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.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Virus Research 108 (2005) 207–212
Virus Research
www.elsevier.com/locate/virusres

Short communication

Molecular analysis of S gene of spike glycoprotein of winter dysentery bovine coronavirus circulated in Korea during 2002–2003

Jae-Ho Jeong[a], Gye-Yeop Kim[b], Soon-Seek Yoon[c], Su-Jin Park[a], You-Jung Kim[a], Chang-Min Sung[a], Sung-Shik Shin[d], Bong-Joo Lee[e], Mun-Il Kang[f], Nam-Yong Park[f], Hong-Bum Koh[g], Kyoung-Oh Cho[a,*]

[a] *Laboratory of Animal Diseases, College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, South Korea*
[b] *College of Oriental Medicine, Dongshin University, Naju, Chonnam 520-714, South Korea*
[c] *Pathology Division, National Veterinary Research and Quarantine Service, Anyang, Kyunggi 430-824, South Korea*
[d] *Laboratory of Veterinary Parasitology, College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, South Korea*
[e] *Laboratory of Infectious Diseases, College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, South Korea*
[f] *Laboratory of Veterinary Pathology, College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, South Korea*
[g] *Laboratory of Veterinary Microbiology, College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, South Korea*

Received 26 April 2004; received in revised form 5 July 2004; accepted 10 July 2004
Available online 26 August 2004

Abstract

Since the molecular analysis of spike (S) glycoprotein gene of bovine coronavirus (BCoV) has been conducted and compared mainly among American and Canadian isolates and/or strains, it is unclear whether BCoV circulated in the other countries are distinctive in genetic characteristics. In the present study, we analyzed the S glycoprotein gene to characterize 10 winter dysentery (WD) coronavirus strains circulated in Korea during 2002–2003 and compared the nucleotide (nt) and deduced amino acid (aa) sequences with the other known BCoV. The phylogenetic analysis of the entire S glycoprotein gene revealed that the aa sequences of all Korean WD strains were more homologous to each other and were very closely related to respiratory bovine coronavirus (RBCV) strain OK and enteric bovine coronavirus (EBCV) strain LY-138, but were distinct from the other known BCoVs. Based on the phylogenetic analysis of the hypervariable region of the S1 subunit, all Korean WD strains clustered with the respiratory strain OK, BCQ3994 and the enteric strain LY-138, while the Canadian BCQ calf diarrhea and WD strains, and the American RBCV LSU, French EBCV F15 and avirulent VACC, L9, and Mebus strains clustered on a separate major branch. These data suggest that the WD strains circulated in Korea had a genetic property of both RBCV and EBCV and were significantly distinct from the ancestral enteric strain.

Keywords: Spike glycoprotein; Bovine coronavirus; Genetic characteristics

Bovine coronavirus (BCoV), a member of the family *Coronaviridae*, order Nidovirales (Cavanagh, 1995) is associated with severe diarrhea in newborn calves (CD), winter dysentery (WD) in adult cattle, and respiratory tract infections in calves and feedlot cattle (Cho et al., 2000; Clark, 1993; Lathrop et al., 2000; Saif et al., 1991; Storz et al., 2000). BCoV possesses a single-stranded, enveloped, non-segmented RNA genome of positive polarity (De Vries et al., 1997). The virion contains five major structural proteins: the nucleocapsid (N) protein, the transmembrane (M) protein, the hemagglutinin/esterase (HE) protein, the spike (S) protein, and the small membrane (E) protein (Lai and Cavanagh, 1997).

The variation in host range and tissue tropism of coronaviruses is largely attributable to variations in the S glycoprotein (Gallagher and Buchmeier, 2001). The BCoV had point mutations or deletions especially in the S1 subunit of S glycoprotein (Chouljenko et al., 1998; Hasoksuz et al., 2002; Gelinas et al., 2001; Rekik and Dea, 1994). Moreover,

* Corresponding author. Tel.: +82 62 530 2845; fax: +82 62 530 2809.
E-mail address: choko@chonnam.ac.kr (K.-O. Cho).

