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Comparisons of envelope through 5B sequences of infectious bronchitis coronaviruses indicates recombination occurs in the envelope and membrane genes

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Received 3 April 2003; received in revised form 20 November 2003; accepted 20 November 2003

Abstract

A 1.78 kb sequence, including the E, M, 5a and 5b genes, and the intergenic region between the M and 5a genes, of six US strains of infectious bronchitis (corona)virus (IBV) were sequenced and compared to the published sequences for two additional strains. The overall identities as determined through pairwise analyses of nucleotide sequences of the entire 1.78 kb region ranged from 90 to 99%, with the 5b open reading frame (ORF) having the greatest identity (94–99%) while the identities of the E, 5a and M ORFs ranged from 87 to 100%.

Nucleotide sequencing of recent field isolates from Alabama (Ala1) and California (Cal3) revealed distinct shifts in homology in the M gene, indicating two apparent recombination events between the Holland 52/Mass41-like strain and an Ark-like strain, both origins of commonly used vaccine strains. Putative sites of recombination could also be identified in both the E and M ORFs of laboratory strains of IBV.

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Keywords: Infectious bronchitis virus; Membrane ORF; Recombination; Nucleotide sequence comparisons

1. Introduction

Infectious bronchitis virus (IBV) is a linear, positive-sense RNA coronavirus that is responsible for recurring economic losses to the poultry industry. First described by Schalk and Hawn (1931) as a "gaping disease" of baby chicks, the clinical signs of IBV infection include respiratory manifestations, such as tracheal rales and coughing, diarrhea, egg loss and nephritis (Beaudette and Hudson, 1937; Winterfield and Hitchner, 1962; Crinion and Hofstad, 1972; Gilchrist, 1963; Cumming, 1963; Julian and Willis, 1969). Different strains of the virus may cause distinct illness. The Gray and Holte strains, for example, have been shown to cause both nephrogenic and respiratory disease (Winterfield and Hitchner, 1962), whereas the Mass41 and Ark99 strains cause primarily respiratory disease (Beaudette and Hudson, 1937; Winterfield and Hitchner, 1962; Cumming, 1963; Darbyshire et al., 1978; Johnson and Marquardt, 1976; Sutou et al., 1988). The pathogenesis and genome arrangement of IBV are similar to the recently described severe acute respiratory syndrome coronavirus or SCoV (Peiris et al., 2003; Drosten et al., 2003; Ksiazek et al., 2003; Marra et al., 2003; Rota et al., 2003).

The RNA genome of IBV is about 27.6 kilobases (kb) in length (Bournef et al., 1987). IBV, like other coronaviruses, has a unique discontinuous transcription which forms a nested set of genomic and subgenomic mRNAs with common 3′ termini and the smaller mRNAs. The 5′ end of each mRNA contains the leader sequence found only in the 5′ unique untranslated region of the genome (Schaap et al., 1983; Lai et al., 1984; Stern and Kennedy, 1990a,b, Gorbelenya, 2001). Due to the economic importance of IBV for much of the last 72 years, an accumulated wealth of genetic IBV variants with distinct serotypes and pathotypes are available.

Four major structural proteins make up the virion of IBV and SCoV: the glycosylated spike (S) protein,
post-translationally cleaved in IBV but not ScCoV, the hydrophobic envelope or small membrane (E) protein, the membrane (M) protein, glycosylated at the amino termini, and the phosphorylated nucleocapsid (N) protein, associated with the viral RNA (Collisson et al., 1992; Cavanagh and Naqvi, 1997). A lipid bilayer membrane formed by budding from intracellular membranes surrounds the nucleocapsid. The genome of the Beaudette (Beau) strain of IBV was the first coronavirus genome completely sequenced (Binnis et al., 1985; Boursnell et al., 1984, 1985a,b; Boursnell and Brown, 1984; Brown et al., 1984). The conserved and variable regions of the S and N genes and 3′ untranslated region from a number of US strains of IBV have been identified, as well as potential recombination sites within the S protein (Binnis et al., 1986a,b; Niesters et al., 1986a; Boursnell et al., 1985b; Williams et al., 1992, 1993; Wang et al., 1993, 1994; Kwon and Jackwood, 1995; Jia et al., 1995; Gelb et al., 1997; Keeler et al., 1998; Collison et al., 1999; Nix et al., 2000; Kingham et al., 2000). However, comparisons of genomic sequences between the S and N genes on the IBV genome have not been examined with most US strains. Only the nucleotide sequences of the Mass41 (Niesters et al., 1986b; Cavanagh and Davis, 1988; Liu et al., 1991) and Holl52 (Cavanagh and Davis, 1988) E and M genes have been reported.

