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Protective effect of vaccination in chicks with local infectious bronchitis viruses against field virus challenge

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Avian infectious bronchitis virus (IBV) causes tremendous economic losses to the poultry industry worldwide. Different serotypes of this virus show little cross-protection. The present study investigated the relationship between differences in the genotype based on the N-terminus of the spike protein and the protection provided by vaccination with IBVs. Cross-immunization tests were performed using both killed and live viruses in specific-pathogen-free chicks. One-day-old chicks were immunized with killed or high [10^5 50% embryo infectious dose (EID_{50})] or low (10^3 EID_{50}) doses of live IBV viruses and challenged with homologous or heterologous IBV strains. The immunization efficacy was evaluated by virus isolation from the challenged chicks. In the killed-virus and high-dose live-virus test groups, IBV vaccination protected against challenge by homologous but not heterologous IBV strains. However, a low dose of live IBV showed no protection against virulent IBV challenge. These results indicate that both the genotype based on the N-terminus of the spike protein and the virus dose are essential to IBV protection from immunization. Thus, development of vaccines from different local strains is necessary to control infectious bronchitis in poultry.

Key words: Chickens, coronavirus spike glycoprotein, infectious bronchitis virus, virus vaccination

Infectious bronchitis virus (IBV) is a major cause of economic losses in the poultry industry worldwide. IBV also poses a constantly changing threat to the poultry industry because new serotypes of the virus continue to be isolated even from vaccinated flocks [1-3]. Numerous serotypes or IBV variants have been identified worldwide, against which little or even no cross-protection exists [4,5]. Thus, developing vaccines from local field isolates is necessary to control this disease.

IBV has been present in Taiwan since at least 1958, and numerous nephrotropic strains have been isolated since that time [3]. In spite of the extensive use of vaccines, IBV outbreaks remain frequent in Taiwan [6]. Infected broilers show clinical signs of depression, dehydration, and polyuria — swelling of the kidneys with severe urate deposition that results in death. Infected breeders or layers show signs of decreased egg production. The failure of vaccines is typically due to differences in the serotypes of vaccine strains and field viruses [2,5], a situation that has been proven to occur in Taiwan [6]. Sequence differences in the S1 gene between Taiwan IBV strains and commercially available vaccine strains reach 20-30%. Based on differences in the N-terminus of the spike protein, the Taiwan isolates can be classified into 2 genetic groups: Taiwan group I (TW I) and Taiwan group II (TW II), which differ serologically from each other [7,8]. The fragment used for differentiating these 2 groups contains the hypervariable region 1, which is thought to be closely associated with major neutralization epitopes [9].

It is well known that the spike protein is responsible for virus neutralization and hemagglutination inhibition [9-12]. However, the role of the spike protein in protecting against the virus remains unclear. The purpose of the present study was to determine whether differences in the N-terminus of the spike protein in vaccine plays a role in protection provided to chicks against IBV infections.

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Materials and Methods

Viruses

The viruses used in this study were isolated from chickens infected with IBV, and were identified as strains 1171/92, 1246/92, 1449/92, 1960/94, 2330/96, and 2389/97. All of these strains belong to genotype TW I, except for 2012/94, 2296/95 and 2300/95, which belong to TW II. Strains 2296/95 and 2300/95 are highly

Table 1. Antibody titers of chicks after vaccination with killed virus[a]

Vaccinated viruses	Challenge virus	ELISA antibody titers			
		1 day	2 weeks	4 weeks	5 weeks
-[b]	2330/96 (TW I)	0	0	0	0.2 ± 0.1
H120+H120	2330/96 (TW I)	0	1.3 ± 0.4	2.1 ± 1.3	2.4 ± 1.3
H120+2389/97	2330/96 (TW I)	0	0.9 ± 1.3	1.2 ± 1.1	3.5 ± 0.8
-	2389/97 (TW I)	0	0	0	0.4 ± 0.3
H120+H120	2389/97 (TW I)	0	1.2 ± 0.4	1.6 ± 1.2	3.8 ± 0.2
H120+2389/97	2389/97 (TW I)	0	1.1 ± 0.3	1.5 ± 1.4	3.8 ± 0.4

Abbreviations: ELISA = enzyme-linked immunosorbent assay; TW I = Taiwan group I

[a]Six chicks in each group were vaccinated with live H120 at 1 day old and revaccinated with killed 2389/97 (TW I) at 2 weeks old. Chicks vaccinated with live H120 twice or without vaccination were used as control groups. All chicks were challenged with 2330/96 or 2389/97 at 4 weeks old and then sacrificed at 5 weeks for protection evaluation by virus isolation. Blood samples collected before vaccination, pre-challenge, and 1-week post-challenge were used for infectious bronchitis virus antibody measurement.