0168-1702/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2004.07.003

molecular analysis of S gene of BCoV has been conducted and compared mainly between American and Canadian isolates and/or strains (Chouljenko et al., 1998; Hasoksuz et al., 2002; Gelinas et al., 2001; Rekik and Dea, 1994). In this study, we performed molecular analysis on the S glycoprotein gene to characterize WD coronaviruses circulated in Korea during 2002–2003, compared the nucleotide, and predicted aa sequences with the other known BCoV.

Ten WD strains, KWD1–KWD10, were originally isolated in the G clone of human rectal tumor cells (HRT-18G) from fecal samples of adult dairy or beef cows, with WD in South Korea, and identified as a BCoV by immunoelectron microscopy, ELISA with BCoV-specific antisera and monoclonal antibodies, and RT-PCR specific for the part of BCoV N gene (Jeong et al., 2004). RNA was extracted from the supernatant fluids of HRT-18G cells infected with 10 KWD strains, based on the acid quanidinium–phenol–chloroform RNA extraction method as described by Cho et al. (2001a). As a negative control, RNA was extracted from mock-infected HRT-18G cells. The oligonucleotide primers used in the RT-PCR were designed from the published sequence of the S gene of the Mebus strain (GenBank accession no. U00735). The sequence of primers (positions calculated from the start codon of the S gene) are as follows: 1st upstream primer, 5′-ATGTTTTTGATACTTTTAATTTCC-3′ (1–24); 1st downstream primer, 5′-ACACCAGTAGATGGTGCTAT-3′ (901–920); 2nd upstream primer, 5′-GGGTTACACCTCT-CACTTCT-3′ (782–801); 2nd downstream primer, 5′-GCAGGACAAGTGCCTATACC-3′ (1531–1550); 3rd upstream primer, 5′-CTGTCCGTGTAAATTGGATG-3′ (1459–1478); 3rd downstream primer, 5′-TGTAGAGTAATC-CACACGT-3′ (2268–2286); 4th upstream primer, 5′-TTC ACGACAGCTGCAACCTA-3′ (2151–2170); 4th downstream primer, 5′-CCATGGTAACACCAATCC CA-3′ (3003–3022); 5th upstream primer, 5′-CCCTGT-ATTAGGTTGTTTAG-3′ (2691–2710); 5th downstream primer, 5′-ACCACTACCAGTGAACATCC-3′ (3587–3606); 6th upstream primer, 5′-GTGCAGAATGCT-CCATATGGT-3′ (3439–3459); 6th downstream primer, 5′-TTAGTCGTCATGTGATGTTT-3′ (4073–4092). A one-step RT-PCR assay was performed as described by Cho et al. (2001a,b). The RT-PCR products were purified using a GenClean II kit (BIO 101, Inc., LaJolla, CA) according to the manufacturer's instructions. The DNA sequencing was done using an automated DNA sequencer (ABI system 3700, Applied Biosystem Inc., Foster City, CA).

Using the DNA Basic module (DNAsis MAX, Alameda, CA), nucleotide sequences of our BCoV strains were first compared for the S sequence of BCoV as follows: the CD strains including Mebus (GenBank accession no. U00735), BCQ2070 (GenBank accession no. U06090), BCQ571 (GenBank accession no. U06093), BCQ20 (GenBank accession no. U06092), BCQ9 (GenBank accession no. U06091), and BCQ1523 (GenBank accession no. AAG40601); the WD strains including BCQ7373 (GenBank accession no. AAG40595) and BCQ2590 (GenBank accession no. AK01071); the RBCV strains including BCQ3994 (GenBank accession no. AAK14398), LSU (GenBank accession no. AAF25509), and OK (GenBank accession no. AAF25519), and EBCV strains including LY-138 (GenBank accession no. AAF25499) and F15 (GenBank accession no. D00731). The deduced aa sequences were then assembled and analyzed on the Amino Acid Basic module (DNAsis MAX, Alameda, CA). A sequence similarity search was performed for the BCoV S glycoprotein using the LALIGN Query program of the GENESTREAM network server at Institut de Génétique Humaine. Phylogenetic analyses were conducted using PhyloDraw program at the Graphics Application Laboratory, Pusan National University.

