortality and resulting in severe economical losses to the affected farms (Saif and Wesley, 1999).

TGEV belongs to the family Coronaviridae. It is a pleomorphic enveloped virus that contains a large positive-sense, single-stranded RNA genome (Siddell et al., 1983). TGEV has three major structural proteins which are spike (S) glycoprotein, membrane (M) glycoprotein and nucleocapsid (N) protein. The S glycoprotein forms the peplomers on the virion envelope, induces neutralizing antibodies, contains receptor binding regions, and contains four major antigenic sites (A, B, C and D) (Correa et al., 1988; Delmas et al., 1986; Gebauer et al., 1991). The sites A and D are known to be regions inducing major neutralizing antibodies (Correa et al., 1988; Gebauer et al., 1991). The M glycoprotein is mostly embedded in the lipid virion envelope and the N phosphoprotein is associated with the genomic RNA to form the nucleocapsid (Kapke and Brian, 1986; Laude et al., 1987).

Porcine respiratory coronavirus (PRCV) was first reported in Europe (Pensaert et al., 1986; Callebaut et al., 1988) and has been reported in US (Wesley et al., 1990b; Vaughn et al., 1994). By sequence data, PRCV has a large deletion in the 5′-region of the S glycoprotein gene which may be related to the differences observed in tissue tropism or pathogenicity between PRCV and TGEV strains (Rasschaert et al., 1990; Wesley et al., 1990c; Britton et al., 1991).

The sequence variation in the S glycoprotein gene and antigenic diversity of TGEV isolates have been reported (Hohdatsu et al., 1987; Sanchez et al., 1992; Vaughn and Paul, 1993; Paton and Lowings, 1997). Based on partial sequence analysis of S glycoprotein gene, TGEV strains isolated in Korea were different from foreign TGEV isolates (Kwon et al., 1998).

The purpose of the present study was to genetically characterize TGEV strains (KT2, KT3, KT4 and 133 strains) isolated in Korea and compare them to a highly passaged TGEV KT2 strain (HKT2) as well as other non-Korean published TGEV and PRCV sequences. The 5′-end of the S glycoprotein genes including antigenic sites A, B, C and D of the Korean TGEV isolates were amplified by RT-PCR, cloned, and sequenced. Comparisons with the sequences of other published TGEVs showed that the nucleotide and amino acid sequences of Korean TGEV isolates differed by 1.0–3.5 and 2.4–5.1%, respectively, from non-Korean TGEV strains in the amino terminal half of the S glycoprotein gene.

2. Materials and methods

2.1. Viruses, cell culture and viral passages

The Korean TGEV field strain 133 and three other TGEV field strains (KT2, KT3 and KT4) were isolated from small intestines of TGE suspected piglets using swine testicular (ST) cells in 1997 and 2000, respectively. The ST cells were grown in Dulbecco’s modified...
Eagle medium (DMEM, GIBCOBRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, GIBCOBRL) and maintained in maintenance medium (MM) which was DMEM supplemented with 2% FBS at 37 °C in a 5% CO₂ incubator.

The Korean TGEV KT2 field strain was passaged serially in ST cells. The KT2 strain (HKT2) passaged 104 times on ST cells was analyzed in this study.

2.2. Preparation of RNAs

Virus-infected ST cells showing cytopathic effects were frozen, thawed and cell debris pelleted by centrifugation. The clarified cell culture supernatant was collected and used for preparation of viral RNA. Viral RNAs were extracted and purified as described previously (Kwon et al., 1993). Briefly, sodium dodecyl sulfate (final concentration 2% w/v) and proteinase K (final concentration 250 µg/ml) were added to clarified cell culture supernatant, incubated for 5 min at 55 °C, extracted with acid phenol (pH 4.0) and chloroform/isoamyl alcohol (49:1), and further purified using the RNaid kit (BIO101, La Jolla, CA, USA). Finally, the RNA was resuspended in diethyl-pyrocarbonate (DEP) treated water and stored at −70 °C until used in the reverse transcriptase reaction.

