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Evaluation of the protection conferred by commercial vaccines and attenuated heterologous isolates in China against the CK/CH/LDL/97I strain of infectious bronchitis coronavirus

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Abstract

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 genotypic relationship between CK/CH/LDL/97I-type strains and reference IBVs based on S1 gene comparisons and the protection provided by vaccination with commercial vaccines and attenuated homologous and heterologous strains. Phylogenetic analysis and the comparison of S1 showed that CK/CH/LDL/97I-type virus might be a new serotype compared to vaccine strains and other types of IBV isolates in China. Protection efficacy was evaluated by morbidity, mortality, and virus re-isolation from the challenged chicks. Complete protection by IBV vaccination was provided by the homologous strain but sufficient respiratory protection was not provided by the commercial vaccines. Heterologous strains against CK/CH/LDL/97I challenge and the development of a vaccine against CK/CH/LDL/97I-type IBV will be necessary to control infectious bronchitis disease in poultry. Further development of the attenuated CK/CH/LDL/97I strain may provide a valuable contribution towards this goal.

Keywords: Infection bronchitis coronavirus; Spike protein; Cross-protection; Homologous; Heterologous

Introduction

Infectious bronchitis virus (IBV), the prototype of the family Coronaviridae in the order Nidovirales, causes an acute and highly contagious disease of the respiratory and urogenital tracts of chickens worldwide (Cavanagh, 1997). Different IBV strains may be responsible for distinct illnesses. Based on virus neutralisation (VN) tests, IBV exists as dozens of serotypes. Although vaccines have generally been used in controlling the clinical disease, infectious bronchitis (IB) outbreaks occur frequently owing to the antigenic differences between serotypes (Arvidson et al., 1991; Bochkov et al., 2006; Cook et al., 2001; Gelb et al., 1981, 2005; Hofstad, 1981; Ladman et al., 2006; Marquardt et al., 1982) and the development of vaccines from local field isolates is necessary for control.

It is well known that the spike (S) protein of IBV induces antibodies for VN and haemagglutination inhibition (Cavanagh, 2007). The S glycoprotein is proteolitically processed into two non-covalently bound peptide chains known as S1 and S2 (Stein and Sefton, 1982). The S1 protein of IBV has serotype-specific and neutralisation-specific epitopes, and serotypic evolution and the genetic diversity of IBV are mainly monitored by analysis of the S1 gene. As might be expected, cross-protection tends to diminish as the degree of amino acid identity between the S1 proteins of two IBV strains decreases (Cavanagh et al., 1997; Gelb et al., 2005). It has been reported that comparing

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IBV S1 gene sequences is a better predictor of immune response to challenge in chickens than serotyping by VN (Ladman et al., 2006), and gene sequence comparisons were used in these studies for virus typing. However, VN is probably a more practical method in terms of control strategies for performing protection studies (protectotype) with the field isolates (Cook et al., 1999).

In spite of the extensive use of vaccines, nephrotropic IBV outbreaks are frequent in China (Li and Yang, 2001; Liu and Kong, 2004; Liu et al., 2006b; Yu et al., 2001a, 2001b). Vaccine failure may be due to differences in the genotypes/serotypes of vaccine strains and field viruses (Liu et al., 2006a). Based on differences of the entire S1 gene, the Chinese IBV isolates can be classified into several genetic groups (Li and Yang, 2001; Liu et al., 2006b; Yu et al., 2001a, 2001b).

IBV CK/CH/LDL/97I-type is a "novel" IBV genotype in China (Liu et al., 2006b, 2007; Yu et al., 2001a) that was first isolated in the mid-1990s and was associated with proventriculitis (Yu et al., 2001a). This genotype of IBV was consistently isolated in vaccinated and non-vaccinated chicken flocks from 1995–2001 (Liu et al., 2006b; Yu et al., 2001a) and has also been found in Taiwan (Huang et al., 2004). CK/CH/LDL/97I is representative of CK/CH/LDL/97I-type IBV, sharing only 73.1% nucleotide and 75.4% amino acid homology of the entire S1 gene with that of another strain of "novel" genotype, LX4-type strain (Liu and Kong, 2004).

