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Experimental confirmation of recombination upstream of the S1 hypervariable region of infectious bronchitis virus

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Abstract

Chimeric infectious bronchitis virus (IBV) genomes with cross-over sites in the S1 gene were generated by co-infection with two distinct IBV strains. Recombinant viruses were collected from chicken embryos, embryonic cultured cells and chickens co-infected with Ark99 and Mass41 strains and purified by differential centrifugation. The recombinant S1 genes were identified by reverse transcription polymerase chain reaction (RTPCR) using heterologous primers and confirmed by nucleotide sequencing. The recombinants with Ark99 5′ and Mass41 3′ sequences were identified following the in vitro, in ovo and in vivo co-infections. Mixed RNA extracted from Ark99 and Mass41 did not produce RTPCR products with these primers at the PCR conditions used. Cross-over sites within the amplified 580 (Mass41) or 604 (Ark99) bases of the 5′ S1 gene could only be detected between nucleotides 50 and 155. While this region, lying upstream of the S1 hypervariable region, corresponded with sites commonly identified in naturally occurring isolates, recombination sites identified in these studies could not be detected within the HVR of S1 of the genomes of chimeric viruses. © 1997 Elsevier Science B.V.

Keywords: Coronavirus; Infectious bronchitis virus; Recombination; Co-infection

Infectious bronchitis virus (IBV), with large positive-sense RNA genome of 27.6 kb, is a prototype virus of the Coronaviridae family (Boursnell et al., 1987; Collisson et al., 1992). IBV is an agriculturally important pathogen of poultry and despite routine use of vaccines, variant stains continue to cause outbreaks in field situations (personal observations, King and Cavanagh, 1991; Wang et al., 1993). The continuing emergence of new strains may be explained in part by the apparent common occurance in nature of recombination between IBV strains (Wang et al., 1993, 1994).

Both in vitro and in vivo RNA recombination between mouse coronavirus genomes in the presence and absence of selective biological pressure has been experimentally demonstrated (Lai et al.,

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1985; Makino et al., 1986; Keck et al., 1988; Banner and Lai, 1991; Liao and Lai, 1992). The frequency of recombination throughout the mouse hepatitis virus (MHV) genome has been estimated to be as high as 25% (Baric et al., 1990). Targeted RNA recombination in the nucleocapsid (N) gene of MHV has also been reported by Peng et al. (1995) following transfection of infected cells with in vitro transcribed donor RNA.

The glycosylated S protein, a major structural protein of IBV, is posttranslationally cleaved into S1 and S2. The S1 protein has been shown to be responsible for attachment to cellular host membranes (Collisson et al., 1992), and for the induction of neutralizing and hemagglutination inhibiting antibodies (Koch et al., 1992; Kant et al., 1992). We have recently also demonstrated that the S1 can induce IBV specific CTL responses in the chick (Seo and Collisson, manuscript in preparation). Comparisons of the S1 from a number of distinct IBV strains have identified a hypervariable region (HVR) between 50–150 amino acids from the amino terminus (Cavanagh et al., 1988; Kusters et al., 1989; Wang et al., 1994).

We have shown that sequences lying within the S1 of the spike of IBV, upstream of the HVR, are a common site for major shifts in sequence homology in naturally occurring strains (Wang et al., 1993, 1994). This frequently used cross-over site immediately adjacent to the HVR included up to 153 bases at the 5′ end of the S1 gene (Wang et al., 1994). Whereas variations within the S1 correlates with serotype, the HVR is thought to be closely associated with major neutralization epitopes (Neister et al., 1987; Lenstra et al., 1989; Ignjatovic and Galli, 1994). Recombination in the IBV genome has recently been experimently demonstrated in the N gene following co-infection of embryonated chicken eggs (ECE) infected with the closely related Mass41 and Beaudette strains (Kottier et al., 1995).

The following experiments were designed to identify experimentally generated chimeric viruses with cross-over sites lying in the first 560 or 580 bases (Mass41 or Ark99 strains, respectively) of the S1 gene, which includes the identified commonly used cross-over region and the HVR. The Mass41 and Ark99 strains were used as parental strains for co-infection because they have extensive differences in the nucleotide sequences of the S1 HVR, have been implicated in the generation of field isolates, and have a number of nucleotide differences within the flanking regions of the targeted sequences (Wang et al., 1993, 1994).