All of the S genes of the 10 KWD strains contained an ORF of 4092 nucleotides, encoding a predicted protein of 1363 aa residues and having a molecular weight of approximately 150 kDa. Spike protein further divided it into S1 and S2 segments of approximately 86 and 65 kDa, respectively, at the cleavage site aa 768. Among all strains analyzed, a total of 77 and 52 polymorphic nucleotides were identified in the S1 and S2 subunit of the spike glycoprotein of BCoV, respectively, compared with the Mebus strain (data not shown). These polymorphisms led to 46 and 24 aa changes at 41 and 20 distinct sites, respectively. We have shown recently that the respiratory and enteric isolates collected concurrently from two different calves in USA were significantly distinct from the ancestral enteric strain, Mebus (Hasoksuz et al., 2002). By drawing comparisons of deduced aa between our strains and other known BCoV, the two most similar sequences were those of KWD10 strain and RBCV LSU strain (96.6%) in paired comparisons, and the most distant were those of the KWD5 strain and the Mebus strain (93.4%). All the virulent-KWD strains tended to be distant from the ancestral enteric strain, Mebus, suggesting that allelic variation resulted in genetic drift over time (Hasoksuz et al., 2002).

Based on the total number of aa substitutions, a phylogenetic tree of all S gene sequences was analyzed with prototype strain Mebus, RBCV strains LSU and OK, EBCV strains F15 and LY-138, and avirulent EBCV L9 (Fig. 1). The aa sequences of 10 KWD strains were more homologous to each other and were very closely related to the virulent RBCV OK and EBCV LY-138 strains, among the other known BCoV studied in paired comparisons (Fig. 1). These data showed that the WD BCoVs circulated in Korea were genetically similar to both RBCV and EBCV. In addition, the phylogenetic comparison of Korean WD strains with other known BCoVs, on the sequences of the hypervariable region, revealed that all Korean WD strains were also clustered in groups with the virulent RBCV OK and BCQ3994 strains, and the virulent EBCV LY-139 strain (Fig. 2). The Canadian BCQ CD and WD strains, the American RBCV LSU strain, French EBCV F15 strain and avirulent VACC, L9 and Mebus strains were clustered on a separate major branch (Fig. 2). Therefore, these results indicated that the BCoV strains may be diverging from an enteric tropism to a dual (respiratory and