The primary objective of the present study was to compare the genotypes of CK/CH/LDL/97I-type IBV with those of vaccine strains and other IBV field isolates. The second objective was to examine the protection induced by these vaccines and embryo-passaged, attenuated homologous and heterologous IBV strains against the virulent CK/CH/LDL/97I strain. The results of the present study should provide information for future IBV vaccination strategies in China.

Materials and methods

IB vaccines, attenuated strains and challenge strain

Eight different commercially available IB vaccines were selected for comparison of the S1 gene with those of IBV CH/CK/LDL/97I-type isolates (Table 1). Each was produced by different manufacturers. Five out of eight IB vaccine strains used in vaccination-challenge tests in this study (Table 1) were recovered from the commercially available vaccines and propagated once in 9–11-day-old embryonated chicken specific pathogen free (SPF) eggs as described for field isolates (Liu et al., 2006a), to yield a titre of 10^6–10^8 EID_{50} per 0.1 mL, before being used in challenge-protection tests.

IBV isolate CH/CK/LDL/97I, a nephropathogenic strain, was isolated, identified and characterised as previously described (Liu et al., 2006b) and was used as the challenge IBV strain (Liu et al., 2007). The virulent CH/CK/LDL/97I was passaged by inoculating 9–11-day-old embryonating SPF chicken eggs by the chorioallantoic sac route 115 times (designated P_{115}) and was determined to be attenuated by clinical response to inoculation of 15-day-old SPF chickens (Liu et al., 2007). Two heterologous IBV isolates, tl/CH/LDT3/03 (Liu et al., 2005) and CK/CH/LHLJ/04V (Liu et al., 2006b), each representing different IBV genotypes in China, were also passaged as mentioned above 120 and 110 times, respectively (designated P_{120} and P_{110}). These isolates were determined to be attenuated by clinical response to inoculation of 15-day-old SPF chickens that were protected against the homologous pathogenic virus. The IBV strains were also propagated in 9–11-day-old embryonated chicken SPF eggs to yield a titre of 10^6–10^8 EID_{50} per 0.1 mL. The IBV vaccine strains, the three embryo-passaged, attenuated strains and the pathogenic CH/CK/LDL/97I strain, were confirmed by negative contrast electron microscopy (JEM-1200, EX) in the allantoic fluids of inoculated eggs.

Eggs and chicks

Fertile White Leghorn SPF chicken eggs were obtained from the Laboratory Animal Center, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences. The White Leghorn SPF chicks were also from this Center. The birds were maintained in isolators with negative pressure and food and water were provided ad libitum.

The study was approved by the Animal Welfare Committee of Heilongjiang Province.

Comparison of S1 gene

For phylogenetic analysis and comparison of S1 genes, IBVs representing different genotypes in the country, eight vaccine strains used in the country, and four other related IBV isolates were included in comparison with CH/CK/LDL/97I-type strains. All of the S1 gene sequences were obtained from the GenBank database. The sequences were analysed using EditSeq software of the Lasergene Software Suite (Windows 4.05, DNA star). The phylogenetic tree was constructed using the neighbour-joining method (Huang et al., 2004; Saitou and Nei, 1987; Schikora1 et al., 2003) based on the amino acids sequences of S1 subunit of spike protein (the first 1656 nucleotides starting at the AUG translation initiation codons of the S protein genes). Bootstrap values >60% are displayed above branch nodes.

The accession numbers for the S1 genes of IBV strains were: LX4 (AY189157), tl/CH/LDT3/03 (AY702975), CK/CH/LHLJ/04V (DQ167139), QXIBV (AF193423), J2 (AF286303), A (AF151953), A2 (AY043312), J (AF352312), HN99 (AY775551), CK/CH/LHN/00I (DQ167143), CK/CH/LDL/01I (DQ167130), CK/CH/LSHH/03I (DQ167149), CK/CH/LDL/98I (DQ167132), CK/CH/LSC/95I (DQ167146), CK/CH/LGD/03I (DQ167134), CK/CH/LDL/97I (DQ068701), H120 (M21970), D41 (AY846836), HK (AY761141), W93 (AY842862), J9 (DQ515802), JAAS (AY839140), Jilin (AY839144), IBN (AY856348), H94 (DQ515801), 2992/02 (AY606323), V5-90 (U29520) and Vic S (U29519).