Cultured chicken embryonic kidney (CEK) cells were co-infected with CEK cell adapted Mass41 and Ark99 strains. The CEK cells were prepared in our laboratory from 19 day-old chicken embryos (Churchill, 1965). Monolayers of confluent CEK grown in 25 cm^2 flasks were inoculated simultaneously or singly with $10^{6.0}$ EID_{50} of Mass41 and $10^{6.0}$ EID_{50} of Ark99 viruses. Progeny viral particles used to detect recombination were harvested 48 h post-infection (pi). Supernatants were collected following repeated freeze-thaw cycles of infected cells and viral particles were concentrated after clarifying the supernatant at $8000 \times g$ for 30 min by centrifuging at $100\,000 \times g$ for 1 h (Williams et al., 1992). The viral RNA was extracted using the proteinase K/chloroform/phenol method (Sambrook et al., 1989) and reverse transcription of RNA was initiated using the downstream strain specific primers described in Table 1 for either Ark99 and Mass41 viruses and Superscript II reverse transcriptase (Gibco-BRL) (Wang et al., 1993). Following the removal of RNA from the hybrids of RNA and newly synthesized first strand cDNA with RNaseH, the remaining single-stranded

Table 1
Primers used for RTPCR amplification of S1 RNA of IBV

Reference	Nucleotide sequence 5′-3′	Location from S1 start codon
Ark-U[a]	CTAAGGAACG-GTAAATTGTTA	−20
Mass-U	ATAGTGTGGT-AAGTTACTGG	−20
Ark-D[b]	CTA-CAACATC-TTCAGTATAG	580
Mass-D	ATGTAACATCT-GTGGTCTCA	604

[a] U refers to upstream primers.
[b] D refers to downstream primers.

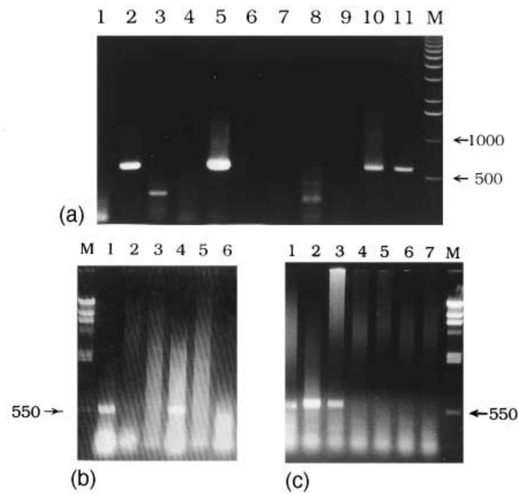

Fig. 1.

Fig. 1. Chimeric RTPCR amplified products from co-infections with Mass41 and Ark99 strains of IBV. Using primers described in Table 1, the region targeted was −20 to nt 604 for Ark99 and 580 for Mass41. The reverse transcription assays were initiated with either primer Mass-D or Ark-D. Conditions for PCR with upstream and downstream primers were as follows: 25 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min. (a) Amplified products from CEK RNA of uninfected CEK generated with Ark-D, Mass-D, Ark-U, and Mass-U primers in lane 1; of Ark99 infected CEK with Ark-U and Ark-D primers in lane 2; of Ark99 infected CEK with Mass-U and Ark-D primers in lane 3; of Ark99 infected CEK with Ark-U and Mass-D primers in lane 4; of Mass41 infected CEK with Mass-U and Mass-D primers in lane 5; of Mass infected CEK with Mass-U and Ark-D primers in lane 6; of Mass infected CEK with Ark-U and Mass-D primers in lane 7; of mixed Mass and Ark RNA with Mass-U and Ark-D primers in lane 8; of mixed Mass and Ark RNA with Ark-U and Mass-D primers in lane 9; of Mass41 and Ark99 co-infected CEK with Ark-U and Mass-D primers in lane 10; of Mass41 and Ark99 co-infected CEK with Mass-U and Ark-D primers in lane 11, and marker *Hin*dIII digested λ DNA ladder marker in lane M with nucleotide size of smaller bands (Gibco-BRL, Grand Island, NY) in lane 12. (b) Amplified chimeric products derived from RNA of Mass41 and Ark99 co-infected CEK cells and allantoic fluid of chicken embryos. *Hin*dIII digested λ phage DNA marker is shown in lane M as in 1a. Amplification of viral RNA from co-infected CEK cells with Ark-U and Mass-D primers is shown in lane 1; viral RNA from co-infected CEK cells with Mass-U and Ark-D primers in lane 2; uninfected CEK RNA with Ark-U, Mass-U, Ark-D and Mass-D primers in lane 3; viral RNA from co-infected ECE with Ark-U and Mass-D primers in lane 4; viral RNA from co-infected ECE with Mass-U and Ark-D primers in lane 5; uninfected chicken embryo RNA with Ark-U, Mass-U, Ark-D and Mass-D primers in lane 6. (c) Amplifed products derived from RNA of particles of Mass and Ark vaccine co-infected chicken lungs using Ark-U and Mass-D primers. Products were derived from an expired co-infected chick (lane 1); from two co-infected healthy chicks (lane 2 and 3); from co-infected sick chicks (lane 4, 5 and 6); from chick lung cellular RNA of an uninfected chick (lane 7), and *Hin*dIII digested lambda phage DNA marker (lane M) as in 1a.

