



## Sequence Comparison of Avian Infectious Bronchitis Virus S1 Glycoproteins of the Florida Serotype and Five Variant Isolates from Georgia and California

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**Abstract.** The infectious bronchitis virus (IBV) spike glycoprotein S1 subunit is required to initiate infection and contains virus-neutralizing and serotype-specific epitope(s). Reported are the S1 gene nucleotide and predicted amino acid sequences for the Florida 18288 strain and isolates GA-92, CV-56b, CV-9437, CV-1686, and 1013. These sequences were compared with previously published gene sequences of IBV strains, and phylogenetic relationships are reported. The S1 amino acid sequence of Florida 18288 was 94.9% similar to the Connecticut strain, and GA-92 was 92.8% similar to the Arkansas 99 strain. S1 amino acid sequences of the California variants, CV-56b, CV-9437, and CV-1686, were 97.6–99.3% similar to one another and only 76.6%–76.8% similar to the Arkansas-type strains. Isolate 1013, also from California, was 84.0% similar to Ark DPI and 77.9% similar to CV-56b. When comparing 19 viruses isolated from the United States, sequence variations were observed between amino acids 55–96, 115–149, 255–309, and 378–395. Similar regions are reported to be involved in virus-neutralizing and/or serotype-specific epitopes. These data demonstrate that variant IBV strains continue to emerge, and unique variants may circulate among poultry in geographically isolated areas.

**Key words:** infectious bronchitis virus, spike glycoprotein, coronavirus, phylogenetic analysis

### Introduction

Avian infectious bronchitis virus (IBV) causes an economically important upper respiratory tract disease in chickens that results in reduced weight gains and reduction of egg production. Infectious bronchitis is complicated by secondary bacterial infections such as *Mycoplasma* sp. and *Escherichia coli* that may increase mortality (1). Vaccination for infectious bronchitis is hindered because monovalent IBV vaccines do not provide complete protection against heterologous serotypes (2,3).

Infectious bronchitis virus, a member of the

Coronaviridae family, contains four structural proteins. These are the small membrane protein, the integral membrane glycoprotein, the phosphorylated nucleocapsid protein, and the spike (S) glycoprotein (4,5). The S glycoprotein is cleaved posttranslationally by cellular proteases into the S1 and S2 subunits (6). The globular S1 subunit forms the tip of the spike, extending outwardly, whereas S2 anchors S1 to the viral membrane (7).

The S1 subunit is involved with infectivity and contains virus-neutralizing epitopes, serotype-specific sequences, and hemagglutinin activity (7–11). Different serotypes and subtypes of IBV are thought to be generated by nucleotide insertions, deletions, or point mutations in the S1 subunit made by the viral polymerase (12). Another mechanism for variability in the S1 subunit may be RNA recombination (13–16). Consequently, changes in the S1 subunit

The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession numbers 1013 = AF027508, CV-9437 = AF027510, CV-1686 = AF027511, CV56-b = AF027509, FL18288 = AF027512.

can lead to emergence and proliferation of variant serotypes.

Sequence variations in S1 subunits have been identified among European IBV strains and within the Massachusetts (Mass) serotype. These are designated hypervariable region (HVR) I (residues 56–69) and HVR II (residues 117–131) (17,18). Isolates from chickens in the United States (US), other than the Mass serotype, have similar highly variable regions in the S1 subunit between residues 53–148 (15). Other variable regions have been detected between residues 250–310 for the strains H120, D207, D1466, V1397, Mass 41, Mass 42, and residues 269–365 (including the signal sequence) in the strains 6/82, 123/82, and 167/82 (12,19). These variable regions (HVR I, HVR II, and residues 250–365) are similar to sequence regions designated I, II, and III associated with three virus-neutralizing epitopes determined by selection of monoclonal antibody (Mab) neutralization-resistant (NR) mutants (20). HVR I was associated with a virus-neutralizing epitope and a hemagglutinin, whereas amino acids 250–386 are associated with a serotype-specific epitope (17,21).

The objectives of this investigation were to sequence the S1 gene of the Florida 18288 strain and variant IBV isolates GA-92, CV-56b, CV-9437, CV-1686, and 1013. Sequence comparison and phylogenetic analysis were used to determine the relationship of these viruses with other USA isolates of IBV and to further identify variable regions that may be virus-neutralizing epitopes on the S1 glycoprotein subunit.

## Materials and Methods

### *Viruses and Histories*

The S1 nucleotide and predicted amino acids sequences were determined from these viruses isolated in the United States; Florida 18288 (FL-18288), Georgia isolate 1992 (GA-92), and isolates from California CV-56b, CV-9437, CV-1686, and 1013.

FL-18288 strain, representing the Florida serotype, was isolated from layers in 1972, and was obtained from Dr. Pedro Villegas at the University of Georgia (22). GA-92 (Arkansas serotype) was isolated in 1992 at the Poultry Diagnostic and Research Center (PDRC) from a broiler flock in Georgia exhibiting

respiratory distress (23). All isolates from California were from chickens showing respiratory distress. CV-56b (isolated in 1991 from broilers), CV-1686 (isolated in 1995 from broilers), and 1013 (isolated in 1995 from silkies) were obtained from the California Veterinary Diagnostic Laboratory System (Fresno, CA), whereas CV-9437 (isolated in 1995 from broilers) was submitted to PDRC. Isolation of IBV strains from California started in 1984 and are referred to as California variants. California variants in this study are represented by CV-56b. Data from serum neutralization studies suggest that these variant strains may compose a new serotype (23,24).

### *RNA Isolation and RT-PCR*

All procedures for RNA isolation and reverse transcriptase-polymerase chain reaction (RT-PCR) were previously described (25). Briefly, RNA was isolated from IBV infective allantoic fluids after treatment with Proteinase K (250 µg/ml) and SDS (final 2% solution wt/wt), with incubation at 55°C for 5 min. Phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1) extractions were completed, and RNA was isolated from infective allantoic fluids using the RNAid Kit (Bio 101). Primers MIBVPCR and NIBVPCR were used to amplify a conserved region between the membrane and nucleocapsid genes for identification of IBV (26,27). The S1oligo5' (5'-TGAAACTGAACAA-AAGAC-3') and S1oligo3' (5'-CATAACTAACAT-AAGGGCAA-3') primers were used to amplify S1 gene as previously described (25,28). The S1 gene amplification product was used for subsequent restriction fragment length polymorphism (RFLP) or DNA sequencing.

### *Restriction Fragment Length Polymorphism Analysis*

Amplification products were excised from an agarose gel and purified using GeneClean (Bio 101). The restriction endonucleases, *Bst* YI, *Hae* III, and *Xcm* I (New England Biolabs) were used to digest the S1 gene amplification product as previously described (25). RFLPS were determined following agarose gel electrophoresis (25).

### DNA Sequencing and Sequence Analysis

The S1 gene polymerase chain reaction (PCR) products for FL-18288, GA-92, and CV-9437 were cloned into the TA cloning vector (Invitrogen Corp.), and ligated areas of the plasmids were sequenced with the M13 universal forward and reverse primers (Molecular Genetics Instrumentation Facility, University of Georgia). After initial sequence data was obtained from plasmid DNA, the S1 gene for each sample was amplified by RT-PCR in six or more tubes then combined and used as template for subsequent sequencing. Sequencing primers to various regions within the S1 gene for FL-18288, GA-92, CV-56b, CV-9437, CV-1686, and 1013 were designed using OLIGO™ version 4.0 software (National Biosciences, Inc.) and are available upon request.

Double-stranded DNA sequencing was conducted using automated sequencing with the Prism™ DyeDeoxy terminator cycle sequencing kit as recommended by the manufacturer (Perkin Elmer; 29,30). All samples were purified for DNA sequencing using Microcon™ 30 columns (Amicon, Inc.). Automated sequencing was conducted at the USDA Southeastern Poultry Research Laboratory (Athens, GA).

The nucleotide sequence of the S1 gene from the ATG start site to the cleavage site for the FL-18288, GA-92, CV-56b, CV-9437, CV-1686, and 1013 were compared with published sequences from Beaudette (accession # X02342), Mass 41 (accession # X04722), Connecticut (Conn) (accession # L18990), Arkansas (Ark) 99 (accession # M85244), Ark DPI (accession # AF006624), CU-T2 (accession # U04739), Pp14 (accession # M99483), Se17 (accession # M99484), Iowa 609 (Dr. Collisson, Texas A & M U., College Station, TX), Holte (accession # L18988), JMK (accession # L14070), Gray (accession # L14069), and De-072 (accession # U90751) (15,16,34–36). Alignments and phylogenetic analysis were conducted using CLUSTAL V method in MegAlign software version 1.03 (DNASar Inc., Madison, WI). This method uses a multiple alignment algorithm, and unweighted pair group method with arithmetic mean algorithm (UPGMA) to derive a preliminary phylogeny (31,32). The final phylogeny is produced by applying the neighbor-joining method (33). The CLUSTAL V method was compared with UPGMA with MacDNASIS ProV3.0 software (Hitachi Software Engineering Co., Ltd) and IntelliGenetics GeneWorks 2.45™ (IntelliGenetics, Inc. Mountain View, CA).

### Results

#### *IBV Isolate Identification and RFLP Analysis of Amplification Products*

Reverse-transcriptase PCR in conjunction with RFLP were conducted for initial characterization of IBV strain FL-18288 and isolates, GA-92, CV-56b, CV-9437, CV-1686, and 1013 (25). All samples were identified as IBV by the amplification of a 1.02 kb PCR product with the MIBVPCR and NIBVPCR primers (data not shown). The S1 gene PCR product (1.7kb) was subsequently amplified with the S1oligo5' and S1oligo3' primers for all isolates (data not shown).

Using the restriction enzymes *Bst* YI, *Hae* III, and *Xcm* I, the RFLP pattern for amplification products from FL-18288 corresponded to a pattern reported by Kwon *et al.* (25). The RFLP patterns for CV-9437 and CV-1686 amplification products were identical to one another and to patterns for other California variants represented by CV-56b. However, the RFLP pattern for GA-92 and 1013 differed from one another and from other IBV strains using the restriction enzyme, *Bst* YI (Fig. 1).

#### *S1 Nucleotide Sequence Comparison among IBV Isolates*

The S1 nucleotide sequence alignments and pair distances data are presented in Fig. 2 and Table 1 respectively. The S1 gene nucleotide sequence of FL-18288 was most similar to the Conn strain (97.3%). Isolates CV-9437, CV-1686, and CV-56b had S1 genes most similar (98.7%–99.7%) to one another, and 78.5% to 79.0% similar to Ark 99. The IBV isolate 1013 was most similar to Ark 99 (84.7%) and Ark DPI (85.1%), whereas GA-92 was most similar to Ark 99 (95.3%). All methods used to create phylogenetic trees produced similar dendrograms when comparing S1 genes and had similar topology to the trees based on S1 amino acid sequences as illustrated in Fig. 3.