[b]Unvaccinated birds (-).

virulent; 1449/92 is moderately virulent; and the others exhibit low virulence [7]. Viruses were purified by terminal dilution for 3 passages in specific-pathogen-free (SPF; National Institute for Animal Health, Tamsui, Taiwan) chicken embryos and 3 to 8 passages in SPF chicken embryos. The purified viruses were propagated in SPF chicken embryos. After 48 h, the viruses in allantoic fluid were pooled. The pooled viruses were titrated in 10-day-old SPF chicken embryos, placed into ampules, rapidly frozen, and stored at –70°C.

H120 is the most popular vaccine strain in the world as well as in Taiwan. It derives from a Holland strain and is attenuated by passing through chicken embryos 120 times; at high passage number it loses its pathogenicity.

Killed-virus preparation

Strain 2389/97 was inoculated into the allantoic cavity of SPF chicken embryos, and the allantoic fluid was harvested 48 h later. The titer of the IBV in the fluid was 10^6 50% embryo infectious dose (EID_{50}) before inactivation. IBV was inactivated using 0.2% formalin with continuous stirring at 37°C for 10 h. The test for inactivation consisted of 2 passages in SPF chicken embryos using inoculations of 0.2 mL with 10 replicates per passage. After complete inactivation, the killed virus was mixed with 5 volumes of 5% aluminum gel (6.8 g $AlCl_3$, 16.8 g CH_3COONa, 920 mL D_2W, and 80 mL 10% NaOH) as an adjuvant.

Efficacy of vaccination with killed virus

Thirty six 1-day-old SPF chicks used in the killed-virus efficacy test were divided into 6 groups with 6 birds in each group (Table 1, Table 2). Chicks were vaccinated intranasally with live H120 (10^3 EID_{50}) at 1 day old and revaccinated intramuscularly with killed 2330/96 (0.5 mL) or 2389/97 at 2 weeks old. Chicks vaccinated with live H120 (10^3 EID_{50}) twice or without vaccination were used as control groups. These birds were raised in separate rooms and supplied with food and water ad libitum. After challenge, the chicks were observed daily for 1 week. At the end of the experiments, chicks were humanely killed and necropsied. Kidneys of SPF chick were used for virus isolation to evaluate the isolation rates. Positive virus isolations were recorded when characteristic dwarfing and/or mortality of the SPF chicken embryos occurred in the absence of bacteria. Reverse-transcription polymerase chain reaction (RT-PCR) and sequencing were also performed to ensure that the isolated viruses were indeed the ones used for the challenge. The primer set used for RT-PCR was modified as previously described [13] from C2U/C3L [7,8], and consisted of rC2U (forward): 5'-TGGTTGGCA(T/C)TTACA(A/C/T)GG(A/G/T)-3' and

Table 2. Protective efficacy of killed-virus vaccine against homologous or heterologous viruses[a]

Vaccinated virus		Challenge virus 4 weeks	Virus isolation 5 weeks
1 day	2 weeks		
-[b]	-	2330/96 (TW I)	6[c]
H120	H120	2330/96 (TW I)	5
H120	2389/97 (TW I)	2330/96 (TW I)	2[d]
-	-	2389/97 (TW I)	6
H120	H120	2389/97 (TW I)	3
H120	2389/97 (TW I)	2389/97 (TW I)	2[d]

Abbreviation: TW I = Taiwan group I

[a]The 6 chicks in each group are the same as described in Table 1.

[b]Unvaccinated birds (-).

[c]The number of chicks from which virus was isolated is indicated.

[d]$p<0.05$ compared with the corresponding control group without vaccination.