cDNA was used as template for PCR with homologous and/or heterologous primers. Amplified PCR products were observed by agarose gel electrophoresis (Fig. 1). Chimeric S1 could be identified by agarose gel electrophoresis of RTPCR amplified products generated with strain specific heterologous primers.

The agarose gel separated amplified cDNA from infected and uninfected CEK cells are shown in Fig. 1a. Similar results were obtained with chicken embryonic lung cells (data not shown). The products from control experiments showed that homologous primers could amplify viral cDNA of the predicted size from particles isolated from either Ark99 or Mass41 infected cells. No recombinant RTPCR product was detected using heterologous primers with either Mass41 or Ark viral RNA, or with mixture of Ark and Mass41 viral RNAs. Nor was any RTPCR product generated from uninfected cellular RNA using both Ark99 and Mass41 specific primers. However, RTPCR amplified recombinant cDNA products could be detected from the viral RNA of Mass and Ark co-infected CEK cells using both sets of heterologous primers. The amounts of viral RNA used for RNA extraction in the experimental and control groups were equivalent.

Recombination was also evaluated in embryonating chicken eggs (ECE). Allantoic sacs of 11-day old ECE were inoculated with about $10^{5.7}$ EID_{50} of ECE adapted Ark99 and Mass41 viruses. The allantoic fluids from infected ECE were harvested 48 h pi and concentrated by differential centrifugation as described above (Williams et al., 1992). Recombinant RTPCR products were clearly detected following amplification with the Ark-U and Mass-D primer set (Fig. 1b). No recombinant RTPCR product was observed with RNA from the uninfected embryos.

```
Ark
Mass

Cross
over

ATG       100       200       300       400       500

          S start                                                                                          100
C1 11                                                       CTTG
C3 11                                                       CTTG
ECE 19                                          C                                                     A     C
Ark99     ATGTTGGTGAAGTCACTGTTTCTAGTGACCATTTTGTTTGCACTATGTAGTGCTAATTTATATGACAACGAATCTTTTGTGTATTACTACCAGAGTGCTT
Mass41            A CAC T  T  A        TC      G  T             GC   G       GTAGT    AC  T  C        A     C
TC 20                                             T             GC   G       GTAGT    AC  T  C        A     C
TC 26                                                       CTTGGC   G       GTAGT    AC  T  C        A     C
TC 25                                                                                         T  C        A     C
TC 6                                                                                           C                C
TC 7                                                                                                            C
TC 30
TC 33                                                       CTG

          101                                                                                              200
C1 11                                                          A T     C   TC           ACT  T A  TG G  T T  T
C3 11                                                          A T     C   TC            CT  T A  TG G  T.....
ECE 19        A   CCTA                C  G           G         A T     C   TC            .....................
Ark99     TTAGGCCAGGACATGGTTGGCATTTACATGGAGGTGCTTATGCAGTAGTTAATGTGTCTAGTGAAAATAATAATGCAGGTACTGCCCCAAGTTGCACTGC
M41           A   CCTA                C  G           G         A T     C   TC            CT  T A  TG G  T T  T
TC 20         A   CCTA                C  G           G         A T     C   TC            .....................
TC 26         A   CCTA                C  G           G         A T     C   TC           ACT  T A  TG G  T T  T
TC 25         A   CCTC                C  G           G         A T     C   TC           ACT  T G  TG G  T T  T
TC 6          A   CCTA                C  G           G         A T     C   TC            CT  T A  TG G  T T  T
TC 7          A   CCTA                C  G           G         A .............................................