#### *S1 Amino Acid Sequence Comparison*

A dendrogram was generated following alignment of the S1 glycoprotein sequences to determine phylogenetic relationships among IBV strains in the United States (Fig. 3). Similar topologies were obtained when

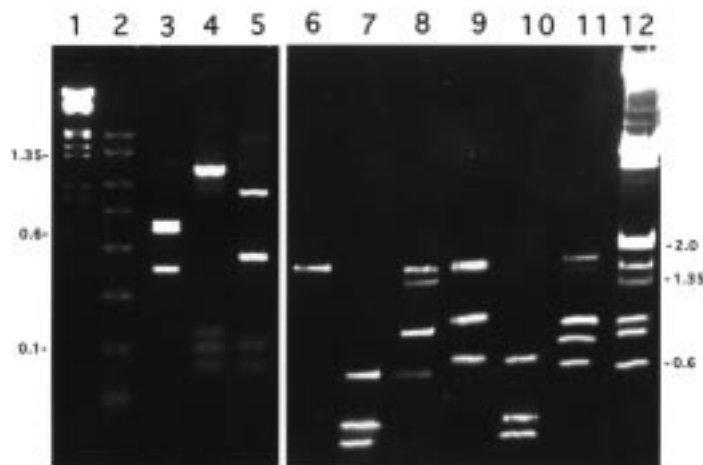


Fig. 1. Variation of infectious bronchitis virus S1 genes demonstrated by RFLP of RT-PCR amplification products using viral genomic RNA as a template. Lane 3 = GA-92 cut with *Bst* YI; Lane 4 = GA-92 cut with *Hae* III; Lane 5 = GA-92 cut with *Xcm* I; Lane 6 = CV-9437 cut with *Bst* YI; Lane 7 = CV-9437 cut with *Hae* III; Lane 8 = CV-9437 cut with *Xcm* I; Lane 9 = 1013 cut with *Bst* YI; Lane 10 = 1013 cut with *Hae* III; Lane 11 = 1013 cut with *Xcm* I. Numbers at the left and right are molecular-weight markers in kilobase pairs. Lanes 1 and 12 = molecular-weight marker, lambda cut with *Eco* RI and *Hind* III giving the fragments of the following sizes: 25.7 kb, 4.3 kb, 2.0 kb, 1.65 kb, 1.35 kb, 0.95 kb, 0.90 kb, and 0.60 kb; Lane 2 = molecular-weight marker (BioVenture, Inc., Murfreesboro, Tenn.).

generating phylogenetic trees using UPGMA, PAUP, and the neighbor-joining method. The S1 amino acid sequence alignments and pair distances data are presented in Fig. 4 and Table 2 respectively. The

FL-18288 strain S1 protein sequence was 94.9% similar to that of the Conn strain. California variants, CV-56b, CV-9437, and CV-1686, were 97.6% to 99.3% similar to one another, and 76.6% to 76.8%

Table 1. S1 nucleic acid sequence alignment pair distances

		Per cent Similarity																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		
1	■	69.9	71.3	71.8	70.0	71.8	93.1	94.8	97.3	70.5	76.0	71.1	75.3	77.6	77.0	75.6	78.6	78.9	49.2	1	FL-18288
2	24.0	■	76.0	76.3	81.8	76.1	74.5	74.4	72.7	95.3	93.2	93.6	92.5	81.9	76.9	76.3	78.3	78.1	45.9	2	GA-92
3	23.1	23.3	■	98.7	81.1	98.8	76.1	75.8	73.9	78.5	78.6	78.1	77.2	76.1	77.1	74.5	75.4	75.5	46.4	3	CV-56b
4	22.7	23.0	1.2	■	81.3	99.7	76.3	75.9	74.3	79.0	79.0	78.5	77.7	76.5	77.2	74.8	75.5	75.9	46.7	4	CV-1686
5	24.3	16.9	18.0	17.8	■	81.1	76.6	75.3	72.7	84.7	85.1	84.2	82.1	80.4	78.3	77.2	78.2	78.3	46.0	5	1013
6	22.8	23.2	1.1	0.3	18.1	■	76.1	75.7	74.1	78.8	78.8	78.4	77.5	76.2	77.0	74.7	75.3	75.7	46.3	6	CV-9437
7	5.4	23.8	23.0	22.9	23.2	23.1	■	97.7	94.1	75.0	78.6	76.9	77.5	78.5	76.4	80.7	79.7	49.2	7	Beaudette	
8	4.5	23.7	22.9	22.9	23.2	23.0	2.2	■	94.9	74.7	78.3	76.8	77.7	79.6	78.5	76.2	80.6	79.8	52.1	8	Mass 41
9	1.2	24.7	24.2	23.7	25.0	23.9	5.2	4.3	■	72.9	76.8	75.2	76.7	78.4	76.5	75.4	78.0	78.2	51.9	9	Conn
10	23.9	4.5	20.3	20.0	14.2	20.2	23.6	23.3	24.6	■	96.1	96.6	94.3	84.4	79.0	79.0	79.5	79.6	47.3	10	Ark 99
11	21.6	6.0	20.6	20.2	14.1	20.5	21.3	21.1	22.4	3.3	■	95.4	92.6	85.4	79.5	80.0	82.3	81.9	48.0	11	Ark DPI
12	23.3	5.3	20.6	20.6	14.6	20.8	22.9	22.9	23.6	2.7	4.1	■	93.7	84.3	79.1	79.2	79.7	79.6	48.2	12	CU-T2
13	22.2	7.1	21.6	21.2	17.5	21.5	22.1	21.7	22.3	5.3	7.0	6.0	■	83.3	79.0	77.9	80.2	80.1	47.9	13	PP14
14	19.9	18.6	23.9	23.7	19.5	24.0	20.3	19.7	20.1	15.8	15.0	15.9	17.4	■	80.9	79.7	80.6	85.8	45.8	14	SE17
15	21.2	23.9	22.5	22.5	21.9	22.7	21.4	21.1	21.7	21.9	21.5	21.8	22.2	20.6	■	79.0	80.1	79.8	47.7	15	Iowa 609
16	23.6	22.5	24.2	23.7	22.2	23.9	23.5	23.4	24.3	20.2	19.1	20.2	21.8	19.7	20.8	■	77.6	78.9	47.4	16	Holte
17	18.8	21.8	23.8	23.7	21.4	24.0	18.7	18.5	19.6	20.7	17.9	20.6	20.3	14.0	21.1	20.7	■	98.8	46.2	17	JMK
18	19.0	21.9	24.0	23.8	21.6	24.1	18.5	18.4	19.6	20.6	18.4	20.8	20.3	13.9	21.3	20.6	1.1	■	46.2	18	Gray
19	51.2	53.1	53.4	53.3	55.0	35.8	51.0	51.7	51.3	51.4	51.0	51.1	50.8	51.6	54.4	53.3	54.4	55.0	■	19	De-072
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		



ACTGCTTCAAGTTGTA CTGCTGGTGCTATTGGTTGTAGTAAGAATTTT AGTGCTGCTTCT Majority

172 - - - - - A G . C A G . . . . T . C T . . . . A T . . . . . G . . G A . C G T G T . G . . . A . . . . T . . . . . Fl-18233  
 181 . . . . . C . . . . . C . . . . . C . . . . . C . A C . . . . . A . . . . . C . C . . . . . G . . . . . C . . . . . A  
 172 T . . . . . A C . . . . . C . . . . . C . . . . . T C . . T . . . . . A . . . . . A . . . . . A . . . . . CV-56b  
 172 T . . . . . A C . . . . . C . . . . . C . . . . . T C . . T . . . . . A . . . . . A . . . . . A . . . . . CV-1656  
 181 G G A . . . . C . . . . . C . . . . . C . . . . . C . A C . . . . . A . . . . . C . . . . . G . . . . . A . . . . . 1013  
 172 T . . . . . A C . . . . . C . . . . . C . . . . . T C . . T . . . . . A . . . . . A . . . . . A . . . . . CV-9437  
 181 T . . . . . T A . . . . . G . . . . . . . . . . . C A . Beaudette  
 181 T . . . . . T A C . T G . . . . . . . . . . . C A . Mass 41  
 172 - - - T T A . . . . G . Conn  
 181 . . . . . C C . . . . . C . . . . . C . . . . . C . A C . . . . . C . . . . . C . . . . . G . . . . . C . . . . . A Ark 99  
 181 . . . . . C C . . . . . C . . . . . C . . . . . C . A C . . . . . C . C . . . . . G . . . . . C . . . . . A Ark DPI  
 181 . . . . . T C C A . . . . . C . . . . . C . . . . . C . A C . . . . . C . . . . . C . . . . . G . . . . . A CU-T2  
 181 . . . . . C T . C C A . . . . . C . . . . . C . . . . . C . A C . . . . . C . . . . . C . . . . . G . . . . . A PP14  
 181 C A . . . . . A C A G G . . . . . C G . . . . . C . . . . . C . A C . . . . . C . . . . . C . . . . . A SE17  
 181 . . . . . G A A . . . . C . . . . . A . . . . . A . . . . . C . . . . . G . . . . . A . . . . . C . . . . . A . . . . . Iowa 609  
 181 . . . . . A . . . . . G . . . . . C T . . . . . A . . . . . A . . . . . G . . . . . G . . . . . C . . . . . T C . . . . . Holte  
 181 G G C A A . . . . T G . . . . . G . G . . . . C . . . . . T T . . . . G . C . . . . C . . . . . C . . . . . JMK  
 181 G G C A A . . . . T G . . . . . G . G . . . . C . . . . . T T . . . . G . C . . . . C . . . . . C . . . . . Gray  
 175 C A A . . . . . - - - - - . . . . . A . . . . . A . . . . . G . . . . . A . . . . . G T . . . . C . A . . . . A . A G . . . . De-072

GTAGCCATGACTGCACCAACCAAGTGGTATGTCATGGTCTACCAACAATTTTGTACGGCT Majority

226 A . . . . . T . . . . . G . . . . . G . A . C C A . . . . . A A T . . . . . G . . G C . . T . . . . . T . . . . . A Fl-18233  
 241 . GA-92  
 232 . . . . . G . CV-56b  
 232 . . . . . G . CV-1656  
 241 . . . . . G . 1013  
 232 . . . . . G . CV-9437  
 241 . Beaudette  
 241 . Mass 41  
 229 . Conn  
 241 . Ark 99  
 241 . Ark DPI  
 241 . CU-T2  
 241 . PP14  
 241 . . . . . G . . . . . G . . . . . . . . . . . T T . . . . . G . . . . . A . . T . . . . . A . . . . . A . . . . . SE17  
 241 . . . . . G . . . . . A . . . . . . . . . . . T . . . . . A . G . C . . . . . C . . . . . T . . T . . . . . A . . . . . Iowa 609  
 241 . . . . . G T . . . . . A . . . . . . . . . . . T T . . . . . G . . . . . G T C . A . . . . . C . . . . . C . . . . . Holte  
 241 . . . . . . . . . . . T . . . . . . . . . . . T . . . . . G . . . . . G T C . G . . . . . C . . . . . C . . . . . JMK  
 241 . . . . . . . . . . . T . . . . . . . . . . . T . . . . . G . . . . . G T C . G . . . . . C . . . . . C . . . . . Gray  
 226 C . A T . G C A T T . . . . . T . . . . . G . . . . . T . . . . . A . . T . . . . . A . . . . . A . A G C . . G G C G . . . . . De-072

C A C T G T A A T T T T A C T T A T T T T A T A G T G T T T G T T A C A C A T T G T T T T A A G A G C G G G A C C T A A T Majority

