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A portable point-of-entrance system for the detection of two trans-boundary viral diseases using recombinase polymerase amplification assay

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Trans-boundary viral diseases in livestock cause huge economic losses and constitute a serious threat worldwide. Early diagnosis of the infectious agents helps to diminish their impact by adequate outbreak management. Samples collected from animals in the field or at quarantine stations are sent long distances to the laboratory for PCR analysis because portable PCR is neither available nor suitable for on-site screening. The recombinase polymerase amplifications (RPA) assay is an isothermal DNA amplification and detection technology. In contrast to PCR, RPA is performed at a single temperature (42°C) and yield a result after only 5-15 minutes. In this study, we describe the development of a real-time RPA assay for the detection of lumpy skin (LSDV) and foot and mouth (FMDV) disease viruses.
Molecular DNA and RNA standards representing a part of the GPCR gene of LSDV and the 3D gene of FMDV were prepared. The assay sensitivity was determined by probit analysis (N=8 runs). The assay specificity was evaluated against a panel of viruses considered for differential diagnosis with LSDV and FMDV. The assays were validated using 22 skin nodule samples from LSDV-infected cattle and 110 samples including vesicular material, sera and swabs from FMD-infected animals. Results were compared to real-time PCR. In addition, the FMDV RPA was used in field during the recent FMDV outbreak in Egypt. The LSDV and FMDV RPAs were rapid (max. 15 minutes) and showed an analytical sensitivity of 179 and 1436 molecules detected, respectively. No cross reactivity with other viruses causing similar clinical pictures were observed. LSDV and FMDV RPAs sensitivity was 100% and 91%, respectively. In conclusion, LSDV and FMDV RPAs were quicker and much easier to handle in the field than real-time PCR. Thus RPA could be easily implemented to perform diagnostics at quarantine stations or farms for rapid on-site viral detection.

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