日本の伝染性ファブリキウス嚢病(IBD)ワクチン未接種鶏におけるワクチン様IBDウイルスの検出

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Detection of Vaccine-Like Infectious Bursal Disease (IBD) Virus in IBD Vaccine-Free Chickens in Japan

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ABSTRACT. The prevalence of infectious bursal disease virus (IBDV) was studied in chickens, which had not been vaccinated against IBD. Fifty sera and forty-six bursae of Fabricius from chickens showing impaired growth, collected from 7 IBD vaccination-free farms in Japan were used for virus neutralization (VN) tests and RT-PCR for detection of IBDV genome corresponding to the VP2 hypervariable region. Of the fifty sera, 39 sera (78%) from 6 farms were VN antibodies positive. Of the forty-six bursae, 37 bursae (80.4%) from 6 farms were positive in the RT-PCR assay. The sequences of all the RT-PCR products detected in this study were closely related or identical to those of the vaccine strains. These results show that vaccine-like IBDV is prevalent even in IBD vaccine-free chicken farms in Japan.

KEY WORDS: chickens, IBDV, vaccine.

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Infectious bursal disease virus (IBDV) is a member of the family *Birnaviridae*, genus *Avibirnavirus* [12]. The viral genome consists of two segments of double stranded RNA, called segments A and B. The IBDV capsid protein VP2 is known as the major host immunogenic component of IBDV and encoded by segment A as a precursor polyprotein, N-VP2-VP4-VP3-C [5]. The amino acid sequence ranging from positions 206 to 350 in VP2 represents a major epitope for virus neutralization. Furthermore, amino acid sequence variations among IBDV strains were found in this region. Therefore, this region is named the VP2 hypervariable region [13]. The VP2 hypervariable region nucleotide sequence has frequently been used for molecular epidemiological studies and genotypic characterization of IBDV [3, 16, 22].

Two serotypes of IBDV (1 and 2) are recognized based on a cross-virus neutralization test [8]. Serotype 1 IBDV infects young chickens and induces immunosuppression by destruction of lymphocytes in the bursa of Fabricius [6], whereas serotype 2 strains are naturally avirulent for chickens [7]. Until the 1980s, prevention of the disease was achieved mainly by using attenuated live vaccines. However, at the end of the 1980s, a very virulent IBDV (vvIBDV) emerged in Europe [4, 17] and later in the early 1990s in Japan [19]. After the emergence of vvIBDV in vaccinated flocks [14], intermediate type vaccines have been introduced and widely used in Japan [15]. After introduction of such intermediate type vaccines, severe outbreaks of vvIBD have subsided. However, extensive usage of live vaccines in the field complicates the epidemiological situation of IBDV, because many vaccine-like viruses were detected in Japan as reported previously [21]. Recently, the numbers of IBDV vaccination-free poultry farms in Japan has increased due to the occurrence of fewer outbreaks and the increasing demand for drug-free food products. In the present study, we examined chickens that had not been immunized with IBD vaccine, in order to define the prevalence of IBDV field strains other than vaccine or vaccine-like viruses and to better understand the epidemiological situation of IBD in Japan.

Forty-three 52- to 57-day-old broiler chickens (Cobb and Chunky) and seven 99-day-old indigenous chickens that had impaired growth rate were randomly collected from 7 farms (named as A to G), in Gifu and Shizuoka Prefectures from 2003 to 2004 (Table 1). None of the chickens had been immunized with IBD vaccine, but they had been vaccinated against Marek's disease, Newcastle disease, and pox vaccines. Serum samples and bursal tissues were collected from each chicken for virus neutralization test and RT-PCR for detection the IBDV genome in the bursa.

Serum samples used for virus neutralization (VN) test were prepared as described previously [19]. VN was tested using serotype 1 IBDV GBF1-E strain as described previously [19]. Because there are very weak or no cross-reactions between serotypes 1 and 2 strains in the VN test [8, 9], it is expected that the test detects the antibodies against serotype 1 IBDV. Prior to the test, serum samples were heat inactivated for 30 min at 56°C, serially diluted in 96-well plates and incubated with 100 $TCID_{50}$/well of GBF1-E for 1 hr. Subsequently, CEF cells adjusted to 5×10^5 cells/m*l* was added and cultured at 37°C in 5% CO_2. Cytopathic effect was monitored for 7 days. VN titer was expressed as the reciprocal of highest dilution that completely inhibited CPE. VN titers of 80 or higher were considered as VN antibody-positive [11].

