Acta virologica **54**: 61 – 73, 2010 doi:10:4149/av_2010_01_61

# Analysis of the gene 3 region sequences of Chinese field strains of Transmissible gastroenteritis virus

C.B. WANG, L. FENG*, J.F. CHEN, H.Y. SHI, H.J. QIU, F. XUE, Y.M. ZHU, S.W. LIU

Division of Swine Infectious Diseases, National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, P.R. China

*Received June 30, 2009; accepted January 11, 2010*

**Summary. –** The genome of Transmissible gastroenteritis virus (TGEV) displays genetic diversity especially in gene 3 region. Sequence and comparative analysis of 3a and 3b genes of eight Chinese field strains with reference TGEV strains indicated that these strains shared 87.0–100% and 51.5%–100% identities at the nucleotide level, respectively, and 86.1%–100% and 66.2%–100% identities at the amino acid level, respectively. Moreover, in one of the strains (CH/SDQ/08), a 51 nt deletion in the gene 3 region was found. Phylogenetic analysis showed that the eight Chinese strains were more closely related to TGEV strains H165, H16, Miller M6, Miller M60, TS, and CHV than to other reference strains. In addition, this study indicated the presence of different TGEV strains within the same pig herds in China.

**Keywords:** gene 3; genomic diversity; sequence analysis; Transmissible gastroenteritis virus

## Introduction

Transmissible gastroenteritis (TGE) caused by TGEV is the major clinical disease in neonatal pigs accompanied by significant economic losses (Enjuanes *et al*., 1985). In United States, TGEV was initially identified as an etiological agent of TGE in pigs (Doyle and Hutchings, 1946; Doyle *et al*., 1951). In neonates, TGEV infects the epithelial cells of the small intestine leading to the potentially fatal gastroenteritis. The infection also occurs in the upper respiratory tract and less often, in the lungs. On the other hand, TGEV causes mild disease in adult pigs that was reported in many pig-farming countries between late 1980s and 1990s (Kemeny and Woods, 1977; Wood *et al*., 1981; Woods and Wesley, 1986; Pritchard, 1987; Saif, 2004). In China, TGE outbreak was first reported in the 1970s. Since then, it has been prevalent in many provinces and has become one of the most important viral diarrhea diseases in China.

*Corresponding author. E-mail: fl@hvri.ac.cn; fax: +86451-82733132.

**Abbreviations:** E = envelope; M = membrane; N = nucleoprotein; PRCV = Porcine respiratory coronavirus; S = spike; TGE = transmissible gastroenteritis; TGEV = Transmissible gastroenteritis virus; TRS = transcription regulatory sequence

TGEV is a member of the genus *Coronavirus*, group 1, within the family *Coronaviridae*. The spherical virion is enveloped and contains positive-sense, single-stranded RNA of about 28.5 kb. The genome contains 9 ORFs that encode 4 structural proteins, e.g. spike (S), envelope (E), membrane (M), nucleoprotein (N) and 5 non-structural proteins, replicases 1a and 1b, proteins 3a, 3b, and protein 7 in the order (5'-replicase 1a/1b-S-3a-3b-E-M-N-7-3'). Porcine respiratory coronavirus (PRCV), strain PRCV-ISU-1, is an attenuated variant of TGEV that has a reduced pathogenicity and predominantly respiratory tropism (Pensaert *et al*., 1986; Wesley *et al*., 1990; Saif and Sestak, 2006). The genome of PRCV-ISU-1 is in 96% identical with TGEV with the exception of deletions of various lengths (nt 45–752) within 5'-end of the S gene (Woods, 1976). Moreover, sequence analysis revealed some heterogeneity also in the genes S, 3a, and 3b of other PRCV isolates (Vaughn *et al*., 1995). Sequence analysis of TGEV isolates also revealed that there was a series of large deletions downstream of the S gene or large deletions/insertions in the gene 3 region (Chen *et al*., 1995; Ballesteros *et al.,* 1997*;* Kwon *et al*., 1998; McGoldrick *et al*., 1999; Kim *et al*., 2000*)*.

Genetic diversity among coronaviruses is generated by point mutations, insertions, and deletions introduced into the viral genome by viral RNA-dependent RNA polymerase

that lacks a proofreading capacity and by genetic recombination that occurs *via* genomic template-switching mechanism (Masters, 2006). It was reported that 3a, 3b protein as well as their RNAs are not essential for the replication of TGEV (Sola *et al.*, 2003), but the precise functions of these proteins have not been completely understood so far.

In the present study, the fragments of 8 Chinese field TGEV strains containing a part of the S gene, 3a gene, 3b gene, and a part of the E gene (part of S-3a-3b-part of E) were sequenced and compared with respect to their genetic diversity in the gene 3 region. The sequences of 3a and 3b genes were aligned and compared with the sequences of Chinese vaccine strain H165 and other reference TGEV strains.

## Materials and Methods

*Fecal samples*. Samples were taken from piglets with watery diarrhea and dehydration in six different pig-raising farms in China. The fecal samples were confirmed as positive for TGEV using Antigen Rapid TGE Ag Test Kit (Animal Genetics Inc.). They were handled as described previously (Chen *et al.*, 2008) and supernatants were collected for amplification of the gene 3 region by RT–PCR.

*RT-PCR*. Total RNA was isolated from the supernatant (200 μl) containing TGEV with Simply P Total RNA Extraction Kit (Bioer Technology) according to the manufacturer's instructions. RT step was performed with reverse transcriptase primer FP3 (5'-GGAG GGTTATGGGGTTGAAG-3') and MMLV reverse transcriptase reagent kit (TaKaRa). PCR was performed using the specific primers FP1 (5'-ATTGAAAAAGTGCACGTCC-3') and FP2 (5'-CAACAG GAACCAGAAAATG-3') with *Ex Taq* DNA polymerase (TaKaRa) for 30 cycles at 95°C for 45 secs, 50°C for 1 min, and 72°C for 75 secs followed by a final extension step at 72°C for 10 mins. The PCR products were analyzed on 1% agarose gels and purified with Biospin Gel Extraction Kit (Bioer Technology) according to the manufacturer's instructions.