2.3. Oligonucleotide primers

The amino-terminal half of the S gene, including antigenic sites A, B, C and D of TGEV, was amplified with forward 2 (5' - AAGGAAGGTAAGTTGCTCA-3', −71 to −59)/reverse 3 (5'-GGTCCATCAGTTACGCCGAA-3', 1159–1184) primer pair (1255 bp PCR product) and forward 4 (5'-AAGGAAGGTAAGTTGCTCA-3', 1064–1183)/reverse 6 (5'-GGTCCATCAGTTGCGCCGAA-3', 2344–2363) primer pair (1300 bp PCR product), which were partially overlapped (Rasschaert and Laude, 1987).

2.4. Reverse transcriptase-polymerase chain reaction (RT-PCR)

The RT reaction to synthesize cDNA contained 3 µg random primers (cat. no. 48190-011, GIBCOBRL), 10 mM dNTPs (Promega, Madison, WI, USA) and purified RNA. The mixture was heated at 65 °C for 5 min. The 5× first-strand buffer (250 mM Tris–HCl (pH 8.3 at room temperature), 375 mM KCl, 15 mM MgCl₂), 0.2 M DTT (GIBCOBRL) and 20–40 units RNase inhibitor (Promega) were added to the mixture and incubated at 25 °C for 10 min and 42 °C for 2 min. Two hundred units Superscript II reverse transcriptase (GIBCOBRL) was added, heated at 42 °C for 50 min then at 70 °C for 15 min. One unit ribonuclease H (GIBCOBRL) was added, heated at 37 °C for 20 min and 80 °C for 10 min.

The PCR reaction mixture to amplify cDNA contained 25 µl PCR premix (1.25 unit/25 ul TaKaRa Ex Taq polymerase, 0.4 mM dNTPs, Ex TaqTM buffer including 4 mM Mg²⁺) (TaKaRa, Shiga, Japan), forward and reverse primers and cDNA. A 50 µl total reaction volume was obtained by adding distilled water. The PCR was performed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 51 °C for 90 s, and polymerization at 74 °C for 90 s. The initial denaturation and polymerization steps were at 94 °C for 5 min and 74 °C for 6 min, respectively. The final annealing and polymerization steps were at 51 °C for 2 min and 74 °C for 10 min, respectively.
2.5. Cloning and DNA sequencing

The PCR products amplified with each primer pair were purified using GENECLEAN Turbo kit (BIO101, La Jolla, CA, USA) according to the manufacturer’s recommendation. The purified DNA was ligated into the pCR2.1-TOPO (Invitrogen, Calsbad, CA, USA) cloning vector and transformed into competent cells (TOP10) (Invitrogen). Cells carrying recombinant plasmid were selected on LB agar plates containing kanamycin and X-gal. Plasmid DNA for sequencing was prepared by Plasmid Maxi kit (Qiagen, Santa Clarita, CA, USA). The sequences were determined using the ALFexpress automatic DNA sequencer (Pharmacia Biotech, Uppsala, Sweden). For each TGEV strain, two or three independent clones originated from different PCR products were sequenced because of the possibility of errors arising during RT, PCR, or the cloning procedure.

2.6. Sequence analysis

Nucleotide and predicted amino acid sequences were analyzed with the MegAlign software (DNAStar Inc., Madison, WI, USA). Phylogenetic tree for partial S glycoprotein was generated by the maximum parsimony method with 100 bootstrap replicates in a heuristic search with the PAUP 4.0b software program (Sinauer Associates Inc., Sunderland, MA, USA). The tree was rooted to an sequence of the TGEV KT2 isolate.

The sequence data were deposited in the GenBank database under the accession numbers AF481360 (KT2), AF481361 (KT3), AF481362 (KT4), AF481365 (133) and AF481366 (HKT2). The origin and history of sequences used for comparison in this study were described elsewhere (Britton and Page, 1990; Chen et al., 1995; Sanchez et al., 1992; Wesley, 1990a). Sequences used for comparison in this study were from the following GenBank database accession numbers: TFI83 (Z35758) (Chen et al., 1995), PUR46-MAD (M94101), NEB72 (M94099), TOY56 (M94103), HOL87 (M94097) (Sanchez et al., 1992), BRI70-FS (X53128) (Britton and Page, 1990), MIL65-AME (S51223) (Wesley, 1990a).

