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Serological Studies of Infectious Bronchitis Vaccines against Japanese Field Isolates of Homologous and Heterologous Genotypes

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ABSTRACT. The genetic diversity of the partial S1 gene involving the hyper variable region for infectious bronchitis (IB) vaccine strains in Japan were compared with those of IB virus isolated from the field in Japan. Field isolates have mainly been classified into three major genotypes, JP-I, JP-II and JP-III, since 2003; however, the 4/91 genotype was detected from recent field isolates in Japan. The virus neutralization (VN) activity with vaccine immunized serum was investigated to evaluate the protective effects of vaccines against Japanese field isolates. In the results of the VN test, antiserum immunized with the GN and C78 (JP-I), TM-86w and Miyazaki (JP-II) and 4/91 (793B) vaccine strains could neutralize a high rate of field isolates of homologous genotype (75% of field isolates of JP-I, 100% of that of JP-II and 100% of that of 793B, respectively). For field isolates of JP-III, even though there are no homologous genotype vaccine strain, some strains of JP-III were neutralized with immune serum from vaccine strains of the heterologous genotype. In this study, a correlation between serological property and genotype was found for JP-I, JP-II and 793B. Our results suggested that an effective vaccine could be predicted in accordance with the genotype of field isolates.
KEY WORDS: genotype, infectious bronchitis virus, Japan, serotype, vaccine.

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Infectious bronchitis virus (IBV), the etiological agent of infectious bronchitis (IB), is a highly contagious pathogen that causes respiratory disease, increasing mortality and production losses in layers and breeders and sometimes induces renal disease in young birds. In spite of the use of vaccines for control of IB, the disease continues to be a problem in commercial poultry because IBV readily undergoes mutation in chickens and some serotypes do not cross-protect against antigenically unrelated serotypes, including new variant serotypes [7, 12].

IBV, a member of the family Coronaviridae, contains single-stranded, positive-sense RNA of 27.6 kb. The genome encodes four major structural proteins, the spike glycoprotein (S), the membrane glycoprotein, the nucleocapsid protein and the envelope protein [1]. The spike glycoprotein is post-translationally cleaved into S1 and S2 subunits [11]. The S1 and S2 associate to form the spike, present in the viral envelope in which S1 is exposed on the virion surface, anchored by S2 [6]. The S1 glycoprotein is responsible for infection to the host cell, inducing virus-neutralizing antibody. Consequently, the S1 gene is usually used for IBV antigenic typing.

Monoclonal antibody analysis has revealed that most of the antigenic sites of virus-neutralization antibody-inducing epitopes are formed by a few amino acids that occur in the first quarters of the S1 subunit, especially in the hyper-variable regions (HVR) [2, 9, 10]. Hence, classification by genotype has been done on the basis of the antigenic site, especially for HVR, on the S gene [15, 16].

On the basis of results of phylogenetic analysis of the HVR, Japanese field strains isolated from 1958 to 2003 have been classified into five genetic groups (Mass, Gray, JP-I, JP-II and JP-III) [16]. Furthermore, IBVs of the 793B genotype, the same as the 4/91 vaccine strain, have been identified since 2003 [20].

In Japan, more than twenty isolates have been used for live or inactivated vaccines to prevent and control IBV. Vaccine selection is important since outbreaks of IB still occur in vaccinated flocks. Identification of the serotype of the IBV field isolate is essential for selection of a vaccine that is antigenically similar to the field isolates. To decide the antigenicity of field isolates, classically, IBV strains have been placed into serologically related groups on the basis of virus-neutralization (VN) tests, which are very laborious and time-consuming. On the other hand, genotyping is a technique for faster identification. Mase *et al.* [16] have reported the genotyping for Japanese isolates (Mass, Gray, JP-I, II and III) by phylogenetic analysis based on partial S1 gene including the sequence of the HVR. However, the relationship between the genotypes and serotypes of Japanese isolates is unknown. The objective of this study was to elucidate the relationship between genotypes and serotypes for IBV vaccines and IBV field isolates in Japan.

