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Antigenicity characterization of four representative natural reassortment IBDVs isolated from commercial three-yellow chickens from Southern China reveals different subtypes co-prevalent in the field

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ABSTRACT

The antigenic relationships between the natural reassortment field strains of infectious bursal disease virus (IBDV), and between the field strains and the vaccine strains are poorly understood. In the present study, the antigenicity of four representative natural reassortment IBDV isolates designated JS7, GD10111, NN1005 and NN1172 from southern China during the years 2005–2011 and their antigenic relationship with the most commonly used vaccine strain B87 were investigated *in vivo*. For this purpose, cross-challenge studies were performed on 28-day-old birds, which were 2 weeks post-vaccination by oil-emulsion vaccines (OEVs) prepared from the four field viruses and B87, respectively. The protection related values (PRV) were evaluated based on the protection rate measured by clinical signs and mortality, bursa/body weight (B/BW) ratio and the viral load in the bursal samples at 3 and 7 days post challenge. As a result, the PRV showed that the isolates NN1172 and GD10111 belonged to the same antigenic subtype, while the isolates NN1005 and JS7 belong to another subtype. The vaccine strain B87 was grouped with the isolates NN1005 and JS7 but actually belongs to another small subgroup and provided only 60–80% protection against the challenge of the four field strains. The results demonstrated that different antigenic subtypes co-existed among the field natural reassortment IBDV strains and the commonly used vaccine strain B87 was antigenically different from the prevalent IBDVs in southern China.

1. Introduction

Infectious bursal disease (IBD) is an acute, highly contagious and immunosuppressive disease in young chickens affecting the poultry industry worldwide (Müller et al., 2003; Eterradossi and Saif, 2013). The disease is caused by infectious bursal disease virus (IBDV). There are two serotypes of IBDV, serotype 1 and serotype 2, but only serotype 1 viruses cause clinical signs in chickens. The serotype 1 viruses are classified in increasing order of virulence as mild, intermediate, classical virulent, variant and very virulent strains (Van Den Berg, 2000). IBDV is a non-enveloped, icosahedral, two-segmented (segment A and segment B) double-stranded RNA virus, a member of the *Birnaviridae* Family. There are two open reading frames (ORF) in segment A which is about 3.3 kb. The smaller ORF encodes a non-structural protein VP5 while the larger ORF encodes the virion proteins as a polyprotein in the following order: VP2, VP4, VP3 (Eterradossi and Saif, 2013). VP2 is the major protective immunogen of IBDV and induces virus-neutralizing antibodies (Fahey et al., 1989). Segment B is about 2.8 kb and contains only one ORF and encodes viral protein VP1. It is reported that the VP1 is essential for the IBDV virulence *in vivo* (Le Nouën et al., 2012).

IBD was first reported in Beijing, China more than 30 years ago. Although extensive attenuated live vaccines and inactivated vaccines have been used to control the disease, the emergence of very virulent (vv), variant and reassortment IBDV in recent years makes the situation complicated. Yuwen et al. (2009) investigated 20 IBDV isolates during 1998–2007 from 11 provinces in China and revealed that 19 out of 20 isolates were a vvIBDV genotype based on the VP2 gene. In our previous work on investigating the molecular epidemiology of IBDV in southern China during the years 2000–2012, 72 out of 91 isolates were vvIBDV genotype based on the variable region of the VP2 gene (vVP2),

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while the phylogenetic analysis of both segments A and B revealed that the majority of the isolates (85.71%, 78/91) were a natural genetic segment reassortment (He et al., 2014). More and more groups (Abed et al., 2018; Cui et al., 2013; Felice et al., 2017; Jackwood et al., 2016; Lu et al., 2015; Wei et al., 2006) have revealed that the natural reassortment in the IBDV genome has become a new prevalent trend for IBDV molecular evolvement. Further studies indicated that both of the IBDV genome segments play an important role in pathogenicity of IBDV in chickens (Escaffre et al., 2013; He et al., 2016; Le Nouën et al., 2006; Li et al., 2015; Lu et al., 2015; Wei et al., 2006). However, the antigenicity of these reassortment IBDV field isolates and their antigenic relationship with the commonly used vaccine strains has remained unknown. Since vaccination and development of new vaccines are still the key measures for the control of IBD in practice, the antigenic investigation of the field strains would be important for the selection of new vaccine candidate strains.