For RT-PCR, total RNA was extracted from bursal tissue

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Table 1. Distribution of virus neutralization (VN) titers and results of RT-PCR for VP2 hypervariable domain of IBDV in IBD vaccine-free chickens

Farm (Prefectures)	Prefectures	Breed	Age (days)	No. tested	VN titers (RT-PCR positive no)							VN-antibody positive no. / tested no. (%)	RT-PCR positive no./ tested no. (%)
					≤40	160	640	1,280	2,560	5,120	≥10,240		
A	Gifu	Cobb	56	9					1 (1)	3 (3)	5 (5)	9/9 (100)	9/9 (100)
B	Gifu	Cobb	55	10			2 (2)	3 (3)	3 (3)		2 (2)	10/10 (100)	10/10 (100)
C	Gifu	Cobb	53	5					3 (3)		2 (0)	5/5 (100)	3/5 (60)
D	Gifu	Cobb	57	6	4 (0)			1 (1)		1 (1)		2/6 (33)	2/6 (33)
E	Shizuoka	Chunky	53	7	1 (1)	1 (1)	1 (1)	2 (2)		2 (2)		6/7 (85)	7/7 (100)
F	Gifu	Chunky	52	6	6 (0)							0/6 (0)	0/2 (0)
G	Gifu	Indigenous	99	7				1 (1)		2 (2)	4 (3)	7/7 (100)	6/7 (85)
Total (7)				50	11 (1)	1 (1)	3 (3)	7 (7)	7 (7)	8 (8)	13 (10)	39/50 (78%)	37/46 (80.4%)

with TRIZOL Reagent (Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer's instructions. The extracted RNA was kept at –30°C until used for RT-PCR. Forward and reverse primers, V1 (5'-CCA GAG TCT ACA CCA TAA-3') and V2 (5'-CCT GTT GCC ACT CTT TCG TA-'3), designed for the VP2 hypervariable region of serotype 1 [20] were used. These primers amplify a 472-bp fragment from position 739 to 1,210, according to the nucleotide number reported by Bayliss *et al.* [2]. cDNA was synthesized at 42°C for 30 min by using one-step Ready-to-Go RT-PCR beads (Amersham, Piscataway, NJ, U.S.A.) after denaturation of RNA mixed with dimethyl- sulfoxide and incubated at 97°C for 5 min. Subsequently, PCR cycle including 40 cycles of 94°C for 30 sec, 57°C for 30 sec and 72°C for 40 sec, and final extension cycle for 5 min at 72°C was conducted. RT-PCR products were subjected to electrophoresis on 1.2% agarose gel and visualized under UV light after staining with ethidium bromide.

Nucleotide sequences of the RT-PCR products were determined after cloning in the pGEM-T vector (Promega, Madison, WI, U.S.A.). The nucleotide sequence was determined by using DYEnamic ET dye terminator sequencing kit (Amersham Biosciences Co., Piscataway NJ, U.S.A.). At least three independent clones were sequenced, and the consensus sequence was determined. The nucleotide sequence analysis was analyzed with the aid of the GENETYX-MAC/Version 12.2.5 program (GENETYX-MAC Co., Tokyo, Japan). Submission of the nucleotide sequence to define identity to the reported sequence of IBDV was obtained by the aid of BLAST search.

The seroprevalence of IBD in IBD vaccine-free farms was examined by VN test using serotype 1 IBDV. Of the fifty samples from 7 farms, 39 chicken sera derived from 6 farms (78%) were positive in VN test. All of the chicken farms except those from farm F were VN antibody positive. VN titers ranged from <40 to >10,240 and were present in most of the chickens except at farms D and F (Table 1).

Thirty-nine VN antibody positive chickens from farms A, B, C, D, E and G, and 7 VN antibody negative chickens from farms D, E and F were examined for IBDV RNA by RT-PCR (Table. 1). Of the thirty-nine VN antibody-positive chickens, 36 samples were also positive in RT-PCR. On the other hand, of the 7 VN antibody-negative samples, only one sample derived from farm E was positive in RT-PCR. Thirty-seven samples (80.4%) were positive for the RT-PCR in total. Both the VN antibody and RT-PCR negative samples were detected only in farms D and F. None of the samples from farm F were positive in either of the tests. Most of the VN antibody-positive chickens were also positive in RT-PCR. These chickens may be persistently infected with IBDV as reported previously [1]. A cross VNT using serotype 2 was not done. The finding that in RT-PCR, the primer pair V1-V2 amplifies serotype 1 but not serotype 2 suggests that detected RT-PCR products belong to serotype1. The results obtained by VN test and RT-PCR indicate that most of the chickens in this study that were not vaccinated against IBD were infected with IBDV.