Table 3. Antibody titers of chicks after vaccination with a low dose of live virus[a]

Vaccinated virus	Challenge virus	ELISA antibody titers		
		1 day	4 weeks	5 weeks
-[b]	2296/95 (TW II)	0	0	0.5 ± 0.4
1171/92 (TW I)	2296/95 (TW II)	0	0.5 ± 0.3	2.3 ± 1.6
1246/92 (TW I)	2296/95 (TW II)	0	0.4 ± 0.3	1.3 ± 0.8
1960/94 (TW I)	2296/95 (TW II)	0	0.9 ± 0.5	2.4 ± 1.8
2012/94 (TW II)	2296/95 (TW II)	0	0.2 ± 0.1	1.4 ± 0.9
-	2300/95 (TW II)	0	0	1.1 ± 0.6
1171/92 (TW I)	2300/95 (TW II)	0	0.4 ± 0.3	1.0 ± 0.6
1246/92 (TW I)	2300/95 (TW II)	0	0.4 ± 0.3	1.8 ± 1.2
1960/94 (TW I)	2300/95 (TW II)	0	1.0 ± 0.6	2.4 ± 1.8
2012/94 (TW II)	2300/95 (TW II)	0	0.2 ± 0.1	0.4 ± 0.1

Abbreviations: ELISA = enzyme-linked immunosorbent assay; TW I = Taiwan group I; TW II = Taiwan group II

[a]Six specific-pathogen-free chicks in each group were vaccinated with the live virus (10^3 50% embryo infectious dose; EID_{50}) at 1 day and at 2 weeks old, and then challenged with the virulent virus (10^6 EID_{50}) 2 weeks after the second vaccination.

[b]Negative control without vaccination or challenge with the virulent IBV strain.

rC3L (reverse): 5'-(A/G)CAATGTGTAACAAA(T/C) ACT-3'. The size of the expected PCR product was 231 bp. The PCR product of the reisolated virus was sequenced for identifying the challenged virus strain. Chicks in which virus was not isolated after 1 SPF chicken embryo passage were considered to be protected. Blood samples before vaccination, pre-challenge, and 1-week post-challenge were taken for IBV antibody measurement using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Kirkegaard & Perry Labs, Gaithersburg, MD, USA).

Efficacy of the low dose of live virus

Thirty 1-day-old SPF chicks used in the efficacy test of the low dose, live virus vaccinations were divided into 4 vaccinated groups and 1 unvaccinated control group, with 6 birds in each group (Table 3, Table 4). Chicks were vaccinated using eyedrops containing 10^3 EID_{50} of the low-virulent strains, 1171/92, 1246/92, 1960/94, or 2012/94, at the ages of 1 day and 2 weeks. Two weeks after the second vaccination, all chicks were challenged with the virulent strain, 2296/95. One week after virus challenges, chicks were humanely killed and necropsied, and virus was isolated from kidneys for evaluation of the vaccination efficacy. In the unvaccinated control group, chicks were also inoculated with 0.02 mL tryptose phosphate buffer through eyedrops. Chicks from which virus was not isolated after 1 SPF chicken embryo passage were considered to be protected. Blood samples before vaccination, pre-challenge, and 1-week post-challenge were taken for IBV antibody measurement using a commercial ELISA kit (Kirkegaard & Perry Labs). Another thirty 1-day-old SPF chicks were tested in the same way as described above except that the challenge virus used was 2300/95 (TW II).

Efficacy of the high dose of live virus

Forty 1-day-old SPF chicks used in the efficacy test of high-dose live virus vaccinations were divided into 3 vaccinated groups and 1 unvaccinated control group, with 10 birds in each group (Table 5, Table 6). Chicks were vaccinated intraocularly with 10^5 EID_{50} of the low-virulence virus strains, 1171/92, 1960/94, or 2012/94, at the ages of 1 day and 2 weeks. After the second vaccination, all birds were challenged with 10^6 EID_{50} of 1449/92. The efficacy of the high dose of live virus was evaluated as described above. Blood samples were collected before vaccination, pre-challenge, and 1-week

Table 4. Protective efficacy of vaccination with low-dose live IBV against homologous or heterologous viruses[a]

Vaccinated virus	Challenge virus	Virus isolations
-[b]	2296/95 (TW II)	6[c]
1171/92 (TW I)	2296/95 (TW II)	4
1246/92 (TW I)	2296/95 (TW II)	4
1960/94 (TW I)	2296/95 (TW II)	4
2012/94 (TW II)	2296/95 (TW II)	3
-	2300/95 (TW II)	6
1171/92 (TW I)	2300/95 (TW II)	4
1246/92 (TW I)	2300/95 (TW II)	6
1960/94 (TW I)	2300/95 (TW II)	5
2012/94 (TW II)	2300/95 (TW II)	6

Abbreviations: TW I = Taiwan group I; TW II = Taiwan group II

[a]The 6 specific-pathogen-free chicks in each group are the same as described in Table 3.

[b]Negative control without vaccination (-).