3. Results

The nucleotide and deduced amino acid sequences of the S glycoprotein gene including four antigenic sites (A, B, C and D) of four TGEV strains (133, KT2, KT3 and KT4) isolated in Korea and KT2 strain (HKT2) passaged 104 times on ST cells were determined and compared with the sequences of published TGEV and PRCV strains (Fig. 1).

Korean TGEV isolates had nucleotide sequence similarity between 98.5% (KT2 and 133) and 99.5% (KT3 and KT4) with each other and they had nucleotide sequence similarity between 96.5% (KT2 and TFI83) and 99.0% (133 and NEB7 and PUR46-MAD) with non-Korean TGEV strains (Table 1). Korean TGEV isolates had amino acid sequence similarity between 97.2% (KT2 and 133) and 99.0% (KT3 and KT4) with each other and they had amino acid sequence similarity between 94.9% (KT2 and TFI83) and 97.6% (133 and NEB72) with foreign TGEV strains (Table 1).

Korean TGEV strains had six and five specific nucleotide and amino acid sequence differences, respectively, compared to foreign TGEV or PRCV strains. The seven Korean
Fig. 1. The deduced amino acid sequence comparisons of the amino terminal half region of S glycoprotein gene in Korean TGEV isolates KT2, KT3, KT4 and HKT2 strains with Korea field TGEV 133 strain isolated in 1997 (Kwon et al., 1998), TF83 (Chen et al., 1995), MIL65-AME and BR170-FS (Britton and Page, 1990; Wesley, 1990a), PUR46-MAD, NEB72, TOY56 and PRCV isolate HOL87 strains (Sanchez et al., 1992). The shaded regions indicate four antigenic site amino acids. Symbol (–) indicates positions where the sequences are identical to those of KT2. Deletions within the sequences are shown with asterisks. The five Korean TGEV-specific amino acids changes were underlined.
TGEV-specific nucleotide changes were 1212 (T to C), 1234 (T or C to A), 1241 (C to T), 1321 (T to C), 1689 (C to G) and 1798 (G to C), and five amino acid changes were 412 (L to I), 414 (T to I), 441 (F to L), 563 (N to K) and 600 (D to H) (Fig. 1). Korean TGEV KT2 strain had nine and seven specific nucleotide and amino acid sequence changes, respectively, compared to other Korean TGEV isolates and non-Korean TGEV or PRCV isolates. The nine Korean TGEV KT2-specific nucleotide changes were 926 (T), 1369 (T),
Table 1
Comparison of the nucleotide and deduced amino acid sequences of the S glycoprotein gene of Korean TGEV isolates (KT2, KT3, KT4, HKT2 and 133) and non-Korean TGEV or PRCV strains\(^a\), \(^b\)