Experimental design

Experiment 1

Ninety 1-day-old white Leghorn chicks were divided into nine groups of 10 birds each and housed in different isolators. Birds in groups 1–8 were inoculated intranasally with the different IBV strains ($\log_{10}4.7$–$\log_{10}5.5$ median embryo infectious doses, [EID_{50}] per chick) (Table 2) when 15 days of age. Group 9 was mock inoculated with sterile allantoic fluid and served as controls. The actual vaccination dose per bird, as determined by egg-titration of inocula immediately following vaccination, is summarised in Table 2. At 20 days post-vaccination, blood samples were collected from all chicks and the birds were then challenged oculonasally with $10^{5.6}$ EID_{50} virulent CK/CH/LDL/97I strain. The chicks were examined daily for signs of infection (morbidity) and mortality for up to 30 days post-challenge.

Experiment 2

The experimental set-up was identical to that described in Experiment 1 except that all the chicks were euthanased at 5 days post-challenge and tracheas and kidneys were collected for virus isolation at necropsy. Blood samples from chicks of all groups were collected at 20 days post-vaccination and 5 days post-challenge and stored at −20 °C.

Table 1
Nucleotide and amino acid homology of IBV S1 protein gene sequences[a]: comparison of the CK/CH/LDL/97I strain with five vaccine strains, CK/CH/LHLJ/04V and tl/CH/LDT3/03 strains

Strain	CK/CH/LDL/97I	H120	IBN	J9	JAAS	Jilin	CK/CH/LHLJ/04V	tl/CH/LDT3/03
Amino acid identity (%)								
CK/CH/LDL/97I		74.3	74.1	78.3	79.2	76.3	75.7	73.0
H120	76.5		99.5	79.7	80.8	77.0	76.1	79.3
IBN	76.5	99.7		79.7	80.8	77.0	76.1	79.3
J9	78.5	83.1	83.1		83.3	79.7	75.5	75.4
JAAS	78.5	81.4	81.4	84.2		81.3	77.2	77.5
Jilin	76.5	79.7	79.7	80.9	80.7		75.4	75.9
CK/CH/LHLJ/04V	75.5	77.0	76.9	77.3	75.8	75.8		83.5
tl/CH/LDT3/03	76.2	81.6	81.6	79.0	79.4	77.8	84.4	
Nucleotide identity (%)								

Top right, amino acid identity (%); bottom left, nucleotide identity (%).
[a] The first 1636 nucleotides, starting at the AUG translation start codon, of the S1 protein genes were compared.

Virus recovery and detection

The tissue samples collected after challenge were used for virus isolation individually as previously described (Liu et al., 2006a). The individual samples, containing 10,000 U penicillin and 10,000 μg streptomycin, were inoculated into at least four SPF embryos via the allantoic cavity (0.2 mL per egg). The eggs were candled daily, and allantoic fluids from two of the inoculated embryos were collected 72 h post-inoculation (PI) for reverse transcription polymerase chain reaction (RT-PCR) amplification. The remaining embryos were examined 1 week later for characteristic IBV symptoms such as dwarfing, stunting, or curling of embryos.

Two hundred microlitres of allantoic fluid were used from each inoculated embryo for RT-PCR amplification (Liu and Kong, 2004). Briefly, RNA was extracted and RT was conducted using IBV oligonucleotide N(−), 5′- ACGCGGAGTACGATCGAGGGTACA-3′, which was specific for the 3′ untranslated region (UTR) (Liu et al., 2006a). Oligonucleotides N(−) and N(+), 5′- GATGCCCCAGCGCCAGTCATTAAA-3′, were used to amplify the majority of the N gene and parts of the 3′-UTR yielding an approximately 1600 bp product (Liu et al., 2006a) from the allantoic fluids inoculated with samples collected after challenge. Both RT and PCR were carried out with the same conditions as described by Liu et al. (2006a). The PCR products were analysed on a 1.0% agarose gel.