*Cloning, sequencing, sequence and phylogenetic analysis.* The purified PCR products were cloned into a T-tailed vector, pMD18-T (TaKaRa). Three to five independent DNA clones from each TGEV strain were sequenced using M13 sequencing primers (Shanghai Sangon Biological Engineering Technology & Services). The sequences were compiled and ORFs were determined using Gene Runner program version 3.00 (http://www.generunner.com). The nucleotide and deduced amino acid sequences of 8 Chinese TGEV strains were deposited in GenBank: CH/HLJB/08 (FJ718993), CH/HLJH/08 (FJ718988), CH/HNH/08 (FJ718991), CH/SDQ/08 (FJ718990), CH/HBQ/08 (FJ718989), CH/JLY1/08 (FJ718992), CH/JLY2/08 (FJ718994), and CH/JLY3/08 (FJ718995). The nucleotide and deduced amino acid sequences of studied strains were aligned and compared with those of the reference TGEV strains using DNAMAN and DNAStar software. The reference strains used for the sequence alignment, sequence analysis and phylogenetic tree construction were: virulent Purdue (DQ811789), Purdue P115 (DQ811788), PUR46-MAD (AJ271965), Miller M60 (DQ811786), Miller M6 (DQ811785), BW021898B (AF179886), CHV TGEV (U26210), TS (DQ201447), SC-Y (DQ443743), H165 (EU074218), H16 (FJ755618), TFI (Z35758), KT2 (AF481368), and 96-1933 (AF104420).

## Results

### *Sequence and homology analyses*

Sequence analysis showed that 7 of 8 Chinese field TGEV strains (except for CH/SDQ/08) contained transcription regulatory sequence (TRS) 5'-CUAAAC-3' upstream from the initiator ATG of 3a and 3b genes as was previously observed in Purdue strains (Alonso *et al.*, 2002). Compared with the TGEV Purdue strains, 8 Chinese TGEV strains had two large deletions, e.g. 16 and 29 nt sequences in the 3a gene as previously observed in Miller strains (Zhang *et al.*, 2007). A sequence of 31 nt upstream from ATG codon and the first 50 nt sequence of 3a gene were deleted in CH/SDQ/08 strain disrupting a predicted 3a protein (Fig. 1). Compared with Chinese vaccine strain H165, CH/JLY2/08 strain had 7 nucleotide mutations resulting in 2 amino acid changes (V4I, K5L). Furthermore, a mutation (T2C) in ATG codon of 3a gene also disrupted 3a protein of CH/JLY1/08 strain. Similarly, each strain of CH/HLJB/08, CH/HBQ/08, CH/HNH/08, and CH/JLY3/08 contained a common nucleotide mutation (T173C) in the 3b gene relative to the sequence of Chinese vaccine strain H165 resulting in the amino acid change (V58A). CH/SDQ/08 strain also had a nucleotide mutation (A430G) that resulted in amino acid change (T144A). CH/JLY2/08 strain had two nucleotide mutations (T71C, T173C) that resulted in two amino acid changes (T24I, V58A). CH/HLJH/08 strain contained two nucleotide mutations (T509C, T730C) and the latter one resulted in amino acid change (S244P). CH/JLY1/08 strain had 4 nucleotide mutations and 2 of them (A733T, G734T) occurred at the stop codon of 3b gene disrupting 3b protein of CH/JLY1/08, what led to a 3b protein that was 7 amino acids longer than those of other TGEV strains.

Characteristics of the nucleotide and amino acid sequences of the 3a and 3b genes are presented in Tables 1 and 2, respectively. The 3a genes of new Chinese TGEV strains shared nucleotide sequence identities of 96.8%–100% compared with each other and 87.0%–100% compared with the reference TGEV strains. At the amino acid level of protein 3a, Chinese TGEV strains shared 97.3%–100% identities among themselves and 85.9%–100% in comparison with the reference strains. Similarly, the 3b genes of new Chinese TGEV strains shared nucleotide sequence identities of 99.0%–100% compared with each other and 96.7%–100% with the reference TGEV strains except for considerably lower identities found with Miller M60 strain (51.5%–52.9%). At the amino acid level of protein 3b, Chinese TGEV strains shared

```
H165       ATTGAAAAAGTGCACGTCCATTAAATTTAAAATGTTAATTTTATTATCTGCTATAATAGC   60
CH/HNH/08  ------------------------------------------------------------   60
CH/HLJB/08 ------------------------------------------------------------   60
CH/HLJH/08 ------------------------------------------------------------   60
CH/HBQ/08  ------------------------------------------------------------   60
CH/SDQ/08  ------------------------------------------------------------   60
CH/JLY3/08 ------------------------------------------------------------   60
CH/JLY1/08 ------------------------------------------------------------   60
CH/JLY2/08 ------------------------------------------------------------   60

H165       ATTTGTTGTTAAGGATGATGAATAAAGTCCTTAAGAACTAAACTTTCGAGTCATTACAGG   120
CH/HNH/08  ------------------------------------------------------------   120
CH/HLJB/08 ------------------------------------------------------------   120
CH/HLJH/08 ------------------------------------------------------------   120
CH/HBQ/08  ------------------------------------------------------------   120
CH/SDQ/08  ------------------------------------..........................    96
CH/JLY3/08 ------------------------------------------------------------   120
CH/JLY1/08 ------------------------------------------------------------   120
CH/JLY2/08 ------------------------------------------------------------   120
H165       TCCTGTATGGACATTGTCAAATCCATTAATACATCCGTAGATGCTGTACTTGACGAACTT   180
CH/HNH/08  ------------------------------------------------------------   180
CH/HLJB/08 ------------------------------------------------------------   180
CH/HLJH/08 ------------------------------------------------------------   180
CH/HBQ/08  ------------------------------------------------------------   180
CH/SDQ/08  ..........................................................---    99
CH/JLY3/08 ------------------------------------------------------------   180
CH/JLY1/08 ---A---C---------------------------------------------------   180
CH/JLY2/08 --------------CA-ACTT--------------------------------------   180

H165       GATTGTGCATACTTTGCTGTAACTCTTAAAGTAGAATTTAAGACTGGTAAATTACTTGTG   240
CH/HNH/08  ------------------------------------------------------------   240
CH/HLJB/08 ------------------------------------------------------------   240
CH/HLJH/08 ------------------------------------------------------------   240
CH/HBQ/08  ------------------------------------------------------------   240
CH/SDQ/08  ------------------------------------------------------------   159
CH/JLY3/08 ------------------------------------------------------------   240
CH/JLY1/08 ------------------------------------------------------------   240
CH/JLY2/08 ----------------------------G-------------------------------   240
```