To define the evolution of recent IBV in Japan, first we investigated the genotypes of recent IBV isolates in addition to field isolates in a previous report [16]. For field strains in each genotype, we examined serological reactivity with various vaccine immunized sera to evaluate the likely protective effect of vaccines against IBV field isolates.

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MATERIALS AND METHODS

IB vaccines and field isolates: The IB vaccine strains and field isolates used in this study are shown in Table 1. Twelve different commercially available live vaccines were used. Of those, KU, Nerima, Kita-1, Miyazaki, TM-86w, GN, C78 and ON were derived from field cases of IB in Japan. Other strains, H120 and Ma5 of Massachusetts (Mass) type, L2 of Connecticut (Conn) type and 4/91 of 793B type, were derived from foreign isolates. Vaccine virus was propagated in 10-day-old embryonated specific pathogen-free (SPF) eggs, and the virus stock was used for preparation of antiserum and challenge-protection trials. For field isolates, twelve IBVs described in a previous report [16] were obtained from the National Institute of Animal Health, and seven isolates were obtained from regional laboratories in Japan. These samples were provided as allantoic fluid that was recovered from IB clinical samples. The viruses were propagated once by injection into the chorioallantoic cavities of 10-day-old embryonated SPF eggs to yield a titer of 10^5 to 10^7 median embryo infective dose (EID_{50}) per 0.1 m*l*. These virus fluids were used for virus neutralization tests.

Chicken embryos and chickens: SPF White Leghorn embryonated chicken eggs and chickens were obtained from Nisseiken Co., Ltd. (Tokyo, Japan). Embryonated eggs were used for production of viral seed stocks and VN assays. Chickens for antiserum production were housed in separate isolators and were provided with food and water *ad libitum*.

Viral RNA extraction and reverse transcriptase (RT)-polymerase chain reaction (PCR) amplification: For the recent field isolates, sequences were determined according to the method of Mase *et al.* [16]. Viral RNA was extracted from infected allantoic fluids using ISOGEN-LS (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. The RNA was air-dried for 10 min, redissolved in 0.05 m*l* RNase-free water and stored at –70°C until use. Reverse transcription was carried out with a kit (Takara AMV RT-PCR kit, Tokyo, Japan) using random 9 mers. PCR was performed to amplify the partial S1 gene using IBV-S1 and IBV-S2 primers, yielding an amplicon of approximately 670 nucleotides [16].

Sequence analysis of the S1 protein gene: PCR products were cut from 1.2% agarose gels and purified with a QIAquick Gel Extraction Kit (Qiagen, Inc.). Purified RT-PCR products were sequenced in the forward and reverse directions using PCR primers and others to various regions within the S gene of each strain. Purified PCR products were used as a template for sequencing on an Applied Biosystems 373S automated DNA sequencer using dye terminator cycle sequencing chemistry (Perkin-Elmer/Applied Biosystems, Foster City, CA, U.S.A.). Analysis of the partial S1 sequence obtained by our laboratory and from GenBank was performed using GENETYX-WIN ver.4.0 (Software Development Corp., Tokyo, Japan). Phylogenetic analysis with available sequences from GenBank was conducted using the Clustal X program [21], and the tree was constructed by the neighbor-joining (NJ) method [19].

Table 1. IBV live vaccine strains and field isolates examined in this study

IBV	Genotype	Sequence accession no.
Vaccine strain		
H120	Mass	M21970
KU	Mass	AB120657
Ma5	Mass	AY561713
Nerima	Mass	AB363962
Kita-1	Mass	AB120656
GN	JP-I	AB363960
C78	JP-I	AB120653
Miyazaki	JP-II	AB120654
TM-86w	JP-II	AB120655
ON	Gray	AB120658
4/91	793B	AF093793
L2	Conn	AB363961
Field isolate		
JP/Miyazaki/89[a)]	JP-II	AB120635
JP/Yamanashi/93	JP-II	AB120538
JP/Yamanashi/95	JP-I	AB120640
JP/Chiba/98	JP-I	AB120643
JP/Shizuoka/98	JP-I	AB120641
JP/Toyama/2000	JP-I	AB120644
JP/Aichi/2000	JP-III	AB120645
JP/Fukui/2000	JP-III	AB120646
JP/Osaka/2000	JP-II	AB120649
JP/Kanagawa/2001	JP-II	AB120650
JP/Shimane/2002	JP-III	AB120651
JP/Ibaraki/2003	JP-I	AB120652
JP/Wakayama/2003	793B	AB363949
JP/Wakayama-3/2004	JP-I	AB363952
JP/Iwate-1/2004	JP-III	AB363956
JP/Iwate-3/2004	JP-I	AB363958
JP/Nara/2005	JP-I	AB363954
JP/Iwate/2005	793B	AB363959
JP/Shiga/2005	JP-II	AB363955

