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NOTE

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Detection and isolation of QX-like infectious bronchitis virus in Japan

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ABSTRACT. The antigenic variant QX-like infectious bronchitis virus (IBV) is endemic in several countries. In Japan, the QX-like genotype is classified as the JP-III genotype based on the partial S1 gene and as the GI-19 genotype based on the complete S1 gene. This study showed that QX-like IBVs and JP-III IBVs can be identified based on the amino acid polymorphism of the S1 glycoprotein. Furthermore, genetic analysis of several IBV field strains detected in commercial broiler farms across the Kyushu area in 2020 revealed Japanese QX-like IBVs, which are highly homologous to the QX-like IBVs recently detected in China and South Korea. Herein, QX-like IBV field strains were isolated for evaluating commercial vaccine efficacy in our future studies.

KEYWORDS: genotype, infectious bronchitis virus, Japan, QX-like

The QX-like infectious bronchitis virus (IBV) was first isolated from diseased chickens showing proventriculitis in China in 1996 and currently represents the predominant IBV genotype in China [29]. QX-like IBVs have now been reported worldwide, including in Europe, Asia, Africa and the Middle East, and have been detected in cases of infectious bronchitis (IB)-associated laying abnormalities, false layer and nephritis [6, 8, 12, 15, 23, 26]. To date, many QX-like IBV strains have emerged, posing a major threat to the global poultry industry.

IBV, the causative agent of IB, is a positive-sense single-stranded RNA virus with an envelope of 100–150 nm in diameter; it belongs to the family *Coronaviridae*, subfamily Coronavirinae and genus *Gammacoronavirus* of the order Nidovirales [11]. Although the genome of coronaviruses is the largest among RNA viruses, the genome length of IBV is reported to be approximately 27.6 kb [11]. Genomic RNA of IBV encodes four structural proteins, including the spike (S), envelope, membrane, nucleoprotein, and six non-structural proteins, including replicase 1a and 1b and proteins 3a, 3b, 5a, and 5b of unknown function. The genome contains ten open reading frames (ORFs) in the following order: 5′-replicase (1a/1b)-S-ORF3a-ORF3b-E-M-ORF5a-ORF5b-N-3′ [2]. The S glycoprotein of IBV is approximately 175–200 kDa in size and consists of 1,431 amino acid residues (aa); it forms a large trimeric spike-like peplomer on the surface of the viral envelope. The S glycoprotein is formed by post-translational cleavage into S1 (92 kDa) and S2 (84 kDa) subunits by cellular proteases [4]. The S1 glycoprotein determines antigenicity and tissue tropism of IBVs, playing a vital role in the induction of neutralising antibodies and attachment to the host cell receptors [24, 28].

The S glycoprotein of IBV is associated with the serotype [10, 14]. Diversity of IBV serotypes exists due to point mutations and recombination of the S1 gene [11, 27]. Although live and inactivated vaccines have been used to prevent IB, vaccine efficacy is reduced when the field strain and the vaccine strain serotypes do not match [7, 9]. Therefore, when novel antigenic variants emerged, corresponding vaccines were developed. Since field strains of diverse serotypes exist in the field, it is important for poultry farms to establish a vaccine programme that is effective against them [22].

Antigenicity-based classification methods for IBVs include serotyping and genotyping [11]. Serotyping classifies the antigenicity of IBVs based on neutralisation testing using antisera; however, this method is laborious and time-consuming. In contrast, genotyping based on the S1 gene is a relatively simpler method [16]. As per the genotyping results of the S1 gene, there are three hypervariable regions (HVRs) in the S1 gene of IBV, corresponding to aa 38–67 (HVR-1), 91–141 (HVR-2) and 274–387 (HVR-3) [3, 21]. In the IBV classification method, using the complete S1 gene sequence proposed by Valastro *et al.*, IBV strains were defined by 6 genotypes that together comprise 32 distinct viral lineages (GI-1 to GI-27 and GII to GVI), and QX-like IBVs were classified as GI-19 [27].

In Japan, genotypes of IBV strains are classified based on the partial nucleotide sequence (nt 1–621) of the S1 gene, including the HVR-1 and 2 regions, into seven genotypes, JP-I, JP-II, JP-III, JP-IV, Mass, 4/91 and Gray, which correspond practically to GI-18,

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GI-7, GI-19, GVI-1, GI-1, GI-13 and GI-3 in classification by Valastro *et al*. based on the complete S1 gene [18–20]. Classification of the partial S1 genotypes has been used for vaccine selection and field strain characterisation [25].