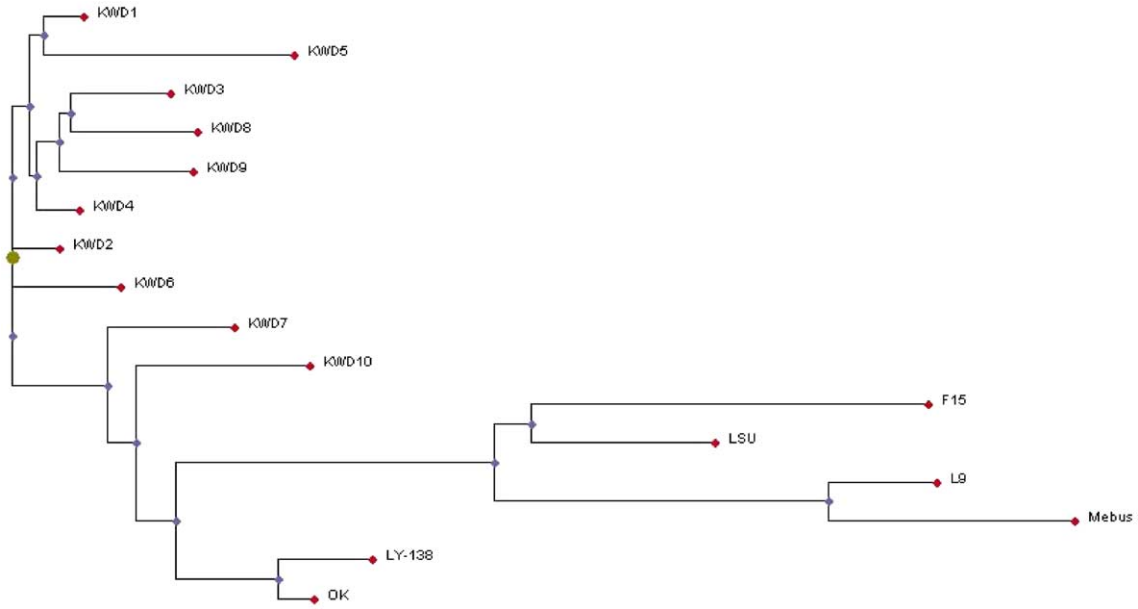

Fig. 1. The phylogenetic tree of the S gene of respiratory (RBCV), enteric (EBCV), calf diarrhea (CD), winter dysentery (WD), and avirulent strains or isolates was made using PhyloDraw at the Graphics Application Laboratory, Pusan National University. KWD1–KWD10: Korean WD strains; F15 and LY-138: EBCV strains; LSU and OK: RBCV strains; L9: avirulent vaccine strain; Mebus: prototype CD strain.

