Development and Characterization of Probe-Based Real Time Quantitative RT-PCR Assays for Detection and Serotyping of Foot-And-Mouth Disease Viruses Circulating in West Eurasia.

Rapid and accurate diagnosis of foot-and-mouth disease (FMD) and virus serotyping are of paramount importance for control of this disease in endemic areas where vaccination is practiced. Ideally this virus characterization should be achieved without the need for virus amplification in cell culture. Due to the heterogeneity of FMD viruses (FMDVs) in different parts of the world, region specific diagnostic tests are required. In this study, hydrolysable probe-based real time reverse transcription quantitative polymerase chain reaction (RTqPCR) assays were developed for specific detection and serotyping of the FMDVs currently circulating in West Eurasia. These assays were evaluated, in parallel with pan-FMDV diagnostic assays and earlier serotype-specific assays, using field samples originating from Pakistan and Afghanistan containing FMD viruses belonging to different sublineages of OPanAsia-A-Iran05 and Asia-1 (Group-II and Group-VII (Sindh-08)). In addition, field samples from Iran and Bulgaria, containing FMDVs belonging to the O-PanAsia-ANT-10 subline-age were also tested. Each of the three primer/probe sets was designed to be specific for just one of the serotypes O, A and Asia-1 of FMDV and detected the RNA from the target viruses with cycle threshold (CT) values comparable with those obtained with the serotype independent pan-FMDV diagnostic assays. No cross-reactivity was observed in the assays between the heterotypic viruses circulating in the region. The assays reported here have higher diagnostic sensitivity (100% each for serotypes O and Asia-1, and 92% [95%CI = 81.4–100%] for serotype A positive samples) and specificity (100% each for serotypes O, A and Asia-1 positive samples) for the viruses currently circulating in West Eurasia compared to the serotyping assays reported earlier. Comparisons of the sequences of the primers and probes used in these assays and the corresponding regions of the circulating viruses provided explanations for the poor recognition of some of the viruses by the earlier assays. These new assays should help in the early detection and typing of serotype O, A and Asia-1 FMDVs circulating in West Eurasia to enable improved disease control.