[c]The number of chicks in which virus was isolated is indicated.

Table 5. Antibody titers of chicks after vaccination with a high dose of live viruses[a]

Vaccinated virus	Challenged virus	ELISA antibody titers		
		1 day	4 weeks	5 weeks
-[b]	1449/92 (TW I)	0	0	0
1171/92 (TW I)	1449/92 (TW I)	0	1.3 ± 0.4	2.6 ± 0.6
1960/94 (TW I)	1449/92 (TW I)	0	0.3 ± 0.1	3.6 ± 0.8
2012/94 (TW II)	1449/92 (TW I)	0	0.2 ± 0.03	3.6 ± 1.0
-[b]	2296/95 (TW II)	0	0	0.6 ± 0.3
1171/92 (TW I)	2296/95 (TW II)	0	0.4 ± 0.2	1.4 ± 1.3
1960/94 (TW I)	2296/95 (TW II)	0	1.0 ± 0.3	0.5 ± 1.4
2012/94 (TW II)	2296/95 (TW II)	0	0.4 ± 0.2	1.4 ± 1.3

Abbreviations: ELISA = enzyme-linked immunosorbent assay; TW I = Taiwan group I; TW II = Taiwan group II

[a]Ten 1-day-old specific-pathogen-free chicks in each group were vaccinated with live virus (10^5 50% embryo infectious dose; EID_{50}) at 1 day and at 2 weeks old, and then challenged with the virulent viruses (10^6 EID_{50}) 2 weeks after the second vaccination.

[b]Negative control without vaccination (-).

post-challenge for IBV antibody measurement using a commercial ELISA kit (Kirkegaard & Perry Labs). Another forty 1-day-old SPF chicks were tested in the same way as described above except that the challenge virus used was strain 2296/95.

Statistical analysis

Fisher's exact test was used to compare the virus isolation rate from the kidneys to determine the efficacy of the killed and live virus vaccinations at a 5% significance level [14].

Results

Protective efficacy of vaccination with killed viruses

The ELISA antibody titers of all chicks were 0 before vaccination (Table 1). The average log titers of chicks vaccinated with H120 reached 0.9 to 1.3. Two weeks after the second vaccination with either H120 or 2389/97 killed virus, the titers reached 1.2 to 2.1. One week after virulent virus challenge, the titers reached 2.4 to 3.8. The titers in birds vaccinated with killed virus were no higher than those vaccinated with H120 ($p>0.05$).

The protective efficacy of vaccination with H120 alone or combined with 2389/97 killed virus is shown in Table 2. The sequences of the re-isolated viruses were verified to be the ones used for the challenge, and not the ones used for the vaccination. The virus isolation rates from the kidneys of vaccinated chicks were less than those of the control groups ($p<0.05$) in the 2300/95-challenged groups. In addition, another TW I strain (2389/97) protected chicks against a TW I strain challenge ($p<0.05$) in the 2389/97-challenged groups. Thus, the killed vaccines were effective against challenge with homologous IBVs.

Table 6. Protective efficacy of high-dose live virus vaccination against homologous or heterologous viruses[a]

Vaccinated virus	Vaccinated virus	Virus isolations
-[b]	1449/92 (TW I)	10[c]
1171/92 (TW I)	1449/92 (TW I)	1[d]
1960/94 (TW I)	1449/92 (TW I)	2[d]
2012/94 (TW I)	1449/92 (TW I)	6
-[b]	2296/95 (TW II)	10
1171/92 (TW I)	2296/95 (TW II)	4[d]
1960/94 (TW I)	2296/95 (TW II)	8
2012/94 (TW I)	2296/95 (TW II)	1[d]

Abbreviations: TW I = Taiwan group I; TW II = Taiwan group II

[a]The ten 1-day-old specific-pathogen-free chicks in each group are the same as described in Table 5.

[b]Negative control without vaccination (-).

[c]The number of chicks from which virus was isolated is indicated.

[d]Significantly different from the corresponding control groups without vaccination ($p<0.05$).

Protective efficacy of vaccination with live viruses

In the low-dose vaccinated groups, the ELISA antibody titers of all chicks were 0 before vaccination (Table 3). The average log titers in vaccinated chicks after the 2 vaccinations (pre-challenge) reached 0.2 to 1.0. One week after challenge, the titers reached 0.5 to 2.4.