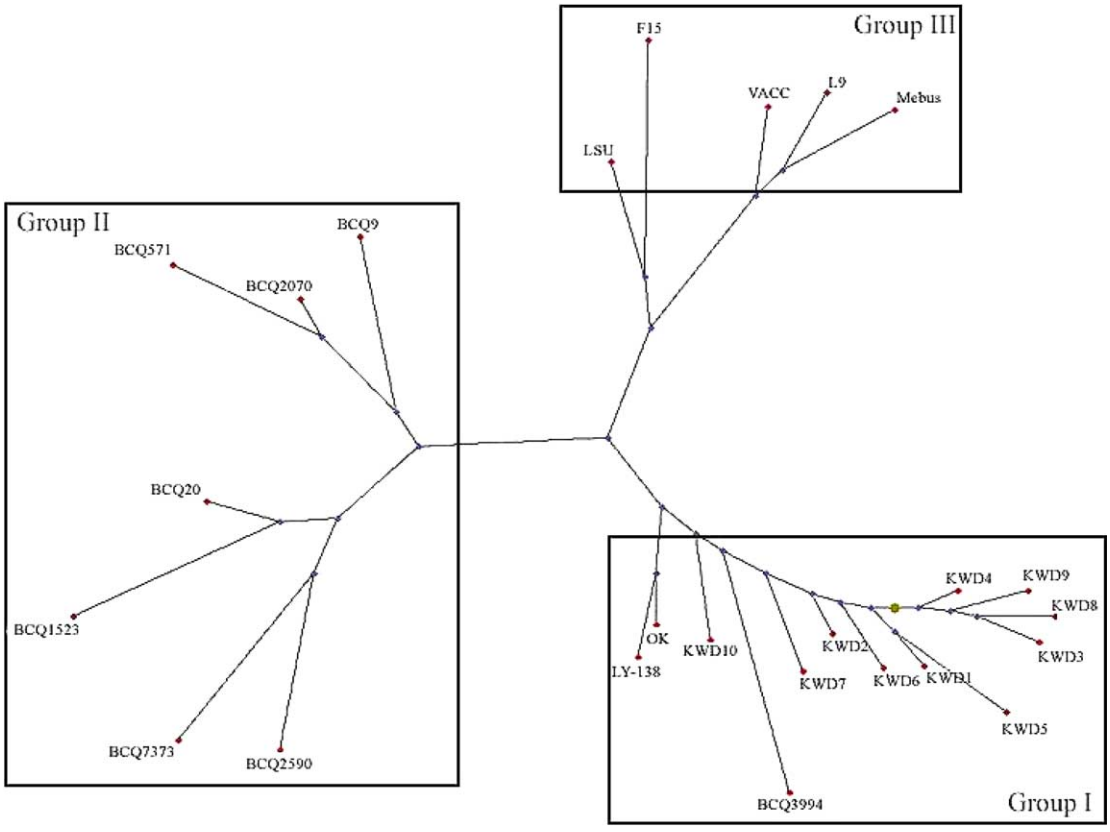

Fig. 2. The phylogenetic tree of the hypervariable region (aa 456–592; Rekik and Dea, 1994) of respiratory (RBCV), enteric (EBCV), calf diarrhea (CD), winter dysentery (WD), and avirulent strains. The CD strains including Mebus, BCQ2070, BCQ571, BCQ20, BCQ9, and BCQ1523; the WD strains including BCQ7373, BCQ2590, and KWD1-KWD10; the RBCV strains including BCQ3994, LSU, and OK; EBCV strains including LY-138 and F15. Phylogenetic analyses were conducted using PhyloDraw at the Graphics Application Laboratory, Pusan National University.

enteric) tropism over time via intermediates (Hasoksuz et al., 2002). This speculation could be supported by our previous paper (Cho et al., 2001a) in which gnotobiotic and colostrum-deprived calves were inoculated with respiratory isolates of BCoV shed viruses, both nasally and rectally. This subsequently induced diarrhea. Moreover, feedlot cattle, which had shown respiratory disease signs at arrival time, developed diarrhea 4 days after arrival, which peaked at 4–7 days post arrival (Cho et al., 2001b). Further study will be needed on the differences in pathogenicity, antigenicity, or any other physicochemical characteristics between the KWD strains.

In the S glycoprotein, the hypervariable region spanning aa 452–593 of the S1 subunit contains the S1B immunoreactive epitope which is the target for virus neutralizing monoclonal antibodies (Mabs) (Cavanagh, 1995). The hypervariable region showed varied antigenicity when reacting with Mabs directed against the S glycoprotein. Therefore, Chouljenko et al. (1998) reported that four RBCV-specific aa substitutions at aa 510, 531, 543, and 578 can be predictive and Mabs-specific strains for this region may also be distinguished among respiratory, enteric, and vaccine BCoV strains. In the present study, three aa sites including aa 510, 543, and 578 of RBCV OK and LSU strains were identical in all Korean WD strains (Fig. 3). In our previous paper (Hasoksuz et al., 2002), enteric and respiratory BCoV pairs collected concurrently from two feedlot calves in USA also had identical aa substitution sites at the same sites. Furthermore, aa 531 (N → G) substitution of RBCV OK and LSU strains compared with the Mebus strain was also detected in the CD strain BCQ 1523 (data not shown). Since these changes were observed in WD, CD, and RBCV strains, our data does not support the speculation of Chouljenko et al. (1998). In addition, aa 13 and 744 of the S1 subunit have been reported for EBCV-specific sites, which were observed in EBCV strains F15 and LY (Chouljenko et al., 1998). However, aa 13 and 744 of the S1 subunit of all Korean WD strains were identical