286 . . . . . C . . . . . T . A G . . . . . A . . . . . C . Fl-18233  
 301 . . . . . . . . . . . C . . . . . A . GA-92  
 292 . . . . . . . . . . . A . C . . . . . G . CV-56b  
 292 . . . . . . . . . . . A . C . . . . . G . CV-1656  
 301 . . . . . . . . . . . A . G C . . . . . G . . . . . . . . . . . C . . . . . G C A T C A . . . . . G . . . . . G G A 1013  
 292 . . . . . . . . . . . A . C . . . . . G . CV-9437  
 301 . Beaudette  
 301 . Mass 41  
 289 . Conn  
 301 . . . . . . . . . . . C . . . . . A . Ark 99  
 301 . . . . . . . . . . . C . . . . . A . Ark DPI  
 301 . . . . . . . . . . . C . . . . . A . CU-T2  
 301 . . . . . . . . . . . C . . . . . C . . . . . A . PP14  
 301 . . . . . C . . . . . . . . . . . C . SE17  
 301 . . . . . T . . . . . . . . . . . C . Iowa 609  
 301 . . . . . T . . . . . . . . . . . C . Holte  
 301 . . . . . . . . . . . C . JMK  
 301 . . . . . . . . . . . C . Gray  
 286 T . . . . . . . . . . . A . . . . . A G C . . . . . T A T . . . . . T . . . . . G . . . . . C . . . . . G G . G G A . C . . . . . C . . . . . De-072

Fig. 2. (Continued)

	A G T T G T C C T T T A A C A G G T C T T A T T C C A C A A G G T T A T A T T C G T A T T G C T G C T A T G A A A A A T	Majority	
340	G . G . . . . . A . . . . T . C A . C . . . . . G C A . . . . . A . . . . G . . T . . . . .	A	Fl-18288
361	. . . . . G . . C . . . . . G . . . . . C A G C . . . . .	C	GA-92
352	T A . . . . . T . . . . .	G	CV-56b
352	T A . . . . . T . . . . .	G	CV-1686
361	C A . . . . . T . . . . .	G	1013
352	T A . . . . . T . . . . .	G	CV-9437
355	. . . . . G C . . . . . A . . . . . A . . . . . C T . . . . .		Beaudette
355	. . . . . G C G . . . . . A . . . . . A . . . . . T . . . . . T . . . . .		Mass 41
343	. . . . . T C . . . . .		Conn
361	. . . . . G . . . . .	C	Ark 99
361	. . . . . G . . . . .	C	Ark DPI
358	. . . . . G . . . . .	C	CU-T2
361	. . . . . C . . . . .	C	PP14
361	. . . . . G . . . . . T . . . . . A . . . . . T C . . . . .	G	SE17
361	. . . . . A . . . . . C . . . . . T . . . . . A . . . . . T C T . . . . .	T	Iowa 609
355	. . . . . C . . . . . C . . . . . T . . . . . A . . . . . G A . . . . . A . . . . . C . . . . . C . . . . . T . . . . . T . . . . .	G	Holte
361	C T . . . . .	G	JMK
361	C T . . . . .	G	Gray
343	. . . . . C . . . . . T . . . . . T . . . . . C A . . . . . G . . . . . A C . . . . . G G C G . . . . . T C . . . . . C . . . . . T . . . . . G . . . . . G . . . . . C . . . . .	C	De-072

	G G - - - T G G T A T T - - - C C T G G T C A C T T A T T T T A T A A T T T A A C A G T T T C T G T G A C T A A A T A T	Majority	
400	. . . . . - - - - - C . G G C . T . . C . . . . .	C	Fl-18288
421	. . . . . A A G . . C . . G - - - - -	C	GA-92
412	A A - - - . . . . C . G G C . A . G . . . . .	C	CV-56b
412	A A - - - . . . . C . G G C . A . G . . . . .	C	CV-1686
421	A . T G G . A C . G G C - - - . A . G . . . . .	C	1013
412	A A - - - . . . . C . G G C . A . G . . . . .	C	CV-9437
415	. . . . .	G	Beaudette
415	. . . . .	G	Mass 41
403	. . . . .		Conn
421	. . . . . A A G . C . . C G - - - . . . . .	C	Ark 99
421	. . . . . A A G . C . . G - - - . . . . .	C	Ark DPI
418	. . . . . A A G . C . . G - - - . . . . . T . . . . .	C	CU-T2
421	. . . . . A A G . T . A . . - - - . . . . . T . . . . .	C	PP14
421	. . . . . A T G . A A . A C - - - G . T C . T . T A . . . . .	T	SE17
421	. . . . . C G G . A . . G . . - - - T C . G . . . . .	C	Iowa 609
415	A A G A G . T . G . . G G G . T C . G . . . . .	C	Holte
421	. . . . . - - - T - A A . G . . - - - G . C T . T . . . . .		JMK
421	. . . . . - - - T - A A . G . . - - - G . C C . T . . . . .		Gray
397	T A - - - - - . A C G A - - . A . T . T . T . . . . . A A . G . . C - - C T T . C G . . C C G G . C T T . . . . .	C	De-072

	C C T A A A T T T A G A T C A C T T C A A T G T G T T A A T A A T C A T A C A T C T G T A T A T T T A A A T G G T G A T	Majority	
442	. . . . . C T . . . . A . . . . T . . . . G . . . . . T T . . . . C . . . . .	C	Fl-18288
478	. . . . . G . . . . . G . . . . A . . . . . T . . . . .	C	GA-92
469	. . . . .	C	CV-56b
469	. . . . .	C	CV-1686
478	A A . C G . . . . .	C	1013
469	. . . . .	C	CV-9437
457	. . . . .	A	Beaudette
457	. . . . .	A	Mass 41
445	. . . . .		Conn
478	. . . . . G . . . . . G . . . . A . . . . . T . . . . .	C	Ark 99
478	. . . . . G . . . . . G . . . . A . . . . . T . . . . .	C	Ark DPI
475	. . . . . G . . . . . G . . . . A . . . . . T . . . . . G . . . . .	C	CU-T2
478	. . . . . G . . . . . G . . . . A . . . . . T . . . . .	C	PP14
478	. . . . . G . . . . . G . . . . A . . . . . T . . . . . G . . . . C . . . . .	C	SE17
478	. . . . . G . . . . . G . . . . A . . . . . T . . . . . G . . . . C C . . . . .	C	Iowa 609
475	. . . . . C T . . . A . . . G . . . . .	G	Holte
475	. . . . . T T . . . G . . . C . . . . . A . . . G . . . . .	C	JMK
475	. . . . . T T . . . G . . . C . . . . . A . . . G . . . . .	C	Gray
448	G . A . . . . . G G . . . G G . . . . . T . A . G . . . . . T A G . . . T . T C . . . . . C A . C . . . . .	C	De-072

Fig. 2. (Continued)

CTTGTTCACATCTAATGAGACTGTAGATGTTGCAGCTGCAGGTGTTCAATTTAAAGCT Majority

502 . . . . . A . . . C . . . . . C A C . . . . . A . T . . . . . T . . . . . FL-18288  
 538 . . . . . . . . . . . C T T . . . A . . . . . T . . . . . C . C . . . A G . . . GA-92  
 529 . . . . . T . T . . . . . . . . . A . C T . . . G . T . . . C . . . . . C CV-56b  
 529 . . . . . T . T . . . . . . . . . A . . . . . T . . . . . C . . . . . C CV-1686  
 538 . . . . . . . . . . . C T T . . . A . . . . . T . . . . . G . . . . . T . . . . . 1013  
 529 . . . . . T . T . . . . . . . . . A . T . . . G . . . T . . . C . . . . . G C . . . CV-9437  
 517 . Beaudette  
 517 . Mass 41  
 505 . T . . . Conn  
 538 . . . . . . . . . . . C T T . . . A . . . . . T . . . . . C . . . . . A G . . . Ark 99  
 538 . . . . . . . . . . . C T T . . . A . . . . . T . . . . . C . . . . . A G . . . Ark DPI  
 535 . . . . . . . . . . . C T T . . . A . . . . . T . . . . . C . . . . . A G . . . CU-T2  
 538 . . . . . . . . . . . C T T . . . A . . . . . T . . . . . C . . . . . A G . . . PP14  
 538 . . . . . T . . . . . C . C . . . . . . . . . G T G . . . . . . . . . . SE17  
 538 . . . . . T . T . . . . . . . . . C G T A G A . . . . . . . . . . A Iowa 609  
 535 . . . . . T . T . . . . . C . A . . . A . . . . . G T A . . . . . . . . . . Holte  
 535 . . . . . C . T T . . . . . . . . . . . T . . . . . G . . . . . . . . . . JMK  
 535 . . . . . C . T T . . . . . . . . . . . T . . . . . G . . . . . . . . . . Gray  
 508 . . . . . C . . . T . T . . . T . T . . A . A . . . . . A . . . . . A . . . G - C . . . A A De-072

GGTGGACC-TATAACTTATAAAGTTATGAGAGAAGTTAAAGCCTTGGCTTATTTTGTTAA Majority

556 . . . . . T . G . . . FL-18288  
 598 . C . . . GA-92  
 589 . . . C . C CV-56b  
 589 . . . C . C CV-1686  
 598 . C 1013  
 589 . . . C . C CV-9437  
 577 . Beaudette  
 577 . Mass 41  
 565 . Conn  
 598 . G . . . Ark 99  
 598 . G . . . Ark DPI  
 595 . G . . . CU-T2  
 598 . . . . . . . . . . . G . C PP14  
 598 . . . . . . . . . . . C . SE17  
 598 . . . . . . . . . . . C . A Iowa 609  
 595 . . . . . G . . . . . . . . . . . G . C Holte  
 595 . . . . . C . A JMK  
 595 . . . . . C . A Gray  
 567 A . . T A . . G G . T . . G A A C G C . . G . . . . . C . . . T A C A C C T . T T A . . . A . . . G . . De-072

TGGTACTGCACAAGATGTTATTCTGTGTGATGGTTACCTAGAGGTTTGTTAGCATGCCA Majority

615 . C . . . FL-18288  
 657 . . . . . C . . . . . T . . . . . C . . . . . A . . . . . A C A . . . . . T . . . . . GA-92  
 648 . . . . . C . C CV-56b  
 648 . . . . . C . C CV-1686  
 657 . . . . . . . . . . . G . . . . . . . . . . . G . . . . . C . . . . . A . . . . . 1013  
 648 . . . . . C . C CV-9437  
 636 . Beaudette  
 636 . Mass 41  
 624 . Conn  
 657 . . . . . . . . . . . T . . . . . C . . . . . A . . . . . A C A . . . . . Ark 99  
 657 . . . . . . . . . . . T . . . . . C . . . . . A . . . . . A C A . . . . . Ark DPI  
 654 . . . . . . . . . . . T . . . . . C . . . . . A . . . . . A C . . . . . CU-T2  
 657 . . . . . . . . . . . T . . . . . C . . . . . A . . . . . A C A . . . . . PP14  
 657 . T . . . SE17  
 657 . G . . . Iowa 609  
 654 . . . . . . . . . . . T . . . . . T . . . . . A . . . . . G . . . . . G . . . . . T . . . Holte  
 654 . T . . . JMK  
 654 . T . . . Gray  
 627 . . . C . . A T G . . . . . . . . . . . C . . A . . A . . . . . A C A G T . . . . . A . . . A G A . . G . . T . . De-072

Fig. 2. (Continued)



