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Live attenuated nephropathogenic infectious bronchitis virus vaccine provides broad cross protection against new variant strains

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ABSTRACT Infectious bronchitis virus (IBV) infections cause great economic losses to the poultry industry worldwide, and the emergence of new variant strains complicates disease control. The present study investigated the genetic and protectotypic features of newly emerged Korean IBV strains. A phylogenetic analysis showed that several recent isolates formed 2 different clusters (new cluster 1 and 2), which were distinct from other preexisting clusters. New cluster 1 IBV strains represented recombinants between Korean nephropathogenic strain KM91 and the QXIBV strain. New cluster 2 IBV strains showed low amino acid homology (<58.7%) compared with previous isolates. We evaluated the protective efficacy of commercial IBV vaccines (H120 and K2 strain) against these new isolates. In cross-protection studies, the H120 strain did not provide sufficient protection against these variants. However, highly attenuated nephropathogenic IBV vaccine, K2 strain, provided significantly higher levels of protection against variants compared with chickens vaccinated with H120 ($P < 0.05$ or better). These results indicate that the K2 vaccine could be helpful for the reduction of economic losses caused by newly evolving IBV recombinants (new cluster 1) and variants (new cluster 2).

Key words: infectious bronchitis virus, vaccine, spike glycoprotein, phylogenetic analysis, cross-protection

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INTRODUCTION

Infectious bronchitis virus (**IBV**) causes an acute and highly contagious viral disease of chickens that is characterized by respiratory signs, nephritis, and reduced egg reproduction (Cavanagh and Naqi, 2003). Among the IBV structural proteins, the spike glycoprotein S1 subunit is responsible for attachment to the host cellular membrane, induction of neutralization, and hemagglutination of inhibiting antibodies (Casais et al., 2003; Cavanagh, 2007). Because of the high rates of *S1* gene mutation, several IBV serotypes or antigenic variant strains have been reported in many countries (Cavanagh, 2007). In general, different serotypes of the virus do not confer cross protection against each other (Ignjatović and Sapats, 2000), and cross protection tends to diminish as the degree of amino acid identity between the S1 proteins of 2 IBV strains decreases (Gelb et al., 2005). However, some strains of the virus are effective at inducing cross protection against other serotypes or genotypes; these are referred to as protectotypes. Protectotypes have been suggested as a valuable method for the development of strategies to control IBV infections (Dhinakar Raj and Jones, 1996).

A variant IBV strain identified as QXIBV that was first reported in China (YuDong et al., 1998) appears to have become widespread in Eurasia (Gough et al., 2008; Monne et al., 2008). In addition, QX-like nephropathogenic strains have increased the genetic diversity of IBV in the field and caused economic losses in the poultry industry (Worthington and Jones, 2006). Our previous study revealed that QXIBV strain originating from China has been introduced in Korea and has formed a variant virus by recombination with the Korean nephropathogenic strain (KM91 strain; Lim et al., 2011). Furthermore, more recently, some IBV strains formed a new cluster that is very distant from all major genogroups in the phylogenetic tree of the IBV *S1* gene. Because the emergence of new variant strains complicates disease control, there is a concern about whether commercial IBV vaccines could provide protection against these new IBV strains. Nevertheless, genetic and protectotypic characterization of new variant IBV strains remains largely undone.

The present study involved a phylogenetic analysis of the *S1* gene to compare new IBV isolates with commer-

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cial vaccine strains and other Korean IBV field isolates. Moreover, we investigated the cross protective efficacy of 2 commercial IBV vaccine strains against new variant IBV strains. The results of the present study might provide information for future IBV vaccination strategies.

MATERIALS AND METHODS

Viruses

Twenty-three new variant IBV isolates obtained from natural outbreak cases of IBV and 2 different commercially available vaccines (K2 strain of the nephropathogenic type and H120 strain of the Mass serotype) were used (Table 1). All isolates were propagated in 10-d-old specific-pathogen-free (**SPF**) embryonated chicken eggs (Hy-Vac, Adel, IA) at 37°C for 48 h. The allantoic fluid from eggs infected with each isolate was harvested and frozen at −70°C until use.

Chickens

The SPF White Leghorn chickens (Nam-Deog Sanitek Co., Icheon, Korea) were maintained in positive pressure high-efficiency particulate air-filtered stainless steel isolation cabinets (Three Shine, Daejeon, Korea) under constant illumination within a biosafety level-2 laboratory. All study procedures and animal care activities were conducted in accordance with the national and institutional guidelines for the care and use of laboratory animals (Seoul, Korea).