	11	13	33	40	100	115	118	147	149	173	179	248	465	470	510	531	543	578	617	744	769	965	984	988	1026	1030	1241	1341
F15	*	L	V	T	*	H	*	*	*	*	R	M	*	D	*	*	*	*	*	A	*	E	W	A	*	*	K	K
LY	*	L	V	T	*	N	*	*	*	*	R	M	*	D	*	*	*	*	*	A	*	E	W	A	*	*	K	K
G95	*	*	V	T	T	H	*	*	*	*	R	M	A	D	*	D	*	*	*	*	*	E	W	A	G	*	K	K
LSU	T	*	V	T	T	D	K	*	*	N	Q	M	A	D	T	G	A	S	*	*	S	E	W	A	G	*	K	K
OK	T	*	V	T	T	D	K	*	*	N	Q	M	A	D	T	G	A	S	*	*	S	E	W	A	G	*	K	K
L9	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	D	*	*	*	*	*	*	W	A	*	*	*	*
BCQ7373	T	*	V	T	T	D	*	*	*	*	Q	M	A	D	*	*	A	*	*	*								
BCQ2590	T	*	V	T	T	D	K	*	*	N	Q	M	A	D	*	*	A	*	*	*								
M	M	F	A	I	I	K	M	L	N	H	K	L	V	H	S	N	S	T	T	V	A	V	L	V	D	E	I	I
KWD1	T	*	V	T	T	N	*	F	S	N	R	M	A	D	T	D	A	S	I	*	S	E	W	A	G	*	K	K
KWD2	T	*	V	T	T	N	*	F	S	N	R	M	A	D	T	D	A	S	I	*	S	E	W	A	G	*	K	K
KWD3	*	*	V	T	T	N	*	F	S	N	R	M	A	D	T	D	A	S	I	*	S	E	W	A	G	*	K	K
KWD4	*	*	V	T	T	N	*	F	S	N	R	M	A	D	T	D	A	S	I	*	S	E	W	A	G	*	K	K
KWD5	T	*	V	T	T	N	*	F	S	N	R	M	A	D	T	D	A	S	I	*	S	E	W	A	G	*	K	K
KWD6	*	*	V	T	T	N	*	F	S	N	R	M	A	D	T	D	A	S	I	*	S	E	W	A	G	*	K	K
KWD7	*	*	V	T	T	D	*	F	S	N	R	M	A	D	T	D	A	S	I	*	S	E	W	A	G	D	K	K
KWD8	*	*	V	T	T	N	*	F	S	N	R	M	A	D	T	D	A	S	I	*	S	E	W	A	G	*	K	K
KWD9	*	*	V	T	T	N	*	F	S	N	R	M	A	D	T	D	A	S	I	*	S	Q	W	A	G	*	K	K
KWD10	T	*	V	T	T	D	*	F	S	N	Q	M	A	D	T	H	A	S	I	*	S	E	W	A	G	D	K	K

Fig. 3. Comparison of the predicted amino acid sequences of the BCoV S glycoprotein specified by different strains. Light-gray boxes contain aa sites known for respiratory bovine coronavirus-specific (Chouljenko et al., 1998), dark boxes contain virulent-specific (Chouljenko et al., 1998), and clear boxes contain Korean strains-specific aa changes.

with those of LSU and OK strains of RBCV, BCQ7373, and BCQ2590 of WD isolates, Mebus, and L9 avirulent strains (Fig. 3). In particular, aa 744, reporting its sequence in many isolates and/or strains, are the same as other known BCoV including RBCV, CD, WD, and EBCV. These results imply that these aa sites are EBCV F15 and LY strain-specific, or that there are no EBCV-specific sites in the S gene.

The N-terminal region of the S1 subunit of 10 KWD strains (aa 1–330; Laude et al., 1995), being shown to function as a receptor-binding domain in mouse hepatitis virus, had a total of 15 aa changes compared with the Mebus strain (data not shown). Amino acid substitutions in this region could alter the tropism of the coronavirus. For example, the first 200 aa of the S1 subunit of the PRCV were associated with respiratory tropism and reduced enteropathogenicity (Wesley et al., 1991). Therefore, these 15 aa substitutions in the Korean WD strains compared with that of the Mebus strain may induce either an alteration of the receptor-binding ability during viral invasion or pathogenicity to cattle.

The S protein of BCoV has a cleavage site located at aa 763–768 (KRRSRR) and is divided into the peripheral S1 glycoprotein from the transmembrane domain of the S2 glycoprotein (Abraham et al., 1990). The sequence KRRSRR at the predicted proteolytic cleavage site was identified in all KWD strains. Interestingly, all KWD strains had an Ala to Ser change at aa 769, immediately upstream of the cleavage site. Since this change was observed exclusively in the respiratory isolates LSU and OK, it was speculated that the change in aa 769 was a potential marker of respiratory tropism (Chouljenko et al., 1998). Although changes in aa surrounding the cleavage site of the HA molecule of the influenza A virus and Sendai virus have been demonstrated to modulate its cleavability (Rott et al., 1984; Hsu et al., 1987), Rekik and Dea (1994) reported that this change (769; A → S) did not appear to modulate cleavability, the rate of viral replication or the type of cytopathic changes induced in HRT-18 cells for their enteric BCoV isolates. In our previous paper (Hasoksuz et al., 2002), this change (769; A → S) was observed in both respiratory and enteric strains. Taken all together, the aa substitution (A → S) at aa 769 does not appear to be a potential marker of respiratory tropism.