The Serotypes and the partial S1 genotypes of IBV have a tendency to match, but serological diversity has been identified among IBVs of the same partial S1 genotype [1, 25]. Both foreign QX-like IBVs and Japanese JP-III IBVs are classified as GI-19, suggesting relatively high homology of the S1 gene. However, there are no reports evaluating the antigenic differences between them. Since the protective effect of commercially available JP-III IBV vaccines against QX-like IBVs is unknown, we considered it necessary to identify these and evaluate the efficacy of commercially available JP-III IBV vaccines in preventing and controlling QX-like IBVs. However, no identification method has been established for foreign QX-like IBVs and Japanese JP-III IBVs, and it is unknown whether foreign QX-like IBVs have already been introduced into Japan. The findings on the pathogenicity of QX-like IBV and the efficacy of IB vaccines against QX-like IBVs will become important information for IB control in Japan. Therefore, this study aimed to identify QX-like IBVs from JP-III IBVs and survey the prevalence of QX-like IBVs in Japan and viral isolation of QX-like IBVs for future investigation.

First, to identify QX-like IBVs and Japanese JP-III IBVs, we used twenty-eight foreign QX-like IBV field strains from GenBank as reference data for the S1 gene; these included 22 strains (and their sequence data) detected in China and South Korea between 1996 and 2020 and 6 strains from outside these countries. Sequence data of strains outside these countries included those from QX-like IBVs detected in Europe and the Middle East. Seventeen Japanese JP-III IBV field strains from GenBank were used as reference data for the S1 gene. These were detected in Japan between 1998 and 2015. Accession numbers of the reference data are shown in Table 1. The obtained nucleotide sequences (nt 1–621) from the S1 gene and the deduced amino acid sequences were aligned and compared using Genetyx ver. 14 (Genetyx Corp., Tokyo, Japan). Comparison of the amino acid polymorphisms (aa 1–207) in S1 glycoprotein of foreign QX-like IBV (28 strains) with those of Japanese JP-III IBV (17 strains) suggested that QX-like IBV and JP-III IBV can be identified based on the three positions of amino acid residues as shown in Tables 1 and 2. The three positions were as follows: (1) at 21 aa, all QX-like IBVs contained phenylalanine (F), while JP-III IBVs contained valine (V) or tyrosine (Y). (2) At 128 aa, all QX-like IBVs contained arginine (R), while all JP-III IBVs contained glutamine (Q). (3) At 199 aa, all QX-like IBVs contained serine (S), while all JP-III IBVs contained asparagine (N), except for JP/Shimane/98 strain, which contained lysine (K). The results indicated that QX-like IBV could be identified by defining 21 aa as F, 128 aa as R, and 199 aa as S, whereas the JP-III IBV can be identified by defining 21 aa as V or Y, 128 aa as Q and 199 aa as K or N.

Besides (1)–(3), the following amino acid trends designated as (4)–(7) were also identified between QX-like IBV and JP-III IBV, as shown in Tables 1 and 2, with some exceptions. (4) At 127 aa, most Chinese and South Korean QX-like IBVs had alanine (A), and only two had prolines (P). Most European QX-like IBVs had proline (P), and only one strain had alanine (A), whereas all JP-III IBVs had proline (P), and only one had lysine (K). (5) At 129 aa, most QX-like IBVs had aspartic acid (D) and two strains had glycine (G); most JP-III IBVs had asparagine (N), and one had glycine (G). (6) At 151 aa, all QX-like IBVs had lysine (K), while eleven JP-III IBVs had asparagine (N), and six had lysine (K). (7) At 200 aa, QX-like IBVs had equally mixed valine (V) and isoleucine (I), whereas all JP-III IBVs had isoleucine (I).

The sequence data were analysed, aligned and assembled using Genetyx version 14 (Genetyx Corporation, Tokyo, Japan). A phylogenetic tree was constructed using MEGA-X (Version 10.2.5) via the neighbour-joining method; distance calculations were performed using the Kimura two-parameter model, and the bootstrap value was 1,000. A phylogenetic tree was constructed using a portion of the S1 gene (nt 1–621). Classification of the genotypes was based on previous reports [18] and our present study. In addition to those of QX-like IBVs and JP-III IBVs shown in Table 1, the S1 gene sequences of several field and vaccine strains of IBV, obtained from GenBank, were used for the phylogenetic tree. Phylogenetic tree analysis showed that all foreign QX-like IBVs were included in the JP-III group according to the classification by Mase *et al*. (Fig. 1A). Foreign QX-like IBVs and Japanese JP-III IBVs were divided into different groups in this study (Fig. 1B), consistent with the different amino acid polymorphism patterns of the S1 glycoprotein.