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GTATAATACTGGCAATTTTTTCAGATGGCTTCTATCCT-TTTACTAATAGTAGTATTGTTA Majority
675 . . . . . T . C . . . . . T . C . . . . . T . A . . . . . FL-18288
717 A . . . T . C . . . . . C . . . . . C . . . . . C . . . . . GA-92
708 A . . . . . C . . . . . . . . . . . . . . . . . . . . . CV-56b
708 A . . . . . . . . . . . . . . . . . . . . . . . . . . . CV-1686
717 A . . . . . . . . . . . . . . . . . . . . . . . . . . . 1013
708 A . . . . . . . . . . . . . . . . . . . . . . . . . . . CV-9437
696 . . . . . . . . . . . . . . . . . . . . . . . . . . . Beaudette
696 . . . . . . . . . . . . . . . . . . . . . . . . . . . T . . . . Mass 41
684 . . . . . . . . . . . . . . . . . . . . . . . . . . . Conn
717 A . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . Ark 99
717 A . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . Ark DPI
714 A . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . CU-T2
717 A . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . PP14
717 . . . . . T . C . . . . . T C . . . . . T A . . . . . C . . . . . SE17
717 . . C . . . . . T . C . . . . . T . . . . . T T . . . . . G A C . C C . . . . . Iowa 609
714 . . . . . T . C . . . . . C . T . . . . . . . . . . . G A . C . . . . . Holte
714 . . . . . C . . . . . T . C . . . . . T . . . . . . . . . . . G . C . C . . . . . JMK
714 . . . . . C . . . . . T . C . . . . . T . . . . . . . . . . . G . C . C . . . . . Gray
687 . . . . . T . C . . . . . C . . . . . T . A . . . . . G . . . . . T G . . . . . G A C . C . G . . . . . De-072

AGGATAAGTTTATTGTTTATCGTGAAAATAGTGTTAATACTACTTTGACATTAACATAATT Majority
734 . . C . G . . . . . C C . . . . . G . . . . . A . . . . . C . T . A G . . . . . C A C . . . . . FL-18288
776 . . . . . . . . . . . G . . . . . C . . . . . . . . . . . G . . . . . GA-92
767 . . . . . A . G . . . . . . . . . . . . . . . . . . . . . C . T . . . . . G T T . . . . . G CV-56b
767 . . . . . A . G . . . . . . . . . . . . . . . . . . . . . C . . . . . G T T . . . . . G CV-1686
776 . . . . . . . . . . . G . . . . . C . . . . . . . . . . . . . . . . . . . . . 1013
767 . . . . . A . G . . . . . . . . . . . . . . . . . . . . . C . . . . . G T T . . . . . G CV-9437
755 . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . Beaudette
755 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Mass 41
743 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Conn
776 . . . . . . . . . . . G . . . . . C . . . . . . . . . . . . . . . . . . . . . Ark 99
776 . . . . . . . . . . . G . . . . . C . . . . . . . . . . . A . . . . . Ark DPI
773 . . . . . . . . . . . G . . . . . G . . . . . . . . . . . A . . . . . CU-T2
776 . . . . . . . . . . . G . . . . . T . C . . . . . . . . . . . . . . . . . . . . . PP14
776 . . . . . G . . . . . . . . . . . . . . . . . . . . . C . . . . . SE17
776 . . . . . A . . . . . G . . . . . . . . . . . . . . . . . . . . . C G T . . . . . Iowa 609
773 . . . . . . . . . . . T . . . . . C . . . . . . . . . . . C . . . . . Holte
773 . . . . . G . . . . . . . . . . . C . . . . . C . . . . . C . . . . . JMK
773 . . . . . G . . . . . . . . . . . C . . . . . C . . . . . C . . . . . Gray
746 G T A . . T T T A C . T . . . . A . C A . T . . . . T . T . T C . T C . . . . G C T A . G G . G T T . . G . . . . De-072

T C A C G T T T A G T A A T G A A A G T G G T G C C C T C C T A A T A C A G G T G G T G T T G A C A C T T T T A T T T Majority
794 . . . . . T . . . . . C A . . . . . G . C . . . . . C . . . . . A A C . . . . . C T T . . . . . C . G . A . . . . . C FL-18288
836 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . GA-92
827 . . . . . T . . . . . C T T . . . . . A . . . . . A . . . . . C G G . . . . . A . C . . . . . A T G . . . . . A A . T . . . . . CV-56b
827 . . . . . T . . . . . C T T . . . . . A . . . . . A . . . . . C G G . . . . . A . C . . . . . A T G . . . . . A A . T . . . . . CV-1686
836 . . . . . . . . . . . C . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . G C . . . . . G 1013
827 . . . . . T . . . . . C T T . . . . . A . . . . . A . . . . . C G G . . . . . A . C . . . . . A T G . . . . . A A . T . . . . . CV-9437
815 . . . . . T . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . Beaudette
815 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . Mass 41
803 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . . . Conn
836 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . Ark 99
836 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . Ark DPI
833 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . CU-T2
836 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . PP14
836 . . . . . . . . . . . T T . . . . . C C . . . . . A . . . . . T . . . . . G . . . . . T . . . . . G . . . . . SE17
836 . . . . . A . . . . . T C . . . . . C . . . . . C . . . . . . . . . . . C A A C . . . . . C . . . . . G . . . . . Iowa 609
833 . . . . . . . . . . . T . . . . . C . . . . . A . A . . . . . G . . . . . . . . . . . C . . . . . T . . . . . G . . . . . Holte
833 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . T . . . . . A C . . . . . C . . . . . JMK
833 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . T . . . . . A C . . . . . C . . . . . Gray
806 . . . . . T . . . . . . . . . . . T T . . . . . T A G . G . . . . . G G . A A A C A . A . C . C T . . . . . G G . . . . . De-072

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Fig. 2. (Continued)

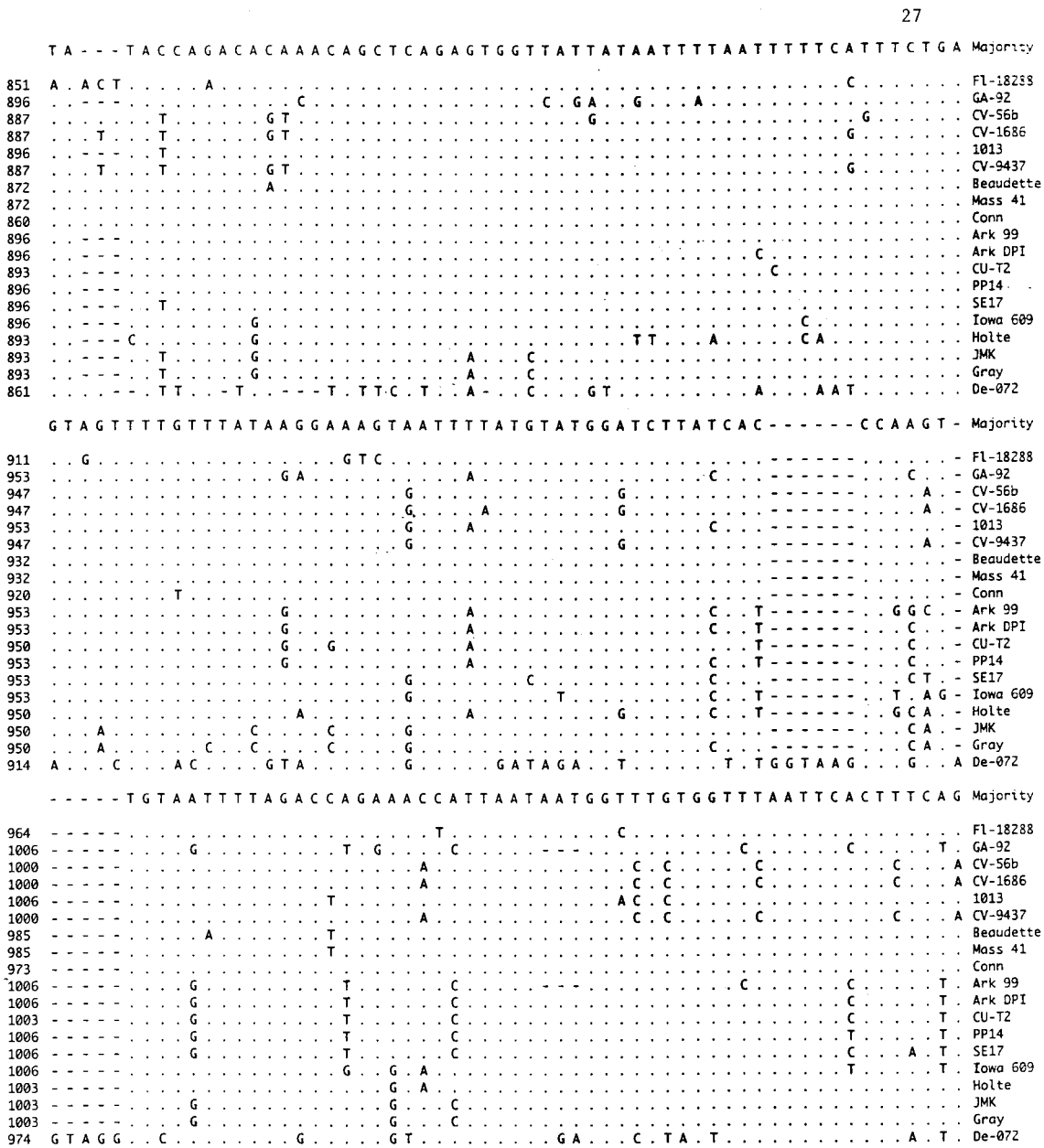


Fig. 2. (Continued)