Viral RNA Extraction, Reverse-Transcription PCR, and DNA Sequencing

Viral RNA was extracted from virus-infected allantoic fluid using an RNeasy minikit (Qiagen, Gaithersburg, MD) following the manufacturer's instructions. The primer sets (forward: 5′- TAG TGA CCC TTT TGT GTG CAC TAT -3′; and reverse: 5′- GTT TGT ATG TAC TCA TCT GTA AC -3′) were designed for the amplification of the IBV *S1* gene. Reverse transcription (**RT**) was done using Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA) and a reverse primer. For the PCR reaction, EX-Taq polymerase (Takara, Otsu, Shiga, Japan) and the forward primer were added to the RT reaction. The PCR was performed by 40 cycles of denaturation (94°C for 10 s), annealing (53°C for 90 s), and polymerization (72°C for 90 s). The predenaturation step was at 94°C for 20 s and the postpolymerization step was at 72°C for 5 min. The amplified sequences were analyzed by 1.5% agarose gel electrophoresis.

The amplified DNA products were purified using a gel extraction kit (Elpis Biotech, Daejeon, Korea) and the purified products were ligated into a pGEM-T vector (Promega, Fitchburg, WI). All 23 clones were sequenced with a BigDye Terminator v3.1 Cycle Sequencing Kit and products were analyzed on the ABI PRISM 3730xl genetic analyzer (Applied Biosystems, Foster City, CA). The nucleotide sequences for the *S1* genes have been submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/) under the accession numbers listed in Table 1.

Table 1. Field isolates of infectious bronchitis virus (IBV) used in this study

IBV isolate (new cluster)	Type of bird	Year of isolation	Lesion[1]	Tissue[2]	Accession no.
K716 (1)	Broiler	2005	RS	Tra, Kid, CT	HM486962
K1585 (1)	Broiler breeder	2007	RS, ED	CT	HM486953
K183 (1)	Korean native chicken	2007	RS, ED	CT	HM486954
K426 (1)	Broiler	2008	NS	Tra	HM486955
K33 (1)	Broiler	2009	RS, N	Kid	HM486956
K40 (1)	Broiler	2009	RS, N	Tra, Kid, CT	HM486957
K74 (1)	Broiler	2009	RS, N	Kid	HM486958
K119 (2)	Broiler breeder	2009	RS	Tra	JF804680
K273 (2)	Broiler breeder	2009	RS	Tra, CT	JF804687
K308 (2)	Layer	2009	RS	Tra, CT	JF804689
K344 (1)	Broiler	2009	RS	Tra, CT	HM486959
K23 (2)	Broiler	2010	RS	Tra	JF804677
K26 (2)	Broiler breeder	2010	RS, ED	Tra, Kid, CT	JF804678
K46 (2)	Broiler breeder	2010	RS	Tra	JF804679
K88 (1)	Broiler	2010	RS, N	Tra, Kid, CT	HM486960
K147 (1)	Broiler	2010	RS, N	Tra, Kid, CT	HM486961
K194 (1)	Korean native chicken	2010	RS, N	Tra, Kid, CT	JF804681
K201 (1)	Broiler	2010	RS, N	Tra, Kid, CT	JF804682
K226 (1)	Broiler	2010	RS, N	Tra, Kid, CT	JF804683
K235 (1)	Broiler	2010	RS, N	Tra, Kid, CT	JF804684
K236 (1)	Broiler	2010	RS, N	Tra, Kid, CT	JF804685
K245 (1)	Korean native chicken	2010	RS, N	Tra, Kid, CT	JF804686
K307 (1)	Broiler	2010	RS, N	Tra, Kid, CT	JF804688

[1]RS = respiratory signs; ED = egg drop; N = nephritis.

[2]Tra = trachea; Kid = kidney; CT = cecal tonsil.

Comparison of S1 Genes and Phylogenetic Analyses

The nucleotide sequences of the *S1* gene of the IBV isolates were compiled, and the amino acid sequences were deduced using Bioedit v7.0.9.0 (Hall, 1999). The 69 IBV reference strains were retrieved from the GenBank database. Sequence analysis of partial *S1* gene nucleotide positions 25 to 1611 (numbering is in reference to the Mass41 strain) were performed with the ClustalW multiple alignment method. The phylogenetic tree was constructed with the neighbor-joining method with 1,000 bootstrap replicates using MEGA version 4 (Tamura et al., 2007).