Of the two cleavage products of S, the S2 subunit is highly conserved among coronaviruses (Gallagher and Buchmeier, 2001). In the present study, the aa sequences of the S2 subunit of Korean WD strains were comparatively well conserved compared with the S1 subunit of the Mebus strain. The notable aa changes were observed only in the first hydrophobic region of all KWD strains. In this region, there were four aa substitutions (aa 959, 965, 984, and 988), resulting in a marked increase of hydrophilicity in comparison with that of the Mebus strain (data not shown). Interestingly, the other known virulent BCoV, including RBCV LSU and OK strains, EBCV LY and F15 strains revealed an increase in hydrophilicity in the first hydrophobic region compared with avirulent strains Vacc, L9, and Mebus (data not shown). The membrane fusion activity is most likely conferred by the internal hydrophobic sequences within the S2 subunit (Luo and Weiss, 1998; Yoo et al., 1991). Therefore, aa substitutions inducing an increase in hydrophilicity observed in our strains and other virulent BCoV may alter the fusion activity or pathogenicity of the viruses.

Putative virulent-specific sites of BCoV were reported to exist at 10 aa sites (six within the S1 subunit and four within the S2 subunit) in the S gene through comparison with avirulent and virulent strains (Zhang et al., 1991). In the present study, the S glycoprotein of all KWD strains contained seven aa substitutions (aa 33, 40, 248, 470, 965, 1241, and 1341) which were common for all virulent strains (Fig. 3). This is in agreement with Chouljenko et al. (1998), who identified seven virulent-specific aa changes in the S gene by comparison with the RBCV, EBCV, and vaccine strains. Since these virulent-specific sites were observed in all virulent BCoV, but not in avirulent BCoV, these aa changes may affect the structure and function of the S glycoprotein and alter the pathogenetic potential of these viruses (Chouljenko et al., 1998; Zhang et al., 1991).

Acknowledgements

This study was supported by the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

References

Abraham, S., Kienzle, T.E., Lapps, W., Brian, D.A., 1990. Deduced sequence of the bovine coronavirus spike protein and identification of the internal proteolytic cleavage site. Virology 176, 296–301.

Cavanagh, D., 1995. The coronavirus surface glycoprotein. In: Siddell, S.G. (Ed.), The *Coronaviridae*. Plenum Press, New York, pp. 73–113.

Cho, K.O., Halbur, P.G., Bruna, J.D., Sorden, S.D., Yoon, K.J., Janke, B.H., Chang, K.O., Saif, L.J., 2000. Detection and isolation of coronavirus from feces of three herds of feedlot cattle during outbreaks of winter dysentery-like disease. J. Am. Vet. Med. Assoc. 217, 1191–1194.

Cho, K.O., Hasoksuz, M., Nielsen, P.R., Chang, K.O., Lathrop, S., Saif, L.J., 2001a. Cross-protection studies between respiratory and calf diarrhea and winter dysentery coronavirus strains in calves and RT-PCR and nested PCR for their detection. Arch. Virol. 146, 2401–2419.

Cho, K.O., Hoet, A., Lorech, S.C., Wittum, T.E., Saif, L.J., 2001b. Evaluation of concurrent shedding of bovine coronavirus via the respiratory tract and enteric route in feedlot cattle. Am. J. Vet. Res. 62, 1436–1441.

Chouljenko, V.N., Kousoulas, K.G., Lin, X., Storz, J., 1998. Nucleotide and predicted amino acid sequences of all genes encoded by the 3′ genomic portion (9.5 kb) of respiratory bovine coronaviruses and comparisons among respiratory and enteric coronaviruses. Virus Genes 17, 33–42.

Clark, M.A., 1993. Bovine coronavirus. Br. Vet. J. 149, 51–70.

De Vries, A.A.F., Horzinek, M.C., Rottier, P.J.M., De Groot, R.J., 1997. The genome organization of the Nidovirales: similarities and differences between Arteri-, Toro-, and Coronaviruses. Semin. Virol. 8, 33–47.