a) Japan/Location/Year of isolation.

Production of antisera: The antisera to vaccine strains were taken from immunized SPF chickens reared in isolators. Four-day-old chickens were inoculated once oculonasally with $10^{4.0}EID_{50}$ of vaccine strains and bled 4 weeks later. Non-immunized SPF chickens at the same age were also bled for control serum. The collected serum was inactivated at 56°C for 30 min and stored at –20°C.

Virus neutralizing (VN) test: The VN test was performed according to the method of Ohta *et al.* [17]. In brief, IB virus was diluted (10-fold) with PBS containing 0.1% BSA and antibiotics (penicillin, 100 U/m*l*; streptomycin, 0.1 mg/m*l*; kanamycin, 1 mg/m*l*). Each dilution was added to an equal volume of immune serum, and these samples were incubated at 37°C for 1 hr. After incubation, 0.1 m*l* of each sample was inoculated into five 10-day-old SPF eggs via the allantoic cavity, and they were then incubated for 7 days at 37°C. Embryos were examined for dwarfing and death on the final day, and virus titers (EID_{50}) were calculated by the method of Reed and Muench [18]. VN titer was determined

as follows: VN titer against IBV=log(EID_{50} against antiserum)–log(EID_{50} against control serum). The relatedness value (r value) was calculated as the percentage of VN titer against field isolates in the VN titer of homologous vaccine strains: (VN titer against field isolates/VN titer against homologous vaccine strains) × 100 (%).

RESULTS

Grouping IBV vaccine strains and field isolates: Vaccine strains commercially available in Japan were divided into three major groups, JP-I (C78 and GN), JP-II (TM-86w and Miyazaki) and Mass (Mass: Kita-1, Nerima, H120, Ma5 and KU). Of the field isolates from recent outbreaks in Japan, two isolates in 2003 and 2005 were classified into 793B as a new genotype in Japan, and the field strains were classified into 4 genotypes (JP-I, II, III and 793B; Fig.1).

HVR sequence analysis: Partial S1 genes (strain H120, GenBank accession number M21970, nucleotide position 1 to 621, amino acid position 1 to 207) of IBV field isolates and vaccine strains were compared (Table 2). The similarities of nucleotides and amino acids of vaccine strains to field isolates in the same genotype ranged from 86 to 99% and 80 to 95%, respectively, for JP-I and from 96 to 99% and 94 to 99%, respectively, for JP-II. For JP-III, in which there are no vaccine strains of homologous genotype, the similarities of nucleotides and amino acids to vaccine strains ranged from 67 to 78 and 59 to 75%, respectively. The amino acid similarities of two strains in the 793B genotype were 87 and 88% against the 4/91 vaccine strain. For vaccine strains of the Mass, Gray and Conn genotypes, the amino acid similarities to field isolates ranged from 59 to 78%.