The genetic relatedness among the amplified RT-PCR products was determined after sequence readings. Of five representative RT-PCR products detected from broiler chickens from farms A, B, C, D and E, respectively, those from farms B, C, D and E were 100% identical. The sequence from farm A differed from the others by only one nucleotide, which was a silent substitution. However, all of the deduced amino acid sequences showed 100% identity each other. The accession numbers of representative RT-PCR products A, B, C, D, E, and G are AB425321, AB425825, AB425826, AB425827, AB425829, and AB425828, respectively. In BLAST search, the sequences from farms B, C, D, and E were found to be identical to 12 sequences including 2512 strain and derivatives (accession numbers AF279288, AF457105 and DQ355819) which are used as live vaccine in other countries. Although the live vaccine derived from 2512 strain is also used in Japan, nucleotide sequence of that vaccine was not available. So, we subsequently determined the sequence for the VP2 hypervariable region of the vaccine used in Japan. That sequence was identical to the sequence from farms B, C, D, and E.

Another RT-PCR product detected from an indigenous chicken sample from farm G showed 19 to 20 nucleotide sequence differences from the sequence detected from farms A, B, C, D and E. In a BLAST search, the sequences closest to the sequence from farm G (98.6% identity) were tissue

culture attenuated strains CT (Y14961), BJ836 (AF413069), PBG98 (D00868), and D78 (Y14962). Of these strains, D78 is used as a live vaccine in Japan. In addition, the deduced amino acids sequences of the four isolates included three amino acids (H253, N279, and T284) that are thought to be critical for tissue culture adaptation [10, 20] were found in sequence from farm G.

In this survey, most of the chickens raised under IBDV vaccine-free condition were infected with IBDV that is genetically close to vaccine strains used in Japan. This suggests that vaccine-like IBDV was prevalent even in IBD vaccine-free chicken farms in Japan. In addition, we previously detected a sequence in other layer chickens that were vaccinated with another live vaccine [21] that is identical to the sequence in broiler chickens in this study. The vaccine-like strains detected in this study may be widely distributed in Japan. The fact that these IBDVs were detected in apparently healthy but impaired growth chickens suggests that the viral infection reduced growth performance in commercial chickens. Because genetic assortment among attenuated and vvIBDVs has recently shown to have an important role in the emergence of virulent strains [18], application of strict biosecurity measures for using live vaccine and monitoring the molecular properties of field strains should be conducted for effective control of IBD.