The protective efficacy of vaccination with low-virulence IBV strains against challenge with virulent strains of IBV is shown in Table 4. Because the virus isolation rates from kidneys of vaccinated chicks were no less than the rates from unvaccinated chicks ($p>0.05$), IBV vaccination at low doses did not protect chicks from virulent IBV infection, regardless of whether homologous or heterologous strains were used.

In the high-dose vaccinated groups, the ELISA antibody titers of all chicks were 0 before vaccination

(Table 5). The average log titers in vaccinated chicks after the 2 vaccinations (pre-challenge) reached 0.2 to 1.3. One week after challenge, the titers reached 0.5 to 3.6.

Considering virus isolation rates from the kidney, the vaccine was only effective against homologous challenge (Table 6). In the TW I-challenged groups, 1171/92 and 1960/94 (TW I) but not 2012/94 (TW II) vaccination protected chicks from 1449/92 (TW I) challenge ($p<0.05$). Although 2012/94 showed some protection, this effect was not significant ($p>0.05$). In the TW II-challenged groups, although 1171/92 vaccination provided protection against 2296/95 challenge, 2012/94 vaccination provided the greatest protection. Although some partial protection occurred in chicks vaccinated with heterologous strains, the most effective protection was provided in homologous-challenged groups.

Discussion

IBV contains 3 structural proteins: spike protein, membrane protein, and nucleocapsid protein. The spike protein is post-translationally cleaved into S1 and S2 subunits [15]. S1 protein is correlated with viral neutralization [4,9,10,16]. The location on the spike protein has been reported to be related to neutralization and hemagglutination-inhibiting activities [10,11,17]. Antigenic studies alone cannot adequately define immunological relationships between strains. Thus, a different approach to IBV typing based on protectotypes was suggested by Lohr [18]. Since the spike protein is responsible for virus neutralization [9-11,17], it is also likely to be responsible for virus protection [19]. In the present work, we found that the N-terminus of the spike protein of IBV vaccine was related to the degree of protection against virulent IBV challenge. This information should provide a useful hypothesis for use in the design of future studies for determining the protectotype of new isolates.

Because chicks do not die after challenge due to age resistance, the protection provided by experimental vaccines in laboratory trials is assessed on the basis of IBV re-isolation attempts following challenge, not on the disease-causing capability of the virulent strains used for challenge [2,20]. Thus, virus re-isolation from challenged birds was used to evaluate the protective ability of the vaccination in the present study. Only re-isolated vaccinated virus and no challenge virus was found in our experiments, as evidenced by sequencing of re-isolated viruses. The influence of the virulence of different IBVs on their protective efficacy when used in vaccine needs further study.

In the high-dose vaccine experiments of this study, we observed that vaccination with TW I viruses provided better protective efficacy against homologous than heterologous viruses (Table 6). However, vaccination with 1171/92 (TW I) protected chicks against 2296/95 (TW II) challenge. This might have been due to cross-immunity between different IBV serotypes, some of which are likely to be located on proteins other than S1, e.g., the other half of the S protein (S2), N, and/or M, may be responsible. It is likely that the N-protein contributes to cross-immunity since this protein of different IBV isolates differed by only about 5% among IBV isolates [21]. In spite of the cross-immunity between 1171/92 and 2296/95, vaccination with 2012/94 (TW II) protected more chicks than 1171/92 against 2296/95.

This study demonstrated that the dose is important to the protective effect of avian IBV vaccination in chicks. The EID_{50} at 10^3 was not sufficient to provide protection against virulent virus challenge, even against homologous strains (Table 4). According to international standards, live vaccine should contain no less than $10^{3.5}$ EID_{50} per dose per bird [14]. Ineffective results have been previously reported to result from insufficient vaccination dose in chicks [22]. Valid comparisons between the vaccinated viruses could be made in this study as they were compared at the same level using the same vaccine and challenge doses in each experiment.

In this study, TW I and TW II were typed by the sequence of the N-terminus of the spike protein as previously described [8,17]. Our results clearly showed the relationship between this genotyping and the protectotypes of IBV strains. In conclusion, the genotype based on the N-terminus of the S protein is responsible for the protective effect of vaccination with different IBV strains against viral challenge in chicks, although some cross-immunity is present in strains from different genotypes.

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References

1. Chang PC, Kuo TY, Chueh LL, Wang CH. Sequencing and analysis of S1 gene of infectious bronchitis viruses isolated in Taiwan. J Chin Soc Vet Sci 1998;24:179-87.