Cross-Protection Study

In total, 120 three-wk-old SPF chickens were divided into 12 groups of 10 chicks. Eight groups were immunized intraocularly with H120 or K2 strain at $10^{3.0}$ 50% egg infective dose (**EID_{50}**), whereas the other 4 groups were kept as nonimmunized controls. Three weeks after immunization, all of the birds were challenged intraocularly with $10^{4.5}$ EID_{50} of 3 recombinant strains belonging to the new genetic cluster 1 (K716/05, K40/09, and K245/09) and one variant strain belonging to the new genetic cluster 2 (K26/09; Table 2 and Figure 1). Five days after the challenge, the challenge virus was reisolated from the trachea and kidney of the birds by inoculating 9- to 11-d-old embryonated SPF chicken eggs to evaluate the cross-protective efficacy of the commercial vaccines.

Statistical Analysis

The reisolation rate of the challenge virus among groups was analyzed using one-tailed Fisher's exact test. A P-value of < 0.05 was considered to be statistically significant.

RESULTS

Phylogenetic Analysis of IBV Isolates

A phylogenetic tree was generated to describe the relationship between nucleotide sequences of the *S1* gene of IBV isolates and other field strains. The Korean IBV strains were divided into 2 distinct genetic groups: Korean group 1 (K-1) and Korean group 2 (K-2). The K-1 included respiratory strains, whereas all nephropathogenic strains were included in K-2. In addition, K-2 was divided into 2 separate subgroups: KM91-like and QX-like. A phylogenetic analysis showed that recent IBV isolates formed 2 different clusters, new genetic cluster 1 and 2, which were distinct from other preexisting clusters of Korean IBV (Figure 1). The IBV isolates belonging to the new cluster 1 shared 88.7 to 90.7% nucleotide similarity to the *S1* gene of the KM91 and QXIBV strains. The IBV strains belonging to the new cluster 2 showed nucleotide identities of <66% compared with all reference strains used in this study, but they were closely related to Chinese strains, with 95.7 to 98.6% similarity (CK/CH/GD/KP10, CK/CH/GD/NC10, CK/CH/SD09/005, and TC07–2).

Comparison of Amino Acid Sequences of S1 Gene

The nucleotide and predicted amino acid sequences of the S1 glycoprotein from 23 IBV field isolates were determined and compared. The IBV isolates of new genetic cluster 1 had 91.7 to 99.8% similarity within the same cluster, and amino acid identities of the *S1* gene among the IBV isolates of new genetic cluster 2 ranged from 92.3 to 99.8%. The IBV isolates belonging to new genetic cluster 1 shared 78.9 to 80.6% amino acid similarity to S1 from the H120 strain and 88.1 to 90.9% similarity to that of the K2 strain. The IBV isolates belonging to new genetic cluster 2 possessed low

Table 2. Protective effects in 3-wk-old specific-pathogen-free chickens immunized with H120 and K2 against a challenge with new variant infectious bronchitis virus (IBV) isolates[1]

IBV strain immunized	Genogroup of challenge virus	IBV strain of challenge virus	No. of challenge virus isolated/ no. of challenged: Trachea, Control	Trachea, Vaccinated	Kidney, Control	Kidney, Vaccinated
H120	New cluster 1	K245/09	10/10	10/10	10/10	8/10
		K40/09	10/10	10/10	10/10	9/10
		K716/05	10/10	9/10	10/10	5/10
	New cluster 2	K26/10	9/10	7/10	3/10	1/10
K2	New cluster 1	K245/09	10/10	1/10***,†††	10/10	0/10***,†††
		K40/09	10/10	5/10*,†	10/10	1/10***,††
		K716/05	10/10	3/10**,†	10/10	0/10***,†
	New cluster 2	K26/10	9/10	0/10***,††	3/10	0/10

[1]Three-wk-old chickens were immunized with IBV, H120, and K2 ($10^{3.0}$ 50% egg infective dose per bird) via the intraocular route. At 3 wk postimmunization, all birds were challenged with $10^{4.5}EID_{50}$ of 4 new Korean IBV strains via the intraocular route. Five days after challenge, protection was evaluated by the absence of the challenge virus in the kidney and trachea.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, by Fisher's exact test, compared with nonvaccinated control group.