As early as 1988, Cavanagh and Davis postulated that IBV recombination could have occurred between two Dutch strains. Sequence analysis of a portion of the S1 and M genes indicated that one recombinant could have the M gene of one parent strain and the S1 gene of the other (Cavanagh and Davis, 1988; Niesters et al., 1986a). Kusters et al. (1989, 1990) and Zwaagstra et al. (1992) used phylogenetic trees to illustrate that some strains may have evolved by recombination. Cavanagh et al. (1990) demonstrated that a Portuguese isolate was a recombinant, and that part of the S1 gene of this isolate was very similar to that of the vaccine strain H120. Wang et al. (1993) found evidence of natural recombination in the S1 gene of field isolates of IBV from Texas, concluding that vaccine strains may be involved in recombination of IBV and that recombination occurred in nature. Later studies by Wang et al. (1994) concluded that shifts in homology, as well as point mutations within the S1 gene were common and contribute to the evolution of IBV. Jia et al. (1995) sequenced the isolate CU-T2 and observed recombination events between three different IBV strains within the S2 gene and the N gene with evidence that the Holl52 vaccine strain N contributed to half that gene in IBV. Jia et al. (1995) sequenced the isolate CU-T2 and observed recombination events between three different IBV strains within the S2 gene and the N gene with evidence that the Holl52 vaccine strain N contributed to half that gene in IBV. Recombination continues to be identified in recent isolates from the United States and Southeast Asia (Lee and Jackwood, 2000a,b; Yu et al., 2003). However, to our knowledge, recombination within the M gene has not been previously reported from a field isolate.

This report compares the genomic sequences from the 5′ end of the E gene through the 3′ end of the 5b gene of eight strains of IBV. Six of these were completely sequenced in this region for the first time (Mass41, Iowa, Conn, Arkansas Delmarva Peninsula (Ark DPI, Gray, and Holl52)). In addition, the E, M, 5a, and 5b genes of one field isolate from Alabama (Ala1) and most of the M gene of two additional field isolates from California (Cal2 and Cal3) were sequenced and analyzed. Apparent recombinations in the genome of two recent IBV field isolates are described.

2. Materials and methods

2.1. Viral stocks

The Gray strain of IBV was obtained from Dr. R.W. Winterfield at Purdue University, and the Arkansas DPI strain of IBV was obtained from Dr. J.K. Rosenberger at the University of Delaware. Dr. Fred Hoerter kindly provided a field isolate (9906426 P-3, referred to in this manuscript as Ala1). Dr. Peter Woolcock provided two field isolates used in this study (F9601491, referred to as Cal2, and F9700100, as Cal3).

2.2. RT-PCR amplification

Virus was propagated in embryonating chicken eggs (Williams et al., 1992). The protocol described by Burlison et al. (1992) was used to extract RNA. Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed, using the protocol for the SuperScript Preamplification System for First Strand cDNA Synthesis from GibcoBRL (Rockville, MD). Primers were made in the DNA Core Facility, Department of Pathobiology, College of Veterinary Medicine, Texas A&M University (Dr. James Derr, Director, College Station, TX). Table 1 shows the sequences of the primers that were used for PCR amplification and sequencing. In addition, primer D1, complementary to the sequence of the 3′ end of the genome, was used for the RT reactions. A 1–5 μg RNA sample was mixed with 2 mM primer in DEPC-treated water and incubated for 10 min at 70 °C. After a 1 min incubation on ice, the RNA-primer mixtures were mixed with 10× PCR buffer, 25 mM MgCl2, 10 mM dNTP mix, and 0.1 M dithiothreitol (DTT). This mixture was incubated for 5 min at 42 °C. SuperScript II reverse transcriptase (RT) was added to each tube, and this mixture was incubated at 42 °C for 50 min. The reaction was terminated first by incubating at 70 °C for 15 min and then by chilling on ice. RNase H was added, and the reactions were incubated for 20 min at 37 °C. The first strand complementary DNA (cDNA) was either used immediately for PCR amplification or stored at −20 °C.