**Fig. 1**

```
H165        TGTATAGGTTTTGGTGACACACTTCTTGCGGCTAGGGATAAAGCATATTCTAAGCTTGGT  300
CH/HNH/08   ------------------------------------------------------------  300
CH/HLJB/08  ------------------------------------------------------------  300
CH/HLJH/08  ------------------------------------------------------------  300
CH/HBQ/08   ------------------------------------------------------------  300
CH/SDQ/08   ----------------------------------------------G-------------  219
CH/JLY3/08  ------------------------------------------------------------  300
CH/JLY1/08  ------------------------------------------------------------  300
CH/JLY2/08  ------------------------------------------------------------  300

H165        CTCTCCATTATTGAAGAAGTAAACACACAAAATCCAAAGCATTAAGTGTTACAAAACAAT  360
CH/HNH/08   ------------------------------------------------------------  360
CH/HLJB/08  ------------------------------------------------------------  360
CH/HLJH/08  ----------------------------------------------------------G---  360
CH/HBQ/08   ------------------------------------------------------------  360
CH/SDQ/08   ------------------------------------------------------------  279
CH/JLY3/08  ------------------------------------------------------------  360
CH/JLY1/08  ------------------------------------------------------------  360
CH/JLY2/08  ------------------------------------------------------------  360
H165        TAAAGAGAGATTATAGAAAAACTGTCGTTCTAAACTTCATGCGAAAATGATTGGTGGACT  420
CH/HNH/08   ------------------------------------------------------------  420
CH/HLJB/08  ------------------------------------------------------------  420
CH/HLJH/08  ------------------------------------------------------------  420
CH/HBQ/08   ------------------------------------------------------------  420
CH/SDQ/08   ------------------------A-----------------------------------  339
CH/JLY3/08  ------------------------------------------------------------  420
CH/JLY1/08  ------------------------------------------------------------  420
CH/JLY2/08  ------------------------------------------------------------  420

H165        TTTTCTTAATACTCTGAGTTTTGTAATTGTTAGTAACCATTCTATTGTTAATAACACAGC  480
CH/HNH/08   ------------------------------------------------------------  480
CH/HLJB/08  ------------------------------------------------------------  480
CH/HLJH/08  ------------------------------------------------------------  480
CH/HBQ/08   ------------------------------------------------------------  480
CH/SDQ/08   ------------------------------------------------------------  399
CH/JLY3/08  ------------------------------------------------------------  480
CH/JLY1/08  ------------------------------------------------------------  480
CH/JLY2/08  ----------------------------------------------------------T---  480
```

**Fig. 1 (continued)**

```
H165        AAATGTGCATCATATAAAACAAGAACGTGTTATAGTACAACAGCATCAGGTTGTTAGTGC   540
CH/HNH/08   ------------------------------------------------------------   540
CH/HLJB/08  ------------------------------------------------------------   540
CH/HLJH/08  ------------------------------------------------------------   540
CH/HBQ/08   ------------------------------------------------------------   540
CH/SDQ/08   ------------------------------------------------------------   459
CH/JLY3/08  ------------------------------------------------------------   540
CH/JLY1/08  ------------------------------------------------------------   540
CH/JLY2/08  ------------------------------------------------------------   540

H165        TAGAACACAAAATTATTACCCAGAGTTCAGCATCGCTGTACTTTTTGTATCTTTTCTAGC   600
CH/HNH/08   ------------------------------------C-----------------------   600
CH/HLJB/08  ------------------------------------C-----------------------   600
CH/HLJH/08  ------------------------------------------------------------   600
CH/HBQ/08   ------------------------------------C-----------------------   600
CH/SDQ/08   ------------------------------------------------------------   519
CH/JLY3/08  ------------------------------------C-----------------------   600
CH/JLY1/08  ------------------------------------C-----------------------   600
CH/JLY2/08  ------------------------------------C-----------------------   600
H165        TTTGTACCGTAGTACAAACTTTAAGACGTGTGTCGGCATCTTAATGTTTAAGATTTTATC   660
CH/HNH/08   ------------------------------------------------------------   660
CH/HLJB/08  ------------------------------------------------------------   660
CH/HLJH/08  ------------------------------------------------------------   660
CH/HBQ/08   ------------------------------------------------------------   660
CH/SDQ/08   ------------------------------------------------------------   579
CH/JLY3/08  ------------------------------------------------------------   660
CH/JLY1/08  ------------------------------------------------------------   660
CH/JLY2/08  ------------------------------------------------------------   660
↵
H165        AATGACACTTTTAGGACCTATGCTTATAGCATATGGTTACTACATTGATGGCATTGTTAC   720
CH/HNH/08   ------------------------------------------------------------   720
CH/HLJB/08  ------------------------------------------------------------   720
CH/HLJH/08  ------------------------------------------------------------   720
CH/HBQ/08   ------------------------------------------------------------   720
CH/SDQ/08   ------------------------------------------------------------   639
CH/JLY3/08  ------------------------------------------------------------   720
CH/JLY1/08  ------------------------------------------------------------   720
CH/JLY2/08  ------------------------------------------------------------   720
```

**Fig. 1 (continued)**

```
H165        AACAACTGTCTTATCTTTAAGATTTGCCTACTTAGCATACTTTTGGTATGTTAATAGTAG   780
CH/HNH/08   ------------------------------------------------------------   780
CH/HLJB/08  ------------------------------------------------------------   780
CH/HLJH/08  ------------------------------------------------------------   780
CH/HBQ/08   ------------------------------------------------------------   780
CH/SDQ/08   ------------------------------------------------------------   699
CH/JLY3/08  ------------------------------------------------------------   780
CH/JLY1/08  ------------------------------------------------------------   780
CH/JLY2/08  ------------------------------------------------------------   780

H165        GTTTGAATTTATTTTATACAATACAACGACACTCATGTTTGTACATGGCAGAGCTACACC   840
CH/HNH/08   ------------------------------------------------------------   840
CH/HLJB/08  ------------------------------------------------------------   840
CH/HLJH/08  ------------------------------------------------------------   840
CH/HBQ/08   ------------------------------------------------------------   840
CH/SDQ/08   ----------------------------------------------------G-------   759
CH/JLY3/08  ------------------------------------------------------------   840
CH/JLY1/08  ------------------------------------------------------------   840
CH/JLY2/08  ------------------------------------------------------------   840
H165        GTTTAAGAGAAGTTCTCACAGCTCTATTTATGTCACATTGTATGGTGGCATAAATTATAT   900
CH/HNH/08   ------------------------------------------------------------   900
CH/HLJB/08  ------------------------------------------------------------   900
CH/HLJH/08  ------------------------------------------------------------   900
CH/HBQ/08   ------------------------------------------------------------   900
CH/SDQ/08   ------------------------------------------------------------   819
CH/JLY3/08  ------------------------------------------------------------   900
CH/JLY1/08  ------------------------------------------------------------   900
CH/JLY2/08  ------------------------------------------------------------   900

H165        GTTTGTGAATGACCTCACGTTGCATTTTGTAGACCCTATGCTTGTAAGCATAGCAATACG   960
CH/HNH/08   ------------------------------------------------------------   960
CH/HLJB/08  ------------------------------------------------------------   960
CH/HLJH/08  ------------------------------------------------------------   960
CH/HBQ/08   ------------------------------------------------------------   960
CH/SDQ/08   ------------------------------------------------------------   879
CH/JLY3/08  ------------------------------------------------------------   960
CH/JLY1/08  ------------------------------------------------------------   960
CH/JLY2/08  ------------------------------------------------------------   960
```