TC 30                                    G           G         A T     C   TC           ACT  T A  TG G  T T  T
TC 33                                 C  G           G         A T     C   TC            CT  T A  TG G  T T  T
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Fig. 2. Schematic representation of shifts and nucleotide sequences of cloned products. Chimeric products were amplified with the Mass-D and Ark-U primers covering nucleotide −20 to 580 in the S1 genes. DNA samples were purified with spin columns (Qiagen, Chatsworth, CA) and the DNA was sequenced using the dideoxy method according to instructions (Sequenase Kit, USB, Cleveland, OH) or by the dye terminator cycle method (Ready Reaction, Perkin-Elmer, Foster City, CA) with the Applied Biosystems/Perkin-Elmer automated DNA/RNA sequencer (model 377). Sequences labelled TC (tissue culture) were derived from RNA of co-infected CEK, ECE from RNA of co-infected ECE and C from RNA of co-infected chicks. The sequences of the first 200 nucleotides of S1 genes from recombinants and their parental strains are compared. The sequences of parental strains Mass41 and Ark99 were derived from published data (Binns et al., 1985; Wang et al., 1993).

Recombination was confirmed and the approximate cross-over sites were identified by cloning and sequencing of cDNA generated from the Ark-U and M41-D primers which consistently produced a product from in vitro or in ovo infection that could be easily seen on an agarose gel (Fig. 2). RTPCR generated cDNA bands with the predicted 600 nt size were excised from agarose gels and the DNA extracted with GeneClean (Bio101, Vista, CA). The DNA was sequenced after cloning into a PCR cloning vector (Invitrogen, San Diego, CA). The sequence information for the recombinant viruses were compared with the published sequences of the parental strains (Binns et al., 1985; Wang et al., 1993). Cross-over sites in the in vitro generated recombinant viruses were detected within cDNA corresponding to nucleotides 50–60 and nucleotides 95–100 of the S1 gene. Recombination in the chimeric RNA derived from in ovo co-infection occurred at about nucleotide 99. These sites lie within the putative recombination 'hot spot' indicated from sequencing of the S1 of a number of natural isolates (Wang et al., 1993, 1994). No shifts in homology could be detected within the HVR which extends from nucleotides 160–560. An identical change at nucleotide 52–55 (TGCT to CTTG) from our original Ark sequence or the

Mass41 sequence occurred in three chimeric molecules (Binns et al., 1985; Wang et al., 1993). This change may reflect mutations in our stocks.

In order to reproduce recombination in the more natural in vivo setting, chicks were also co-infected with Mass41 and Ark99 viruses. Two-day old specific pathogen-free chicks were inoculated intranasally/intraorbitally with 500 times the recommended dose of both Mass41 and Ark99 vaccine strains. The infected chicks were sacrificed by cervical dislocation six days pi. Five of seven chicks co-infected with 500 doses suffered severe respiratory distress with sneezing, labored breathing, and rales, and two of the five succumbed three and five days pi. Lungs were collected, minced and homogenized in Eagle's MEM (Gibco, BRL, Bethesda, MD) and the viruses were collected from the supernatant as described above. The RNA of viruses from the lungs of one chick that died, and three sick and two healthy chicks were extracted for RTPCR using the Ark-U and Mass-D primer set. Fig. 1c shows the agarose gel with amplified chimeric cDNA products of RNA from the lungs of one co-infected sick chick that died, and two healthy chicks (Fig. 1c). No products were detected from the lungs of the remaining infected chicks nor uninfected chicks.

cDNA clones were generated from the RTPCR products amplified from the chick that died (C-1) and one clinically healthy chick (C-3). Nucleotide sequencing of the viral RNA from the in vivo co-infected lungs indicated that cross-over sites of recombination for both RNA were at approximately nucleotide 155 of the S1 gene, a region immediately upstream of the HVR. Again, no cross-over sites were identified within the HVR (Fig. 2).

