**Detection of very virulent infectious bursal disease virus in vaccinated broilers**

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## Infectious bursal disease (IBD), usually referred as Gumboro disease, is an acute and highly contagious immunosuppressive disease of young chickens. The etiological agent is infectious bursal disease virus (IBDV), a nonenveloped, icosahedral virus of the Birnaviridae family with a bisegmented, double-stranded RNA genome. The clinical signs are usually observed between 3 and 6 weeks and include whitish or watery diarrhea, anorexia, depression, ruffled feathers, trembling, and severe prostration. Necrosis of the bursa of Fabricius and depletion of B lymphocytes, resulting in immunosuppression is also observed. Vaccination is used by the poultry industry to control IBD, but it is necessary to vaccinate poultry at an early age, before the disappearance of maternal antibodies. IBDV can be grouped into classical, very virulent or variant strains. The morbidity usually reaches 100 % for all strains while the mortality varies widely with strain, 30 % to 60 % in classic strains and 70 % to 100 % in very virulent strains. There is usually no mortality in chickens infected with variant strains, although bursal atrophy is severe. The contribution to virulence was initially identified in the structural protein VP2 but VP1 and VP4 have proven to be also involved. The major neutralizing epitope of VP2 is located within the 145 amino acid fragment known as the hypervariable region (HVR) and certain amino acid changes in this site may cause antigenic variation.

## A vaccination programme *in ovo* with a live attenuated variant strain was recently introduced in Portugal and in order to monitor the spread of the virus in the bursa of vaccinated birds, the molecular detection of IBDV genome was performed. The bursas collected regularly during time were used for RNA extraction. An amplicon of 491 pb of the hypervariable region of VP2 encoding gene was amplified by RT-PCR and the nucleotide sequence of the amplified fragment was determined. IBDV was detected in almost all birds, but mainly around the age of 21 days. The alignment of the predicted amino acid sequence showed two distinct populations. In one population the amino acid sequence is similar to that of the vaccine strain, but in the other population, the mutations characteristic of very virulent strains were observed. Additionally to the described changes P222A, V256I, and N299S also mutations V242I and T270A could be detected.

## The identification of different IBDV genotypes in field isolates allowed the detection of emerging IBDV mutants with the ability to break through the immunity to commercial IBDV vaccines.