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REFERENCES

1. Abdel-Alim, G. A. and Saif, Y. M. 2001. Detection and persistence of infectious bursal disease virus in specific-pathogen-free and commercial broiler chickens. *Avian Dis.* **45**: 646–654.
2. Bayliss, C. D., Spies, U., Shaw, K., Peters, R. W., Papageorgiou, A., Muller, H. and Boursnell, M. E. 1990. A comparison of the sequences of segment A of four infectious bursal disease virus strains and identification of a variable region in VP2. *J. Gen. Virol.* **71**: 1303–1312.
3. Brown, M. D., Green, P. and Skinner, M. A. 1994. VP2 sequences of recent European 'very virulent' isolates of infectious bursal disease virus are closely related to each other but are distinct from those of 'classical' strains. *J. Gen. Virol.* **75**: 675–680.
4. Chettle, N., Stuart, J. C. and Wyeth, P. J. 1989. Outbreak of virulent infectious bursal disease in East Anglia. *Vet. Rec.* **125**: 271–272.
5. Fahey, K. J., McWaters, P., Brown, M. A., Erny, K., Murphy, V. J. and Hewish, D. R. 1991. Virus-neutralizing and passively protective monoclonal antibodies to infectious bursal disease virus of chickens. *Avian Dis.* **35**: 365–373.
6. Hirai, K., Funakoshi, T., Nakai, T. and Shimakura, S. 1981. Sequential changes in the number of surface immunoglobulin-bearing B lymphocytes in infectious bursal disease virus-infected chickens. *Avian Dis.* **25**: 484–496.
7. Ismail, N. M., Saif, Y. M. and Moorhead, P.D. 1988. Lack of pathogenicity of five serotype 2 infectious bursal disease viruses in chickens. *Avian Dis.* **32**: 757–759.
8. Jackwood, D. J., Saif, Y. M. and Moorhead, P.D. 1985. Immunogenicity and antigenicity of infectious bursal disease virus serotypes I and II in chickens. *Avian Dis.* **29**: 1184–1194.
9. McFerran, J. B., McNulty, M. S., McKillop, E. R., Conner, T.J., McCracken, R. M., Collins, D. S. and Allan, G. M. 1980. Isolation and serological studies with infectious bursal disease viruses from fowl, turkey, and duck: Demonstration of a second serotype. *Avian Pathol.* **9**: 395–404.
10. Mundt, E. 1999. Tissue culture infectivity of different strains of infectious bursal disease virus is determined by distinct amino acids in VP2. *J. Gen. Virol.* **80**: 2067–2076.
11. Ogawa, M., Wakuda, T., Yamaguchi, T., Murata, K., Setiyono, A., Fukushi, H. and Hirai, K. 1998. Seroprevalence of infectious bursal disease virus in free-living wild birds in Japan. *J. Vet. Med. Sci.* **60**: 1277–1279.
12. Pringle, C. R. 1998. The universal system of virus taxonomy of the International Committee on Virus Taxonomy (ICTV), including new proposals ratified since publication of the Sixth ICTV Report in 1995. *Arch. Virol.* **143**: 203–210.
13. Schnitzler, D., Bernstein, F., Muller, H. and Becht, H. 1993. The genetic basis for the antigenicity of the VP2 protein of the infectious bursal disease virus. *J. Gen. Virol.* **74**: 1563–1571.
14. Tsukamoto, K., Tanimura, N., Hihara, H., Shirai, J., Imai, K., Nakamura, K. and Maeda, M. 1992. Isolation of virulent infectious bursal disease virus from field outbreaks with high mortality in Japan. *J. Vet. Med. Sci.* **54**: 153–155.
15. Tsukamoto, K., Tanimura, N., Kakita, S., Ota, K., Mase, M., Imai, K. and Hihara, H. 1995. Efficacy of three live vaccines against highly virulent infectious bursal disease virus in chickens with or without maternal antibodies. *Avian Dis.* **39**: 218–229.
16. Ture, O., Saif, Y. M. and Jackwood, D. J. 1998. Restriction fragment length polymorphism analysis of highly virulent strains of infectious bursal disease viruses from Holland, Turkey, and Taiwan. *Avian Dis.* **42**: 470–479.
17. van den Berg, T. P., Gonze, M. and Meulemans, G. 1991. Acute infectious bursal disease in poultry: isolation and characterization of a highly virulent strain. *Avian Pathol.* **20**: 133–143.
18. Wei, Y., Li, J., Zheng, J., Xu, H., Li, L. and Yu, L. 2006. Genetic reassortment of infectious bursal disease virus in nature. *Biochem. Biophys. Res. Commun.* **350**: 277–287.
19. Yamaguchi, T., Iwata, K., Kobayashi, M., Ogawa, M., Fukushi, H. and Hirai, K. 1996. Epitope mapping of capsid proteins VP2 and VP3 of infectious bursal disease virus. *Arch. Virol.* **141**: 1493–1507.
20. Yamaguchi, T., Ogawa, M., Inoshima, Y., Miyoshi, M., Fukushi, H. and Hirai, K.1996. Identification of sequence changes responsible for the attenuation of highly virulent infectious bursal disease virus. *Virology* **223**: 219–223.
21. Yamaguchi, T., Kasanga, C.J., Terasaki, K., Maw, M.T., Ohya, K. and Fukushi, H. 2007. Nucleotide sequence analysis of VP2 hypervariable domain of infectious bursal disease virus detected in Japan from 1993 to 2004. *J. Vet. Med. Sci.* **69**: 733–738.
22. Zierenberg, K., Raue, R. and Muller, H. 2001. Rapid identification of "very virulent" strains of infectious bursal disease virus by reverse transcription -polymerase chain reaction combined with restriction enzyme analysis. *Avian Pathol.* **30**: 55–62.