|        | KT2   | KT3   | KT4   | HKT2  | 133   | TFI83 | PUR46-MAD | NEB72 | TOY56 | BRI70-FS | MIL65-AME | HOL87 |
|--------|-------|-------|-------|-------|-------|-------|-----------|-------|-------|----------|-----------|-------|
| KT2    | ***   | 99.3  | 99.2  | 99.0  | 98.5  | 96.5  | 98.1      | 97.9  | 97.5  | 97.2     | 97.7      | 95.9  |
| KT3    | 98.6  | ***   | 99.5  | 99.3  | 98.8  | 96.7  | 98.3      | 98.1  | 97.8  | 97.4     | 97.9      | 96.3  |
| KT4    | 98.4  | 99.0  | ***   | 99.3  | 98.7  | 96.7  | 98.2      | 98.1  | 97.7  | 97.4     | 97.9      | 96.5  |
| HKT2   | 98.0  | 98.6  | 98.5  | ***   | 98.5  | 96.4  | 98.0      | 97.9  | 97.5  | 97.1     | 97.6      | 96.0  |
| 133    | 97.2  | 97.8  | 97.6  | 97.2  | ***   | 97.0  | 99.0      | 99.0  | 98.3  | 97.6     | 98.0      | 96.5  |
| TFI83  | 94.9  | 95.3  | 95.1  | 94.8  | 95.4  | ***   | 96.9      | 96.8  | 97.7  | 98.2     | 98.1      | 96.1  |
| PUR46-MAD | 96.3 | 96.7  | 96.4  | 96.2  | 97.5  | 95.7  | ***       | 99.9  | 98.5  | 97.7     | 98.1      | 96.1  |
| NEB72  | 98.1  | 96.4  | 96.2  | 95.9  | 97.6  | 95.5  | 99.6      | ***   | 98.5  | 97.6     | 98.0      | 96.0  |
| TOY56  | 95.6  | 96.1  | 95.8  | 95.6  | 96.6  | 96.3  | 97.3      | 97.2  | ***   | 98.1     | 98.4      | 96.4  |
| BRI70-FS | 95.8 | 96.2  | 95.9  | 95.7  | 96.3  | 96.7  | 96.8      | 96.7  | 97.2  | ***      | 98.9      | 97.1  |
| MIL65-AME | 95.9 | 96.3  | 96.1  | 95.8  | 96.6  | 96.8  | 97.1      | 96.9  | 97.2  | 98.2     | ***       | 97.2  |
| HOL87  | 93.6  | 94.3  | 94.5  | 93.8  | 94.5  | 95.4  | 94.9      | 94.7  | 94.9  | 96.1     | ***       | 96.1  |

\(^a\) Percentage of nucleotide similarity in upper triangle.

\(^b\) Percentage of amino acid similarity in lower triangle.
Fig. 2. Phylogenetic relationship based on amino acid sequences of the S glycoprotein of the Korean TGEV field isolates (KT2, KT3, KT4, HKT2 and 133) and non-Korean TGEV or PRCV strains generated by the maximum parsimony method with heuristic search and 100 bootstrap replicates. The tree was rooted to a sequence of the TGEV KT2 isolate. The length of each branch represents the number of amino acid changes between sequences.
1402 (C), 1405 (T), 1411 (T), 1893 (C), 1895 (A), 1930 (G) and 2364 (T), and the seven amino acid changes were 309 (L), 457 (L), 468 (Q), 469 (S), 471 (L), 632 (H) and 644 (G). The deletion of six nucleotides found between 1123 and 1128 nucleotides of TGEV PUR46-MAD, NEB72 and vaccine strains did not occur in any of the Korean TGEV isolates, including HKT2 strain.

The HKT2 strain differed by 0.89% (21/2366) in nucleotide and 2.03% (16/788) in amino acid sequences from original KT2 strain. The four antigenic sites of KT2 strain were not changed in HKT2 strain.

Phylogenetic analysis of the S glycoprotein genes of Korean TGEV isolates and non-Korean TGEV or PRCV strain is shown in Fig. 2. Four distinct branches were observed in the phylogenetic trees. Korean TGEV KT3, KT4 and HKT2 strains were branched into one group. Non-Korean TGEV or PRCV strains (TFI83, HOL87, BR170-FS, MIL65-AME, TOY56, PUR46-MAD and NEB72) were branched into another group in which NEB72 and PUR46-MAD strains formed a subgroup different from the other foreign strains. Korean TGEV KT2 and 133 strains was separated distinctly from the other two groups.

4. Discussion

The amino-terminal half of the S glycoprotein gene of recent Korean TGEV isolates were sequenced to determine the genetic diversity among Korean TGEV isolates and differences with foreign TGEV isolates.

The three TGEV strains (KT2, KT3 and KT4) isolated in 2000 had high similarity, showing 99.2–99.5% nucleotide sequence similarity and 98.4–99.0% amino acid sequence similarity among themselves. But Korean TGEV field strain 133 isolate had low similarity compared to TGEV strains isolated in 2000. TGEV strain 133 showed 98.5% (KT2) to 98.8% (KT3) nucleotide sequence similarity compared to other Korean TGEV isolates and 97.0% (TFI83) to 99.0% (NEB72 and PUR46-MAD) nucleotide sequence homology compared to non-Korean TGEV strains. In amino acid sequences, TGEV 133 strain 97.2% (KT2) to 97.8% (KT3) similarity compared to other TGEV isolates and 95.4% (TFI83) to 97.6% (NEB72) similarity. These data showed that TGEV strains differentiated from non-Korean TGEV strains in genetic composition have existed in Korea.

