Journal of Animal and Veterinary Advances 2 (11): 607-610, 2003

Simultaneous Detection and Identification of Epizootic Hemorrhagic Disease Virus Serotype 1 and 2 using A Multiplex RT PCR

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Abstract: A multiplex reverse transcriptase (RT) polymerase chain reaction (RT-PCR)-based assay, for simultaneous serogroup-specific detection and serotype-specific identification of North American serotypes of epizootic hemorrhagic disease virus (EHDV) in cell culture and clinical samples, was developed. For detection of EHDV serogroup-specific, a pair of primers (EG1 & EG4) was designed from a conserve region of non-structural protein 1 (NS1) genome of EHDV serotype 2 (EHDV-2). For serotype-specific identification, two pairs of primers (ES1 and ES4) and (ESa and ESb) were designed from variable regions of genome segment 2 (L2) of EHDV-1 and that of EHDV-2. The multiplex RT-PCR-based assay utilized a single tube-PCR amplification in which EHDV serogroup-specific and serotype-specific primers were used simultaneously in a multiplex format. The EHDV serogroup-specific primers generated a 387 base pair (bp) PCR product from RNA samples of EHDV-1 and EHDV-2. The EHDV serotype-specific primers generated a 821-bp PCR product and a 1054-bp PCR product from RNA samples of EHDV-1 and EHDV-2, respectively. However, RNAs from BTV serotypes 2, 10, 11, 13 and 17; or total nucleic acid extract from non infected Vero cells failed to demonstrate the specific EHDV PCR products. The described multiplex RT-PCR-based assay could be used to facilitate rapid detection and differentiation of EHDV serotypes 1 and 2. In addition, it could also be used to monitor incursion of new serotypes of EHDV

Key words: Epizootic hemorrhagic disease, virus, PCR, assay