Target cDNA was amplified by PCR, using the SuperScript Preamplification System for First Strand cDNA Synthesis from GibcoBRL (Rockville, MD). The following reaction was mixed in PCR tubes: 10× PCR buffer, 25 mM MgCl2, 10 mM dNTP mix, 10 mM forward and reverse amplification primers, Taq DNA polymerase, cDNA, and autoclaved, distilled water. PCR amplification was performed at 94 °C for 3 min, then 30 cycles of 95 °C for 1 min, 50 °C
Table 1

| Primer | Sequence | Sense | Position       |
|--------|----------|-------|----------------|
| E1     | ACTATTGTTATTACAGAGGA | Positive | 24,141–24,160  |
| E2     | TGCATAGCCATACTG | Negative | 24,613–24,627  |
| M1     | CTCAACATCGGGAATTGA | Positive | 24,420–24,440  |
| M2     | TTTCCTAAGAACGGTTGGAATA | Positive | 24,459–24,480  |
| M3     | ACTCTTTAACGCGTGTAGTTC | Positive | 24,810–24,830  |
| M4     | GCCACAAAGAAGTGCGAAAAGT | Negative | 6,479–6,499    |
| M5     | CTGTCTTTCCAGTTGCCTTACC | Negative | 25,882–25,903  |
| M6     | TCCCAGGTGACCCTCTAGCTA | Positive | 25,901–25,922  |
| D1     | GATGGCTGTTGACCTCTAGCTA | Negative | 27,580–27,608  |

Primers are written 5'–3'. All primer positions are in relationship to primer locations in the Beaudette genome except primer M4, which is within a deleted region of the Beaudette genome. The position of primer M4 is relative to the start codon of the S gene of strain KB8523.

2.3. Nucleotide sequencing

The cDNA samples were purified with the QiAquick PCR Purification Kit from Qiagen (Santa Clarita, CA). PCR products were directly sequenced or were sequenced after cloning into the pCR 2.1 plasmid from the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA). Sequencing was performed using the ABI PRISM dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Sequencing PCR reactions were performed for 25 cycles, with 10 s at 96 °C, 5 s at 50 °C, and 4 min at 60 °C. Residual primers were removed from these samples by ethanol precipitation or Bio-Rad Micro Bio-Spin chromatography columns (Hercules, CA). The products of these reactions were sent to the DNA Core Facility, Department of Pathobiology, College of Veterinary Medicine, Texas A&M University (College Station, TX) for dideoxy sequence analysis. Each region was sequenced at least twice from at least two PCR products from different RT reactions.

2.4. Computer analysis

Computer analysis of the sequences was performed using the CLUSTLW, SIXFRAME, and DRAWGRAM programs, all from Biology Workbench 5.2 (San Diego Supercomputer Center, University of California, San Diego, CA, 2000). GenBank submissions were made with the BankIt software program through the National Center for Biotechnology Information (NCBI), US National Library of Medicine (Bethesda, MD).

2.5. GenBank accession numbers

GenBank accession numbers for the genes submitted through this study included: Gray E, AF318282; Ark DPI E, AF317209; Holl52 E, AF317210; Conn E, AF317471; Iowa E, AF467921; Gray M, AF286180; Mass41 M, AF286184; Ark DPI M, AF286181; Holl52 M, AF286185; Conn M, AF286182; Iowa M, AF286183; Gray intergenic through 5b, AF469011; Mass41 intergenic through 5b, AF469015; Ark DPI intergenic through 5b, AF469012; Holll52 intergenic through 5b, AF469016; Conn intergenic through 5b, AF469013; Iowa intergenic through 5b, AF469014; Alabama 1 E, AF470627; Alabama 1 M, AF470628; Alabama 1 5a and 5b, AF470629; California 2 M, AF470629; California 3 M, AF470630.

