Comparison of the S genes and the biological properties of respiratory and enteropathogenic bovine coronaviruses

Brief Report

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Summary. The nucleotide sequence of the S gene of the bovine respiratory coronavirus (BRCV) strain G95, which was isolated from nasal swabs of a calf suffering from respiratory disorders, was determined and compared with the S gene of the enteropathogenic bovine coronavirus (BECV) strain LY138. Sequence analysis revealed 98.7% nucleotide and 98.3% deduced amino acid identities between the S genes of BRCV-G95 and BECV-LY138 without any deletions or insertions. Nucleotide substitutions were distributed randomly throughout the gene. Five monoclonal antibodies specific for the S protein distinguished BRCV-G95 from BECV-L9, but failed to differentiate it from BECV-LY138 in Western blots under denatured and native conditions. BRCV-G95 induced cytopathic changes in cell cultures that were similar to BECV-LY138 but different from BECV-L9. These results suggest that strain BRCV-G95 is more closely related to the virulent strain BECV-LY138 than to the avirulent, cell culture-adapted strain BECV-L9.

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Bovine coronavirus (BCV) causes severe enteritis of newborn calves [3, 11, 12], and it is also recognized as an etiological factor of respiratory disease of calves [5, 6, 9, 13, 15]. Comparative studies revealed that coronavirus isolates from the intestinal or respiratory tracts of calves both replicated in intestinal and upper respiratory cells of gnotobiotic calves [17, 18]. Serological tests failed to distinguish between bovine respiratory coronavirus (BRCV) and entero-pathogenic bovine coronavirus (BECV). A porcine respiratory coronavirus (PRCV) had a deletion in the S gene when compared with the transmissible

gastroenteritis virus. This deletion was considered most likely responsible for the differences in tissue tropism and pathogenicity of the two viral strains [2, 16].

BCV possesses a single-stranded, nonsegmented RNA genome of positive polarity [22]. The virion contains four major structural proteins: the nucleocapsid protein (N), the transmembrane protein (M), the hemagglutinin esterase protein (HE) and the spike protein (S) [1]. The S glycoprotein is the predominant peplomere responsible for the characteristic coronavirus morphology. It is synthesized as a high molecular weight precursor (190 kDa) which is cleaved to yield two comigrating subunit polypeptides: the N-terminal half (S1) and the C-terminal half (S2) with approximate molecular weights of 100–110 kDa [23, 24]. The S glycoprotein functions in virus attachment to permissive cells, virus-induced cell fusion, and elicitation of neutralizing antibodies [22].

The BRCV strain Giessen 89-4595-(BRCV-G95) was originally isolated in human rectal tumor (HRT-18) cells [25] from nasal swabs of a calf affected with respiratory disease, and tentatively identified as a BCV by immunoelectron microscopy and inhibition of hemagglutination with BCV-specific antisera. We report here the comparative analysis of biological and antigenic properties as well as the S gene sequences of the BRCV-G95 and of BECV.

Viral RNA was isolated from BRCV-infected HRT-18 cells using isothiocyanate/cesium chloride gradients [10, 26]. cDNA synthesis, PCR amplification and single-stranded cDNA synthesis were performed as reported [19, 26]. Nucleotide sequences of the complete S gene of the BRCV-G95 were determined from PCR-products in both directions, and were analyzed as described in the legend of Fig. 1.