2. Cook J. Coronoaviridae. In: Jordan F, Pattison M, Alezander D, Faragher T, eds. Poultry diseases. 5th ed. London: WB Saunders; 2002:298-306.
3. Tseng CC, Li NZ, Yao CH, Wang CH. Isolation and adaptation of infectious bronchitis virus in Taiwan from 1993 to 1995. J Chin Soc Vet Sci 1996;22:113-20.
4. Cowen BS, Hitchner SB. Serotyping of avian infectious bronchitis viruses by the virus-neutralization test. Avian Dis 1975;19:583-95.
5. Hofstad MS. Cross-immunity in chickens using seven isolates of avian infectious bronchitis virus. Avian Dis 1981;25:650-4.
6. Wang CH, Hsieh MC, Chang PC. Isolation, pathogenicity and H120 protection efficacy of infectious bronchitis viruses isolated in Taiwan. Avian Dis 1996;40:620-5.
7. Wang CH, Huang YC. Relationship between serotypes and genotypes based on the hypervariable region of the S1 gene of infectious bronchitis virus. Arch Virol 2000;145:291-300.
8. Wang CH, Tsai CT. Genotypic grouping for the isolates of avian infectious bronchitis virus in Taiwan. Arch Virol 1996; 141:1677-88.
9. Koch G, Kant A, Cook JKA, Cavanagh D. Epitopes of neutralizing antibodies are localized within three regions of the S1 spike protein of infectious bronchitis virus. In: The Second International Symposium on Infectious Bronchitis. Rauischholzhausen, Germany; 1991:154-60.
10. Kant A, Koch G, van Roozelaar DJ, Kusters JG, Poelwijk FAJ, van der Zeijst BAM. Location of antigenic sites defined by neutralizing monoclonal antibodies on the S1 avian infectious bronchitis virus glycopolyprotein. J Gen Virol 1992;73:591-6.
11. Kusters JG, Jager EJ, Lenstra JA, Koch G, Posthumus WPA, Meloen RH, et al. Analysis of an immunodominant region of infectious bronchitis virus. J Immunol 1989;143:2692-8.
12. Lambrechts C, Pensaert M, Ducatelle R. Challenge experiments to evaluate cross-protection induced at the trachea and kidney level by vaccine strains and Belgian nephropathogenic isolates of avian infectious bronchitis virus. Avian Pathol 1993;22: 577-90.
13. Tseng ZM. Analysis of nucleotide and preparation of inactivated vaccine of infectious bronchitis virus isolated in Taiwan. Taichung, Taiwan: Master thesis, National Chung-Hsing University; 1999:13.
14. Rosner B. Hypothesis testing: categorical data. In: Fundamentals of biostatistics. 4th ed. Belmont, CA: Duxbury Press, Wadsworth Publishing; 1995:345-442.
15. Cavanagh D. Coronavirus IBV: structure characterization of the spike protein. J Gen Virol 1983;64:2577-83.
16. Cavanagh D, Davis PJ, Darbyshire JH, Peters RW. Coronavirus IBV: virus retaining spike glycoprotein S2 but not S1 is unable to induce virus-neutralization or haemagglutination-inhibiting antibody, or induce chicken tracheal protection. J Gen Virol 1986;46:1435-42.
17. Koch G, Hartog L, Kant A, van Roozelaar DJ. Antigenic domains on the peplomer protein of avian infectious bronchitis virus: correlation with biological functions. J Gen Virol 1990; 71:1929-35.
18. Lohr JE. Differentiation of IBV strains. In: Kaleta EF, Heffels-Redmann U, eds. Proceedings of the First International Symposium on Infectious Bronchitis. Rauischholzhausen, Germany: World Veterinary Poultry Association; 1988:199-207.
19. Cavanagh D, Ellis MM, Cook JK. Relationship between sequence variation in the S1 spike protein of infectious bronchitis virus and the extent of cross-protection in vivo. Avian Dis 1997;26:63-74.
20. OIE. Avian infectious bronchitis. In: Manual of Standards for Diagnostic Tests and Vaccines. 2nd ed. Paris: Office International des Epizooties; 1997:539-48.
21. Williams A, Wang L, Sneed LW, Collisson EW. Comparative analyses of the nucleocapsid genes of several strains of infectious bronchitis virus and other coronaviruses. Virus Res 1992;25:213-22.
22. Toro H, Espinoza C, Ponce V, Rojas V, Morales MA, Kaleta EF. Infectious bronchitis: Effect of viral doses and routes on specific lacrimal and serum antibody responses in chickens. Avian Dis 1997;41:379-87.