†$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$, by Fisher's exact test, compared with the H120 vaccinated group.

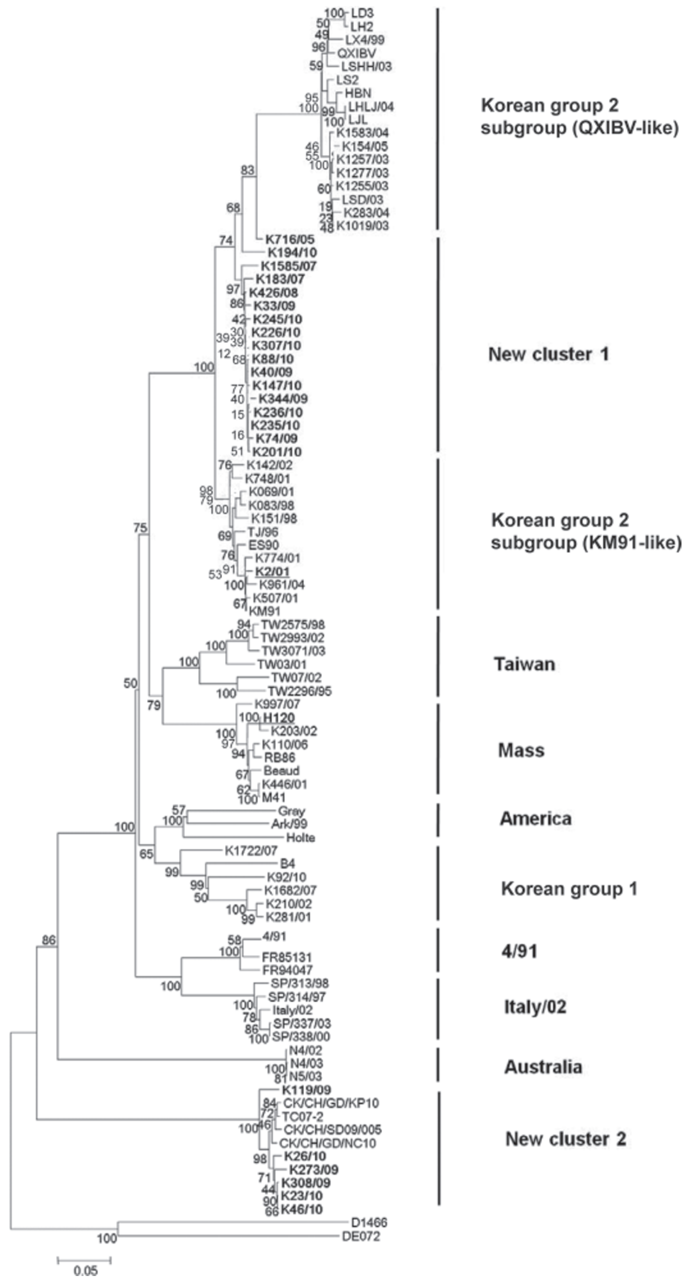

Figure 1. Infectious bronchitis virus (IBV) phylogenetic tree of *S1* genes. The tree is based on the sequence of the *S1* genes from the 23 IBV isolates and the 69 reference strains. ClustalW alignment method for *S1* nucleotide positions 25 to 1611 corresponding to those of strain Mass41 (GenBank accession number X04722; http://www.ncbi.nlm.nih.gov/genbank/). The IBV isolates used in this study are marked in bold and 2 vaccine strains (K2 and H120) are underlined. The provisional designations, including genogroups and subgenogroups, are indicated on the right.

amino acid identity (<58.7% similarity) to the 2 vaccine strains (H120 and K2). In particular, the challenge strains K716/05, K40/09, and K245/09 of new cluster 1 shared 79.6, 80.4, and 78.9% amino acid similarity to S1 from the H120 strain and 88.8, 90.9, and 89.6% similarity to that of the K2, respectively. The challenge strain K26/10 of new cluster 2 had 57.9% and 58.1% amino acid similarity to S1 from the H120 and K2 vaccine strains, respectively.