ELISA determination of specific serum IgG

Serum samples were assayed in single dilutions using a commercial total antibody ELISA (IDEXX) according to the manufacturer's instructions. Serum-to-positive ratios (*S*/*P*-ratios) were calculated (Liu et al., 2006a) using the following formula:

$$SP \text{ ratio} = \frac{\text{OD sample} - \text{OD negative control}}{\text{OD positive control} - \text{OD negative control}}$$

where OD stands for optical density. From these S/P-ratios, individual serum titres expressed as $\log_2$ values, were calculated according to the manufacturer's instructions.

Results

Phylogenetic analyses and comparison of S1 protein gene

The published nucleotide and deduced amino acid sequences of the S1 subunit of the eight IB vaccine strains and of 20 field strains obtained from GenBank were compared. The eight vaccine strains were widely used in China for preventing IB. Of the 20 IBV reference field strains in this study, two were from Australia (N5-90 and Vic S), one from New Zealand (A), one from Taiwan (2992/02), and the remaining 16 were all from China and represented different genotypes (Liu et al., 2006b). When phylogenetic analysis was performed based on the entire S1 subunit of the spike gene, the 28 IBV strains were grouped into seven clusters (Fig. 1).

As shown in Fig. 1 and Table 1, viruses in cluster I, LX4-type (Liu and Kong, 2004) and CK/CH/LHLJ/04V viruses, shared 75.5% and 75.7% nucleotide and amino acid similarities, respectively, with strain CK/CH/LDL/97I. Cluster II contained tl/CH/LDT3/03-type viruses and the nucleotide and amino acid homology between strains tl/CH/LDT3/03 and CK/CH/LDL/97I was 76.2% and 73%, respectively. Six IBV vaccine strains were placed into cluster III and viruses in this cluster were all mass-type strains and they shared 76.5% and approximately 74% S1 gene nucleotide and amino acid similarities, respectively, with strain CK/CH/LDL/97I. Among the IBV strains compared, vaccine strains in cluster IV (JAAS) and VI (J9, which is an Australian associated IBV strain) had more S1 nucleotide and amino acid homology (more than 78.3% and 81.4% nucleotide and amino acid similarities, respectively) with CK/CH/LDL/97I than any other reference strain examined. IB vaccine strain Jilin in cluster V shared approximately 76% S1 gene nucleotide and amino acid similarities to that of CK/CH/LDL/97I (Table 1). In addition, the genetic relationships of S1 genes among vaccine strains and tl/CH/LDT3/03 and tl/CH/LHLJ/04V are summarised in Table 1.

Clinical signs and mortality

Complete clinical protection against pathogenic CK/CH/LDL/97I strain was only conferred following vaccination with CK/CH/LDT3/03 P_{120} and J9 strains, the remaining vaccines or the attenuated, heterologous CK/CH/LHLJ/04V P_{110} strain did not afford complete clinical protection against pathogenic CK/CH/LDL/97I strain in Experiment 1 (Table 2.). All chickens in the control group

Table 2
Results of serology and virus recovery after vaccination with attenuated strains (including five IB vaccine strains) and following challenge with CK/CH/LDL/97I P5 strain (20 days post-vaccination). Results are expressed as the number of chicks showing positive results after challenge/number of chicks challenged

Strains[a]	Dose, median embryo infectious doses (log10)[b]	Morbidity	Mortality	Antibody [c]		Virus recovery[e]	
				15 days after vaccination[d]	5 days Post-challenge	Trachea	Kidney
CK/CH/LDL/97I P_{115}	5.2	0/10	0/10	20/20	10/10	0/10	0/10
IBN	5.5	3/10	0/10	20/20	10/10	9/10	0/10
H120	4.8	4/10	0/10	20/20	10/10	7/10	2/10
JAAS	5.3	10/10	0/10	20/20	10/10	7/10	1/10
Jilin	4.8	5/10	0/10	20/20	10/10	6/10	0/10
J9	4.7	0/10	0/10	20/20	10/10	5/10	1/10
CK/CH/LHLJ/04V P_{110}	5.0	5/10	0/10	20/20	10/10	10/10	2/10
tl/CH/LDT3/03 P_{120}	5.0	0/10	0/10	20/20	10/10	5/10	0/10
CK/CH/LDL/97I P_5 (control)	5.6	10/10	1/10	–	10/10	10/10	9/10