3. Results

The nucleotide sequences of a region extending from the E gene through the 5b gene of six US strains of IBV (Gray, Mass41, Ark DPI, Holl52, Conn, and Iowa) and putative amino acid sequences were determined and compared with each other and the published sequences of the Beaudette (Boursnell et al., 1987) and KB8523 (Sutou et al., 1988) strains. The E, M, 5a, and 5b genes were sequenced for one new field isolate (Ala1). In addition, the M genes of two new field isolates from California were sequenced. This region represented a 1780 nucleotide sequence from the KB8523 strain genome (Sutou et al., 1988), but was shorter in the Beaudette strain (Boursnell et al., 1987) and in the Iowa strain due to lengthy deletions. The remaining five strains had about the same number of nucleotides as the KB8523 strain. The longest sequence of complete conservation was a stretch of 38 identical bases near the 5' end of the M gene. For each strain examined, the intergenic (IG) consensus sequence CTTAACAA, found at positions 78 through 85 upstream from the start codon of the M gene, positions 9 through 16 upstream from the start codon of the 5a gene, and positions 96 through 103 upstream from the N gene, was identical and in the same positions in the previously published strains.

3.1. Pairwise sequence homologies

Pairwise nucleotide sequence homologies in the E gene through 5b region of the various strains ranged from 90 to
99% (data not shown). Overall, the 5b ORF, with 94–99% identity among strains, was the most conserved region and the least conserved E ORF, had 87–99% nucleotide sequence homology. In the 5a gene, the nucleotide sequences of the Ark DPI and Conn strains were 100% identical and 97% identical to those of the Gray strain. The intergenic region between the M gene and the 5a gene was much more variable with an overall identity of only 74%. In addition, the Beaudette strain had a large deletion in this region, and the nucleotide sequences for the Ark DPI, Conn, and Gray strains were 97% identical.

Pairwise putative amino acid identities for each individual putative protein ranged from a low of 79% identity for the E, between the Gray and Holl52 strains and between the Conn and Holl52 strains, to 100% identity for M, between the Mass41 and Holl52 strains, and for the 5a gene, between the Conn and Ark DPI strains.

3.2. Phylogenetic trees

The sequence relationships of these eight strains of IBV, strain DE072, and the field isolate Ala1 are illustrated in phylogenetic trees for the E, M, 5a, and 5b genes (Fig. 1). The trees for all four genes indicated that Gray, Ark DPI, and Conn cluster together. The E gene of the recent Ala1 isolate clustered with Beau, Holl52, and Mass41 (Fig. 1A) and the M gene of Ala1 was also grouped closely with Holl52 (Fig. 1B). However, in the 5a and 5b trees (Fig. 1C and D), Ala1 sequences were placed in a cluster with Conn, Ark DPI, and Gray. The inconsistencies in the clustering observed among the individual trees of each gene suggested shifts in sequencing homology.

3.3. Shifts in homology

Analysis of the region from the E gene through the 3’ end of the 5b region located the shifts in homology or potential recombination sites in the E and M genes (Fig. 2), while the remainder of the 1.78 kb sequence did not appear to have any
obvious changes in homology. The E genes of the Mass41, Holl52, and Beau strains were very similar in nucleotide sequence (Fig. 2). The Iowa strain was similar to Mass41, Holl52, and Beau strains were very similar in nucleotide sequence (Fig. 2). The Iowa strain was similar to Mass41, Holl52, and Beau, but from about nucleotide 170 of the E gene, the 3′ end was quite similar to that of Ark DPI and Conn. Another apparent recombination event occurred between nucleotide 150 and nucleotide 215 of the M gene and thereafter, the Ala1 nucleotide sequence most closely resembled the Holl52 strain.

Wang et al. (1993) noted that the sequence CTT(A/T) (A/T)G was located near every putative junction where homology shifts were found. The sequence CTTTTG was identified between bases 171 and 176 of the M gene (Fig. 3). This site is in the middle of a 60-nucleotide region of 100% identity, including the Ala1 isolate. In addition, the second apparent recombination event in the Ala1 isolate occurred within this region of total homology. The nucleotide sequences of the 5a and 5b genes for Ala1 were 99% identical to those of Conn and Ark DPI and only 93 and 96% identical to the respective genes for Holl52, indicating that another recombination event may have occurred within the intergenic region between the M gene and the 5a gene.