Cross-Protective Efficacy of Commercial IBV Against Challenge with New IBV Isolates

We evaluated the efficacy of vaccine strains through reisolation of the challenge strain. Five days after the challenge, we reisolated the challenge virus from the tracheas and kidneys of birds. None of the birds showed clinical signs or mortality. The results shown in Table 2 implied that recombinant strains belonging to new genetic cluster 1 possessed high tropism to the trachea and kidney, whereas variant strains belonging to new genetic cluster 2 displayed high tropism only for the trachea. These findings corresponded to clinical lesions of field cases. Nearly all of the recombinant strains produced respiratory signs and nephropathogenicity in chicken farms, and variant strains showed clinical signs that were limited to the respiratory tract (Table 1).

In groups of chickens vaccinated with H120, incomplete protection against recombinants and variants was observed at both the trachea and kidney. However, chickens immunized with K2 showed significantly higher levels of protection against the challenge with all of the viruses at the trachea and kidney levels ($P < 0.05$ or better) compared with that of chickens immunized with H120.

DISCUSSION

Nephropathogenic strains of different serotypes are a major problem worldwide. In particular, outbreaks of QX-like nephropathogenic strains have been reported in many countries, including China (Liu et al., 2006b), Thailand (Pohuang et al., 2009), Russia (Bochkov et al., 2006), the United Kingdom (Gough et al., 2008), Italy (Beato et al., 2005), Germany, France, and the Netherlands (Landman et al., 2005), in recent years. These strains are continuously evolving and producing new variants as a feature of avian coronaviruses (Lee and Jackwood, 2001). The present study also demonstrates that circulating QXIBV-like strains have increased the diversity of Korean IBV strains through recombination with heterologous strains (KM91-like strains) that are classified into different genetic groups.

The sequence data of this study demonstrate that the S1 amino acid sequences of IBV strains belonging to the new cluster 2 display nucleotide identities of <65% compared with those of all known IBV strains, except 4 strains that were recently isolated from China (Li et al., 2010). A previous study indicated that a recent Korean IBV isolate (Kr/D64/05) was closely related to the China CK/CH/LDL/97I-type strains (Lee et al., 2008), and the prevalence of QX-like IBV strains in Korea is reminiscent of the spread of the China QXIBV strain in many Eurasian countries (Beato et al., 2005; Gough et al., 2008). The origin of this variant IBV strain is unclear, but the geographical closeness between China and Korea might have fostered IBV transmission and exchange of IBV genetic material. Furthermore, mi-

grating birds are considered to be potential vectors of IBV due to the ability of IBV to replicate even in an asymptomatic teal, which is a member of the largely migratory order Anseriformes (Liu et al., 2005). Therefore, we cannot exclude the possibility of spread of IBV over long distances by wild birds.

The emergence of new infectious bronchitis serotypes creates difficulties in the design of adequate vaccination programs (Liu et al., 2006a). However, development of vaccines against new IBV variants is not generally an option, owing to the high cost and time required for their final approval (Jackwood et al., 2003; Bijlenga et al., 2004). Some IBV vaccine strains from a certain serotype may provide protection against IBV strains not belonging to that serotype (Cook et al., 1999). Thus, performing protection studies (protectotype) with new IBV isolates is a highly relevant and practical method in control strategies of IBV, because it provides direct information about the efficacy of currently available IBV vaccines. In the present study, IBV isolates belonging to new clusters showed low amino acid homology compared with the 2 vaccine strains (H120 and K2 strain), but the K2 strain could provide greater protection against the challenge with recombinant strains K716/05, K40/09, and K245/09 and variant strain K26/09 than that conferred by the commercial H120 vaccine. As already proposed, good protection can be achieved by vaccines containing serotypes that differ both antigenically and genetically from the challenge virus (Cook et al., 1999). The reason might be that, despite extensive differences in the S1 protein, much of the virus genome remains unchanged and there are epitopes in common among different strains of IBV, which play a major role in protective immunity (Cavanagh et al., 1997).