Korean TGEV strains have several unique characteristics. All Korean TGEV strains including the HKT2 strain had six unique nucleotides between positions 1212 and 1798 and five unique amino acid sequences between positions 412 and 600, which were not found in non-Korean TGEV or PRCV isolates. Those nucleotide or amino acid sequences may be used to differentiate Korean TGEV strains from foreign TGEV strains although more TGEV isolates need to be analyzed. Paton and Lowings (1997) reported the nucleotide sequences (250–648 nucleotides) near the amino terminus of the S glycoprotein gene of several TGEV isolates, which are regions deleted in PRCV. TGEV Erica and Slagharen (Dutch isolates) had 15 nucleotide insertions between positions 496 and 497, and TGEV V63 (Belgium isolate) had 5 nucleotide insertions compared to other TGEV isolates (Paton and Lowings, 1997). And TGEV 83-3289 (English isolate) had a deletion of six nucleotides between positions 286 and 291, which is a region known to be one of two B antigenic sites (Correa et al., 1988; Gebauer et al., 1991). The additions or deletions described above were
not observed in Korean TGEV isolates. Sanchez et al. (1992) reported that the protein domain including antigenic subsites Aa and Ab and site D (Gebauer et al., 1991) showed more amino acid changes than other areas of the S glycoprotein. Six Korean TGEV-specific amino acids were found in that domain. A higher number of amino acid changes in Korean and non-Korean TGEV strains were fixed in that antigenic domain because it is involved in the neutralization of virus infectivity.

TGEV isolates showed biologic and antigenic diversity (Vaughn and Paul, 1993). Some TGEV isolates had cytopathic effect differences in swine testis cell culture and virus neutralization differences were observed when monoclonal antibodies were used in the virus neutralization. The virus neutralization test using polyclonal antibodies revealed only one serotype among the isolates as reported by Kemeny (1976). Although the difference in amino acids forming antigenic sites were not found between Korean TGEV isolates and non-Korean TGEV strains, the virus neutralization test using monoclonal or polyclonal antibodies against TGEV need to be used to test antigenicity of Korean TGEV strains.

Differences in the virulence of TGEV strains have been reported (Furuuchi et al., 1979; Cubero et al., 1992; Kim and Chae, 2002). One Korean TGEV strain was less virulent than two American TGEV strains (Purdue and Miller) (Kim and Chae, 2002). The relationship between nucleotide and amino acid sequence differences and virulence may be revealed when several TGEV strains are tested under identical conditions.

The Korean TGEV KT2 strain was highly passaged in ST cells. The HKT2 strain, which was the KT2 strain passaged 104 times on ST cells, had several changes in nucleotide and amino acid sequences when compared to the parent TGEV KT2 strain. However, nucleotides or amino acids known to form four antigenic sites were not changed (Correa et al., 1988; Gebauer et al., 1991). The HKT2 strain had a deletion in genes 3a and 3b, which may be involved in virulence of TGEV (Woods, 1978; Wesley et al., 1990c), and it was non-pathogenic in piglets (unpublished data). Therefore, the HKT2 strain may be used for a live vaccine to prevent TGE in piglets after testing the pathogenicity and immunogenicity in piglets and sows.

In the phylogenetic tree, all Korean TGEV strains formed a different cluster from foreign TGEV or PRCV strains. Although Korean TGEV strains except for the KT2 and 133 strains formed one cluster, several sequence differences were found among Korean TGEV strains isolated in the same year. The Korean TGEV KT2 and 133 strains formed distinct clusters that were different from other Korean TGEV strains or non-Korean TGEV or PRCV strains. Recent European TGEV isolates were quite different from earlier TGEV types because they appear to originate from a different ancestor and result from accumulation of mutations but some isolates showed genetic stability over years (Paton and Lowings, 1997). It has been suggested that reemergence of similar viruses into the field, laboratory cross-contamination, point mutations or recombination could affect the appearance of various TGEV strains (Banner and Lae, 1991; Paton and Lowings, 1997; Sanchez et al., 1999). Those mechanisms may contribute to the emergence of TGEV strains with the differences in nucleotides and amino acids in Korea.