Overall, the nucleotide sequence of the M for the recent isolate Cal2 was similar to that of the Gray strain (data not shown). However, the Cal3 isolate had putative sites of recombination within the M gene. From nucleotide 170 of the E gene through nucleotide 50 of the M gene, the Cal3 nucleotide sequence was similar to that of Mass41, Holl52, KB8523, and Beau group. However, from approximately nucleotide 60 of the M gene through 250 of the M gene, the Cal3 sequence was similar to that of Ark DPI. From about 250 to the Cal3 then resembled the M gene of Mass41 and Beau. Thus, Cal3 had two apparent recombination events within the M gene.

4. Discussion

This is the first report of nucleotide sequence comparisons, representing the E through the 5b region of the IBV genome. The comparisons of sequences from eight strains of IBV showed abrupt shifts in homology that suggested recombination had occurred in several locations within this region. Williams et al. (1992, 1993) analyzed the nucleocapsid gene sequences and the 3′ non-coding regions and found that this isolate had similar to the respective genes for Holl52, indicating that another recombination event may have occurred within the intergenic region between the M gene and the 5a gene.

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Wang et al. (1994) produced a phylogenetic tree that showed the relationships of 25 different IBV strains and field isolates based on their S1 amino acid sequences. These were divided into five genotypic groups based on the homologies.
among the strains. The Iowa 609 and Gray strains were in one group; the KB8523, H52, Beaudette, Mass41, and Conn strains were in another; Ark99 strain was in still another group. Like the Williams et al. (1992, 1993) studies of the nucleocapsid and 3′ UTR sequences, the phylogenetic trees of the genes of the 1.78 kb region clustered Gray and Ark at one end of the tree, while Beau, Holl52, and Mass41 were placed at the other end. Wang et al. (1994), however, found that the S1 genes of the Iowa and Gray strains were clustered together and the Ark99 strain was in another group. As with the present study and the Williams et al. (1992, 1993) studies, Wang et al. (1994) noted that the S1 gene of Beau, Holl52, and Mass41 formed a common group. Combined with the present work, these results indicated a homology shift for the Ark, Gray, and Iowa strains somewhere in the region between the S1 gene and the E gene. Moreover, the phylogenetic trees in this study suggested that putative recombination occurred to generate the recently isolated Ala1
CTTTTG, that Wang et al. (1993) described adjacent to the IG consensus sequences (CTTAACAA). The sequence in the present study, no recombination “hot spots” were found in the M gene and once within the 5b gene for every strain studied in this paper. In addition, this sequence was found once within the E gene, once within the intergenic region between the M and 5a genes, and once within the 5a gene for several of the strains. Data from the present study demonstrated one putative recombination site in the M gene of the Alai isolate that was 40 nucleotides downstream from the first CTTTTG sequence in the M gene. These so-called “hot spots” could possibly promote recombination because they act as T-rich regions. However, recombination events among the eight strains would seem to occur randomly rather than at specific “hot spots”, because only one apparent recombination event occurred near the CTTTTG sequence. Whereas there were no detectable recombination events in the 5a or 5b genes, the nucleotide sequence of the 5b gene is well conserved. Sequencing of field isolates demonstrated recombination within the M gene. A recombination sequence could be identified as related to a portion of the H0152 vaccine. Such natural recombination is apparently a widespread event and occurs throughout the genome, providing a natural process for the construction of genetic variants that produce more virulent strains of IBV.

Acknowledgements

This work was funded by grants from the US Poultry and Egg Association (#297), USDA Formula Animal Health Funds (#1433), and USDA Cooperative State Research Service (#97-35204-5069).

References

Bourne, M.E.G., Binns, M.M., Foulds, I.J., Brown, T.D.K., 1985a. Sequencing of coronavirus IBV genomic RNA: three open reading frames in the 5′ 'unique' region of mRNA D. J. Gen. Virol. 66, 2255–2259.

Jia, W., Karaca, K., Parrish, C.R., Naqi, S.A., 1995. A novel variant of avian infectious bronchitis virus. J. Gen. Virol. 66, 573–580.

Bourne, M.E.G., Binns, M.M., Brown, T.D.K., 1985b. Sequences of the nucleocapsid genes from two strains of avian infectious bronchitis virus. J. Gen. Virol. 66, 2251–2254.

References

Bourne, M.E.G., Binns, M.M., Brown, T.D.K., 1984. Sequencing of coronavirus IBV genomic RNA: a 195-base open reading frame encoded by mRNA B. Gene 29, 37–42.