To date, the Mass strains have been primarily used as live vaccines due to their epizootic distributions and cross-protective ability (Ignjatović and Sapats, 2000; Bijlenga et al., 2004). Despite the widespread use of vaccines, outbreaks related to nephropathogenic strains of different serotypes have increased in many countries (Li and Yang, 2001; Liu et al., 2006b). In Europe, a combination of the Mass and 4/91 vaccine regimen have prevented nephropathogenic IBV infection (Terregino et al., 2008). However, the use of multiple strains of live vaccines raises concerns regarding the formation of variant viruses by recombination with field strains resulting from the spread of vaccine strains (Farsang et al., 2002). Furthermore, use of 4/91 strains is prohibited in some countries, including Korea, due to the absence of its outbreak in fields. Previous studies demonstrated that the highly attenuated nephropathogenic IBV vaccine strain K2 provides great protection against 2 distinct subgroups of nephropathogenic strains (KM91-like and QX-like; Lee et al., 2010). Moreover, a present study revealed that the K2 strain has a broad cross-protective ability against nephropathogenic recombinant and variant strains. Therefore, a single administration of the K2 strain might be effective and suitable for application in many countries where the nephropathogenic IBV infection is a major problem.

In conclusion, field IBV undergo genetic recombination and evolution, which might lead to disease control difficulty. Thus, examination of the protection conferred by commercially available IBV vaccines against new variants provides important information on control strategies against IBV infection. Based on the data presented in this study, it can be concluded that the K2 vaccine strain confers broad cross protection against a challenge with new variant strains. This information may be helpful for the reduction of economic losses caused by newly evolving nephropathogenic strains and variant IBV.

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REFERENCES

Beato, M. S., C. De Battisti, C. Terregino, A. Drago, I. Capua, and G. Ortali. 2005. Evidence of circulation of a Chinese strain of infectious bronchitis virus (QXIBV) in Italy. Vet. Rec. 156:720.

Bijlenga, G., J. K. Cook, J. Jr. Gelb, and J. J. de Wit. 2004. Development and use of the H strain of avian infectious bronchitis virus from the Netherlands as a vaccine: A review. Avian Pathol. 33:550–557.

Bochkov, Y. A., G. V. Batchenko, L. O. Shcherbakova, A. V. Borisov, and V. V. Drygin. 2006. Molecular epizootiology of avian infectious bronchitis in Russia. Avian Pathol. 35:379–393.

Casais, R., B. Dove, D. Cavanagh, and P. Britton. 2003. Recombinant avian infectious bronchitis virus expressing a heterologous spike gene demonstrates that the spike protein is a determinant of cell tropism. J. Virol. 77:9084–9089.

Cavanagh, D. 2007. Coronavirus avian infectious bronchitis virus. Vet. Res. 38:281–297.

Cavanagh, D., M. M. Elus, and J. K. Cook. 1997. Relationship between sequence variation in the S1 spike protein of infectious bronchitis virus and the extent of cross-protection in vivo. Avian Pathol. 26:63–74.

Cavanagh, D., and S. Naqi. 2003. Infectious bronchitis. Pages 101–109 in Diseases of Poultry. 11th ed. Iowa State University Press, Ames.

Cook, J. K. A., S. J. Orbell, M. A. Woods, and M. B. Huggins. 1999. Breadth of protection of the respiratory tract provided by different live attenuated infectious bronchitis vaccines against challenge with infectious bronchitis virus of heterologous serotypes. Avian Pathol. 28:477–485.

Dhinakar Raj, G., and R. C. Jones. 1996. Protectotypic differentiation of avian infectious bronchitis viruses using an in vitro challenge model. Vet. Microbiol. 53:239–252.

Farsang, A., C. Ros, L. H. Renstrom, C. Baule, T. Soos, and S. Belak. 2002. Molecular epizootiology of infectious bronchitis virus in Sweden, indicating the involvement of a vaccine strain. Avian Pathol. 31:229–236.

Gelb, J., Jr., Y. Weisman, B. S. Ladman, and R. Meir. 2005. *S1* gene characteristics and efficacy of vaccination against infectious bronchitis virus field isolates from the United States and Israel (1996–2000). Avian Pathol. 34:194–203.

Gough, R. E., W. J. Cox, D. de B Welchman, K. J. Worthington, and R. C. Jones. 2008. Chinese QX strain of infectious bronchitis virus isolated in the UK. Vet. Rec. 162:99–100.

Hall, T. A. 1999. A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95–98.

Ignjatović, J., and S. Sapats. 2000. Avian infectious bronchitis virus. Rev. Sci. Tech. 19:493–508.

Jackwood, M. W., D. A. Hilt, and T. P. Brown. 2003. Attenuation, safety, and efficacy of an infectious bronchitis virus GA98 serotype vaccine. Avian Dis. 47:627–632.

