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Main topic : Surveillance and control of emerging diseases

Development of a 4-plex real-time TaqMan® RT-qPCR assay for simultaneous differentiation of equine group A and B rotaviruses and genotyping of equine rotavirus A G3 and G14

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Introduction: Equine rotavirus A (ERVA) is the leading cause of foal diarrhea, posing a significant impact on the equine breeding industry. The most prevalent ERVA genotypes infecting foals include G3P[12] and G14P[12]; other genomic arrangements are very rare. Recently, a group B equine rotavirus (ERVB) has been identified as an emerging cause of diarrhea in the US, with an impending need for development of molecular diagnostic tools for its detection, understanding its molecular epidemiology, and inform future vaccine development. Our group has previously developed a one-step 3-plex TaqMan® real-time reverse transcription polymerase chain reaction (RT-qPCR) assay for rapid detection and G-typing of ERVA directly from fecal specimens. Here, we developed a 4-plex TaqMan® RT-qPCR assay for differentiation of ERVA and ERVB and simultaneous G-typing of epidemiologically relevant ERVA strains.
Methods: Two one-step 4-plex TaqMan® RT-qPCR assays targeting the NSP3 and VP7 genes of ERVA G3 and G14 genotypes, as well as either the VP6 or NSP5 genes of ERVB were designed (ERVA/ERVB VP6 and ERVA/ERVB NSP5). The analytical performance was compared to the previously established ERVA 3-plex RT-qPCR assay and the clinical performance was evaluated using a panel of 193 archived fecal samples and compared to an ERVA VP7-specific or ERVB VP6-specific standard RT-PCR assay and Sanger sequencing.
Results: Two 4-plex assays incorporating ERVB VP6 or NSP5 gene targets were developed. Based on the overall lower efficiency of the ERVA/ERVB NSP5 4-plex assay, the ERVA/ERVB VP6-based 4-plex assay was selected and its clinical performance evaluated. This 4-plex RT-qPCR assay demonstrated high sensitivity/specificity for every target (sensitivity >90% and specificity of 100%) and high overall agreement (>96%) compared to conventional RT-PCR and sequencing. Comparison between the 3-plex and 4-plex assays revealed only a slightly higher sensitivity for the ERVA NSP3 target using the 3-plex format (p-value 0.008) while no significant differences were detected in the sensitivity and specificity of other targets.
Conclusions: This new 4-plex RT-qPCR assay will significantly enhance rapid surveillance of both ERVA and ERVB circulating strains in diarrheic foals.