28

TTTCACTTACTTACGGTCCTCTTCAAGGTGGTTGTAAGCAATCTGTCTTTAATGGTAGAG										Majority										
1019	.	.	A	.	G	.	.	.	C	.	.	.	G	.	.	Fl-18288				
1058	.	.	T	A	T	A	.	.	.	.	.	.	.	.	A	GA-92				
1055	.	C	.	C	.	.	C	A	A	.	G	C	.	G	A	T	CV-56b			
1055	.	C	.	C	.	.	C	A	A	.	G	C	.	A	G	A	T	CV-1686		
1061	.	C	.	C	.	.	C	A	A	.	G	C	.	A	G	A	T	1013		
1055	.	C	.	C	.	.	C	A	A	.	G	C	.	A	G	A	T	CV-9437		
1040	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	Beaudette		
1040	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Mass 41		
1028	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Conn		
1058	.	.	T	A	T	A	.	.	C	A	.	.	.	.	.	A	.	Ark 99		
1061	.	.	T	A	A	.	.	.	C	A	.	.	.	.	.	A	.	Ark DPI		
1058	.	.	T	A	A	.	.	.	C	A	.	.	.	.	.	A	.	CU-T2		
1061	A	.	T	A	A	.	.	.	A	.	.	.	.	.	.	A	.	PP14		
1061	.	.	A	A	.	.	.	.	C	A	.	.	.	.	.	.	.	SE17		
1061	C	.	.	A	A	T	G	.	A	.	G	.	.	.	C	T	.	Iowa 609		
1058	.	.	.	.	.	.	A	C	.	G	.	.	.	.	.	T	.	Ark Holte		
1058	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	JMK		
1058	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Gray		
1034	A	.	T	.	G	.	T	.	A	.	A	A	T	C	.	.	.	De-072		
CAACTTGTTGTTATGCTTATTCATAACA --- GAGGTCCTCCTGTTTGTAAGGTGTTTATT										Majority										
1079	.	.	.	.	.	.	.	G	---	.	.	T	G	T	G	.	.	G	Fl-18288	
1118	.	.	.	.	.	.	.	.	.	.	A	.	G	C	.	.	C	.	GA-92	
1115	.	.	.	.	.	.	.	.	.	.	AT	.	A	C	.	.	C	.	CV-56b	
1115	.	.	.	.	.	.	.	.	.	.	AT	.	A	C	.	.	C	.	CV-1686	
1121	.	G	.	.	.	.	.	.	.	.	AC	.	A	C	.	.	.	.	1013	
1115	.	.	.	.	.	.	.	.	.	.	AT	.	A	C	.	.	C	.	CV-9437	
1100	.	.	.	.	.	.	.	.	T	.	.	.	.	C	.	.	.	.	Beaudette	
1100	.	.	.	.	.	.	.	.	T	.	.	.	.	C	.	.	.	.	Mass 41	
1088	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Conn	
1118	.	.	.	.	.	.	.	.	.	.	.	A	.	G	C	.	C	.	Ark 99	
1121	.	.	.	.	.	.	.	.	.	.	.	A	.	G	G	.	C	.	Ark DPI	
1118	.	A	A	A	.	.	.	.	.	.	.	A	.	G	A	G	.	C	.	CU-T2
1121	.	A	A	A	.	.	.	.	.	.	.	T	.	G	G	.	C	.	PP14	
1121	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	SE17	
1121	.	.	.	.	.	.	.	.	T	.	T	.	AC	.	A	A	.	.	Iowa 609	
1118	.	.	.	.	.	.	.	.	C	T	T	.	C	.	A	ACA	.	.	Ark Holte	
1118	.	.	.	.	.	.	.	.	C	T	T	.	C	.	A	ACA	.	.	JMK	
1118	.	.	.	.	.	.	.	.	C	A	T	.	.	.	.	C	.	.	Gray	
1094	.	A	.	.	C	T	.	.	A	A	.	AT	G	T	.	T	.	G	AA	De-072
GAGGTTGAGTTAAACACATCATTTTGAATGTGGTTTTGTTAGTTTATGTTACTAAGAGCGATG										Majority										
1136	C	.	.	.	.	T	A	G	.	G	.	.	.	.	.	AC	.	.	.	Fl-18288
1175	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	GA-92
1172	.	.	C	.	.	.	A	.	T	.	.	.	C	.	.	A	.	.	.	CV-56b
1172	.	.	C	.	.	.	A	.	T	.	.	.	C	.	.	A	.	.	.	CV-1686
1178	.	.	C	.	T	C	G	T	.	G	.	.	.	.	.	.	.	.	.	1013
1172	.	.	C	.	.	.	A	.	T	.	.	.	C	.	.	A	.	.	.	CV-9437
1157	.	.	.	.	.	G	A	T	.	A	.	.	.	.	.	.	.	G	.	Beaudette
1157	.	.	.	.	G	A	T	.	A	.	.	.	.	.	.	.	G	.	.	Mass 41
1145	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	Conn
1175	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	Ark 99
1178	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	Ark DPI
1175	C	.	.	.	.	C	.	.	G	T	.	.	.	.	.	.	.	.	.	CU-T2
1178	.	.	.	.	.	C	.	.	G	T	.	.	.	.	.	.	.	A	.	PP14
1178	.	.	.	.	.	C	G	T	.	A	.	.	.	.	.	.	.	.	.	SE17
1178	.	.	.	.	.	C	G	T	.	A	.	.	.	.	.	.	.	A	.	Iowa 609
1175	T	.	.	.	C	T	.	G	.	AA	G	.	.	.	.	C	A	.	.	Ark Holte
1175	.	.	.	.	C	T	.	A	.	.	.	.	.	.	.	.	.	.	.	JMK
1175	.	.	.	.	C	T	.	A	.	.	.	.	.	.	.	.	.	.	.	Gray
1154	A	T	T	.	T	A	A	.	G	A	T	G	T	.	A	.	.	.	.	De-072

Fig. 2. (Continued)

GCTCTCGTATACAAACAGCAACAGAACACCTGTATTAAC TCAAATTTTATAATAACA Majority

1196 . . . . . C . T . . . . . G . A . T A . . . . . C . C A A . . . . . T . . . . . FL-18288  
1235 . . . . . C . . . . . T . . . . . C . . . . . G . GA-92  
1232 . . . . . . . . . . . . . . . A . G . . . . . G . . . . . C A C . . . . . . . . . . . . . . . CV-56b  
1232 . . . . . . . . . . . . . . . A . G . . . . . T . G . . . . . C A C . . . . . . . . . . . . . . . CV-1686  
1238 . . . . . C . . . . . T . . . . . C . . . . . T C . 1013  
1232 . . . . . . . . . . . . . . . A . G . . . . . T . . . . . G . . . . . C A C . . . . . . . . . . . CV-9437  
1217 . Beaudette  
1217 . Mass 41  
1205 . Conn  
1235 . . . . . C . . . . . T . . . . . C . . . . . . . . . . . C . Ark 99  
1238 . . . . . C . . . . . T . . . . . C . . . . . . . . . . . C . Ark DPI  
1235 . . . . . C . . . . . T . . . . . C . . . . . . . . . . . C . . . . . G . . . . . . . . . . . . . . . CU-T2  
1238 . . . . . C . . . . . . . . . . T . . . . . C . . . . . . . . . . . C . . . . . . . . . . . . . . . . . PP14  
1238 . . . . . C . . . . . G . . . . . T . . . . . . . . . . . T . SE17  
1238 . . . . . . . . . . . . . . . G A . A . . . . . . . . . . . C . . . . . . . . . . . C . . . . . Iowa 609  
1235 . . . . . . . . . . . . . . . C . . . . . . . . . . . T . . . . . A C . . . . . . . . . . . . . . . . Holte  
1235 . A . . . . . . . . . . . T . . . . . . . . . . . JMK  
1235 . T . . . . . A . . . . . . . . . . . T . . . . . Gray  
1214 . . . . . T . . . . . A A . A . T A G . . . . . T . . . . . T C C . . . . . T . . . . . A T . . . . . A T . . . . . G T . . . . . T . . . . . De-072

TTACTTTAGATACGTTGTTGATTATAATATATATG G C A G A A C T G G C C A A G G T T T T A T T A Majority

1256 . . . . . . . . . . A . . . . . T . FL-18288  
1295 . . . . . . . . . . G . . . . . G . A . . . . . . . . . . . C . G . T . . . . . T . . . . . . . . . . . A . . . . . GA-92  
1292 . . . . . C . . . . . . . . . . . . . . . G . . . . . . . . . . . G . . . . . . . . . . . G T A . . . . . A . . . . . CV-56b  
1292 . . . . . C . . . . . . . . . . . . . . . G . . . . . . . . . . . G . . . . . . . . . . . G T A . . . . . A . . . . . CV-1686  
1298 . . . . . . . . . . . . . . . A . . . . . . . . . . . G . . . . . . . . . . . C . . . . . . . . . . . G T A . . . . . A . . . . . 1013  
1292 . . . . . C . . . . . . . . . . G . . . . . . . . . . . G . . . . . . . . . . . G T A . . . . . A . . . . . CV-9437  
1277 . Beaudette  
1277 . Mass 41  
1265 . Conn  
1295 . . . . . C . . . . . . . . . . G . A . . . . . . . . . . . G . T . . . . . T . . . . . . . . . . . A . . . . . Ark 99  
1298 . . . . . C . A . . . . . G . A . . . . . . . . . . . T . . . . . . . . . . . T . . . . . . . . . . . C . . . . . Ark DPI  
1295 . . . . . . . . . . C . G . A . . . . . T . . . . . . . . . . . G . . . . . T . . . . . T . . . . . A . . . . . CU-T2  
1298 . . . . . . . . . . G . A . . . . . . . . . . G . T . . . . . T . . . . . T . . . . . . . . . . . A . . . . . PP14  
1298 . SE17  
1298 . T . . . . . T . . . . . G T . . . . . C . . . . . Iowa 609  
1295 . . . . . . . . . . G . G . A . T . . . . . T . . . . . G . . . . . Holte  
1295 . JMK  
1295 . Gray  
1274 . . . . . G . . . . . G . G . T T . . . . . . . . . . . G . T . . . . . T . T T . . . . . A G . . . . . A C . . . . . De-072

CTAATGTAAC T G A C T C A G C T G C T A G T T A T A A T T A T T T A G C A G A T G G A G G T T T G G C T A T T T Majority

1316 . . . . . . . . . . C . . . . . . . . . . T . . . . . . . . . . . C . . . . . C . C . . . . . A . . . . . FL-18288  
1355 . . . . . . . . . . . . . . . T . T . . . . . A . A . T C . C . . . . . . . . . . T G . . . . . A . . . . . A . . . . . GA-92  
1352 . . . . . . . . . . . . . . . . . . . A . . . . . G A . A . . . . . A . . . . . CV-56b  
1352 . . . . . T . . . . . . . . . . . . . . . A . . . . . G A . . . . . . . . . . . C . . . . . A . . . . . T . . . . . A C . . . . . C . . . . . CV-1686  
1358 . A . . . . . G A . A . . . . . A . . . . . 1013  
1337 . . . . . . . . . . . G . Beaudette  
1337 . Mass 41  
1325 . Conn  
1355 . . . . . . . . . . . . . . . T . T . . . . . A . T C C C . . . . . C . . . . . G . G . . . . . A . . . . . A . . . . . Ark 99  
1358 . T . . . . . . . . . . . T . . . . . . . . . . . A . . . . . A . . . . . Ark DPI  
1355 . . . . . . . . . . . . . . . T . T . . . . . A . T C . C . . . . . . . . . . G . . . . . . . . . . . A . . . . . A . . . . . CU-T2  
1358 . . . . . . . . . . . . . . . T . T . . . . . A . T C . C . . . . . . . . . . G . . . . . . . . . . . A . . . . . A . . . . . PP14  
1358 . SE17  
1358 . Iowa 609  
1355 T . . . . . A . . . . . A . T . . . . . . . . . . G . . . . . C . . . . . . . . . . . T . Holte  
1355 . JMK  
1355 . Gray  
1334 . . . . . . . . . . A . T . . . . . G . . . . . . . . . . C . . . . . C A . C C A G G . . . . . . . . . . A C C . . . . . T . . . . . T . . . . . T . . . . . G C . . . . . De-072

Fig. 2. (Continued)