Recombination within the S1 gene was experimentally produced from genomes within viral particles rather than cell lysates. Although the viability of the recombinant viruses was not demonstrated in this study, the experimentally produced S1 gene recombination events resembled the shifts in homology observed in nature (Wang et al., 1993, 1994). The approximate locations of all cross-over sites within chimeric RNA were located between nucleotides 50 and 155 (Fig. 2). Comparisons of sequences from the naturally occurring IBV strains had indicated that cross-over sites were concentrated at the 5′ end of the S1 genes (Wang et al., 1994; unpublished data). A second, less often used site was found close to the 3′ end of S1 (Wang et al., 1993, 1994). Although frequencies of recombination are not known, the fact that the HVR and adjacent downstream 600 or more nucleotides may seldom be targets for cross-over events suggests that the region lying upstream, in fact, is more susceptible for recombination. This region may have structures or sequences that promote polymerase interchange of templates. However, because the additional sequences examined were within the HVR, it is also possible that HVR sequences are not favorable sites for recombination. During the process of synthesizing potential chimeric molecules, the variability of the HVR may prohibit necessary RNA–RNA interactions. For example, released, replicating S1 HVR may not be capable of binding to an HVR of new template. An alternative explanation for the absence of detectable recombination events is that chimeric HVR may be less likely to express functional S1 required for viable virus than other chimeric regions, for example, the initial 155 bases of the S1.

The Ark99 and Mass41 strains were chosen because they are most often used in the USA for vaccination programs and consequently, are artificially maintained in the poultry population. The Mass serotype vaccines have historically by far been most often used to induce flock immunity and does demonstrate more cross-reactivity with other IBV strains (Sneed et al., 1989). Because the parental sequences of many recombinant, naturally occurring strains are derived from distinct Mass41-like sequences, it is a concern that the vaccine may be contributing to the evolution of new strains (Wang et al., 1994). These studies confirm that, with excessive doses of parental virus used to accelerate the rate of recombination, RNA from a Mass serotype can participate in cross-over events resulting in progeny with sequences of the Mass origin and sequences derived from a second parent with the Ark serotype. These studies, which were not designed to examine virulence of experimentally produced recombi-

nants, did produce a chimeric S1 from a sick chick and one from a healthy chick. The contribution, if any, of these recombination events to the generation of virulent strains can only be determined with further investigations.

The effect of recombination on immunologic evasion in the vaccinated chick is also not known. A successful escape mutant must certainly be viable, if not virulent, and have altered critical epitopes in order to cause disease in an immunized host. Preliminary studies in which recombinant molecules were detected following transfection of in vitro transcribed RNA from the Gray strain into Mass41 infected embryonic cultured cells have suggested that targeted recombination is also feasible (unpublished data). The capacity for Mass41 strains and Mass vaccines, in particular, to participate in the occurrence of new isolates and the ready experimental generation of Mass41 recombinants in vivo, as well as in vitro and in ovo, may further raise concern about the routine use of live IBV to control disease. Caution should also be encouraged in the use of attenuated vaccines of any virus that can readily undergo recombination.

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References

Banner, L.R. and Lai, M.C. (1991) Random nature of Coronavirus RNA recombination in the absence of selection pressure. Virology 185, 441–445.

Baric, R.S., Fu, K., Schaad, M.C. and Stohlman, S.A. (1990) Establishing a genetic recombination map for murine coronavirus strain A59 complementation groups. Virology 177, 646–656.

