Relative quantification and detection of different types of infectious bursal disease virus in bursa of Fabricius and cloacal swabs using real time RT-PCR SYBR green technology

In present study, different types of infectious bursal disease virus (IBDV), virulent strain DK01, classic strain F52/70 and vaccine strain D78 were quantified and detected in infected bursa of Fabricius (BF) and cloacal swabs using quantitative real time RT-PCR with SYBR green dye. For selection of a suitable internal control gene, real time PCR parameters were evaluated for three candidate genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 28S rRNA and beta-actin to IBDVs. Based on this P-actin was selected as an internal control for quantification of IBDVs in BF. All BF samples with D78, DK01 or F52/70 inoculation were detected as virus positive at day 1 post inoculation (p.i.). The D78 viral load peaked at day 4 and day 8 p.i., while the DK01 and F52/70 viral load showed relatively high levels at day 2 p.i. In cloacal swabs, viruses detectable were at day 2 p.i. for DK01 and F52/70, day 8 p.i. for D78. Importantly, the primers set were specific as the D78 primer set gave no amplification of F52/70 and DK01 and the DK01 primer set gave no amplification of D78, thus DK01 and D78 could be quantified simultaneously in dually infected chickens by use of these two set of primers. The method described here is robust and may serve as a useful tool with high capacity for diagnostics as well as in viral pathogenesis studies.