In southern China, the most commonly raised commercial chicken breeds are local lines, with the Three-Yellow chicken being the most popular line. The farming situations of these local chickens are complicated. Most of the chickens are free-range in the wild with considerably poor biosecurity conditions indicated by incomplete disinfection between each brood, multiple-age flocks, and non-compliance with an all-in-all-out procedure in practice. Further, IBDV live vaccines are commonly used in broiler and breeding chickens at the age of 12 and 24 days (He et al., 2014). These situations easily allow IBDV strains with different virulent, antigenicity, and genotype to co-exist on the farms, resulting in the possibility of segment reassortment among these IBDVs. Our group has confirmed this possible event on those farms in southern China and found that reassortment IBDVs have become the most prevalent strains on those farms (He et al., 2014). Also, we demonstrated that different natural reassortment IBDV field strains exhibited differential pathogenicity in Three-Yellow chickens (He et al., 2016). In the field, even though the program of one vaccination with the OEV in the breeder flock before laying, and one vaccination in the chicks with the attenuated live vaccine strain B87 during 7–14 days of age or the IBDV-VP2 recombinant HVT live vaccine at hatching (Le Gros et al., 2009) was in practice, the IBD outbreaks and immunosuppression from IBD were still commonly reported in the production system in southern China (He et al., 2014; Li et al., 2015; Liu et al., 2013). We hypothesized that the antigenicity of the natural reassortment IBDV field strains are different, and the antigenicity between the field strains and commonly used vaccine strains is also different, which might contribute to the IBD outbreak in southern China even after vaccination. The purpose of this study is to investigate probable different antigenicity of the different representative reassortment IBDV field strains isolated from Three-Yellow chickens in southern China and their antigenic relationship with the most commonly used vaccine strain B87. The analysis might provide important new insights into the antigenicity of the different kinds of natural reassortment IBDV field isolates that cause the major infection in the field and the findings might also be helpful in the effective control of IBD.

2. Materials and methods

2.1. Viruses

The information concerning the four representative natural reassortment IBDV field strains JS7, GD10111, NN1005 and NN1172 used in this study is shown in Table 1 which represents differential partial genome characters, from different places and different years, which we analyzed in our previous work (He et al., 2014). The viruses were isolated from natural outbreaks of IBD that involved previously vaccinated chickens in southern China as described previously (He et al., 2014). The commonly used intermediate virulent commercial live vaccine, strain B87 (HLJ Animal-use Biological Products Co., Ltd., China), was used in the study. The results from our previously phylogenic study showed that the *v*VP2 gene and VP1b gene of B87 were derived from intermediate IBDV and attenuated IBDV respectively (He et al., 2014).

All the viruses were propagated and titrated on Vero cells as previously described (He et al., 2016). Briefly, all the plague-purified viruses were cultured in Vero cells with 1–2 passages, and the viruses were harvested. The viruses were then titrated by inoculating 10-fold serial dilutions (10^{-1}–10^{-10}) into the Vero cells. The cytopathic effect (CPE) characterized by cytoplasmic, cell rounding, detachment and lysis was recorded and the titrations were calculated as a 50% tissue culture infective dose ($TCID_{50}$) using the Reed-Muench method (Reed and Muench, 1938). Viruses used for the OEV preparation were propagated in 9-day-old specific pathogen free (SPF) embryonated chicken eggs and titrated using the method previously described (Abdel-Alim and Saif, 2001). The concentrations of viruses used for OEV preparation were titrated at 10^5 EID_{50}/ml.

2.2. Chicken egg embryos and chickens

SPF embryonated chicken eggs were purchased from Beijing Merial Vital Laboratory Animal Technology Co., Ltd., Beijing, China. Day-old commercial Three-Yellow chickens were purchased from a local commercial farm and transferred to the isolation facility and no vaccination was administered. The animal experiments were conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for the International Organizations of Medical Sciences.

2.3. Virus inactivation and preparing the OEVs

The harvested viruses were inactivated using 0.5% formaldehyde with continuous stirring at 37 °C for 24 h. The inactivation efficiency was performed by 3 blind passages of the inactivated virus in 5 SPF embryonated chicken eggs. After complete inactivation, the inactivated viruses were added, with 4% Tween 80 to be the aqueous phase. The No. 10 mineral oil was added with Span 80 in a ratio of 10/1 to create the oil phase. The aqueous phase was emulsified with the oil phase in a ratio of 40/60 (V/V). The OEVs were stored at 4 °C.

2.4. Experiment arrangement

Two hundred and seventy 7-day-old Three-Yellow chickens were randomly allocated to six groups, groups A to E with 50 birds each and group F with 20 birds. Ten birds from each group were used for experiment 1, and the remaining birds for experiment 2 in the following experiment designs. Birds in the groups A–E were subcutaneously vaccinated with the OEVs containing inactivated viruses JS7, GD10111, NN1005, NN1172 and B87, respectively, at 7-days-old and a second immunization was administered in the exact same way at 14-days-old. Group F was selected as the unvaccinated control.

Within the overall experiment, two sub-experiments were designed. Experiment 1 was designed to determine if each OEV containing the natural reassortment IBDV field strain could induce anti-IBDV antibodies in Three-Yellow birds. In this experiment, serum samples from 10 birds in group A–F were obtained at 0, 7, 14, 21, 28 and 35 days post immunization (DPI) respectively and the anti-IBDV antibody was detected by using a commercial indirect enzyme-linked immunosorbent assay (ELISA) kit (Flock Check Infectious Bursal Disease Antibody Test Kits, IDEXX Laboratory, Inc. USA) following the manufacturer's instructions.

Experiment 2 was designed to determine the antigenicity of the field isolates and their antigenic relationships with the most commonly used vaccine strain B87. In this experiment, cross-challenge studies were performed in groups A–E, while group F served as the non-challenged control (Table 2). At 2 weeks after the secondary vaccination, each group was divided into four subgroups with 10 birds each. Birds of 4