This identification method was used for the surveillance of QX-like IBV genes from commercial broiler farms in the Kyushu area. Since IBV is excreted in faeces for several weeks after infection, faecal screening is useful for rapid virus identification during IBV monitoring [5]. Field samples (n=104 faecal specimens) were examined from broiler farms in three prefectures A, B and C, in the Kyushu area, surveyed between January 2020 and February 2020. Prefecture B was divided into two geographically distant regions, V and W. Prefecture C was divided into three geographically distant regions X, Y and Z. Faecal sample collection was performed according to previously reported methods [13]. Briefly, ten faecal samples were collected per farm and pooled into a single specimen. Faecal specimens were diluted 10-fold (v/v) with phosphate-buffered saline (−) and centrifuged at 500 × *g* for 10 min.

Viral RNA was extracted from field samples obtained from broiler farms using the QIAamp Viral RNA mini-Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All extracted viral RNA was stored at −30°C until use. RT-PCR was performed using PrimeScript One Step RT-PCR Kit Ver.2 (Takara Bio, Kusatsu, Japan) with a primer set that amplifies the partial S1 gene of IBV [20]. Total volume was 25 µL, including 12.5 µL of 2 × 1-step buffer, 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM), 1 µL of PrimeScript 1 step Enzyme Mix, 5 µL of template RNA, and 4.5 µL of RNase-free water. The reaction mixture was incubated at 37°C for 30 min and at 94°C for 1 min. PCR reaction was performed as follows: 35 cycles of denaturation (94°C, 30 sec), annealing (50°C, 30 sec), and extension (72°C, 1 min), followed by the elongation step (72°C, 7 min). Products of PCR reaction corresponding to the predicted size of the target gene (approximately 670 bp) were isolated from 1.5% agarose gels and purified using QIAquick PCR Purification Kit (Qiagen). Purified products were sequenced by Fasmac Co., Ltd. (Atsugi, Japan).

QX-like IBV genes were detected in field samples from 16 of 104 broiler farms, including 5 farms in prefecture B and 11 farms in prefecture C in the Kyushu area (Table 3). A high proportion of QX-like IBV genes was detected in region V of prefecture B (4 of 14 farms) and region Z of prefecture C (8 of 25 farms). We deposited partial S1 gene sequences for all 16 Japanese QX-like IBV field

Table 1. Amino acid polymorphisms in S1 glycoproteins of QX-like infectious bronchitis virus (IBVs) and JP-III IBVs and nt identity to Japanese QX-like IBVs