**Fig. 1 (continued)**

```
H165        TGGCTTAGCTCATGCTGATCTAACTGTAGTTAGAGCAGTTGAACTTCTCAATGGTGATTT  1020
CH/HNH/08   ------------------------------------------------------------  1020
CH/HLJB/08  ------------------------------------------------------------  1020
CH/HLJH/08  ----------------------------------------------------C-------  1020
CH/HBQ/08   ------------------------------------------------------------  1020
CH/SDQ/08   ------------------------------------------------------------   939
CH/JLY3/08  ------------------------------------------------------------  1020
CH/JLY1/08  ------------------------------------------------------------  1020
CH/JLY2/08  ------------------------------------------------------------  1020

H165        TATTTATGTATTTTCACAGGAGCCCGTAGTCGGTGTTTACAATGCAGCCTTTTCTCAGGC  1080
CH/HNH/08   ------------------------------------------------------------  1080
CH/HLJB/08  ------------------------------------------------------------  1080
CH/HLJH/08  ----------------------------------C-------------------------  1080
CH/HBQ/08   ------------------------------------------------------------  1080
CH/SDQ/08   ------------------------------------------------------------   999
CH/JLY3/08  ------------------------------------------------------------  1080
CH/JLY1/08  ------------------------------------------------------------  1080
CH/JLY2/08  ------------------------------------------------------------  1080
H165        GGTTCTAAACGAAATTGACTTAAAAGAAGAAGAAGAAGACCGTACCTATGACGTTTCCTA  1140
CH/HNH/08   ------------------------------------------------------------  1140
CH/HLJB/08  ------------------------------------------------------------  1140
CH/HLJH/08  ----------------------------------------------------C-------  1140
CH/HBQ/08   ------------------------------------------------------------  1140
CH/SDQ/08   ------------------------------------------------------------  1059
CH/JLY3/08  ------------------------------------------------------------  1140
CH/JLY1/08  ----------------------------------------------------G-T-------  1140
CH/JLY2/08  ------------------------------------------------------------  1140

H165        GGGCATTGACTGTCATAGATGACACTGGAATGGTCATTAGCATCATTTTCTGGTTCCTGT  1200
CH/HNH/08   ------------------------------------------------------------  1200
CH/HLJB/08  ------------------------------------------------------------  1200
CH/HLJH/08  ------------------------------------------------------------  1200
CH/HBQ/08   ------------------------------------------------------------  1200
CH/SDQ/08   ------------------------------------------------------------  1119
CH/JLY3/08  ------------------------------------------------------------  1200
CH/JLY1/08  TA----------------___------------------------------------------  1200
CH/JLY2/08  ----------A-------------------------------------------------  1200
```

**Fig. 1 (continued)**


**Fig. 1**

**Sequence alignment of 3a and 3b genes of Chinese field TGEV strains**

Vaccine strain H165 was used as a reference. TRS (gray shaded), start codon (boxed), stop codon (underlined), deletion (dot), identity (dash).

99.2%–100% identities among themselves and 93.0%–100% identities with the reference TGEV strains.

*Phylogenetic analysis*

Phylogenetic trees were constructed according to the available 3a and 3b protein sequences of TGEV strains presented in the GenBank database (Figs. 2, 3). The phylogenetic trees showed quite similar profiles with slight differences. Phylogenetic analysis of TGEV strains showed 4 distinct clusters, in which the new Chinese TGEV strains seemed to be more closely related to the strains H165, H16, Miller M6, Miller M60, TS, and CHV, than to other reference TGEV strains.

## Discussion

Although combined attenuated vaccine against TGEV and Porcine epidemic diarrhea virus infections is authorized for the use in pig-raising farms in many Chinese provinces, TGE still occurs in the pig herds of China. Moreover, the diversity among Chinese TGEV strains and other reference TGEV strains has been reported (Yin *et al.*, 2005). However, this report is a first one dealing with the genetic diversity in gene 3 of Chinese field TGEV strains and reference TGEV strains. Previous studies have shown that there is a genetic diversity in the genomes of TGEV, especially in gene 3 (Chen *et al.*, 1995; Kwon *et al.*, 1998; McGoldrick *et al.*, 1999; Kim *et al.*, 2000; Zhang *et al.*, 2007). Our findings showed that 8 Chinese TGEV strains were genetically diverse in the gene 3 among themselves as well as in comparison with the reference strains. The previous results and our findings presented here suggest that the absence or abnormalities of nonstructural protein genes is tolerated by the TGEV as well as by other coronaviruses (De Haan *et al.*, 2002; Shen *et al.*, 2003; Liu *et al.*, 2008). The results also imply that ORF3 product is not functionally essential as previously demonstrated with a reverse genetics system (Sola *et al.*, 2003).

Coronaviruses as single-stranded RNA viruses are fully equipped for adaptation to ever changing ecological niches, as they have a high substitution rate of $10^{-4}$ substitutions/year/site (Sanchez *et al.*, 1992; Vijgen *et al.*, 2005). Homologous recombination has also been observed *in vitro* and *in vivo* implying that the mutated or recombinant coronavirus variants can emerge anywhere at any time possibly putting public health at risk, as in the case of Severe acute respiratory syndrome coronavirus (Makino *et al.*, 1986; Keck *et al.*, 1988; Wang *et al.*, 1993; Jia *et al.*, 1995). Recombination is undoubtedly a feature of TGEV replication and evolution (Sanchez *et al.*, 1992; Enjuanes *et al.*, 1993; Ballesteros *et al.*, 1995). Sequence analysis demonstrated that the strains CH/JLY1/08, CH/JLY2/08, CH/JLY3/08, CH/HNH/08, CH/HLJB/08, CH/HLJH/08, and CH/HBQ/08 were almost identical to the vaccine strain H165, yet some nucleotide differences were observed. The strain CH/SDQ/08 was identical to strain H16 except for its large deletions. This finding suggested that some kind of recombination occurred in examined Chinese strains and these recombinant events increased the diversity of gene 3. In present study, the new Chinese strains were isolated from the intensive pig farms raising large numbers of pigs. However, under these conditions, intensity of viral spread would contribute to the recombination, since these herds were treated with the live vaccines. The demonstrated natural recombination event revealed, that the continuous use of live vaccines might have actually contributed to natural recombination and TGEV-associated disease. The sequence analysis also revealed that these TGEV strains might be prevalent in