Jia, W., Karaca, K., Parrish, C.R., Naqi, S.A., 1995. A novel variant of avian infectious bronchitis virus. J. Gen. Virol. 66, 573–580.

Bourne, M.E.G., Binns, M.M., Brown, T.D.K., 1984. Sequence of the membrane protein gene from avian coronavirus IBV. Virus Res. 1, 303–313.

Hudson, C.B., Brown, T.D.K., 1987. Completion of the sequence of the genome of the coronavirus avian infectious bronchitis virus. J. Gen. Virol. 68, 57–77.

Bourne, M.E.G., Binns, M.M., 1984. Evolution of avian coronavirus IBV: sequence of the matrix glycoprotein gene and intergenic region of several serotypes. J. Gen. Virol. 69, 621–629.

Callison, S.A., Jackwood, M.W., Hilt, D.A., 1999. Infectious bronchitis virus S2 gene sequence variability may affect S1 subunit specific antibody binding. Virus Genes 19, 147–151.

Bourne, M.E.G., Binns, M.M., 1984. Sequence of coronavirus IBV genomic RNA: three open reading frames in the 5′ 'unique' region of mRNA D. J. Gen. Virol. 66, 2255–2259.

Jia, W., Karaca, K., Parrish, C.R., Naqi, S.A., 1995. A novel variant of avian infectious bronchitis virus. J. Gen. Virol. 66, 573–580.

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

Brown, T.D.K., Bourne, M.E.G., Binns, M.M., 1984. A leader sequence is present on mRNA A of avian infectious bronchitis virus. J. Gen. Virol. 65, 1437–1442.

Callison, S.A., Jackwood, M.W., Hilt, D.A., 1999. Infectious bronchitis virus S2 gene sequence variability may affect S1 subunit specific antibody binding. Virus Genes 19, 147–151.

Bourne, M.E.G., Binns, M.M., Brown, T.D.K., 1984. Evolution of avian coronavirus IBV: sequence of the matrix glycoprotein gene and intergenic region of several serotypes. J. Gen. Virol. 69, 621–629.

Collison, S.A., Jackwood, M.W., Hilt, D.A., 1999. Infectious bronchitis virus S2 gene sequence variability may affect S1 subunit specific antibody binding. Virus Genes 19, 147–151.

Cavanagh, D., Davis, P.J., 1988. Evolution of avian coronavirus IBV: sequence of the matrix glycoprotein gene and intergenic region of several serotypes. J. Gen. Virol. 69, 621–629.

Collison, S.A., Davis, P., Cook, J., Li, D., 1990. Molecular basis of the variation exhibited by avian infectious bronchitis coronavirus (IBV). Adv. Exp. Med. Biol. 276, 369–372.

Cavanagh, D., Davis, P.J., 1988. Evolution of avian coronavirus IBV: sequence of the matrix glycoprotein gene and intergenic region of several serotypes. J. Gen. Virol. 69, 621–629.

Collison, S.A., Davis, P., Cook, J., Li, D., 1990. Molecular basis of the variation exhibited by avian infectious bronchitis coronavirus (IBV). Adv. Exp. Med. Biol. 276, 369–372.

Cavanagh, D., Davis, P.J., 1988. Evolution of avian coronavirus IBV: sequence of the matrix glycoprotein gene and intergenic region of several serotypes. J. Gen. Virol. 69, 621–629.

Collison, S.A., Davis, P., Cook, J., Li, D., 1990. Molecular basis of the variation exhibited by avian infectious bronchitis coronavirus (IBV). Adv. Exp. Med. Biol. 276, 369–372.

Cavanagh, D., Davis, P.J., 1988. Evolution of avian coronavirus IBV: sequence of the matrix glycoprotein gene and intergenic region of several serotypes. J. Gen. Virol. 69, 621–629.

Collison, S.A., Davis, P., Cook, J., Li, D., 1990. Molecular basis of the variation exhibited by avian infectious bronchitis coronavirus (IBV). Adv. Exp. Med. Biol. 276, 369–372.

Cavanagh, D., Davis, P.J., 1988. Evolution of avian coronavirus IBV: sequence of the matrix glycoprotein gene and intergenic region of several serotypes. J. Gen. Virol. 69, 621–629.