[a] Ten chicks per group.
[b] Dose per chick, 100 μL.
[c] Number seroconverted/number inoculated.
[d] The numbers of inoculated chicks included in both Experiments 1 and 2.
[e] Two procedures were used for virus recovery after challenge. First, lesions in embryos that had been inoculated with individual tissue samples (trachea or kidney) were observed. Secondly, RT-PCR using oligonucleotide primers N(+) and N(−) on RNA recovered from allantoic fluid of the same eggs was conducted. The results from the two procedures were identical.

also showed respiratory signs at about 3–13 days after challenge with the virulent CK/CH/LDL/97I strain. The morbidity rates of birds vaccinated with different vaccines and attenuated viruses following pathogenic challenge are shown in Table 2.

Clinically, the chicks appeared depressed with ruffled feathers and were listless and huddled together. One of the diseased birds in the control group died during the experiment. Necropsy of the dead and the sick birds revealed that gross lesions were mainly confined to the kidneys. The kidney parenchyma of the affected birds was pale, swollen and mottled; tubules and urethras were distended with uric acid crystals. In addition, mild respiratory signs (sneezing, rales) were also observed in affected birds. However, only mild proventriculitis was observed in a few very sick chicks. The clinical signs of the inoculated birds tended to disappear gradually after 20 days of challenge.

Protective efficacy of vaccination with attenuated homologous CK/CH/LDL/97I strain (P_{115})

Complete clinical and respiratory protection against pathogenic CK/CH/LDL/97I strain was provided by vaccination with CK/CH/LDL/97I P_{115} (Table 2). Both the trachea and kidney of SPF chickens were used for virus isolation. The kidneys were used for efficacy evaluation because CK/CH/LDL/97I is a nephropathogenic strain that typically causes severe renal damage. As shown in Table 2, no virus was isolated from kidney samples from the CK/CH/LDL/97I P_{115}-vaccinated birds 5 days after challenge and all the birds showed seroconversion after 20 days post-inoculation. Considering the morbidity and virus isolation rates from the tracheas and kidneys, the attenuated virus strain of CK/CH/LDL/97I, was effective against homologous challenge and could be a used as an IB vaccine for controlling CK/CH/LDL/97I-type IBV.

Contrary to the CK/CH/LDL/97I P_{115} vaccinated group, all birds in the control group showed clinical signs associated with IB, and 100% and 90% of birds were positive for virus isolation from tracheas and kidneys, respectively, suggesting that all non-vaccinated controls were susceptible to challenge.

Protective efficacy of vaccination with IB vaccines or attenuated heterologous IBV strains

As shown in Table 2, the cross-protection against virulent CK/CH/LDL/97I provided by different vaccines and two heterologous attenuated IBVs was variable. The respiratory protection conferred by commercial vaccines or the heterologous attenuated IBVs was <50%, as determined by the re-isolation of the virus from tracheas 5 days post-challenge.

These data indicate that respiratory protection was not provided by vaccination with each of the IBV strains in spite of the presence of anti-IBV antibody in the vaccinated chickens. In contrast, commercial vaccines or the two heterologous attenuated IBVs conferred >80% kidney protection as determined by virus re-isolation from kidneys 5 days post-challenge. The data indicate that each of the strains tested was sufficient to provide protection from kidney damage. Of the seven IBV strains, tl/CH/LDT3/03 P_{120} strain, isolated from a teal in 2003 in China's Guangdong province (Liu et al., 2005), provided the best