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	T A G A T A C A T C T G G T G C C A T A G A C A T C T T C G T T G T A C A A G G T G A A T A T G G T C T T A A T T A T T	Majority
1376	. . . . . T . . . . . T . . . . . C . . . . .	Fl-18288
1415	. . . . . C . . . . . C . . . . . C . . . . .	GA-92
1412	. . . . . C . . . . . A . . . . . T . . . . . T . . . . .	CV-56b
1412	. . . . . C . . . . . A . . . . . T . . . . . T . . . . .	CV-1686
1418	. . . . . C . . . . . A . . . . . C . . . . . T . . . . .	1013
1412	. . . . . C . . . . . A . . . . . T . . . . . T . . . . .	CV-9437
1397	. . . . . C . . . . . A . . . . . T . . . . . T . . . . .	Beaudette
1397	. . . . . C . . . . . A . . . . . T . . . . . T . . . . .	Mass 41
1385	. . . . . C . . . . . A . . . . . T . . . . . T . . . . .	Conn
1415	. . . . . C . . . . . C . . . . . C . . . . . C . . . . .	Ark 99
1418	. . . . . C . . . . . C . . . . . C . . . . . C . . . . .	Ark DPI
1415	. . . . . C . . . . . C . . . . . C . . . . . C . . . . .	CU-T2
1418	. . . . . C . . . . . C . . . . . C . . . . . C . . . . .	PP14
1418	. . . . . C . . . . . C . . . . . C . . . . . C . . . . .	SE17
1418	. . . . . G T . . . . . A . . . . . T . . . . . G . . . . . A C . . . . .	Iowa 609
1415	. . . . . G T . . . . . A . . . . . T . . . . . G . . . . . A C . . . . .	Holte
1415	. . . . . G T . . . . . A . . . . . T . . . . . G . . . . . A C . . . . .	JMK
1415	. . . . . G T . . . . . A . . . . . T . . . . . G . . . . . A C . . . . .	Gray
1391	. . . . . G A C A . . . . . A . . . . . C T . . . . . T . . . . . T A . . . . . C C . . . . . C T A A C C . . . . . G . . . . .	De-072
	A T A A G G T T A A T C C T T G T G A A G A T G T T A A C C A A C A G T T T G T A G T T T C T G G T G G T A A A T T A G	Majority
1436	. . . . . C . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	Fl-18288
1475	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	GA-92
1472	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	CV-56b
1472	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	CV-1686
1475	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	1013
1472	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	CV-9437
1457	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	Beaudette
1457	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	Mass 41
1445	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	Conn
1475	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	Ark 99
1478	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	Ark DPI
1475	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	CU-T2
1478	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	PP14
1478	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	SE17
1478	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	Iowa 609
1475	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	Holte
1475	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	JMK
1475	. . . . . T A . . . . . C . . . . . C . . . . . C . . . . . G . . . . .	Gray
1451	. . . . . A . . . . . A . . . . . A G C . . . . . A . . . . . T G . . . . . A . . . . . G . . . . . A . . . . . G . . . . . A . . . . . T . . . . .	De-072
	T A G G T A T T C T T A C T T C A C G T A A T G A A A C T G G T T C T C A G C T T C T T G A G A A C C A G T T T T A C A	Majority
1496	. . . . . G . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	Fl-18288
1535	. . . . . C . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	GA-92
1532	. . . . . C . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	CV-56b
1532	. . . . . C . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	CV-1686
1535	. . . . . T . . . . . C G . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	1013
1532	. . . . . T . . . . . C G . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	CV-9437
1517	. . . . . T . . . . . C G . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	Beaudette
1517	. . . . . T . . . . . C G . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	Mass 41
1505	. . . . . T . . . . . C G . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	Conn
1535	. . . . . C . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	Ark 99
1538	. . . . . C . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	Ark DPI
1535	. . . . . C . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	CU-T2
1538	. . . . . C . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	PP14
1538	. . . . . C . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	SE17
1538	. . . . . G . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	Iowa 609
1535	. . . . . G . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	Holte
1535	. . . . . G . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	JMK
1535	. . . . . G . . . . . C . . . . . C . . . . . C . . . . . A . . . . .	Gray
1511	. . . . . T . . . . . A A . . . . . T A A . . . . . C C . . . . . T . . . . . G . . . . . A . . . . . A G T . . . . . G . . . . . C C G . . . . . A . . . . . T . . . . . G . . . . .	De-072

Fig. 2. (Continued)

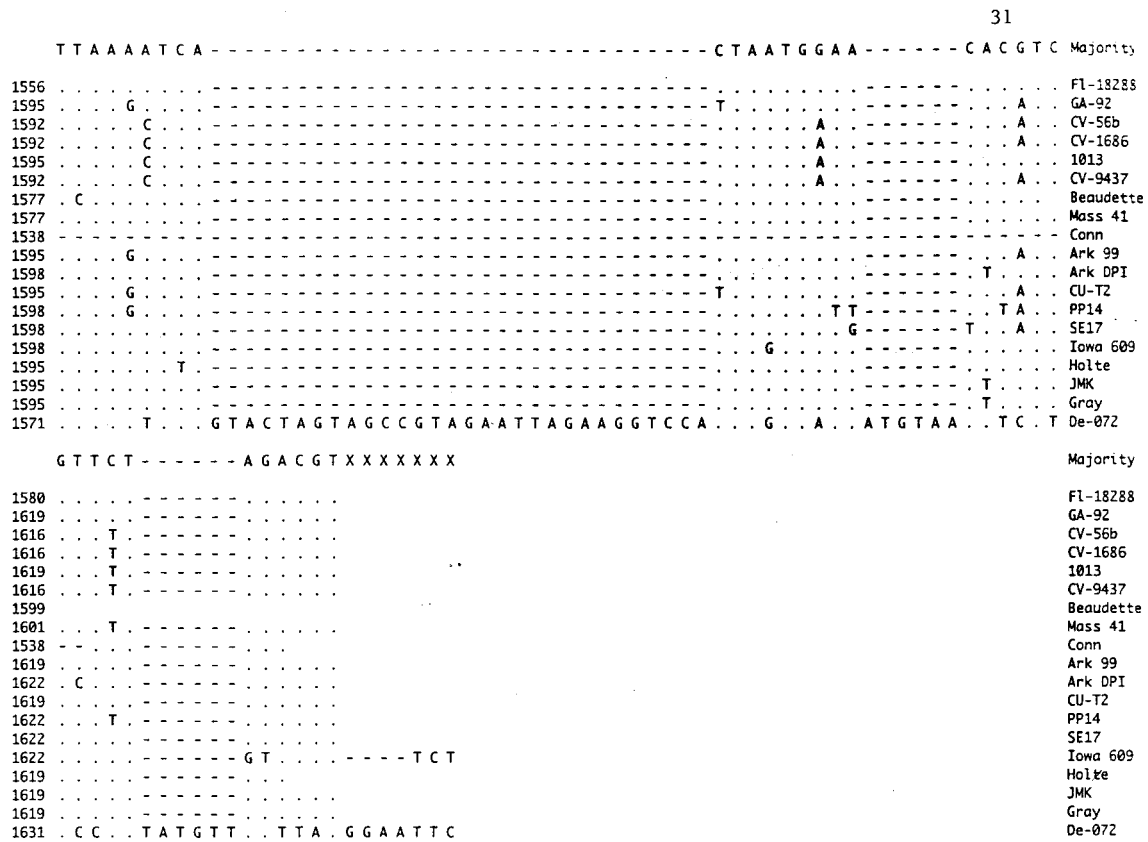


Fig. 2. (Continued)

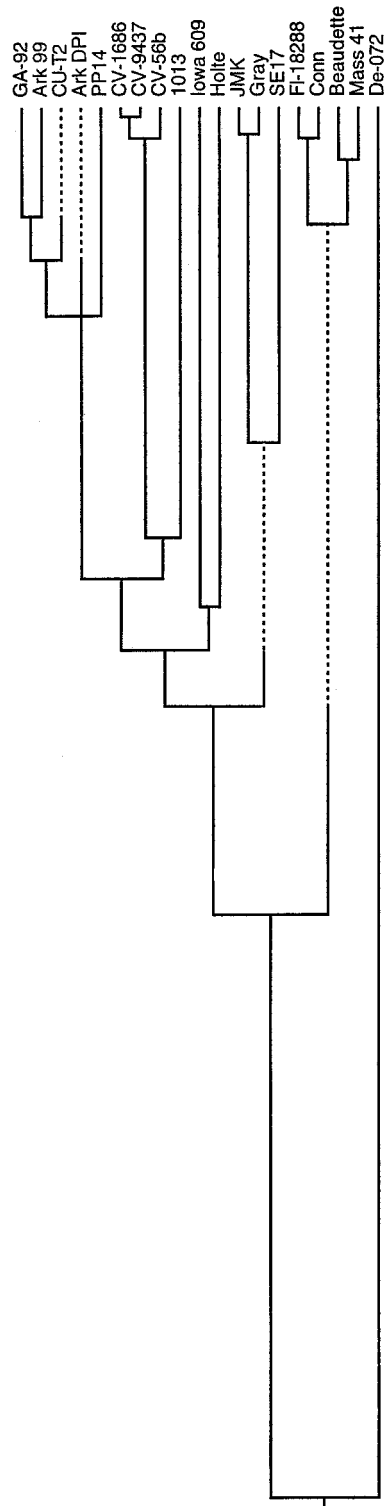
similar to the S1 protein from Ark strains. Another isolate from California, 1013, had an S1 glycoprotein that was 84.0% similar to Ark DPI and only 77.9% similar to CV-56b. The GA-92 IBV isolates S1 protein was 92.8% similar to the Ark 99 glycoprotein.

The predicted S1 glycoprotein amino acid sequences for IBV isolates FL-18288, GA-92, CV-56b, CV-9437, CV-1686, and 1013 were aligned with previously published S1 glycoprotein sequences of other USA strains in the GenBank database. When comparing the S1 sequences, the most variations were observed between residues 55–96, 115–149, 255–309,

and 378–395 (numbering is in reference to Mass 41 S1 and includes the signal sequence). When comparing strains Mass 41, Conn, and FL-18288 representing three serotypes with closely related sequences, variations occurred in the S1 subunit of all three strains at residues 91, 125, and 392 as reported in Table 3.

The S precursor glycoprotein is cleaved post-translationally marking the carboxyl terminus of the S1 subunit and the S2 subunit amino terminus (9). Several IBV isolates reported here had amino acid sequence variations at the cleavage site (residues

Fig. 3. Phylogenetic analysis demonstrating relationships among the S1 glycoprotein amino acid sequences of infectious bronchitis viruses isolated in the United States. The tree contains five major groups. One group contains the Arkansas strains, GA-92 (U16157), Ark 99 (M99482), Ark DPI (AF006624), CU-T2 (U04739), and Pp14 (M99483). Another includes the California variants, CV-1686, CV-9437, CV-56b. Gray (14069), JMK (L14070), Se17 (M99484), and Iowa 609 are grouped together, and Conn (L18990), Florida 18288, Beaudette (M95169), and Mass41 (X04722) form another variety. De-072 (U77298) is substantially different from other IBV strains, whereas Holte (18988) is placed between the California variants and Gray, with isolate 1013 between the Arkansas strains and the California variants.