Serological analysis of vaccine strains and field isolates: The results of the VN test are shown in Table 3. All antiserum prepared with vaccine showed the highest reactions against homologous strains. Of the eight field isolates clas-

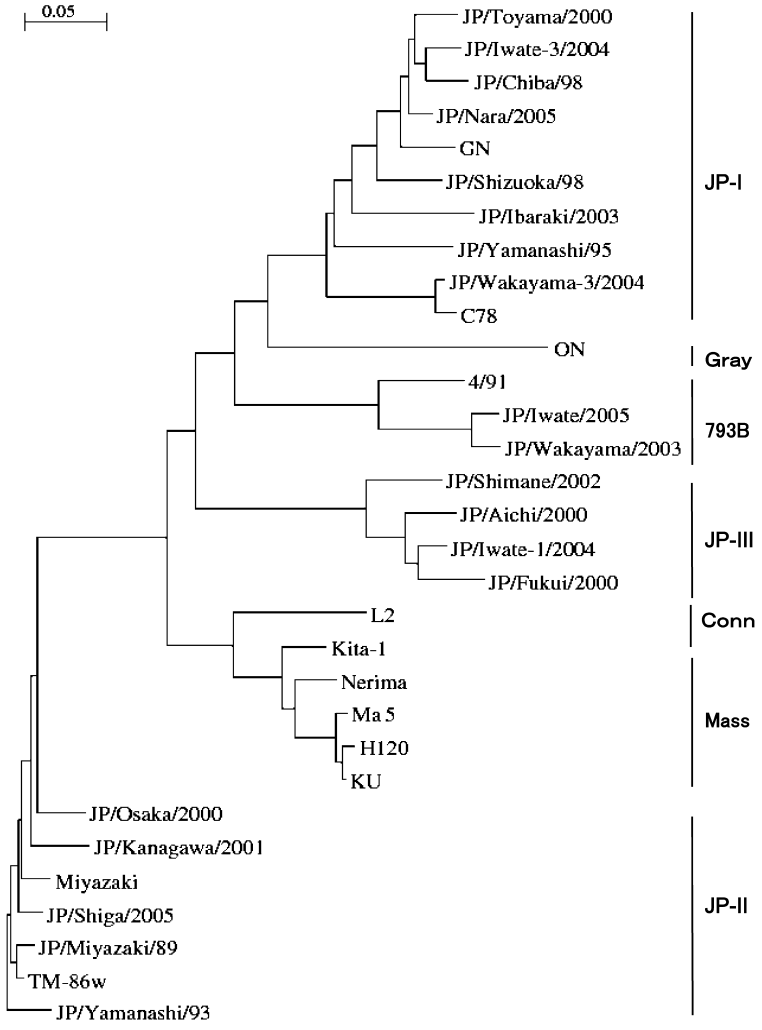


Fig. 1. Phylogenetic tree showing partial S1 gene interrelationships between IBV field isolates and vaccine strains in Japan.

Table 2. Nucleotide and Amino acid similarity (%) of partial S1 gene (1–621bp, 1–207aa)

