Dear Editor,

Infectious bursal disease virus (IBDV) causes an economically significant disease of chickens worldwide (Berg, 2000). The molecular basis for the virulence of very virulent IBDV (vvIBDV) is not fully understood. Previous studies have shown that genome segment A, especially VP2 protein, plays the most important role in the tropism and pathogenicity of serotype 1 IBDV (Brandt et al., 2001). VP2 is, however, unlikely to be the only factor for the virulence of vvIBDV (Boot et al., 2000). A chronophylogenetic study suggested that the worldwide expansion of vvIBDV likely started following the acquisition of a new vvIBDV-related segment B, whereas the vvIBDV-related segment A could have been introduced several years earlier (Hon et al., 2006). Another recent experimental study based on reverse genetics demonstrated that several domains of the IBDV polymerase may contribute to the virulence of vvIBDV (Nouën et al., 2012). Segment B encodes VP1, an RNA-dependent RNA polymerase (RdRp) responsible for the replication of the genome and synthesis of mRNAs (von Einem et al., 2004). The IBDV RdRp could be divided into three domains according to the tri-dimensional structure: an N-terminal domain (residues 1–167), the central polymerase domain (residues 168–658), and a C-terminal domain (residues 659–878) (Pan et al., 2007).

To study the function of different domains of VP1 protein of vvIBDV for its virulence, we constructed and rescued two wild-type and four intra-segment B reassortant IBDVs derived from vvIBDV HuB-1 and attenuated IBDV Gt strains based on the tri-dimensional structure of VP1 protein (Figures S1 and S2 in Supporting Information). The study of viral replication showed that the substitution of the central activity domain and C-terminal domain of VP1 protein of the attenuated IBDV Gt with the corresponding domains of VP1 from vvIBDV HuB-1 had a positive effect on viral replication in CEF cells at the early stage of infection, while the substitution of HuBN with GtN reduced the replication of HuB-1 in chickens significantly (P<0.05), and enhanced its replication in chickens with the substitution of GtN with HuBN (Figure S3 in Supporting Information). These results indicated that the N-terminal domain of VP1 from vvIBDV has a negative effect on viral replication in *vivo* while exhibits a positive effect on viral replication in *vitro*. The molecular basis for this effect needs further study.

*Corresponding author (email: xmw@hvri.ac.cn)
vivo, and the N-terminal domain of VP1 from attenuated IBDVs has a negative effect on viral replication in vivo.

To examine whether the substitution of N-terminus of VP1 could affect the viral pathogenicity, 3-week-old chickens were inoculated with the rescued wild-type and N-terminus substitution viruses based on the parental viruses, attenuated IBDV Gt and vvIBDV HuB-1. Throughout the experimental period, no death and no clinical symptom of IBD were observed for chickens receiving the cell adapted viruses rGtAHuBNGtB, rGtAHuBMGtB and rGtAHuBCGtB. The bursa: body-weight index (BBIX) among these groups were compatible ($P>0.05$) and kept above 0.7 (Figure 1A). The histopathological sections of bursae from group rGtAHuBMGtB showed normal follicles and follicular connective tissues, and no microscopic lesions were observed with the histopathologic bursal lesion scores (HBLS) being 0–1. However, the bursae derived from one chicken out of five in group rGtAHuBNGtB and rGtAHuBCGtB showed scattered or partial bursal damage (HBLS being 2), respectively (Figure 1B). All the bursae derived from the group rHuBAHuB and rHuBAGtNHuB were atrophic with a BBIX value of below 0.7 (Figure 1A), and bursal gross lesions including necrosis of lymphocytes, fibroplasias, atrophy of follicles and follicular depletion were observed (HBLS being 4–5, Figure 1B). The mortality rate caused by rHuBAHuB in chickens was reduced dramatically (from 93.3% to only 6.67%) by substituting the HuBN with the GtN (Figure 1C). These results indicated that the N-terminal domain of VP1 contributes to the virulence of vvIBDV and it might play a more important role than the central and C-terminal domains in the virulence enhancement of vvIBDV. Additionally, our results revealed that the pathogenicity of the IBDV was consistent with its viral replication in vivo.

The polymerase activity of the IBDV VP1 was closely related with the viral replication and pathogenicity. To study the roles played by different domains of VP1 in the polymerase activity, the luciferase-based minigenome derived from the segment B was developed and the relative polymerase activities of the wild-type and different mosaic VP1 of vvIBDV HuB-1 were detected. The results indicated that the N-terminal domain of VP1 from the attenuated IBDV had a positive effect on the polymerase activity in cell cultures while its central polymerase and the C-terminal domains exhibited negative effects, compared with the counterparts in the vvIBDV VP1 protein (Figure S4 in Supporting Information). The levels of all mutant proteins were also estimated by Western blot and showed that there was no significant difference among those mutant protein expression levels (Figure 4S in Supporting Information). These differences of polymerase activity were not caused by protein expression levels but related to the function of the protein itself.

In conclusion, the important role of segment B in the viral pathogenicity of vvIBDV was confirmed. Different domains of VP1 were shown to have different effects on viral replication, virulence as well as the polymerase activity of IBDV. The results of this study further suggested that the N-terminal domain of VP1 might play a more important role in the pathogenicity of vvIBDV compared with the central and