**Table 1 Homology of TGEV strains based on the sequences of 3a gene and protein**

| Virulent Purdue | Purdue P115 | PUR46-MAD | SC-Y | Miller M6 | TS | H16 | H165 | TFI | KT2 | BW021898B | CHV TGEV | 96-1933 | Chinese field TGEV strains | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | CH/HLJB/08 | CH/HBQ/08 | CH/HLJH/08 | CH/JLY1/08 | CH/JLY3/08 | CH/JLY2/08 |
| *** | 99.5 | 99.5 | 99.5 | 90.7 | 90.3 | 89.8 | 90.7 | 97.4 | 88 | 97.7 | 87.5 | 90.7 | 90.3 | 90.3 | 90.3 | 90.3 | 90.3 | 87 |
| 98.6 | *** | 100 | 100 | 90.7 | 90.3 | 89.8 | 90.7 | 97.4 | 88 | 97.7 | 87.5 | 90.7 | 90.3 | 90.3 | 90.3 | 90.3 | 90.3 | 87 |
| 98.6 | 100 | *** | 100 | 90.7 | 90.3 | 89.8 | 90.7 | 97.4 | 88 | 97.7 | 87.5 | 90.7 | 90.3 | 90.3 | 90.3 | 90.3 | 90.3 | 87 |
| 98.6 | 100 | 100 | *** | 90.7 | 90.3 | 89.8 | 90.7 | 97.4 | 88 | 97.7 | 87.5 | 90.7 | 90.3 | 90.3 | 90.3 | 90.3 | 90.3 | 87 |
| 90.3 | 90.3 | 90.3 | 90.3 | *** | 99.5 | 99.1 | 100 | 99.5 | 96.8 | 91.3 | 96.8 | 100 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 96.3 |
| 88.9 | 88.9 | 88.9 | 88.9 | 98.6 | *** | 98.6 | 99.5 | 99 | 96.3 | 90.9 | 96.3 | 99.5 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 95.9 |
| 87.5 | 87.5 | 87.5 | 87.5 | 97.3 | 95.9 | *** | 99.1 | 98.4 | 95.9 | 90.4 | 95.9 | 99.1 | 98.6 | 98.6 | 98.6 | 98.6 | 98.6 | 95.4 |
| 96.9 | 96.9 | 96.9 | 96.9 | 100 | 98.5 | 96.9 | *** | 99.5 | 96.8 | 91.3 | 96.8 | 100 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 96.3 |
| 95.4 | 95.4 | 95.4 | 95.4 | 98.5 | 96.9 | 95.4 | 98.5 | *** | 95.9 | 98.4 | 95.9 | 99.5 | 100 | 100 | 100 | 100 | 100 | 96.4 |
| 88.9 | 88.9 | 88.9 | 88.9 | 98.6 | 97.3 | 95.9 | 98.5 | 96.9 | *** | 88.1 | 95.4 | 96.8 | 96.3 | 96.3 | 96.3 | 96.3 | 96.3 | 93.2 |
| 94.4 | 94.4 | 94.4 | 94.4 | 94.4 | 93.1 | 91.7 | 98.5 | 96.9 | 93.1 | *** | 88.1 | 91.3 | 90.9 | 90.9 | 90.9 | 90.9 | 90.9 | 87.7 |
| 86.1 | 86.1 | 86.1 | 86.1 | 95.9 | 94.5 | 93.2 | 95.4 | 93.8 | 97.3 | 90.3 | *** | 96.8 | 96.3 | 96.3 | 96.3 | 96.3 | 96.3 | 93.2 |
| 90.3 | 90.3 | 90.3 | 90.3 | 100 | 98.6 | 97.3 | 100 | 98.5 | 98.6 | 94.4 | 95.9 | *** | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 96.3 |
| 88.9 | 88.9 | 88.9 | 88.9 | 98.6 | 97.3 | 95.9 | 98.5 | 100 | 97.3 | 93.1 | 94.5 | 98.6 | *** | 100 | 100 | 100 | 100 | 96.8 |
| 88.9 | 88.9 | 88.9 | 88.9 | 98.6 | 97.3 | 95.9 | 98.5 | 100 | 97.3 | 93.1 | 94.5 | 98.6 | 100 | *** | 100 | 100 | 100 | 96.8 |
| 88.9 | 88.9 | 88.9 | 88.9 | 98.6 | 97.3 | 95.9 | 98.5 | 100 | 97.3 | 93.1 | 94.5 | 98.6 | 100 | 100 | *** | 100 | 100 | 96.8 |
| 88.9 | 88.9 | 88.9 | 88.9 | 98.6 | 97.3 | 95.9 | 98.5 | 100 | 97.3 | 93.1 | 94.5 | 98.6 | 100 | 100 | 100 | *** | 100 | 96.8 |
| 88.9 | 88.9 | 88.9 | 88.9 | 98.6 | 97.3 | 95.9 | 98.5 | 100 | 97.3 | 93.1 | 94.5 | 98.6 | 100 | 100 | 100 | 100 | *** | 96.8 |
| 86.1 | 86.1 | 86.1 | 86.1 | 95.9 | 94.5 | 93.2 | 95.4 | 96.9 | 94.5 | 90.3 | 91.8 | 95.9 | 97.3 | 97.3 | 97.3 | 97.3 | 97.3 | *** |

Nucleotide identity (%) in the upper triangle; deduced amino acid identity (%) in the lower triangle.