Genotype	Virus	JP-I		JP-II		Mass		Conn	Gray	793B
		GN	C78	TM-86w	Miyazaki	H120	Kita-1	L2	ON	4/91
JP-I	JP/Yamanashi/95	89.3 (86)[a)]	89.1 (85)	74.0 (70)	74.8 (69)	74.9 (70)	73.9 (70)	73.2 (66)	76.9 (71)	73.4 (71)
	JP/Chiba/98	96.9 (94)	89.9 (84)	74.0 (71)	73.9 (72)	74.1 (70)	74.6 (71)	73.0 (67)	76.4 (71)	73.4 (72)
	JP/Shizuoka/98	94.5 (91)	88.6 (83)	73.1 (71)	73.2 (72)	73.9 (70)	74.5 (71)	72.9 (69)	77.2 (71)	73.2 (71)
	JP/Toyama/2000	95.6 (93)	90.5 (86)	73.8 (71)	74.1 (70)	74.3 (70)	73.9 (70)	72.1 (67)	76.4 (70)	73.8 (72)
	JP/Ibaraki/2003	89.9 (87)	86.4 (80)	73.6 (70)	73.3 (69)	73.6 (70)	73.6 (71)	71.9 (66)	75.5 (71)	72.7 (70)
	JP/Iwate-3/2004	95.6 (93)	89.6 (86)	74.1 (71)	73.5 (71)	74.0 (70)	73.7 (71)	72.4 (67)	76.6 (72)	73.6 (72)
	JP/Nara/2005	96.4 (95)	90.3 (86)	74.1 (71)	74.5 (71)	73.5 (70)	73.9 (70)	72.9 (68)	76.3 (71)	73.2 (72)
	JP/Wakayama-3/2004	90.6 (85)	99.2 (98)	74.3 (71)	73.9 (70)	74.8 (69)	74.0 (69)	72.6 (67)	77.3 (71)	74.7 (73)
JP-II	JP/Miyazaki/89	73.1 (68)	74.1 (70)	99.5 (99)	98.6 (96)	78.8 (75)	78.3 (76)	78.2 (75)	70.1 (65)	75.1 (68)
	JP/Yamanashi/93	72.4 (67)	73.9 (68)	98.4 (96)	97.8 (95)	78.3 (74)	78.0 (76)	77.2 (72)	69.3 (63)	74.8 (67)
	JP/Osaka/2000	73.1 (69)	74.1 (71)	97.4 (96)	97.1 (94)	79.5 (77)	78.8 (78)	78.5 (75)	70.1 (63)	75.3 (68)
	JP/Kanagawa/2001	72.1 (68)	73.8 (70)	96.0 (94)	96.3 (95)	79.0 (75)	79.1 (77)	78.2 (75)	69.6 (62)	74.6 (68)
	JP/Shiga/2005	73.1 (69)	73.9 (71)	99.0 (98)	98.4 (97)	79.3 (76)	79.1 (77)	78.7 (77)	69.9 (64)	75.1 (68)
JP-III	JP/Aichi/2000	71.2 (64)	71.4 (65)	76.4 (72)	76.2 (72)	75.8 (70)	75.7 (72)	74.4 (75)	67.6 (61)	74.7 (71)
	JP/Fukui/2000	70.8 (63)	71.3 (64)	75.8 (70)	75.6 (71)	75.4 (68)	75.0 (69)	74.6 (66)	67.1 (59)	74.1 (68)
	JP/Shimane/2002	73.3 (67)	73.8 (69)	78.4 (75)	77.7 (75)	77.5 (71)	77.0 (72)	75.7 (69)	68.0 (60)	75.6 (71)
	JP/Iwate-1/2004	71.1 (65)	71.2 (66)	76.6 (72)	76.4 (73)	77.1 (71)	77.0 (71)	75.2 (67)	67.9 (60)	75.5 (71)
793B	JP/Wakayama/2003	72.4 (67)	74.1 (69)	75.1 (67)	74.6 (67)	74.6 (66)	74.3 (67)	72.5 (65)	70.4 (64)	93.3 (87)
	JP/Iwate/2005	72.1 (67)	73.5 (70)	75.2 (67)	74.6 (67)	74.9 (66)	75.1 (68)	73.0 (64)	70.4 (65)	93.9 (88)

a) Nucleotide similarity (amino acid similarity).

Table 3. Results of VN tests using immunized serum with vaccine and field isolates