Collison, S.A., Davis, P., Cook, J., Li, D., 1990. Molecular basis of the variation exhibited by avian infectious bronchitis coronavirus (IBV). Adv. Exp. Med. Biol. 276, 369–372.

Cavanagh, D., Davis, P.J., 1988. Evolution of avian coronavirus IBV: sequence of the matrix glycoprotein gene and intergenic region of several serotypes. J. Gen. Virol. 69, 621–629.

Collison, S.A., Davis, P., Cook, J., Li, D., 1990. Molecular basis of the variation exhibited by avian infectious bronchitis coronavirus (IBV). Adv. Exp. Med. Biol. 276, 369–372.
Niesters, H.G., Zijderveld, A.J., Seifert, W.F., Lenstra, J.A., 2001. Spike gene analysis of the DE072 strain of infectious bronchitis virus. Avian Dis. 43, 84–88.

Nix, W.A., Tovee, D.A., Kingham, B.F., Keeler Jr., C.L., Gelb Jr., J., 2000. Emergence of subtype strains of the Arkansas serotype of infectious bronchitis virus in Delmarva broiler chickens. Avian Dis. 45, 566–568.

Peiris, J.S.M., Chu, C.M., Cheng, V.C.C., Chan, K.S., Hung, I.F.N., Poon, L.L.M., Low, K.I., Tang, B.S.F., Hon, T.S.W., Chan, C.S., Chan, K.H., Ng, J.C.S., Zheng, B.J., Ng, W.L., Lau, R.W.M., Guan, Y., Yap, K.Y., Members of the HKU/SARS Study Group, 2003. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet 361, 1767–1772.

Rota, P.A., Osterhe, M.S., Monner, S.S., Nix, W.A., Campagnoli, R., Icenogle, J.P., Penaranda, S., Bankamp, B., Maher, K., Chen, M.H., Seng, A., Lowe, L., Frace, M., DeRisi, J., Chen, Q., Wang, D., Erdman, D.D., Peret, T.C., Burns, C., Ksiazek, T.G., Rollin, P.E., Sanchez, A., Lüflick, S., Holloway, B., Limor, J., McCaustland, K., Olsen-Rasmussen, M., Foucher, R., Gunther, S., Osterhaus, A.D., Drosten, C., Pallansch, M.A., Andersen, L.J., Bellini, W.J., 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300 (5624), 1394–1399.

Schuk, A.F., Hara, M.C., 1931. An apparently new respiratory disease of baby chicks. J. Am. Vet. Med. Assoc. 78, 413–422.

Spanu, W., Delius, H., Skinner, M., Armstrong, J., Rottier, P., Smeekens, S., van der Zeijst, B.A.M., Siddell, S.G., 1983. Coronavirus mRNA synthesis involves fusion of non-contiguous sequences. EMBO J. 2, 1839–1844.

Stem, D.F., Kennedy, S.T., 1990b. Coronavirus multiplication strategy. I. Identification and characterization of virus-specific RNA. J. Virol. 34, 665–674.

Stem, D.F., Kennedy, S.T., 1990a. Coronavirus multiplication strategy. II. Mapping the avian infectious bronchitis virus intracellular RNA species to the genome. J. Virol. 36, 440–449.

Suto, S., Sato, S., Okabe, T., Nakai, M., Sasaki, N., 1988. Cloning and sequencing of genes encoding structural proteins of avian infectious bronchitis virus. Virology 165, 589–595.

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

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

Williams, A.K., Wang, L., Sneed, W.L., Collisson, E.W., 1993. Comparison of the nucleocapsid genes of several strains of infectious bronchitis virus and other coronaviruses. Virus Res. 25, 222.

Williams, A.K., Wang, L., Sneed, W.L., Collisson, E.W., 1992. Comparison of the S1 glycoprotein of the Gray and JMK strains of infectious bronchitis virus. Avian Dis. 44, 325–326.

Williams, A.K., Wang, L., Sneed, L.W., Collisson, E.W., 1992. Comparison of the S1 glycoprotein of the Gray and JMK strains of infectious bronchitis virus. Avian Dis. 44, 325–326.

Winterfield, R.W., Hitchner, S.B., 1962. Etiology of an infectious nephritis-nephrosis syndrome of chickens. Avian Dis. 6, 233–236.