An open reading frame (ORF) of 4092 nucleotides encoding a protein of 1363 amino acids was identified for BRCV-G95. This ORF has the same size as the S gene of BECV-LY138 [26]. Differences between the S gene sequences of

Position	55	57	111	124	222	281	317	356	361	423	459	523	544	555	559	829	834	1009	1041	1044	1119	1155	1412	1514	1519	1544	1590	1609	1659	1680	1729	1789	1841	1938	2046	2106	2205	2249	2448	2468	2744	2797	2811	2824	2889	2904	2943	2956	2985	3045	3090	3095	3252	3282	3294	3342	3603	3693	3824	3825	3964
BRCV-G95	T	C	T	A	T	G	C	C	C	T	G	A	C	A	G	C	A	G	G	T	C	T	C	C	C	C	C	C	G	C	C	C	G	G	T	T	C	C	T	C	C	G	G	C	T	G	G	G	T	T	G	T	G	G	T	C	C	T	T	A	C	T
BECV-LY138	C	T	C	G	C	C	T	T	A	C	A	C	T	G	A	T	C	T	T	G	T	A	T	G	T	A	T	A	T	T	T	T	A	A	C	T	T	C	T	T	A	A	T	A	A	A	C	C	C	T	C	A	T	C	T	T	C	C	C	A	C

BRCV-G95: F - I - R T T H - - N - - V - - G - - - - A T P T - D - - H E G - - - - V - T R A - Y - - - - - - - G - - - - - - N F
BECV-LY138: L V T I I N H I C V S S N N Y D D A I K S N D T L

Fig. 1. Comparison of nucleotides and deduced amino acids of the S genes between BRCV-G95 and BECV-LY138. DNA sequencing was carried out with the modified dideoxynucleotide chain termination procedure [19] using Sequenase (USB, Cleveland, OH, U.S.A). Sequence data were analyzed with the aid of the Sequence Analysis Software Package of the Genetics Computer Group of the University of Wisconsin and the MacVector Software (IBI, New Haven, CT, U.S.A). The numbers at the top represent the position of the nucleotide changes in reference to the S gene sequence of BECV-LY138 [23]. The nucleotide sequences of the S gene of BRCV-G95 identical to the S gene of BECV-LY138 are not shown and the complete sequence is deposited in the GenBank/EMBL under accession number M80844. The changes of deduced amino acids are indicated below the nucleotides for the strains listed on the left

BRCV-G95 and BECV-LY138 consisted of randomly distributed point muta-
tions without any deletions or insertions. The nucleotides and deduced amino
acids of the S genes of BRCV-G95, which differ from those of BECV-LY138, are
presented in Fig. 1. Sixty-one nucleotides differed between the S genes of
BRCV-G95 and BECV-LY138, and 34 of these did not result in any amino acid
changes, whereas 27 nucleotide changes coded for 25 different amino acids. The
degree of nucleotide and amino acid identity was 98.7 and 98.3%, respectively.
The predicted S protein of BRCV-G95 had one N-linked glycosylation site and
one cysteine residue less than the S of BECV-LY138. The putative fusogenic
domain and proteolytic cleavage site of the S protein were conserved.

The antigenicity of BRCV-G95 and BECV was compared in Western blots
under denatured and native conditions using a panel of monoclonal antibodies
(MAbs) specific for the S protein (#34B8, #43C2, #43F6, #44, #38, #31) and the
N protein (#36, #46). The characteristics of these MAbs and the procedures for
Western blots were described [8]. As shown in Table 1, MAbs #34B8, 43C2 and
43F6 reacted with gp100 of BRCV-G95, BECV-LY138 and BECV-L9, while
MAbs #44, 38 and 31 reacted with gp 100 of BECV-L9 but not BECV-LY138
and BRCV-G95 in native Western blots. These MAbs did not react with S of
the three viruses under denatured Western conditions, except that MAb 34B8
reacted weakly with BRCV-G95. Two N-specific MAbs reacted with N of all
three viruses under denatured and native conditions.