IBV field strains	Origin	Year of isolation	Accession number	Genetic groups based on this study	Position of amino acids in the S1 glycoprotein[a]							nt identity (%)[b]	
					21	127	128	129	151	199	200	JP/ZK-B7/2020	JP/ZK-B22/2020
QX IBV	China	1996	AF193423	QX-like	**F**	**A**	**R**	**D**	**K**	**S**	**I**	96.9	97.1
CK/CH/LLN/98I	China	1998	DQ167145	QX-like	-	P	-	G	-	-	V	93.9	93.8
LX4	China	1999	AY338732	QX-like	-	-	-	G	-	-	-	94.1	93.9
A2	China	2000	AY043312	QX-like	-	-	-	-	-	-	-	96.9	97.1
CK/CH/LHLJ/05I	China	2005	EF213560	QX-like	-	-	-	-	-	-	V	93.9	93.8
HBN	China	2005	DQ070837	QX-like	-	-	-	-	-	-	V	93.9	93.8
NN07	China	2007	GQ265946	QX-like	-	-	-	-	-	-	V	95.5	95.3
SDZB0804	China	2008	FJ210647	QX-like	-	-	-	-	-	-	V	97.4	97.6
CK/CH/LSD/08-12	China	2008	GQ258327	QX-like	-	-	-	-	-	-	-	93.6	93.4
HB08	China	2008	GQ265934	QX-like	-	-	-	-	-	-	V	97.9	97.9
CK/CH/GD/LZ09	China	2009	HQ018896	QX-like	-	-	-	-	-	-	-	94.2	94.1
GX-NN-6	China	2011	JX291985	QX-like	-	-	-	-	-	-	V	96.1	96.0
CK/CH/ZJ/QZ1301-2	China	2014	KX107847	QX-like	-	-	-	-	-	-	V	97.7	97.6
CK/CH/SD/TA1502-1	China	2015	KX107834	QX-like	-	-	-	-	-	-	V	96.1	96.3
gammaCoV/ck/China/I1116/16	China	2016	MH427450	QX-like	-	-	-	-	-	-	V	99.3	99.2
gammaCoV/ck/China/I0614/17	China	2017	MH427418	QX-like	-	-	-	-	-	-	V	99.3	99.3
CK/CH/LN/0711/2018	China	2018	MN128008	QX-like	-	-	-	-	-	-	V	99.0	98.8
CK/CH/YNKM/191128	China	2019	MW560639	QX-like	-	-	-	-	-	-	V	96.1	96.0
CK/CH/GZGA/201127	China	2020	MW560649	QX-like	-	-	-	-	-	-	V	96.5	96.3
K154/05	South Korea	2005	FJ807922	QX-like	-	P	-	-	-	-	-	96.0	96.1
R18/27K	South Korea	2018	MK510437	QX-like	-	-	-	-	-	-	V	99.3	99.1
IBV/Korea/151/2019	South Korea	2019	MW877636	QX-like	-	-	-	-	-	-	V	99.5	99.3
L-1148	Netherlands	2004	DQ431199	QX-like	-	P	-	-	-	-	-	93.3	93.1
NL/L-1449K/04	Netherlands	2004	EF079115	QX-like	-	P	-	-	-	-	-	94.2	94.1
FR/L-1450L/05	France	2005	EF079117	QX-like	-	P	-	-	-	-	-	94.1	94.2
IBV/Ck/SP/79/08	Spain	2008	GQ253484	QX-like	-	P	-	-	-	-	-	94.1	93.9
CK/SWE/0658946/10	Sweden	2010	JQ088078	QX-like	-	-	-	-	-	-	V	93.5	93.3
IBV/Chicken/Iran/QX-like/MRB03/2015	Iran	2015	MG013974	QX-like	-	-	-	-	-	-	-	93.8	93.6
JP/ZK-B7/2020[c]	Japan	2020	LC701739	QX-like	-	-	-	-	-	-	V	100	99.8
JP/ZK-B22/2020[c]	Japan	2020	LC701742	QX-like	-	-	-	-	-	-	V	99.8	100
JP/Shimane/98	Japan	1998	AB120642	JP-III	Y	P	Q	N	N	K	-	89.6	89.5
JP/Aichi/2000	Japan	2000	AB120645	JP-III	V	P	Q	N	-	N	-	92.3	92.5
JP/Fukui/2000	Japan	2000	AB120646	JP-III	V	P	Q	N	N	N	-	92.5	92.4
JP/Shimane/2002	Japan	2002	AB363956	JP-III	Y	P	Q	G	-	N	-	92.7	92.9
JP/Iwate-1/2004	Japan	2004	AB363956	JP-III	V	P	Q	N	N	N	-	92.8	93.0
JP/Chiba/2004	Japan	2004	LC500567	JP-III	Y	P	Q	N	N	N	-	90.1	90.1
JP/Okayama-5/2004	Japan	2004	LC500571	JP-III	Y	P	Q	N	-	N	-	92.7	92.5
JP/Wakayama-13/2006	Japan	2006	LC701734	JP-III	V	P	Q	N	-	N	-	91.4	91.2
JP/Saitama/2007	Japan	2007	LC500572	JP-III	Y	K	Q	N	-	N	-	91.4	91.2
JP/Fukuoka/2010	Japan	2010	LC701736	JP-III	Y	P	Q	N	N	N	-	90.7	90.6
JP/Gunma/2011	Japan	2011	LC701733	JP-III	V	P	Q	N	-	N	-	92.8	92.6
JP/Kagawa/2011	Japan	2011	LC701735	JP-III	Y	P	Q	N	N	N	-	90.9	90.9
JP/Kagawa/2012	Japan	2012	LC701737	JP-III	Y	P	Q	N	N	N	-	89.8	90.0
JP/Gifu/2015	Japan	2015	LC662587	JP-III	V	P	Q	N	N	N	-	92.4	92.2
JP/Gunma-1/2015	Japan	2015	LC428323	JP-III	V	P	Q	N	N	N	-	91.4	91.2
JP/Niigata-3/2015	Japan	2015	LC428328	JP-III	V	P	Q	N	N	N	-	91.2	91.4
JP/Kagoshima-2/2015	Japan	2015	LC428346	JP-III	Y	P	Q	N	N	N	-	89.8	89.6