Landman, W. J. M., R. M. Dwars, and J. J. de Wit. 2005. High incidence of false layers in (re)production hens supposedly attributed to a juvenile infectious bronchitis virus infection. Page 369 in Proc. of the 14th World Vet. Poult. Congr., Istanbul, Turkey.

Lee, C. W., and M. W. Jackwood. 2001. Origin and evolution of Georgia 98 (GA98), a new serotype of avian infectious bronchitis virus. Virus Res. 80:33–39.

Lee, E. K., W. J. Jeon, Y. J. Lee, O. M. Jeong, J. G. Choi, J. H. Kwon, and K. S. Choi. 2008. Genetic diversity of avian infectious bronchitis virus isolates in Korea between 2003 and 2006. Avian Dis. 52:332–337.

Lee, H. J., H. N. Youn, J. S. Kwon, Y. J. Lee, J. H. Kim, J. B. Lee, S. Y. Park, I. S. Choi, and C. S. Song. 2010. Characterization of a novel live attenuated infectious bronchitis virus vaccine candidate derived from a Korean nephropathogenic strain. Vaccine 28:2887–2894.

Li, H., and H. Yang. 2001. Sequence analysis of nephropathogenic infectious bronchitis virus strains of the Massachusetts genotype in Beijing. Avian Pathol. 30:535–541.

Li, L., C. Xue, F. Chen, J. Qin, Q. Xie, Y. Bi, and Y. Cao. 2010. Isolation and genetic analysis revealed no predominant new strains of avian infectious bronchitis virus circulating in South China during 2004–2008. Vet. Microbiol. 143:145–154.

Lim, T. H., H. J. Lee, D. H. Lee, Y. N. Lee, J. K. Park, H. N. Youn, M. S. Kim, J. B. Lee, S. Y. Park, I. S. Choi, and C. S. Song. 2011. An emerging recombinant cluster of nephropathogenic strains of avian infectious bronchitis virus in Korea. Infect. Genet. Evol. 11:678–685.

Liu, S., J. Chen, J. Chen, X. Kong, Y. Shao, Z. Han, L. Feng, X. Cai, S. Gu, and M. Liu. 2005. Isolation of avian infectious bronchitis coronavirus from domestic peafowl (*Pavo cristatus*) and teal (*Anas*). J. Gen. Virol. 86:719–725.

Liu, S., J. Chen, Z. Han, Q. Zhang, Y. Shao, X. Kong, and G. Tong. 2006a. Infectious bronchitis virus: *S1* gene characteristics of vaccines used in China and efficacy of vaccination against heterologous strains from China. Avian Pathol. 35:394–399.

Liu, S. W., Q. X. Zhang, J. D. Chen, Z. X. Han, X. Liu, L. Feng, Y. H. Shao, J. G. Rong, X. G. Kong, and G. Z. Tong. 2006b. Genetic diversity of avian infectious bronchitis coronavirus strains isolated in China between 1995 and 2004. Arch. Virol. 151:1133–1148.

Monne, I., G. Cattoli, R. Jones, K. Worthington, and W. Wijmenga. 2008. QX genotypes of infectious bronchitis virus circulating in Europe. Vet. Rec. 163:606–607.

Pohuang, T., N. Chansiripornchai, A. Tawatsin, and J. Sasipreeyajan. 2009. Detection and molecular characterization of infectious bronchitis virus isolated from recent outbreaks in broiler flocks in Thailand. J. Vet. Sci. 10:219–223.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24:1596–1599.

Terregino, C., A. Toffan, M. S. Beato, R. De Nardi, M. Vascellari, A. Meini, G. Ortali, M. Mancin, and I. Capua. 2008. Pathogenicity of a QX strain of infectious bronchitis virus in specific-pathogen-free and commercial broiler chickens, and evaluation of protection induced by a vaccination program based on the Ma5 and 4/91 serotypes. Avian Pathol. 37:487–493.

Worthington, K. J., and R. C. Jones. 2006. An update in the European RT-PCR IBV survey and recent findings on a novel IBV genotype. Pages 176–188 in Proc. 5th Symp. Avian Corona and Pneumovirus Infections, Rauischholzhausen, Germany.

YuDong, W., W. YongLin, Z. ZiChun, F. GenChe, J. YiHai, and L. XiangE. 1998. Isolation and identification of glandular stomach-type IBV (QX IBV) in chickens. Zhongguo Dongwu Jianyi 15:1–3.