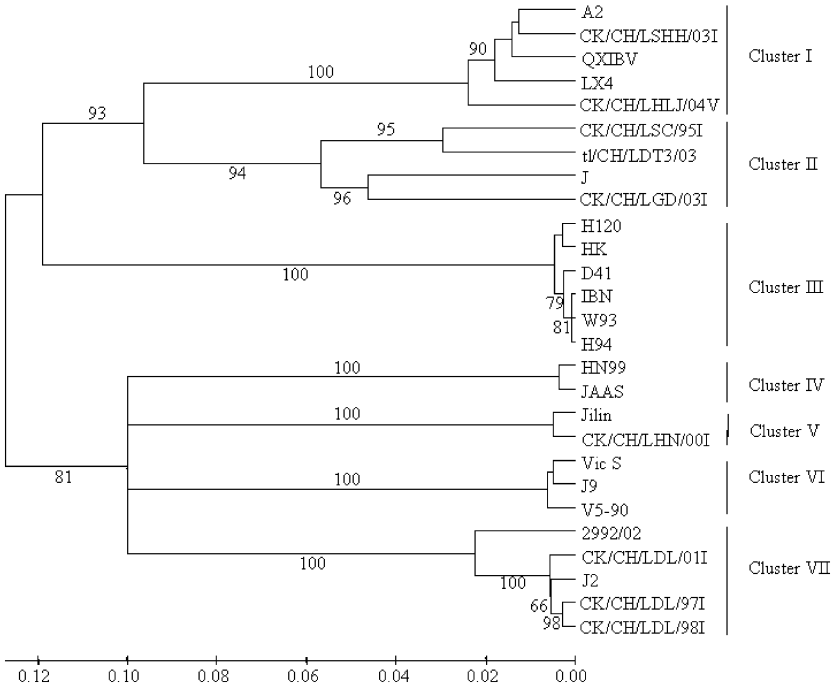

Fig. 1. The phylogenetic tree was constructed using neighbour-joining and bootstrap analysis ($n = 1000$) based on the S1 subunit of spike protein (Huang et al., 2004; Saitou and Nei, 1987; Schikora1 et al., 2003). Bootstrap values >60% are displayed above branch nodes. The tree was rooted with the first 1656 nt of the S protein genes, starting at the AUG translation initiation codon.

protection against challenge, when considering the morbidity and the re-isolation of the virus from tracheas and kidneys 5 days post-challenge.

Discussion

Nephropathogenic IBV has been a major problem in the poultry industry in China in recent years (Li and Yang, 2001; Liu and Kong, 2004; Liu et al., 2006b; Yu et al., 2001a, 2001b). Yu et al. (2001) first isolated three IBV isolates, Q1, J2 and T3, from proventricular tissues of 25–70-day-old H120-vaccinated chicken flocks having outbreaks of so-called "an avian disease associated with the proventriculus" from three different areas of China between 1996 and 1998 (Yu et al., 2001a).

Sequence data demonstrated the complete S1 amino acid sequences of these isolates had 47.3 to 82.3% similarity compared to the 47 published S1 sequences, indicating that the virus might be a new IBV variant (Yu et al., 2001a). Proventriculitis was observed in in vivo pathogenicity experiments, but no kidney swelling was observed (Yu et al., 2001a). These data suggested that the three isolates might be a new emerging IBV variant associated with the alimentary tract, especially the proventriculus (Yu et al., 2001a). We have also previously isolated IBV isolates (CK/CH/LDL/97I-type) between 1997 and 2001, indicating that this type of IBV has continued to circulate in vaccinated and non-vaccinated chicken flocks in recent years. However, as observed previously (Liu et al., 2007) and in the present study, only mild proventriculitis was observed in a few sick chicks and gross lesions were mainly confined to the kidneys. This is similar to the case with a Taiwan IBV isolate, 2992/02, belonging to the CK/CH/LDL/97I-type by S1 gene comparison (Huang et al., 2004). The nephropathogenic 2992/02 strain was isolated from a Ma5-vaccinated broiler farm built near the migratory bird wetland in Yilan in Taiwan in 2002 (Huang et al., 2004). The change in tropism is of interest and should be addressed further in future studies.

To prevent economic losses associated with morbidity, poor weight gain, loss of egg production and mortality, IBV vaccines are routinely administered. Although IB vaccines provide adequate protection against homologous serotypes, vaccination with one serotype does not ensure complete protection against heterologous strains (Hofstad, 1981; Lambrechts et al., 1993; Liu et al., 2006a). Heterologous strains emerge by changes in the IBV genome through point mutations, deletions, insertions or RNA recombination (Cavanagh et al., 1988; Jia et al., 1995; Liu et al., 2007; Wang et al., 1993) and these heterologous strains are often responsible for IB outbreaks in vaccinated chicken flocks.