a) Amino acid positions are based on the IBV M41 strain. Amino acid pattern of QX IBV strain is shown in bold letters, and identical amino acids are indicated by the symbol "-". b) Similarity in partial S1 gene (nt 1–621). c) Japanese QX-like IBV field strains isolated in this study.

strains in GenBank (Accession No. LC701739– LC701754). Analysis of amino acids in the partial S1 glycoproteins of these Japanese QX-like IBVs revealed that the 21 aa was F, 128 aa was R, and 199 aa was S in all cases. Sixteen isolates were closely interrelated (nucleotide similarity, 97.3–100%).

Table 2. Identification of amino acids between QX-like infectious bronchitis virus (IBVs) and JP-III IBVs

Genetic groups based on Valastro *et al*. (2016)	Genetic groups based on Mase *et al*. (2004, 2021)	Genetic groups based on this study	Corresponding position of amino acids in S1 glycoprotein for identification between QX-like and JP-III in this study						
			21	127	**128**	129	151	**199**	200
GI-19	JP-III	QX-like	F	A/P	R	D/G	K	S	I/V
		JP-III	V/Y	P/K	Q	N/G	N/K	N/K	I

The three locations in bold (aa21, 128, and 199) indicate QX-like IBVs and JP-III IBVs. The other four locations (aa127, 129, 151, and 200), with some exceptions, show certain trends for QX-like IBVs and JP-III IBVs, respectively.

Genetic analysis of the complete S1 gene in 61 Japanese IBV strains has been performed by Mase *et al*. [19]. In their study, several foreign QX-like IBVs were used as reference strains for phylogenetic tree analysis and amino acid polymorphism analysis, but QX-like IBVs detected in Japan were not included. Outbreaks of QX-like IBV have been reported in neighbouring countries of Japan (e.g., China and South Korea) [12, 29]. This is the first report of the detection and isolation of QX-like IBVs that are genetically related to QX-like IBVs recently reported in neighbouring countries in Japan. Nevertheless, the introduction route of the QX-like IBV into Japan is still unknown. The Japanese QX-like IBV had possibly been introduced by migratory birds that flew to Japan via these countries.

To isolate QX-like IBVs, five 38-day-old dead broilers were obtained from one farm in each of the V and W regions of B prefecture where the QX-like IBV gene was detected. Although these farms are geographically distant from each other, the epidemiological relevance was unknown. Mild respiratory lesions (mucus retention and mucosal hyperemia) were observed in the trachea of these dead chickens, and no renal lesions were observed. Virus isolation was performed as follows. Tracheas and kidneys from dead chickens were used to prepare a 10% w/v organ homogenate in phosphate-buffered saline (−). A 10% w/v organ homogenate with QX-like IBV gene detected via RT-PCR and sequencing analysis was used for virus isolation. The supernatant of the organ homogenate, obtained after centrifugation, was filtered through a 45-nm filter and inoculated into the allantoic cavity of three 10-day-old specific-pathogen-free (SPF) embryonated eggs (0.1 mL/egg) and incubated for 48–72 hr. Eggs were cooled at 4°C, and the allantoic fluid was harvested. Three blind passages were performed, and specimens with distinct dwarfing, curling, or death of the chicken embryos were considered positive for virus isolation. RT-PCR and sequencing analysis confirmed the presence of QX-like IBV in the allantoic fluid.

We isolated two Japanese QX-like IBVs, namely JP/ZK-B7/2020 strain from V regions and JP/ZK-B22/2020 strain from W regions. In the phylogenetic tree analysis in Fig. 1, these Japanese QX-like IBVs were included in the group of foreign QX-like IBVs. These Japanese QX-like IBVs were a genetically related group to the QX-like IBVs detected in China in 2016–2018 and Korea in 2018–2019 (Supplementary Fig. 1). Sequence similarity of the partial S1 gene (nt 1–621) of these QX-like IBVs was 99.8%. Japanese QX-like IBVs showed higher sequence similarity to foreign QX-like IBVs (93.3–99.5%) than to Japanese JP-III IBVs (89.5–93.0%), especially to QX-like IBVs recently detected in China or South Korea between 2016 and 2020 (Table 1).