Genotype	Virus	Immunized serum of live vaccine strain								
		I		II		Mass		Conn	Gray	793B
		GN	C78	TM-86w	Miyazaki	H120	Kita-1	L2	ON	4/91
JP-I	JP/Yamanashi/95	36.3[a)]	**66.7**	**57.1**	30.8	**63.6**	**53.3**	50.0	43.8	**54.5**
	JP/Chiba/98	**81.8**[b)]	20.0	42.9	50.0	36.4	26.7	27.8	18.8	–9.1
	JP/Shizuoka/98	27.3	40.0	42.9	38.5	36.4	33.3	–11.1	–12.5	18.2
	JP/Toyama/2000	**72.7**	13.3	14.3	**53.8**	27.3	**73.3**	–6.9	18.8	27.3
	JP/Ibaraki/2003	**81.8**	40.0	**64.3**	**92.3**	**81.8**	**66.7**	38.9	**62.5**	NT
	JP/Iwate-3/2004	**63.6**	50.0	**71.4**	**80.8**	22.7	36.7	**66.7**	**62.5**	**86.4**
	JP/Nara/2005	22.7	16.7	50.0	32.7	45.5	26.7	11.1	6.3	18.2
	JP/Wakayama-3/2004	**81.8**	**93.3**	**92.9**	NT[c)]	27.3	NT	44.4	NT	NT
JP-II	JP/Miyazaki/89	**63.6**	26.7	**57.1**	**53.8**	36.4	41.7	22.2	6.3	18.2
	JP/Yamanashi/93	9.1	33.3	**78.6**	**53.8**	36.4	26.7	11.1	0.0	27.3
	JP/Osaka/2000	36.4	46.7	**78.6**	38.5	45.5	26.7	33.3	25.0	NT
	JP/Kanagawa/2001	36.4	40.0	**92.9**	**53.8**	27.3	13.3	38.9	6.3	18.2
	JP/Shiga/2005	**63.6**	26.7	**64.3**	NT	**54.5**	NT	38.9	NT	45.5
JP-III	JP/Aichi/2000	18.2	40.0	50.0	7.7	9.1	20.0	16.7	25.0	0.0
	JP/Fukui/2000	36.4	30.0	50.0	30.8	**63.6**	40.0	22.2	0.0	27.3
	JP/Shimane/2002	**81.8**	**60.0**	35.7	**69.2**	**81.8**	**80.0**	33.3	12.5	**81.8**
	JP/Iwate-1/2004	**81.8**	40.0	35.7	23.1	50.0	36.7	30.6	40.6	50.0
793B	JP/Wakayama/2003	**81.8**	**63.3**	35.7	50.0	**79.5**	50.0	50.0	46.9	**86.4**
	JP/Iwate/2005	0.0	20.0	21.4	7.7	9.1	13.3	0.0	6.3	**54.5**
VN titer against homologous strain		2.2	3.0	2.8	2.6	2.2	3.0	3.6	3.2	2.2

a) r value=(VN titer against field isolate/ VN titer against homologous vaccine strain) × 100 (%).
b) All r values above 50 are shown in boldface.
c) Not tested.

sified as JP-I, six strains reacted well (r>50) with the immune serum of GN or C78 (homologous genotype). For JP/Ibaraki/2003, JP/Yamanashi/95 and JP/Iwate-3/2004, high r-values (r>50) were also recognized in some of the immune sera of different genotype vaccines, such as JP-I type, Mass type, ON, L2 and 4/91. GN and C78 were the same genotype, but the reactivity of each immune serum against field isolates such as JP/Yamanashi/95, JP/Chiba/

Table 4. Relationship between amino acid similarities and r-values in the VN test

Amino acid similarity (%)	Number of samples	Positive samples (r>50)	Positive correlation (% of positive sample)
100–90	15	12	80.0[a)]
80–89	12	5	41.7[b)]
70–79	77	20	26.0[c)]
<70	58	11	19.0[c)]
Total	162	48	29.6[c)]
100–80	27	17	63.0[d)]
<80	135	31	23.0[e)]

a)-e) Different superscripts indicate significant differencees: a>b ($p<0.05$), a>c ($p<0.01$), d>e ($p<0.01$).

98, JP/Toyama/2000 and JP/Ibaraki/2003 did not correlate well with other immune sera.

All field isolates in JP-II showed high reaction (r>50) with TM-86w or Miyazaki type JP-II immune serum. JP/Miyazaki/89 and JP/Shiga/2005 reacted with not only the immune serum of JP-II, but also that of the JP-I or Mass type.

For two isolates of 793B, the r values for immune serum of the 4/91 vaccine were highest. JP/Wakayama/2003 also reacted with immune sera of the JP-I and Mass type vaccines.

There was no vaccine strain for JP-III. However, some field isolates in JP-III were neutralized with immune sera of different genotypes such as JP/Shimane/2002 to GN, C78, Miyazaki, H120, Kita-1 and 4/91, JP/Fukui/2000 to H120 and JP/Iwate-1/2004 to GN.

The relationships of amino acid similarity and r values in the VN tests are shown in Table 4. An r value above 50 was considered to be positive, and the positive correlation (percentage of the positive [r>50] samples) was compared for the respective amino acid similarity to examine the correlation between amino-acid similarity and the results of the VN tests. The positive rate increased in the groups with high amino acid similarity. The positive rate of the group with the highest similarity (≥90) was 80%, and this was significantly different compared with the other groups (Fisher's exact test, $p<0.05$ or $p<0.01$). The percentages of the groups with amino acid similarity above 80% were significantly higher ($p<0.01$) than those of the groups with less than 80% identity.