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DLVFTSNETEDVSGAGVHFKAAGGPITYKVMREVKALAYFVNGTAQDVLCDGSPRGLLAC Majority
499 . . . Y . . . . T . . . T S . . . Y - . . . S . . . . . G . . . . . G . . . . . Fl-18288
535 . . . . . Y . . . V A . . . . S . . . . . A . . . . . G . . . . . P H . . . . . D T . . . . . F GA-92
526 . . . . . V . I . . . . . A . . . . . . . . . . . . . . . . . . . . . T . . . . . CV-56b
526 . . . . . V . I . . . . . T . . . . . . . . . . . . . . . . . . . . . T . . . . . CV-1686
535 . . . . . Y . . . V . . . . . S . . . . . K . N . . . . . . . . . . V . S . . . . . 1013
526 . . . . . V . I . . . . . A . . . . . . . . . . . . . . . . . . . . . T . . . . . CV-9437
514 . . . . . I . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Beaudette
514 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Mass 41
502 . . . . . . . . . . . N . . . . . . . . . . . . . . . . . . . . . . . . . . . Conn
535 . . . . . Y . . . V A . . . . S . . . . . . . . . . . . . . . . . H . . . . . D T . . . . . Ark 99
535 . . . . . Y . . . V A . . . . S . . . . . . . . . . . . . . . . . H . . . . . D T . . . . . Ark DPI
532 . . . . . Y . . . V A . . . . S . . . . . . . . . . . . . . . . . H . . . . . D . . . . . CU-T2
535 . . . . . Y . . . V A . . . . S . . . . . V . . . . . . . . . . . H . . . . . D T . . . . . PP14
535 . . . . . D . I . . . . . S . . . . . V . . . . . K . T . . . . . I . . . . . . . . . . I . . . . . SE17
535 . . . . . K . . . G R . . . . . . . . . . . . . . . . . I . . . . . . . . . . . Iowa 609
532 . . . . . S . . . I . . . . . . . . . . . R . . . Q T . V . . . . . V H . . . . . . . . . . Holte
532 . . . . . S . . . I . . . . . . . . . . . . . . . . . K . T . . . . . . . . . . JMK
532 . . . . . S . . . I . . . . . . . . . . . . . . . . . K . T . . . . . . . . . . Gray
505 N . L . . . . Y . . . . E A . . . . A . Q V N G L E R R . . . . D T P V M . . . . V . . . . . D . . K . R . . De-072

Q Y N T G N F S D G F Y P F T N S S I V K D K F I V Y R E N S V N T T L T L T N F T F S N E S G A P P N T G G V D S - F Majority
673 . . . . . . . . . . . L . Q . . . . H . . . . I . . . . K . H . . . . H . . T . . N . . L . . Q N - I Fl-18288
715 . . . . . Y . A . . . . A . . . . . T . . . . . S . . . . . . . . . . P . . . . . . . . . . GA-92
706 . . . . . . . . . . . . . . . . . . . . . E R . . . . . . . . . . I . V . . . V . . F . . S . . . G . D L N A N . CV-56b
706 . . . . . . . . . . . . . . . . . . . . . E R . . . . . . . . . . V . . . V . . F . . S . . . G . D L N A N . CV-1686
715 . . . . . . . . . . . . . . . . . . . . . T . . . . . S . . . . . . . . . . V . . . V . . F . . Q . . . N . . . . 1013
706 . . . . . . . . . . . . . . . . . . . . . . . . . . . E R . . . . . . . . . . V . . . V . . F . . S . . . G . D L N A N . CV-9437
694 . . . . . . . . . . . . . . . . . . . . . . . . . . . C . . . . . I . . . . . . . . . . P S . . . . . Beaudette
694 . . . . . . . . . . . I . . . . . . . . . . . . . . . . . F . . . . . . . . . . . P S . . . . . Mass 41
682 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . S . . . . . Conn
715 . . . . . . . . . . . T . . . . . . . . . . . S . . . . . . . . . . . . . . . . . . . . . Ark 99
715 . . . . . . . . . . . T . . . . . . . . . . . S . . . . . . . . . . . . . . . . . . . . . Ark DPI
712 . . . . . . . . . . . T . . . . . . . . . . . S R . . . . . . . . . . . . . . . . . . . . . CU-T2
715 . . . . . . . . . . . T . . . . . . . . . . . S I . . . . . . . . . . . . . . . . . . . . . PP14
715 . . . . . . . . . . . L . . . . . Y T . . . . R . . . . . . . . . . . N . . . . F . Q . D . L . . S . . . . . SE17
715 . . . . . . . . . . . L . . . . F . . . . . D T . . . . V . . . . . . . . . . . F V . . . . Q . . . . . N . . . . . Iowa 609
712 . . . . . . . . . . . . . . . . . . . . . D T . . . . F . . . . . . . . . . . A . . . . I . Q . R . D . . . . . Holte
712 . . . . . . . . . . . . . . . . . . . . . G T . . . . R . . . . . T . . . . . . . . . . . R . . . A . . . . . N . . . . . JMK
712 . . . . . . . . . . . . . . . . . . . . . G T . . . . R . . . . . T . . . . . . . . . . . R . . . A . . . . . N . . . . . Gray
685 . . . . . . . . . . . L . . . V Y E E P V A S N F T F . P L D I . S T S Y G V . . . . . N . V . . V E . . G . . . . . De-072

I L Y Q T Q T A Q S G Y Y N F N F S F L S S F V Y K E S N F M Y G S Y H - - - P S C N F R P E T I N N G L W F N S L S Majority
850 Q T . . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . Fl-18288
892 . . . . . H . . . . Q N D Y . . . . . R . . . . . Y . . . . . . . . . . R . S . . . G . L . . . S . . . . GA-92
886 . . . . . V . . . . . D . . . . . V . . . . . . . . . . . D . . . . . . . . . . N . . . . . N . . . . . CV-56b
886 . . . . . I . . . V . . . . . . . . . . . D L . . . . . . . . . . . N . . . . . N . . . . . CV-1686
892 V . . . . . . . . . . . . . . . . . . . . . D Y . . . . . . . . . . . . . . . . . L . . . . . 1013
886 . . . . . I . . . V . . . . . . . . . . . D . . . . . . . . . . . N . . . . . N . . . . . CV-9437
871 . . . . . K . . . . . . . . . . . . . . . . . . . . . . . . . . . K . . . L . . . . . Beaudette
871 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . L . . . . . Mass 41
859 . . . . . . . . . . . . . . . . . . . . . F . . . . . . . . . . . . . . . . . . . . . Conn
892 . . . . . . . . . . . . . . . . . . . . . . . . . . . R . . . Y . . . . . A . S . . . . L . . . . . S . . . . Ark 99
892 . . . . . . . . . . . . . . . . . . . . . . . . . . . R . . . Y . . . . . R . S . . . . L . . . . . Ark DPI
889 . . . . . . . . . . . L . . . . . R G . . . Y . . . . . . . . . . . R . S . . . . L . . . . . CU-T2
892 . . . . . . . . . . . . . . . . . . . . . R . . . Y . . . . . . . . . . . R . S . . . . L . . . . . PP14
892 V . . . . . . . . . . . . . . . . . . . . . . . . . . . D . I . . . . . L . S . . . . L . . . . . T SE17
892 V . . . . . . . . . . . . . . . . . . . . . . . . . . . D . F . . . . . K . . . . . N . . . . . Iowa 609
889 V . H . . . . . . . . F L . T . . . . . . . . . . . Y . . . . . . . . . . . H . . . . . N . . . . . Holte
889 . . . . . . . . . . . N . . . . . D . . . . A . D . . . . . . . . . . H . S . . . . L . . . . . JMK
889 . . . . . . . . . . . N . . . . . D . . . . A . D . . . . . . . . . . H . S . . . . L . . . . . Gray
853 A R F N I S . I P E . . V . . K . N . . N . . T . V . . D D R . . Y G K P G S R . . . . . S . . R . . S . . . T De-072
    
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Fig. 4. (Continued)

		V S L T Y G P L Q G G C K Q S V F S G R A T C C Y A Y S Y - G G P S L C K G V Y S G E L T Q N F E C G L L V Y V T K S D	Majority
1018	. . . I A . . . . .	. . . . . I S D . . . . .	FL-18288
1057	. . . I . . . I . . . . .	. . . . . H . . . . .	GA-92
1054	I . . . . . N R . . . . .	. . . . . R . Q . . . . .	CV-56b
1054	I . . . . . N R . . . . .	. . . . . R . Q . . . . .	CV-1686
1060	. . . . . N R . . . . .	. . . . . R K L . . . . .	1013
1054	I . . . . . N R . . . . .	. . . . . R . Q . . . . .	CV-9437
1039	. . . . . K . . . . .	. . . . . D H . . . . .	G Beaudette
1039	. . . . . . . . . . .	. . . . . D L . . . . .	G Mass 41
1027	. . . . . . . . . . .	. . . . . L . . . . .	Conn
1057	. . . I . . . I . . . . .	. . . . . R . . . . .	Ark 99
1060	. . . I . . . . . N K . . . . .	. . . . . R G . . . . .	Ark DPI
1057	. . . I . . . . . N K . . . . .	. . . . . R S . . . . .	CU-T2
1060	. . . I . . . . . N K . . . . .	. . . . . R S . . . . .	PP14
1060	. . . I . . . . . N K . . . . .	. . . . . R S . . . . .	SE17
1060	. . . I . . . . . T . . . . .	. . . . . R Q . . . . .	Iowa 609
1057	. . . . . K T . . . . .	. . . . . T T . . . . .	Holte
1057	. . . . . . . . . . .	. . . . . L R . . . . .	JMK
1057	. . . . . . . . . . .	. . . . . L R . . . . .	Gray
1033	. . . G . . . I S . . . . .	. . . . . W Q N E . . . . .	De-072
		F . . . K . N . . . S R N . . . N F D K D V . Y . . . V . . . F I S . T .	
		G S R I Q T A T E P P V I T Q N F Y N N I T L N T C V D Y N I Y G R T G Q G F I T N V T D S A V S Y N Y L A D G G L A I	Majority
1195	. . . . . H N . . . . .	. . . . . . . . . . .	FL-18288
1234	. . . . . Q . . . L . . . . .	. . . . . G K . . . . .	GA-92
1231	. . . . . K A . . V . T . . . . .	. . . . . D R . E . . . . .	CV-56b
1231	. . . . . K A L . V . T . . . . .	. . . . . D R . E . . . . .	CV-1686
1237	. . . . . Q . . . L . . . . .	. . . . . K . . . E . . . . .	1013
1231	. . . . . K A L . V . T . . . . .	. . . . . D R . E . . . . .	CV-9437
1216	. . . . . . . . . . .	. . . . . . . . . . .	Beaudette
1216	. . . . . . . . . . .	. . . . . R . . . . .	Mass 41
1204	. . . . . . . . . . .	. . . . . . . . . . .	Conn
1234	. . . . . Q . . . L . . . . .	. . . . . G K . . . . .	Ark 99
1237	. . . . . Q . . . L . . . . .	. . . . . N . G K . . . . .	Ark DPI
1234	. . . . . Q . . . L . . . . .	. . . . . F G K . . . . .	CU-T2
1237	. . . . . Q . . . L . . . . .	. . . . . G N . . . . .	PP14
1237	. . . M . . . . .	. . . . . D . . . . .	SE17
1237	. . . E K . . . L . . . . .	. . . . . I . . . . .	Iowa 609
1234	. . . . . Q . . . . . T . . . . .	. . . . . S K . . . . .	Holte
1234	. . . . . Q . . . . . Y . . . . .	. . . . . I . . . . .	JMK
1234	. . . . . Q . . . . . Y . . . . .	. . . . . Y . . . . .	Gray
1213	. . . . . R . . . . . S . . . . .	. . . . . Y . N . V I . . . . .	De-072
		S . . . G L . . . . . V . . . I . . . R . L . . . I . E . S . H P G . D H . . . V L .	
		L D T S G A I D I F V V Q G E Y G L N Y Y K V N P C E D V N Q Q F V V S G G K L V G I L T S R N E T G S Q L L E N Q F Y	Majority
1375	. . . . . S . . . . .	. . . . . T . . . . .	FL-18288
1414	. . . . . . . . . . .	. . . . . P . . . . .	GA-92
1411	. . . . . . . . . . .	. . . . . V . . . . .	CV-56b
1411	. . . . . . . . . . .	. . . . . V . . . . .	CV-1686
1417	. . . V I W - P . . . . .	. . . . . F . . . . .	1013
1411	. . . . . . . . . . .	. . . . . V . . . . .	CV-9437
1396	. . . . . . . . . . .	. . . . . F . . . . .	Beaudette
1396	. . . . . . . . . . .	. . . . . . . . . . .	Mass 41
1384	. . . . . . . . . . .	. . . . . . . . . . .	Conn
1414	. . . . . . . . . . .	. . . . . P . . . . .	Ark 99
1417	. . . . . . . . . . .	. . . . . P . . . . .	Ark DPI
1414	. . . . . . . . . . .	. . . . . P . . . . .	CU-T2
1417	. . . . . . . . . . .	. . . . . Q . . . . .	PP14
1417	. . . . . . . . . . .	. . . . . P . . . . .	SE17
1417	. . . S . . . . .	. . . . . I . . . . .	Iowa 609
1414	. . . . . . . . . . .	. . . . . R . . . . .	Holte
1414	. . . . . . . . . . .	. . . . . P . . . . .	JMK
1414	. . . . . . . . . . .	. . . . . P . . . . .	Gray
1390	. . . D T . . . . . T . . . . .	. . . . . L H R D H L T S . . . . .	De-072
		S . I . E . Y . . . . . N . . . . . K . . . . . N . Q . V A . Q . A D M . . . . .	