It has been reported that IBV S1 gene sequence comparison is a better predictor of immune challenge in chickens than serotyping by VN (Ladman et al., 2006). In the present study most of the vaccines used in China to prevent IB and IBV isolate representatives of different genotypes circulating in chicken flocks were selected for phylogenetic analysis and S1 gene comparison with those of CK/CH/LDL/97I-type strains. CK/CH/LDL/97I-type strains clearly belonged to different clusters in comparison to the

reference strains (Fig. 1), suggesting that the CK/CH/LDL/97-type strains may differ genotypically not only with the selected vaccine strains, but also with other types of IBVs circulating in China. It has been reported that S1 amino acids of different IBV serotypes usually differ by 20–25% (Gelb et al., 1997). In our study, <80% homology of nucleotides and amino acids in the S1 domain of the spike protein was observed between CK/CH/LDL/97I and the vaccine strains, as well as between CK/CH/LDL/97I and other types of IBV isolates from China. This indicates that CK/CH/LDL/97I-type viruses are a different serotype than the vaccine strains and the other types of IBV isolates examined.

It is possible that some strains with high degrees of S1 homology may not cross-protect against challenge because few amino acid differences located in major immunodominant regions of the gene may be sufficient to cause a discrepancy between sequence and cross-protection (Cavanagh et al., 1988, 1992). However, in most cases, it is reported that IBV isolates with high S1 homology induce a high degree of cross-protection. Accordingly, IBV strains with low S1 homology provide little or inconsistent cross-protection (Cavanagh, 1997; Ladman et al., 2006). Little S1 similarity between CK/CH/LDL/97I and the vaccine strains in this study could explain the poor clinical and respiratory protection conferred against CK/CH/LDL/97I challenge in the chickens vaccinated with heterologous viruses. This might be why the birds from which CK/CH/LDL/971-like isolates were recovered developed disease in spite of being vaccinated with IBV vaccines (Liu et al., 2006b; Yu et al., 2001a).

Interestingly, almost all the vaccines and two attenuated heterologous strains offered kidney protection (>80%) against the nephropathogenic CK/CH/LDL/97I challenge. This was determined by re-isolation of the virus from kidneys 5 days post-challenge and is a useful tool in evaluating protection conferred by IBV commercial vaccines against IBV challenge (Alvarado et al., 2003). It was shown that circulating antibody levels were of minor importance in the protection of the respiratory mucosa against challenge (Hofstad, 1967; Holmes, 1973; Macdonald et al., 1981). However, nephritis can be induced by infection of the respiratory tract presumably following viraemia. Although circulating antibody against IBV played only a minor role in protection of the respiratory tract, humoral immune mechanisms directed against viraemia appeared to be of particular importance in protecting the kidney (Holmes, 1973; Macdonald et al., 1981; Marquardt et al., 1982). All of the vaccinated birds showed seroconversion after 20 days post-inoculation and this antibody contributed to protection of kidneys against the nephropathogenic CK/CH/LDL/97I challenge.

Production of novel commercial vaccines against these new variants is not generally an option owing to the high cost and time required for their final approval. Thus examination of the protection conferred by commercially available IBV vaccines against new variants was important to evaluate the extent of protection that currently available IB vaccines can provide against challenge with IBVs of distinct antigenic types.

Conclusion

Currently available vaccines do not provide sufficient respiratory protection against CK/CH/LDL/97I challenge. If reduction in transmission of IBV is considered to be important, the high rates of challenge virus isolation (50%) in birds vaccinated with a heterologous vaccine indicate that these vaccines do not offer sufficient protection against the IBV CK/CH/LDL/97I strain. This result reveals that it will be necessary to develop a vaccine from CK/CH/LDL/97I-type strains. The embryo-passaged, attenuated CK/CH/LDL/97I P_{115} strain should be further considered for vaccine development and evaluated in field conditions as it was capable of conferring protection against CK/CH/LDL/97I type IBV.

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