The S1 gene of IBV is prone to mutation (e.g., insertions, deletions, and substitutions), and the occurrence of recombinants among IBV field strains has also been reported [27]. In Japan, analysis of the complete S1 gene of JP-III IBV field strains has revealed several recombinant viruses of the GI-19 and GI-13 genotypes [19]. Because of genomic mutation or recombination of the S1 gene, novel IBVs may emerge in the future to which the identification of QX-like IBVs and JP-III IBVs presented in this study is not applicable.

S1 genotyping of IBV based on partial S1 gene sequences (nt 1–621) is frequently performed in Japan. Several S1 genotyping surveys of IBV field strains have been recently performed in Japan, which reported JP-I, JP-II, and JP-III genotypes as relatively predominant [22]. We have shown that QX-like IBVs and JP-III IBVs could be identified based on the amino acid polymorphism of the S1 gene region obtained with the same primers as the S1 genotype. This makes it possible to verify whether QX-like IBV is included among the IBVs that have been determined to be JP-III IBVs in previous studies.

The diverse antigenicity of IBV is due to the diversity associated with genetic variation in the S glycoprotein, which has a neutralising active region [14]. While a small mutation (<2%) in the S1 gene can cause a major change in the conformation of S glycoprotein and alter the neutralising reactivity of the virus [17], the amino acid change associated with the gene mutation might not significantly affect the neutralising reactivity if it occurred at a site unrelated to the neutralising activity of the S glycoprotein. Therefore, the S1 genotype and serotype may not always match. It is unclear whether the changes in the amino acid residues between the QX-like IBVs and JP-III IBVs cause changes in the antigenicity of IBV. Therefore, evaluation in this regard via neutralisation tests and experimental animal studies is warranted.

The commercial broiler farms where QX-like IBV genes were detected in this study did not show significantly reduced productivity, such as stunting or increased mortality, suggesting that the detected QX-like IBV strains may not be highly pathogenic or that the current vaccination programmes may be protective against the QX-like IBVs. A high proportion of QX-like IBV genes was detected in regions V and Z of prefectures B and C, respectively, suggesting that QX-like IBVs are prevalent in these regions. The prevalence of QX-like IBVs should be monitored carefully since mutations in QX-like IBVs may result in the emergence of highly virulent strains during repeated outbreaks in this region.

A correlation has been reported between the S1 genotype and serotype of IBVs [1, 25]. Based on the relatively high nucleotide sequence similarity of the partial S1 gene between QX-like IBVs and JP-III IBVs, the JP-III IBV live vaccine strain may elicit cross-protection against QX-like IBV strains; however, no study has evaluated the cross-neutralisation ability. All surveyed commercial broiler farms in which QX-like IBV genes were detected did not implement the JP-III IBV live vaccine. Additional animal studies using SPF chickens should be performed to clarify the cross-protection effect between Japanese QX-like IBVs and various genotypes