Biological properties such as cytopathic changes with cell fusion, hemagglu-
tination, and acetylesterase activities of BRCV- G95 were compared with those
of BECV-LY138 and BECV-L9 (Table 2). All three viral strains agglutinated
chicken and mouse erythrocytes. BRCV-G95 and BECV-LY138 agglutinated
mouse erythrocytes at 32-fold higher titers than chicken erythrocytes, while
BECV-L9 had only a 4-fold higher titer. Viral acetylesterase activity was
determined by the amount of acetate released from bovine submaxillary mucin

Table 1. Antigenic reactivities of BRCV-G95 and BECV with monoclonal antibodies in
Western blots

Monoclonal antibodies		Denatured western			Native western		
		BRCV	LY-138	L9	BRCV	LY-138	L9
Anti-S	34B8	+	−	−	+	+	+
	43C2	−	−	−	+	+	+
	443F6	−	−	−	+	+	+
	44	−	−	−	−	−	+
	38	−	−	−	−	−	+
	31	−	−	−	−	−	+
Anti-N	36	+	+	+	+	+	+
	46	+	+	+	+	+	+

Table 2. Comparison of biological properties between BRCV-G95 and BECV

Virus	Cytopathic effect				Hemagglutination titer[a]		Acetylesterase activity
	HRT-18	GBK	D2BFS	MDBK	Chicken-RBC	Mouse-RBC	(μg acetate\μl[b])
BRCV-G95	+ +	–	–	–	64	1024	2.02
BECV-LY138	+ +	–	–	—	256	8192	2.86
BECV-L9	+ + +	+ +	+ +	+ +	1024	4096	2.15

HRT-18 Human rectal tumor; *GBK* Georgia bovine kidney; *D2BFS* bovine fetal spleen, clone D2; *MDBK* Madin-Darby bovine kidney

[a] Reciprocal value of highest dilution with complete hemagglutination (HA) by 50 μl of virus preparation in the presence of chicken or mouse red blood cells (*RBC*)

[b] The acetylesterase activity was determined by releasing acetate from substrate bovine submaxillary mucin (BSM) and expressed as μg acetate per μl purified virus preparation as described previously [7, 27]

according to the method described [7]. Acetylesterase activities of these strains were similar, and ranged from 2.02 to 2.86 μg acetate per microliter of purified virus preparation.

BECV-L9 induced cytopathic changes and cell fusion in HRT-18, bovine fetal spleen (D2BFS), Georgia bovine kidney (GBK) and Madin Darby bovine kidney (MDBK) cells while BRCV-G95 and BECV-LY138 induced these changes only in HRT-18 cells (Table 2). The ability of BRCV to replicate in HRT-18 cells which possess the properties of intestinal epithelial cells, indicates this virus may be able to infect both respiratory and intestinal sites of calves. Coronaviruses were frequently isolated from the respiratory as well as intestinal samples of calves with pneumoenteritis when HRT-18 cells were employed (Herbst et al., unpubl. res.) indicating that HRT-18 cell cultures should be included in routine diagnostic viral isolation attempts from respiratory disease of cattle. Comparatively, the porcine respiratory coronavirus replicated in the intestine but at lower titers and without causing enteritis [2, 14].

The envelope proteins of coronaviruses play a major role in interactions with cellular receptors and in infection. A small deletion in the amino terminal of the S protein of the mouse hepatitis virus and PRCV altered their cell tropism and pathogenicity reflecting the function of the S protein in tissue-tropism [4, 16]. Sequence analysis of the BRCV-G95 did not reveal any deletions or insertions in the S gene when compared with BECV strains (Fig. 1). Sixty-one single nucleotide changes occurred in the S genes between BRCV-G95 and BECV. These mutations were found throughout the genes in a random fashion and caused 25 amino acid changes. It is not clear at this point whether these single mutations in the peplomeric proteins contribute to a difference in the pathogenicity of BRCV-G95 and BECV-LY138. Interestingly, the S and the HE glycoproteins of BCV recognized sialic acid-containing receptors [20, 21]. The location of the receptor-binding domains on the S protein of BCV is not known. Whether the single amino acid changes occurring in the S and HE proteins are located within domains involved in virus recognition of cellular receptor determinants remains to be elucidated. The results suggest that the respiratory

isolate of bovine coronavirus BRCV-G95 should be considered as a member of the hemagglutinating BCV group rather than as a separate BRCV group.

Acknowledgements

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