Identifcation of reference genes in chicken intraepithelial lymphocyte natural killer cells infected with very-virulent infectious bursal disease virus

ABSTRACT

Due to the limitations in the range of antibodies recognising avian viruses, quantitative real-time PCR (RT-qPCR) is still the most widely used method to evaluate the expression of immunologically related genes in avian viruses. The objective of this study was to identify suitable reference genes for mRNA expression analysis in chicken intraepithelial lymphocyte natural killer (IEL-NK) cells after infection with very-virulent infectious bursal disease virus (vvIBDV). Fifteen potential reference genes were selected based on the references available. The coefcient of variation percentage (CV%) and average count of these 15 genes were determined by NanoString technology for control and infected samples. The M and V values for shortlisted reference genes (ACTB, GAPDH, HMBS, HPRT1, SDHA, TUBB1 and YWHAZ) were calculated using geNorm and NormFinder. GAPDH, YWHAZ and HMBS were the most stably expressed genes. The expression levels of three innate immune response related target genes, CASP8, IL22 and TLR3, agreed in the NanoString and RNA sequencing (RNA-Seq) results using one or two reference genes for normalisation (not HMBS). In conclusion, GAPDH and YWHAZ could be used as reference genes for the normalisation of chicken IEL-NK cell gene responses to infection with vvIBDV.