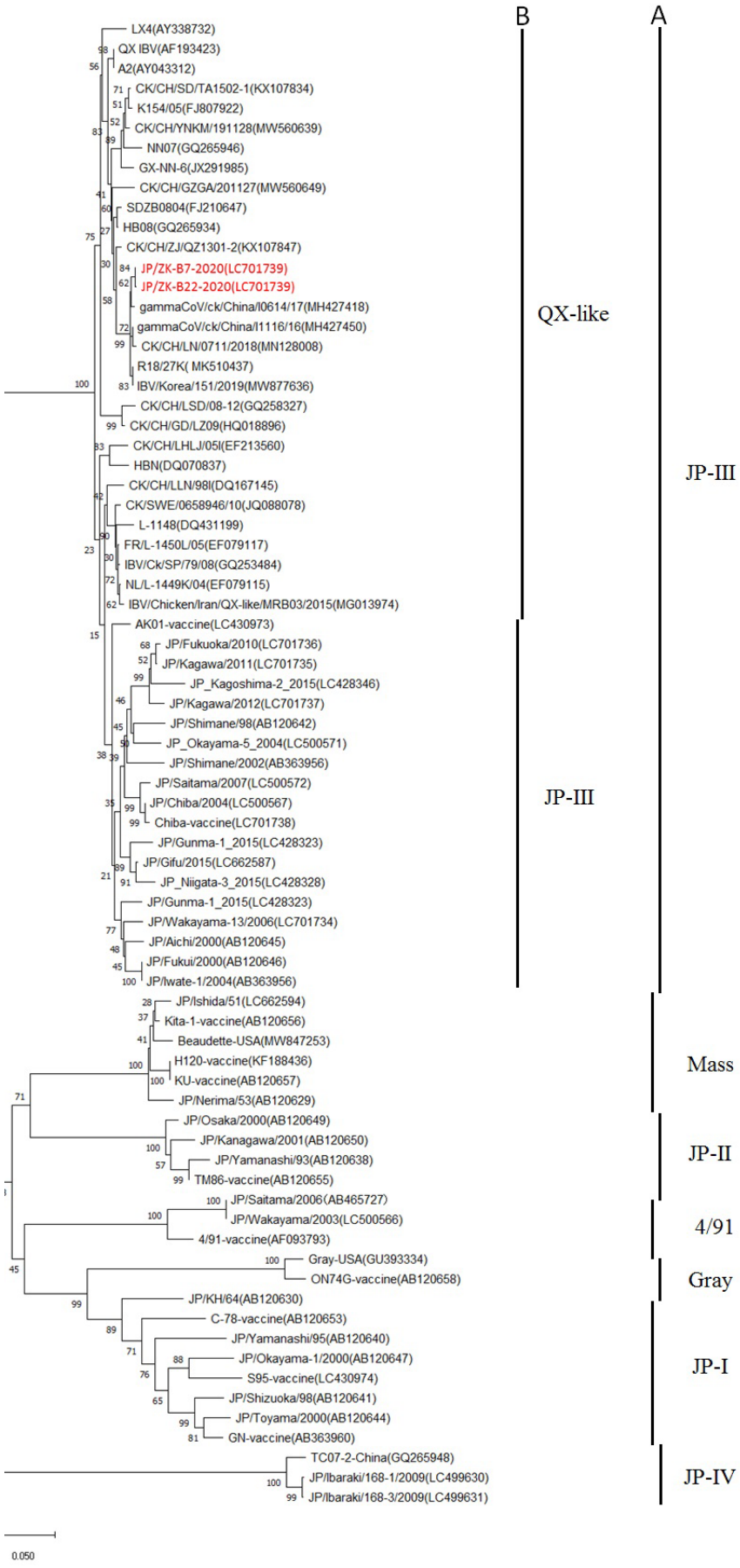

Fig. 1. Phylogenetic tree based on the sequence of partial S1 gene of Infectious bronchitis virus (IBV) (nt 1–621). Phylogenetic trees were generated with MEGA-X (Version 10.2.5) using the neighbour-joining method, distance calculations were performed using the Kimura two-parameter model, and the bootstrap value was 1,000. The Japanese QX-like IBVs isolated in this study showed in red. The sequences retrieved from GenBank are shown in black, and the accession number in GenBank is mentioned after the IBV strain name. A: Genetic groups based on Mase *et al.* (2004, 2021). B: Genetic groups based on this study.

Table 3. Infectious bronchitis virus (IBV) surveillance of commercial broiler farms in Kyushu Area, Japan

Prefecture	Region	Number of surveyed farms	Number of farms where IBV genes were detected	Number of vaccine strains	Number of field strains	Genetic groups of field strain							
						JP-I[a)]	JP-II[a)]	JP-III[a)]		JP-IV[a)]	Mass[a)]	4/91[a)]	Gray[a)]
								JP-III[b)]	QX-like[b)]				
A		10	10	8	2	2	0	0	0	0	0	0	0
B	V	14	14	5	9	0	0	0	4	0	5	0	0
	W	16	15	4	11	0	0	0	1	0	10	0	0
C	X	18	15	12	3	0	0	0	3	0	0	0	0
	Y	21	14	14	0	0	0	0	0	0	0	0	0
	Z	25	15	0	15	6	0	1	8	0	0	0	0

a) Genetic groups based on Mase *et al*. (2004, 2021) [18, 20]. b) Genetic groups based on this study.

of IBV live vaccine strains available in Japan. The data would help develop countermeasures for IB in poultry farms across Japan. In future research, we intend to evaluate the pathogenicity of the Japanese QX-like IBVs isolated in this study and their efficacy against Japanese QX-like IBV using several live vaccines commercially available in Japan.

CONFLICT OF INTEREST. No potential conflict of interest was reported by the authors.

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