Antigenic and Molecular Characterization of Recent Infectious Bursal Disease Virus Isolates in China*

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Abstract. Eleven infectious bursal disease virus (IBDV) strains isolated recently from China were compared with the early classical virulent strain CJ801, the chicken embryo fibroblast-adapted (CEF) variant strain GZ902, and the attenuated vaccine strains BJ836, BK912, and LM to discern the evolutionary characteristics of IBDV in China at both antigenic and genetic levels. Virus neutralization (VN) assay showed that all ten very virulent (vv) IBDV strains belong to the same subtype as attenuated strains, whereas the other variant isolate strain BX could be attributed to other subtype of the variant strain GZ902. Antigen-capture ELISA (AC-ELISA) determined by a panel of monoclonal antibodies (Mabs) against classical and variant strains showed further that among these vv strains, nine strains except for strain NC had no reaction with neutralizing Mab B69. The vv strains SC and YV had no reaction with non-neutralizing Mabs 2B8 and 2C4, respectively, whose epitopes were located in classical IBDV strains. On the other hand, there is no alteration in antigenic epitopes located in the variant strain BX as that of the variant GZ902. Sequence comparison of the highly variable region (HVR) of the VP2 proteins showed that these vv strains had 98.6–100.0% identities to European and Asian vv strains at amino acid level. For the vv strains NC, SC, and YV, all had one amino acid substitution at the major hydrophilic domains, indicating that new vv strains are evolving. In addition, the vv strains DMS and NC had amino acid residue 279N as well, showing that the substitution of amino acid at this position might not be related to the virulence of IBDV. The variant strain BX had one amino acid substitution in the two major hydrophilic domains and two unique amino acids 249K and 254S as the other early variant strains, and shared 97.3% of amino acid identity to the variant strain VarE. Phylogenetic analysis suggests that the recent Chinese vvIBDVs and the previous European and Asian vv strains still belong to a genetic group and the variant strain BX to the other genetic group, which is more closely related to the European classical virulent strain F52/70 and the American classical virulent strain STC than to the early Chinese classical virulent strain CJ801, showing that the recent vv and variant strains that spread widely in the country might be derived from Europe and America than from early Chinese classical virulent strains.

Key words: antigenic variation, highly variable region, IBDV, sequence analysis

Introduction

Infectious bursal disease virus (IBDV) is the etiological agent of infectious bursal disease (IBD), which is a highly contagious disease in 3–15 weeks old chickens that results in significant losses to the poultry industry [1]. Infection causes destruction of lymphoid organs, especially the bursa of Fabricius [2], causing
immunosuppression and increased susceptibility to other diseases [1]. Two distinct serotypes (I and II) are recognized for IBDV. The serotype I virus strains differ markedly in virulence, whereas serotype II viruses are naturally avirulent for chickens [3]. In 1985, variant IBDV strains, which could escape from immunization of classical IBDV vaccines to chickens, emerged in the USA. These strains were of different subtypes to that of classical IBDV strains as determined by virus neutralization (VN) and monoclonal antibody (Mab)-based antigen-capture ELISA (AC-ELISA) assays [4,5]. In contrast to the USA strains, very virulent (vv) strains emerged in Europe and Asia in the late 1980s and early 1990s. These vv strains could break through high-level maternal antibodies induced by classical, mild IBDV vaccines and cause up to 60–100% mortality in chicken of various ages [6–9]. IBDV, especially vvIBDV, has become an economically important pathogen in poultry industries worldwide.

IBDV is a member of the Birnaviridae family, with two classes of double-stranded RNA, designated A (≈3.2 kb) and B (≈2.8 kb), respectively. Segment A has a large open reading frame (ORF) encoding a polyprotein that is co-translationally cleaved into the major structural proteins VP2 and VP3. Other proteins encoded are VP4, a proteolytic enzyme-like protein, and a short second ORF of 435 bp partially overlapping the 5’ end of the large ORF, encoding the small non-structural protein VP5 [10–13]. Segment B encodes VP1 which is known as a multifunctional protein with polymerase and capping enzyme activities [14,15]. VP2 and VP3 are the major structural proteins of the virus, of which VP2 is considered as the major protective antigen that contains the antigenic regions responsible for the induction of neutralizing antibodies [16,17]. The epitopes recognized by neutralizing antibodies are conformational and have been mapped to the highly variable region (HVR) of VP2 between amino acids 206–350 [18,19]. Amino acid changes in the two major hydrophilic peaks in VP2 at positions 212–224 and 314–324 were identified to be closely related to antigenic variation of IBDV strains [19–21]. Three additional minor hydrophilic peaks at amino acid positions 248–252, 279–290, and 299–305 are considered to influence IBDV antigenicity as well [22]. Adjacent to the second hydrophilic peak, a serine-rich heptadecapeptide (SWSASGS) located at amino acids 326–332 might be correlated with the virulence of IBDV strains [19].

In China, IBD has been a major poultry disease throughout the country since the first isolation of IBDV strain, CJ801, in 1982 in Beijing [23]. Although it had been well controlled by the use of a range of conventional attenuated live and inactivated IBD vaccines, a novel kind of IBD resulting from infection by highly pathogenic and/or antigenic variant IBDV strains since the late 1980s and early 1990s has occurred and spread continuously in many regions [24–26], resulting in more severe damage to the poultry industry in China. It has been shown at molecular level that vv and variant Chinese IBDV strains isolated in the early 1990s are closely related to vv European and variant American strains [27,28]. During 1996–2000, we isolated representatives of dominant IBDV field strains from various regions of China. In this study, we show the characterization of these isolates at both antigenic and molecular levels which could help in the proper control of IBD in China.

Materials and Methods

Virus Isolates, Titration, and Pathogenicity

IBDV isolate strains LX, LN, NC, DMS, HB97, HD98, QV, YV, FJ, SC, and BX were isolated from different regions in China. Other IBD vaccine strains BJ836, BK912, and LM are commercial vaccines (Table 1). In addition, the first IBDV isolate strain CJ801 and the first variant GZ902 from China are used in this study as well (Table 1). The strains were separately propagated in the bursae of Fabricius of specific-pathogen-free (SPF) chickens or chicken embryo fibroblast (CEF) cells. For field isolates, they were titrated in 10-day-old SPF embryonating eggs. CEF-adapted strains were titrated in SPF CEF cells. The virus titer was determined as mean embryo infectious dose (EID₅₀) or mean tissue culture infectious dose (TCID₅₀).

Three-week-old SPF chickens were used to carry out the pathogenicity test. Chickens were individually inoculated with different isolates at a dose of 10³.5EID₅₀ or 10⁷.0TCID₅₀ via a combination of nasal drops and oral route. The chickens were observed for 7 days. All the chickens were autopsied and grossly examined during this period and at 7 days post-inoculation (PI).