DISCUSSION

The S1 glycoprotein is the major inducer of virus-neutralizing antibody and protection, and serotypic differences between IBV strains are associated primarily with sequences of the S1 glycoprotein [3, 13, 22]. Based on S1 analysis, Japanese field isolates have been classified into mainly three genotypes (JP-I, JP-II and JP-III) involving the indigenous genotype (JP-I) [16, 20]. In Japan, several original vaccine strains derived from IBV outbreaks in Japan have been developed, but there is little information about relationships between genotype and serotype for Japanese vaccines. Hence, in the present study, sequences of the S1 parts of live vaccine strains used in Japan were compared with those of IBV field isolates in Japan.

IBV readily undergoes mutation in chickens, resulting in the emergence of new variants. Therefore, investigation of the characteristics of the field strains is important. In a previous study, we identified strains of the 793B genotype, which are the same as the 4/91 vaccine strain, as a new variant in Japan [20]. In Japan, field isolates of 793B had not been isolated until 2003, but IBVs of 793B have been widely isolated in several European countries since 1991 [4, 8, 23].

In a study on Taiwan isolates, field isolates were grouped into two genotypes by gene analysis of the HVR, and it was confirmed that the genotype correlates with serotype results [22]. Liu *et al.* [15] and Gelb *et al.* [7] reported the relationship between genotype and efficacy of a vaccine against field isolates and showed that vaccines with high S1 identity values are protective against field isolates.

Our VN test results showed that field isolates of the JP-I, JP-II and 793B genotypes reacted well with immune serum of the homologous genotype. The positive rates of the VN test correlated with amino acid similarity. Amino acid similarity among homologous genotype was more than 80%, but high reactivity in the VN test was observed mainly in strains with more than 90% similarity.

The reactivity to vaccine antisera by field isolates was variable. Vaccine strains of C78 and GN were classified into the same genotype (JP-I), but their r values against some field isolates were slightly different. Amino acid similarity among strains of JP-I was above 80%, which was lower than that among strains of JP-II (above 94%), and the range of field isolates of JP-I was broad compared with that of JP-II. The genetic diversity within JP-I may have influenced the reactivity of the immune serum of both the C78 and GN vaccines against field isolates in the VN test. Further investigation might be needed to clarify the serological diversity in IBVs of JP-I.

In the results of the VN test with sera of heterologous genotype vaccines, 23% were positive. For the isolates of the JP-III genotype, there was no vaccine strain of the same genotype, but heterologous cross-reaction with immune serum of different genotypes has been recognized for field

isolates in JP-III. However, the reactivity to immune sera differed according to the virus, and it was hard to predict the reactivity to immune serum of a heterologous genotype. A VN test with immune sera would be required to confirm the efficacy of vaccines, especially in heterologous genotypes. Immunization with two kinds of vaccine strains could provided cross-protection against challenge with IBV field strains [5]. Therefore, combination of plural vaccines could yield improved performance to induce heterologous cross-protection.

The reactivity of IBVs to immune serum varied; for example, JP/Ibaraki/2003 and JP/Iwate-3/2004 reacted well with various immune serum of heterologous genotype vaccine in addition to the homologous genotype vaccine, but JP/Nara/2005 showed low reactivity with all antiserum. The cause of the varied serological reactivity is unclear, but it is supposed that there is factor influencing virus neutralization in addition to a genotype based on the S1 gene.

In the reports of Lee *et al.* [14], field isolates in the United States showed the highest protection rate with antisera prepared with reference IBV of the same genotype. Our results for Japanese field isolates of IBV agree with their report and showed good correlation between serological properties and genotype classification. Therefore, it may be assumed that a particular genotype vaccine will be effective against homologous genotype field isolates. The present study suggests that genotyping is a useful method for prediction of an efficient vaccine against field isolates in Japan.

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