**Table 2. Homology of TGEV strains based on the sequences of 3b gene and protein**

| Strains | Virulent Purdue | Purdue P115 | PUR46-MAD | SC-Y | Miller M6 | Miller M60 | TS | H16 | H165 | TFI | KT2 | BW021898B | CHV TGEV | 96-1933 | Chinese field TGEV strains | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | CH/ HLJB/08 | CH/ HBQ/08 | CH/ HLJH/08 | CH/ HNH/08 | CH/ SDQ/08 | CH/ JLY1/08 | CH/ JLY3/08 | CH/ JLY2/08 |
| Virulent Purdue | *** | 99.9 | 99.7 | 99.9 | 99.2 | 52.5 | 98.8 | 99.2 | 99 | 98.4 | 98.9 | 99 | 96.9 | 96.3 | 98.9 | 98.9 | 98.6 | 98.9 | 99.2 | 98.5 | 98.8 | 98.9 |
| Purdue P115 | 99.6 | *** | 99.9 | 100 | 99 | 52.5 | 98.6 | 99 | 98.9 | 98.2 | 98.8 | 98.9 | 96.7 | 96.2 | 98.8 | 98.8 | 98.5 | 98.8 | 99 | 98.4 | 98.6 | 98.8 |
| PUR46-MAD | 99.2 | 99.6 | *** | 99.9 | 98.9 | 52.5 | 98.5 | 98.9 | 98.8 | 98.1 | 98.6 | 98.8 | 96.6 | 96.3 | 98.6 | 98.6 | 98.4 | 98.6 | 98.9 | 98.2 | 98.5 | 98.6 |
| SC-Y | 99.6 | 100 | 99.6 | *** | 99 | 52.5 | 98.6 | 99 | 98.9 | 98.2 | 98.8 | 98.9 | 96.7 | 96.2 | 98.8 | 98.8 | 98.5 | 98.8 | 99 | 98.4 | 98.6 | 98.8 |
| Miller M6 | 98.4 | 98 | 97.6 | 98 | *** | 52.9 | 99.6 | 100 | 99.9 | 98.6 | 98.1 | 99.9 | 97.4 | 96.6 | 99.7 | 99.7 | 99.5 | 99.7 | 100 | 99.3 | 99.6 | 99.7 |
| Miller M60 | 64.7 | 63.2 | 63.2 | 63.2 | 66.2 | *** | 52.5 | 52.9 | 52.9 | 51.5 | 44.1 | 52.9 | 51 | 51.5 | 52.9 | 52.9 | 52 | 52.9 | 52.9 | 51.5 | 52.9 | 52.9 |
| TS | 97.1 | 96.7 | 96.3 | 96.7 | 98.8 | 63.2 | *** | 99.6 | 99.5 | 98.2 | 97.8 | 99.5 | 97 | 96.2 | 99.3 | 99.3 | 99 | 99.3 | 99.6 | 98.9 | 99.2 | 99.3 |
| H16 | 98.4 | 98 | 97.6 | 98 | 100 | 66.2 | 98.8 | *** | 99.9 | 98.6 | 98.1 | 99.9 | 97.4 | 96.6 | 99.7 | 99.7 | 99.5 | 99.7 | 100 | 99.3 | 99.6 | 99.7 |
| H165 | 98 | 97.6 | 97.1 | 97.6 | 99.6 | 66.2 | 98.4 | 99.6 | *** | 98.5 | 98 | 99.7 | 97.3 | 96.5 | 99.9 | 99.9 | 99.6 | 99.9 | 99.9 | 99.5 | 99.7 | 99.9 |
| TFI | 97.6 | 97.1 | 96.7 | 97.1 | 98.4 | 63.2 | 97.1 | 98.4 | 98 | *** | 97.7 | 98.5 | 97.4 | 96.9 | 98.4 | 98.4 | 98.1 | 98.4 | 98.6 | 98 | 98.2 | 98.4 |
| KT2 | 93 | 93 | 92.6 | 93 | 91.6 | 52.9 | 90.7 | 91.6 | 91.2 | 91.2 | *** | 98 | 96.1 | 95.3 | 97.8 | 97.8 | 97.8 | 97.8 | 98.1 | 97.8 | 97.7 | 97.8 |
| BW021898B | 98 | 97.6 | 97.1 | 97.6 | 99.6 | 66.2 | 98.4 | 99.6 | 99.2 | 98 | 91.2 | *** | 97.3 | 96.5 | 99.6 | 99.6 | 99.3 | 99.6 | 99.9 | 99.2 | 99.5 | 99.6 |
| CHV TGEV | 94.7 | 94.3 | 93.9 | 94.3 | 95.5 | 63.2 | 94.3 | 95.5 | 95.1 | 95.1 | 89.3 | 95.1 | *** | 96.1 | 97.1 | 97.1 | 96.9 | 97.1 | 97.4 | 96.7 | 97 | 97.1 |
| 96-1933 | 93.5 | 93.1 | 93.5 | 93.1 | 94.3 | 61.8 | 93.1 | 94.3 | 93.9 | 94.7 | 87 | 93.9 | 93.9 | *** | 96.3 | 96.3 | 96.1 | 96.3 | 96.6 | 95.9 | 96.2 | 96.3 |
| CH/HLJB/08 | 97.6 | 97.1 | 96.7 | 97.1 | 99.2 | 66.2 | 98 | 99.2 | 99.6 | 97.6 | 90.7 | 98.8 | 94.7 | 93.5 | *** | 100 | 99.5 | 100 | 99.7 | 99.6 | 99.9 | 100 |
| CH/HBQ/08 | 97.6 | 97.1 | 96.7 | 97.1 | 99.2 | 66.2 | 98 | 99.2 | 99.6 | 97.6 | 90.7 | 98.8 | 94.7 | 93.5 | 100 | *** | 99.5 | 100 | 99.7 | 99.6 | 99.9 | 100 |
| CH/HLJH/08 | 97.6 | 97.1 | 96.7 | 97.1 | 99.2 | 64.7 | 98 | 99.2 | 99.6 | 97.6 | 91.2 | 98.8 | 94.7 | 93.5 | 99.2 | 99.2 | *** | 99.5 | 99.5 | 99 | 99.3 | 99.5 |
| CH/HNH/08 | 97.6 | 97.1 | 96.7 | 97.1 | 99.2 | 66.2 | 98 | 99.2 | 99.6 | 97.6 | 90.7 | 98.8 | 94.7 | 93.5 | 100 | 100 | 99.2 | *** | 99.7 | 99.6 | 99.9 | 100 |
| CH/SDQ/08 | 98.4 | 98 | 97.6 | 98 | 100 | 66.2 | 98.8 | 100 | 99.6 | 98.4 | 91.6 | 99.6 | 95.5 | 94.3 | 99.2 | 99.2 | 99.2 | 99.2 | *** | 99.3 | 99.6 | 99.7 |
| CH/JLY1/08 | 97.6 | 97.1 | 96.7 | 97.1 | 99.2 | 66.2 | 98 | 99.2 | 99.6 | 97.6 | 90.7 | 98.8 | 94.7 | 93.5 | 100 | 100 | 99.2 | 100 | 99.2 | *** | 99.5 | 99.6 |
| CH/JLY3/08 | 97.1 | 96.7 | 96.3 | 96.7 | 98.8 | 64.7 | 97.6 | 98.8 | 99.2 | 97.1 | 90.2 | 98.4 | 94.3 | 93.1 | 99.6 | 99.6 | 98.8 | 99.6 | 98.8 | 99.6 | *** | 99.9 |
| CH/JLY2/08 | 97.6 | 97.1 | 96.7 | 97.1 | 99.2 | 66.2 | 98 | 99.2 | 99.6 | 97.6 | 90.7 | 98.8 | 94.7 | 93.5 | 100 | 100 | 99.2 | 100 | 99.2 | 100 | 99.6 | *** |

Nucleotide identity (%) in the upper triangle; deduced amino acid identity (%) in the lower triangle.