In conclusion, four Korean TGEV field strains were different from published non-Korean TGEV or PRCV strains in nucleotide and amino acid sequences and were clustered into different groups from non-Korean TGEV or PRCV strains in phylogenetic tree. And four Korean TGEV isolates were clustered into three groups according to isolation years in
phylogenetic tree. The highly passaged TGEV HKT2 strain had changes in S glycoprotein gene but did not contain a deletion in the S glycoprotein gene, which was found in PRCV. The pathogenicity and immunogenicity of the HKT2 strain are currently being tested in vivo.

Acknowledgements

The authors thank Dr. Chang-Won Lee for his excellent assistance to make phylogenetic tree and Dr. Mark W. Jackwood for editorial comments. This study was supported by Agricultural R&D Promotion Center, Korea.

References

Banner, L.R., Lae, M.M.C., 1991. Random nature of coronavirus RNA recombination in the absence of selection pressure. Virology 185, 441-445.

Britton, P., Page, K.W., 1990. Sequence of the S gene from a virulent British field isolate of transmissible gastroenteritis virus. Virus Res. 18, 71-80.

Britton, P., Mawditt, K.L., Page, K.W., 1991. The cloning and sequencing of the virion protein genes from a British isolate of porcine respiratory coronavirus, comparison with transmissible gastroenteritis virus genes. Virus Res. 21, 181-198.

Callebaut, P., Correa, I., Pensaert, M., Jimenez, G., Enjuanes, L., 1988. Antigenic differentiation between transmissible gastroenteritis virus of swine and a related porcine respiratory coronavirus. J. Gen. Virol. 69, 1725-1730.

Chen, C.-M., Cavanagh, D., Britton, P., 1995. Cloning and sequencing of a 8.4-kb region from the 3'-end of a Taiwanese virulent isolate of the coronavirus transmissible gastroenteritis virus. Virus Res. 38, 83-89.

Correa, I., Jimenez, G., Sune, C., Bulido, M.J., Enjuanes, L., 1988. Antigenic structure of the E2 glycoprotein from transmissible gastroenteritis coronavirus. Virus Res. 10, 77-94.

Cubero, M.J., Bernard, S., Leon, L., Benton, P., Contreras, A., 1992. Pathogenicity and antigen detection of the Nouzilly strain of transmissible gastroenteritis coronavirus, in 1-week-old piglets. J. Comp. Pathol. 106, 61-73.

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

Furuuchi, S., Shimizu, Y., Kamagai, T., 1979. Multiplication of low and high cell culture passaged strains of transmissible gastroenteritis virus in organs of newborn piglets. Vet. Microbiol. 3, 169-178.

Gebauer, F., Posthumus, W.P.A., Correa, I., Sune, C., Smerdou, C., Sanchez, C.M., Lenstra, J.A., Meloen, R.H., Enjuanes, L., 1991. Residues involved in the antigenic sites of transmissible gastroenteritis coronavirus S glycoprotein. Virology 183, 225-238.

Hohdatsu, T., Esugu, Y., Tsuchimoto, M., Ide, S., Yamagishi, H., Matumoto, M., 1987. Antigenic variation of porcine transmissible gastroenteritis virus detected by monoclonal antibodies. Vet. Microbiol. 14, 115-124.

Kapke, P.A., Brian, D.A., 1986. Sequence analysis of the porcine transmissible gastroenteritis coronavirus nucleocapsid protein gene. Virology 151, 41-49.

Kemeny, L.J., 1976. Antibody response in pigs inoculated with transmissible gastroenteritis virus and cross reactions among ten isolates. Can. J. Comp. Med. 40, 209-214.