Fig. 4. (Continued)

38



Fig. 4. (Continued)

542–546) conforming to the sequence pattern X<sub>1</sub>-Arg-X<sub>2</sub>-Arg-Arg. Strains Ark 99, CU-T2, GA-92, SE17 had the sequence His-Arg-Ser-Arg-Arg, whereas Ark DPI, Gray, JMK, Conn, and FL-18288 had the sequence Arg-Arg-Ser-Arg-Arg (34). Strains Beaudette, Mass 41, and 1013 had the sequence Arg-Arg-Phe-Arg-Arg, whereas CV-56b, CV-9437 and CV-1686 had the sequence His-Arg-Phe-Arg-Arg (6).

**Discussion**

Because of variant RFLP patterns using the RT-PCR/RFLP diagnostic test for IBV, GA-92, CV-56b, CV-9437, CV-1686, 1013 were selected for further characterization. The S1 gene nucleotide and predicted amino acid sequences of FL-18288 strain, GA-92, CV-56b, CV-9437, CV-1686, 1013 were determined, and compared with other IBV sequences.

Table 2. S1 amino acid sequence alignment pair distances

		Percent Similarity																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	FL-18288	█	71.7	73.8	73.8	72.5	73.8	87.9	89.8	94.9	74.7	76.4	74.5	75.7	77.4	77.2	74.7	77.4	77.4	46.0
2	GA-92	31.2	█	72.1	72.5	78.1	72.1	73.2	71.5	71.2	92.8	91.0	90.1	88.6	77.3	75.1	73.6	75.7	75.3	41.1
3	CV-56b	28.2	31.6	█	97.6	77.9	98.0	73.9	73.4	73.9	76.6	76.6	75.8	75.5	74.5	76.2	73.4	74.0	74.0	43.4
4	CV-1686	28.5	31.1	2.4	█	78.2	99.3	73.9	73.0	73.9	77.1	77.1	76.4	76.2	74.7	76.4	73.8	74.2	74.5	43.4
5	1013	29.8	24.2	23.6	23.1	█	77.9	74.7	74.1	72.2	83.1	84.0	82.9	80.7	78.8	79.6	76.2	77.7	77.5	43.3
6	CV-9437	28.5	31.6	2.1	0.7	23.6	█	73.7	73.0	73.9	76.8	76.8	76.0	75.8	74.5	76.2	73.6	74.0	74.4	43.2
7	Beaudette	11.1	30.4	28.4	28.4	28.2	28.6	█	94.9	88.7	76.2	77.9	76.2	76.7	78.2	77.7	73.9	78.6	79.2	46.3
8	Mass 41	9.7	33.0	29.5	29.8	29.1	29.8	5.3	█	90.1	75.0	76.7	74.9	76.2	77.8	77.1	74.5	78.2	78.2	46.9
9	Conn	3.0	32.2	27.7	28.0	30.4	28.0	11.1	9.6	█	74.1	75.7	74.1	75.7	77.0	76.7	74.3	76.7	76.7	46.5
10	Ark 99	26.8	7.6	25.2	24.4	17.6	24.9	26.1	27.7	27.9	█	95.4	94.7	92.6	81.8	79.2	78.0	78.8	78.8	44.0
11	Ark DPI	25.4	9.0	26.1	25.4	17.4	25.9	24.7	26.3	26.7	4.2	█	93.9	92.1	82.7	79.8	78.8	81.8	80.7	45.2
12	CU-T2	27.8	9.2	26.2	25.4	17.9	25.9	27.1	29.0	28.7	4.2	5.7	█	92.3	81.6	79.4	78.4	78.8	78.8	44.6
13	PP14	26.5	11.8	27.7	26.7	21.7	27.2	26.3	27.1	26.7	7.2	8.4	7.6	█	80.7	78.5	76.9	79.4	79.4	43.9
14	SE17	23.8	26.2	29.1	28.8	24.2	29.1	24.2	24.8	24.5	20.2	19.7	20.5	22.4	█	81.1	76.6	84.0	84.2	44.7
15	Iowa 609	24.1	29.4	26.7	26.4	23.2	26.7	25.0	25.8	25.0	23.7	23.6	23.4	25.4	21.9	█	77.9	79.7	79.9	44.8
16	Holte	28.2	29.6	28.9	28.1	26.4	28.4	31.2	30.2	29.2	23.3	23.3	23.8	25.8	26.4	24.5	█	76.2	76.0	44.8
17	JMK	23.8	27.6	29.1	28.9	24.7	29.1	23.7	24.2	25.0	23.2	20.2	23.2	23.4	17.4	22.9	27.2	█	98.2	44.0
18	Gray	23.8	28.4	29.4	28.6	25.3	28.9	22.9	24.2	25.0	23.5	21.9	23.5	23.7	17.1	22.9	27.5	1.9	█	44.0
19	De-072	74.4	87.0	84.3	84.8	82.7	84.8	72.9	73.0	72.2	78.6	76.9	79.1	80.9	79.9	79.6	78.6	82.7	82.2	█

Table 3. Infectious bronchitis virus S1 subunit variation at the same amino acid in strains representing three different serotypes

IBV serotype	Amino acid 91*	Amino acid 125	Amino acid 392
Mass 41	ALA (nonpolar)	MET (nonpolar)	ASP (uncharged polar)
Conn	GLY (nonpolar)	SER (uncharged polar)	LYS (charged polar)
FL-18288	ASN (uncharged polar)	ILE (nonpolar)	ILE (nonpolar)

\*Amino acid numbering is in reference to Mass41 and includes the signal sequence.

Although GA-92 has a unique RFLP pattern, it is serologically related to the Arkansas serotype (23). Consistent with serologic data, we determined that the S1 glycoprotein sequence was 92.8% similar to the Ark 99 strain.

Initial characterization of isolates from California obtained from poultry between 1984 and 1993 (including CV-56b) indicated these variant strains (designated California variants) were serologically distinct from other IBV serotypes (23,24). The epidemiology of California variants is unknown. It is unlikely that these California variants originated directly from a live vaccine virus because the S1 glycoprotein sequences are not closely related to other IBV strains. Recombination of genomic RNA also is unlikely because no crossover sites were observed in the S1 gene. We observed a genetic drift from CV-56b (1991) to CV-9437 (1995) and CV-1686 (1995) with 98.0% and 97.6% similarity, respectively. Based on RFLP patterns and sequence analysis, we predict that CV-9437 and CV-1686 are serologically similar to other California variants represented by CV-56b. Another isolate from California, 1013, has a unique RFLP pattern, and the S1 glycoprotein sequence was more similar to Ark DPI (84.0%) than CV-56b (77.9%). Isolate 1013 may represent a new serotype or may belong to an existing serotype, such as the Ark serotype.

Following alignment of these newly reported S1 glycoprotein sequences with those previously published from other IBV strains, regions of sequence variation were detected between residues 59–96, 115–149, 255–309, and 378–395. Amino acid variations between 59–96 and 115–149 reported herein were similar to HVR I (residues 56–69) and HVR II (residues 117–131) of Mass and European strains as well as residues 53–148 in strains isolated from the United States (15,17). These variable regions were also similar to regions I (residues 38–67) and II (residues 97–141) associated with two separate virus-

neutralizing, conformationally dependent epitopes (20). It appears that regions between residues 59–96 and 115–149 of the S1 glycoproteins reported in this study, may also be involved with epitopes that induce virus-neutralizing and serotype specific antibodies.

Two other regions of S1 glycoprotein variation were also observed. The first region between residues 255–309 corresponds to a previously reported hypervariable region (18,19), and the second domain between amino acids 378–395 has not been previously reported. Amino acid variation between residues 255–309 and 378–395 are similar to region III (274–387) associated with one virus-neutralizing epitope in European strains using monoclonal antibody resistant (MAR) mutants (20). In another study, MAR mutants of the Ark DPI strain had substitutions at Thr 304 and Arg 386 (Thr 306 and Arg 388 in this multiple alignment) of the S1 protein and were associated with one serotype-specific, virus-neutralizing epitope (21). During this investigation, amino acids surrounding 306 and 388 were identified as hypervariable. This suggests that sequences of the S1 glycoprotein between 255–309 and 378–395 may be important in forming serotype-specific, conformationally dependent epitopes.

FL-18288 was 95.8% and 89.4% similar to Conn and Mass 41, respectively. This demonstrates that the S1 subunit may vary by a few amino acids giving rise to different serotypes. Amino acid variation in all three serotypes were observed at amino acids 91, 125, and 392. Amino acids 91 and 125 are within regions of variation, I and II, respectively. These regions are associated with two separate virus-neutralizing, conformationally dependent epitopes (20). Amino acid 392 is within region III that is associated with one virus-neutralizing, serotype-specific epitope.

Amino acid substitutions in the cleavage site among various IBV strains was detected during these studies. As reported previously, the cleavage site sequence for Beaudette and Mass 41 strains in

Arg-Arg-Phe-Arg-Arg (6,17), whereas Gray and JMK strains have Arg-Arg-Ser-Arg-Arg (34). In this study, differences were detected giving a cleavage site sequence of X1-Arg-X2-Arg-Arg. Herein X1 represents His or Arg and X2 represents Ser or Phe. The importance of these variations in the cleavage sequence to virulence is currently not known.

In summary, we observed S1 glycoprotein variations similar to others (residues 55–96, 115–149, and 255–309). However, a newly defined region of variation between amino acids 378–395 may interact with residues between 255–309 to form one conformationally dependent epitope with virus-neutralizing and serotype specificity. In addition, the S1 subunit may vary by 8% and remain the same serotype, whereas, a variation of only 4.2% may also change the serotype because of critical amino acid substitutions in the S1 glycoprotein. These data also demonstrate that variant IBV strains can circulate among poultry in geographically isolated areas.

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