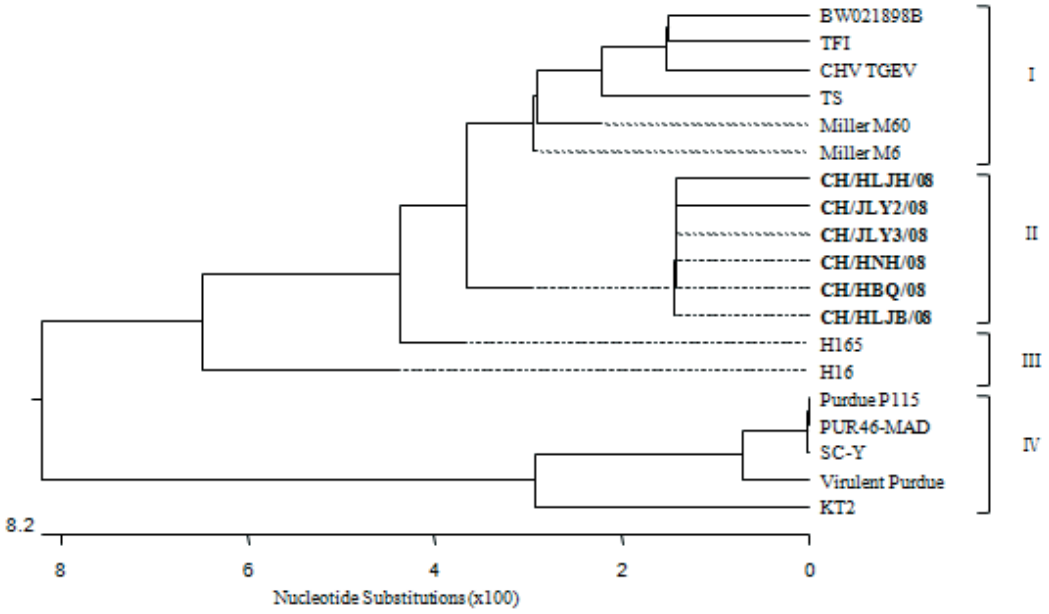


**Fig. 2**

**Phylogenetic tree of TGEV strains based on 3a protein**

New Chinese TGEV strains are boldfaced.

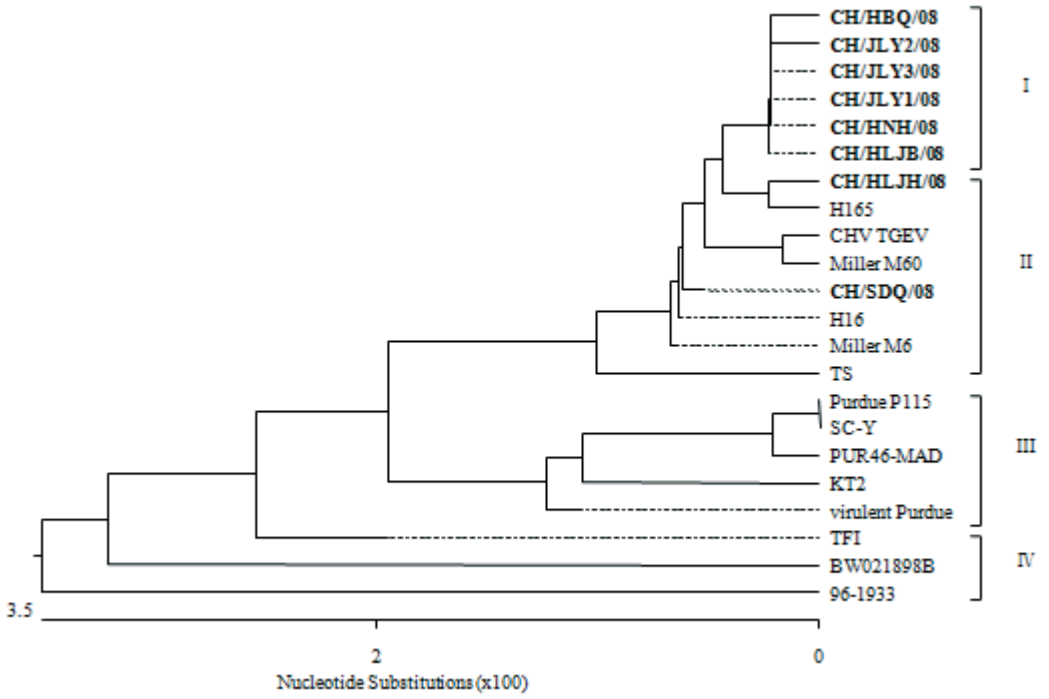


**Fig. 3**

**Phylogenetic tree of TGEV strains based on 3b protein**

New Chinese TGEV strains are boldfaced.

China, especially in herds maintained at high densities and intensively exposed to the live vaccines. The possibility that natural recombination affects efficacy of the vaccination will be tested in subsequent animal experiments.

In this study, we also investigated whether TGEV strains isolated from a single herd or from the same province were identical or similar to each other and evaluated the degree of their relatedness. We collected the strains CH/HLJB/08 and CH/HLJH/08 from the same province and strains CH/JLY1/08, CH/JLY2/08, and CH/JLY3/08 from the same herd. Sequence analysis demonstrated that the sequence homologies between them were relatively low. The nucleotide sequence data discussed above revealed genetic diversity in ORF3 of the Chinese field TGEV strains, even though the viruses were isolated from the same place. These results also indicated that different strains were present in the same farm.

The constructed phylogenetic trees showed that the new Chinese TGEV strains formed a cluster that was separated from other TGEV strains. This analysis also indicated that eight Chinese TGEV strains were more closely related to the H165 and H16 strains than to Korean, American Purdue, European, or SC-Y strains. In spite of the fact that most coronavirus structural proteins have been thoroughly investigated, at present we are investigating the genetic diversity of gene 3 region in Chinese field TGEV strains, because little is known about the 3a and 3b proteins, their homologies and genetic variations. The isolation of new TGEV strains in other countries could increase our understanding of the genetic structure and evolution of TGEV.

**Acknowledgements.** This work was supported by the grants from the National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, and Chinese Academy of Agricultural Sciences.

## References

Alonso S, Izeta A, Sola I, Enjuanes L (2002): Transcription regulatory sequences and mRNA expression levels in the coronavirus transmissible gastroenteritis virus. J. Virol. 76, 1293–1308.

Ballesteros ML, Sanchez CM, Enjuanes L (1997): Two amino acid changes at the N-terminal of the transmissible gastroenteritis coronavirus spike protein result in the loss of enteric tropism. Virology 227, 378–388. doi:10.1006/viro.1996.8344

Ballesteros ML, Sanchez CM, Martin-Caballero J, Enjuanes L (1995): Molecular bases of tropism in the PUR46 cluster of transmissible gastroenteritis coronaviruses. Adv. Exp. Med. Biol. 380, 557–562.