Binns, M.M., Boursnell, M.E.G., Cavanagh, D., Pappin, D.J.C. and Brown, T.D.K. (1985) Cloning and sequencing of the gene encoding the spike protein of the coronavirus IBV. J. Gen. Virol., 66, 719.

Boursnell, M.E.G., Brown, T.D.K., Foulds, I.J., Green, P.F., Tomley, F.M. and Binns, M.M. (1987) Completion of the sequence of the genome of the coronavirus avian infectious bronchitis virus. J. Gen. Virol. 68, 57–77.

Cavanagh, D., Davis, P.J. and Mockett, A.P.A. (1988) Amino acids within hypervariable region 1 of avian coronavirus IBV (Massachusetts serotype) spike glycoprotein are associated with neutralization epitopes. Virus Res. 11, 141–150.

Churchill, A.E. 1965. The use of chicken kidney tissue culture in the study of the avians viruses of Newcastle Disease, infectious laryngo-tracheitis and infectious bronchitis. Res. Vet. Sci. 6, 162–167.

Collisson, E.W., Parr, R.L., Wang, L., and Williams, A.K. (1992) An overview of the molecular characteristics of avian infectious bronchitis virus. Poultry Sci. Rev. 4, 41–55.

Ignjatovic, J., and Galli, L. (1994) The S1 glycoprotein but not the N or M proteins of avian infectious bronchitis virus induces protection in vaccinated chickens. Arch. Virol. 138, 117–134.

Kant, A., Koch, G., van Roozelaar, D.J., Kusters J.G., Poelwijk, F.A. and van der Zeijst B.A. (1992) Location of antigenic sites defined by neutralizing monoclonal antibodies on the S1 avian infectious bronchitis virus glycopolypeptide. J. Gen. Virol. 73, 591–596.

Keck, J.G., Matsushima, G.K., Makino, S., Fleming, J.O., Vannier, D.M., Stohlman, S.A., Lai, M.M. (1988) In vivo RNA–RNA recombination of coronavirus in mouse brain. J. Virol. 62, 1810–1813.

King, D.J. and Cavanagh, D. (1991) Infectious bronchitis. In: B.W. Calnek et al. (Eds.) Diseases of Poultry. Iowa State Press, Ames, IA, pp. 471–484.

Koch, G., Hartog, L., Kant, A. and van Roozelar D.J. (1992) Antigenic domains on the peplomer protein of avian infectious bronchitis virus: Correlation with biological functions. J. Gen. Virol. 71, 1929–1935.

Kottier, S.A., Cavanagh, D. and Britton, P. (1995) Experimental evidence of recombination in coronavirus infectious bronchitis virus. Virology 213, 2201–2213.

Kusters, J.G., Niesters, H.G.M., Lenstra, J.A., Horzinek, M.C. and Van Der Zeijst, B.A.M. (1989) Phylogeny of antigenic variants of avian coronavirus IBV. Virology 169, 217.

Lai, M.M.C., Baric, R.S., Makino, S., Deck, J.G., Egbert, J., Leibowitz, J.L., and Stohlman, S.A. (1985) The recombination between nonsegmented RNA genomes of murine coronaviruses. J. Virol. 56, 449–456.

Lenstra, J.A., Kusters, J.G., Koch, G., and van der Zeijst, B.A.M. (1989) Antigenicity of the peplomer protein of infectious bronchitis virus. Mol. Immunol. 26, 7–15.

Liao, C-L., and Lai, M.M.C. (1992) RNA recombination in a coronavirus: Recombination between viral genomic RNA and transfected RNA fragments. J. Virol. 66, 6117–6124.

Makino, S., Keck, J.G., Stohlman, S.A., and Lai, M.M.C. (1986) High-frequency RNA recombination of murine coronaviruses. J. Virol. 57, 729–737.

Neister, H.G.M., N.M.C. Bleumink-Pluym, A.D.M.E. Osterhaus, M.C. Horzinek, and van der Zeijst, B.A.M. (1987) Epitopes on the peplomer protein of infectious bronchitis virus strain M41 as defined by monoclonal antibodies. Virology 118, 1–7.

Peng, D. Koetzner, C.A. and Masters, P.S. (1995) Analysis of second-site revertants of a murine coronavirus nucleocapsid protein deletion mutant and construction of nucleocapsid protein mutants by targeted RNA recombination. J. Virol. 69, 3449–3457.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sneed, L.W., Butcher, G.D., Parr, R., Wang, L., and Collisson, E.W. (1989) Comparison of the structural proteins of avian infections bronchitis virus as determined by western blot analysis. Virol Immunol. 2, 221–227.

Wang, L., Junker, D., and Collisson, E.W. (1993) Evidence of natural recombination within the S1 gene of infectious bronchitis virus. Virology 192, 710–716.

Wang, L., Junker, D., Hock, L., Ebiary, E., and Collisson, E.W. (1994) Evolutionary implications of genetic variations in the S1 gene of infectious bronchitis virus. Virus Res. 34, 327–338.

Williams, A., Wang, L., Sneed, L. and Collisson, E. (1992) Comparative analyses of the nucleocapsid genes of several strains of infectious bronchitis virus and other coronaviruses. Virus Res. 25, 213–222.