Gallagher, T.M., Buchmeier, M.J., 2001. Coronavirus spike proteins in viral entry and pathogenesis. Virology 279, 371–374.

Gelinas, A.M., Boutin, M., Sasseville, A.M.J., Dea, S., 2001. Bovine coronaviruses associated with enteric and respiratory diseases in Canadian dairy cattle display different reactivities to anti-HE monoclonal antibodies and distinct amino acid changes in their HE, S and ns4. 9 protein. Virus Res. 76, 43–57.

Hasoksuz, M., Sreevatsan, S., Cho, K.O., Hoet, A.E., Saif, L.J., 2002. Molecular analysis of the S1 subunit of the spike glycoprotein of respiratory and enteric bovine coronavirus isolates. Virus Res. 84, 101–109.

Hsu, M.C., Scheid, A., Choppin, P.W., 1987. Protease activation mutants of Sendai viruses: sequence analysis of the mRNA of the fusion protein (F) gene and direct identification of the cleavage-activation site. Virology 156, 84–90.

Jeong, J.H., Kim, G.Y., Yoon, S.S., Park, S.J., Kim, Y.J., Sung, C.M., Shin, S.S., Koh, H.B., Lee, B.J., Lee, C.Y., Kang, M.I., Kim, H.J., Park, N.Y., Cho, K.O., 2004. Detection and isolation of winter dysentery bovine coronavirus circulated in Korea during 2002–2004. J. Vet. Med. Sci., in press.

Lai, M.M.C., Cavanagh, D., 1997. The molecular biology of coronaviruses. Adv. Virus Res. 48, 1–100.

Lathrop, S.L., Wittum, T.E., Loerch, S.C., Saif, L.J., 2000. Antibody titers against bovine coronavirus and shedding of the virus via the respiratory tract in feedlot cattle. Am. J. Vet. Res. 61, 1057–1061.

Laude, H., Talbot, P.J., Levy, G.A., 1995. Corona and Related Virus: Funtional Domains in the Spike Protein of Transmissible Gastroenteritis Virus. Plenum Press, New York, pp. 299–394.

Luo, Z., Weiss, S., 1998. Roles in cell-to-cell fusion of two conserved hydrophobic regions in the murine coronavirus spike protein. Virology 244, 483–494.

Rekik, M.R., Dea, S., 1994. Comparative sequence analysis of a polymorphic region of the spike glycoprotein S1 subunit of enteric bovine coronavirus isolates. Arch. Virol. 135, 319–331.

Rott, R., Orlich, M., Klenk, H.D., Wang, M.L., Skehel, J.J., Wiley, D.C., 1984. Studies on the adaptation of influenza viruses to MDCK cells. EMBO J. 3, 3329–3332.

Saif, L.J., Brock, K.V., Redman, D.R., Kohler, E.M., 1991. Winter dysentery in dairy herds: electron microscopic and serological evidence for an association with coronavirus infection. Vet. Rec. 128, 447–449.

Storz, J., Purdy, W., Lin, X., Burrell, M., Truax, R.E., Briggs, R.E., Frank, G.H., Loan, R.W., 2000. Isolation of respiratory bovine coronavirus, other cytocidal viruses, and *Pasteurella* spp. from cattle involved in two natural outbreaks of shipping fever. J. Am. Vet. Med. Assoc. 216, 1539–1604.

Wesley, R.D., Woods, R.D., Cheung, A.K., 1991. Genetic analysis of porcine respiratory coronavirus, an attenuated variant of transmissible gastroenteritis virus. J. Virol. 65, 3369–3373.

Yoo, D., Parker, M.D., Babiuk, L.A., 1991. The S2 subunit of the spike glycoprotein of bovine coronavirus mediates membrane fusion in insect cells. Virology 180, 395–399.

Zhang, X., Kousoulas, K.G., Storz, J., 1991. Comparison of the nucleotide and deduced amino acid sequences of the S genes specified by virulent strains of bovine coronaviruses. Virology 183, 397–404.