Kim, B., Chae, C., 2002. Experimental infection of piglets with transmissible gastroenteritis virus: a comparison of three strains (Korean, Purdue and Miller). J. Comp. Pathol. 126, 30-37.

Kwon, H.M., Jackwood, M.W., Gell Jr., J., 1993. Differentiation of infectious bronchitis virus serotypes using the polymerase chain reaction and restriction fragment length polymorphism analysis. Avian Dis. 37, 194-202.

Kwon, H.M., Pi, J.H., Seong, H.W., 1998. Sequence of the spike gene containing antigenic sites A and D of transmissible gastroenteritis virus isolated in Korea. Korean J. Vet. Res. 38, 319-327.
Laude, H., Rasschaert, D., Huet, J.C., 1987. Sequence and N-terminal processing of the transmembrane protein E1 of the coronavirus transmissible gastroenteritis virus. J. Gen. Virol. 68, 1687–1693.
Paton, D., Lowings, P., 1997. Discrimination between transmissible gastroenteritis virus isolates. Arch. Virol. 142, 1703–1711.
Pensaert, M., Callebaut, P., Vergote, J., 1986. Isolation of a porcine respiratory, non-enteric coronavirus related to transmissible gastroenteritis. Vet. Quart. 8, 257–261.
Rasschaert, D., Laude, H., 1987. The predicted primary structure of the peplomer protein E2 of the porcine coronavirus transmissible gastroenteritis virus. J. Gen. Virol. 68, 1883–1890.
Rasschaert, D., Duarte, M., Laude, H., 1990. Porcine respiratory coronavirus differs from transmissible gastroenteritis virus by a few genomic deletions. J. Gen. Virol. 71, 2599–2607.
Saif, L.J., Wesley, R.D., 1999. Transmissible gastroenteritis. In: Straw, B.E., D’Allaire, S., Mengeling, W.L., Taylor, D.J. (Eds.), Disease of Swine. Iowa State University Press, Ames, IA, pp. 295–325.
Sanchez, C.M., Gebauer, F., Sune, C., Mendez, A., Dopeno, J., Enjuanes, L., 1992. Genetic evolution and tropism of transmissible gastroenteritis coronaviruses. Virology 190, 92–105.
Sanchez, C.M., Izeta, A., Sanchez-Morgad, J.M., Alonso, S., Sola, I., Balasch, M., Plana-Duran, J., Enjuanes, L., 1999. Targeted recombination demonstrates that the spike gene of transmissible gastroenteritis coronavirus is a determinant of its enteric tropism and virulence. J. Virol. 73, 7607–7618.
Siddell, S.G., Anderson, R., Cavanagh, D., Fujiwara, K., Klenk, H.D., Macnaughton, M.R., Pensaert, M., Stohman, S.A., Sturman, L., Van Der Zeijst, B.A.M., 1983. Coronaviridae Intervirology 20, 181–189.
Vaughn, E.M., Paul, P.S., 1993. Antigenic and biological diversity among transmissible gastroenteritis virus isolates of swine. Vet. Microbiol. 36, 333–347.
Vaughn, E.M., Halbur, P.G., Paul, P.S., 1994. Three new isolates of porcine respiratory coronavirus with various pathogenicities and spike (S) gene deletions. J. Clin. Microbiol. 32, 1809–1812.
Wesley, R.D., 1990a. Nucleotide sequence of the E2-peplomer protein gene partial nucleotide sequence of the upstream polymerase gene of transmissible gastroenteritis virus (Miller strain). Adv. Exp. Med. Biol. 276, 301–306.
Wesley, R.D., Woods, R.D., Hill, H.T., Biwer, J.D., 1990b. Evidence for a porcine respiratory coronavirus, antigenically similar to transmissible gastroenteritis virus, in the United States. J. Vet. Diagn. Invest. 2, 272–277.
Wesley, R.D., Woods, R.D., Cheung, A.K., 1990c. Genetic basis for the pathogenesis of transmissible gastroenteritis virus. J. Virol. 64, 4761–4766.
Woods, R.D., 1978. Small plaque variant transmissible gastroenteritis virus. J. Am. Vet. Med. Assoc. 173, 643–648.