Chen CM, 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. doi:10.1016/0168-1702(95)00046-S

Chen JF, Sun DB, Wang CB, Shi HY, Cui XC, Liu SW, Qiu HJ, Feng L (2008): Molecular characterization and phylogenetic analysis of membrane protein genes of porcine epidemic diarrhea virus isolates in China. Virus Genes 36, 355–364. doi:10.1007/s11262-007-0196-7

De Haan CAM, Masters PS, Shen X, Weiss S, Rottier PJM (2002): The group-specific murine coronavirus genes are not essential, but their deletion, by reverse genetics, is attenuating in the natural host. Virology 296, 177–189. doi:10.1006/viro.2002.1412

Doyle LP (1951): Transmissible gastroenteritis of pigs. North Am. Vet. 32, 477–478.

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

Enjuanes L, Smerdou C, Castilla J, Anton IM, Torres JM, Sola I, Golvano J, Sanchez JM, Pintado B (1985): Development of protection against coronavirus induced diseases. A review. Adv. Exp. Med. Biol. 380, 197–211.

Enjuanes L, Sanchez C, Gebauer F, Mendez A, Dopazo J, Ballesteros ML (1993): Evolution and tropism of transmissible gastroenteritis coronavirus. Adv. Exp. Med. Biol. 342, 35–42.

Jia W, Karaca K, Parrish CR, Naqi SA (1995): A novel variant of avian infectious bronchitis virus resulting from recombination among three different strains. Arch. Virol. 140, 259–271. doi:10.1007/BF01309861

Keck JG, Soe LH, Makino S, Stohlman SA, Lai MM (1988): RNA recombination of murine coronaviruses: recombination between fusion-positive mouse hepatitis virus A59 and fusion–negative mouse hepatitis virus 2. J. Virol. 62, 1989–1998.

Kemeny LJ, Woods RD (1977): Quantitative transmissible gastroenteritis virus shedding patterns in lactating sows. Am. J. Vet. Res. 38, 307–310.

Kim L, Hayes J, Lewis P, Parwani AV, Chang KO, Saif LJ (2000): Molecular characterization and pathogenesis of transmissible gastroenteritis coronavirus (TGEV) and porcine respiratory coronavirus (PRCV) field isolates co-circulating in a swine herd. Arch. Virol. 145, 1133–1147. doi:10.1007/s007050070114

Kwon HM, Saif LJ, Jackwood DJ (1998): Field isolates of transmissible gastroenteritis virus differ at the molecular level from the Miller and Purdue virulent and attenuated strains and from porcine respiratory coronaviruses. J. Vet. Med. Sci. 60, 589–597. doi:10.1292/jvms.60.589

Liu SW, Zhang QX, Chen JF, Han ZX, Shao YH, Kong XG, Tong GZ (2008): Identification of the avian infectious bronchitis coronaviruses with mutations in gene 3. Gene 412, 12–25. doi:10.1016/j.gene.2008.01.004

Makino S, Keck JG, Stohlman SA, Lai MM (1986): High-frequency RNA recombination of murine coronaviruses. J. Virol. 57, 729–737. Masters PS (2006): The molecular biology of coronaviruses. Adv. Virus Res. 66, 193–292. doi:10.1016/S0065-3527(06)66005-3

McGoldrick A, Lowings JP, Paton DJ (1999): Characterisation of a recent virulent transmissible gastroenteritis virus from Britain with a deleted ORF3a. Arch. Virol. 144, 763–770. doi:10.1007/s007050050541

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

Pritchard GC (1987): Transmissible gastroenteritis in endemically infected breeding herds of pigs in East Anglia, 1981–85. Vet. Rec. 120, 226–230.

Yin JC, Ren XF, Li YJ (2005): Molecular cloning and phylogenetic analysis of ORF7 region of Chinese isolate TH-98 from transmissible gastroenteritis virus. Virus Genes 30, 395–401. doi:10.1007/s11262-004-6783-y

Saif LJ (2004): Animal coronaviruses: what can they teach us about the severe acute respiratory syndrome? Rev. Sci. Tech. 23, 643–660.

Sanchez CM, Gebauer F, Sune C, Mendez A, Dopazo J, Enjuanes L (1992): Genetic evolution and tropism of transmissible gastroenteritis coronaviruses. Virology 190, 92–105. doi:10.1016/0042-6822(92)91195-Z

Saif LJ, Sestak K (2006): Transmissible gastroenteritis virus and porcine respiratory coronavirus. In Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ (Eds.): Diseases of Swine. 9th ed. Blackwell Publishing, Iowa, pp. 489–516.

Shen S, Wen ZL, Liu DX (2003): Emergence of a coronavirus infectious bronchitis virus mutant with a truncated 3b gene: functional characterization of the 3b protein in pathogenesis and replication. Virology 311, 16–27. doi:10.1016/S0042-6822(03)00117-X

Sola I, Alonso S, Zuniga S, Balasch M, Plana-Duran J, Enjuanes L (2003): Engineering the transmissible gastroenteritis virus genome as an expression vector inducing lactogenic immunity. J. Virol. 77, 4357–4369. doi:10.1128/JVI.77.7.4357-4369.2003

Vaughn EM, Halbur PG, Paul PS (1995): Sequence comparison of porcine respiratory coronavirus isolate reveals heterogenecity in the S, 3 and 3-1 genes. J. Virol. 69, 3176–3184.

Vijgen L, Keyaerts E, Moes E, Thoelen I, Wollants F, Lemey P (2005): Complete genomic sequence of human coronavirus OC43: molecular clock analysis suggests a relatively recent zoonotic coronavirus transmission event. J. Virol. 79, 1595–1604. doi:10.1128/JVI.79.3.1595-1604.2005

Wang L, Junker D, Collisson EW (1993): Evidence of natural recombination within the S1 gene of infectious bronchitis virus. Virology 192, 710–716. doi:10.1006/viro.1993.1093

Wesley RD, Woods RD, Hill HT, Biwer JD (1990): Evidence for a porcine respiratory coronavirus, antigenically similar to transmissible gastroenteritis virus, in the United States. J. Vet. Diagn. Invest. 2, 312–317.

Wood EN, Pritchard GC, Gibson EA (1981): Transmissible gastroenteritis of pigs. Vet. Rec. 108, 41.

Woods RD (1976): Leukocyte-aggregation assay for transmissible gastroenteritis of swine. Am. J. Vet. Res. 37, 1405–1408.

Woods RD, Wesley RD (1986): Immune response in sows given transmissible gastroenteritis virus or canine coronavirus. Am. J. Vet. Res. 47, 1239–1242.

Zhang XS, Hasoksuz M, Spiro D, Halpin RS, Wang SL, Stollar S, Janies D, Hadya N, Tang YX, Ghedin E, Saif LJ (2007): Complete genomic sequences, a key residue in the spike protein and deletions in non-structural protein 3b of US strains of the virulent and attenuated coronaviruses, transmissible gastroenteritis virus and porcine respiratory coronavirus. Virology 358, 424–435. doi:10.1016/j.virol.2006.08.051