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Main topic : Surveillance and control of emerging diseases

A new pan RT-PCR for the detection of Epizootic Haemorrhagic Disease Virus in cattle and small ruminants

DESPOIS L. [1], SAVARIT L. [1], BIANCHINI E. [1], LIMOZIN A. [1], COMTET L. [1], POURQUIER P. [1]

[1] Innovative Diagnostics, Grabels, France

Background and Objectives.

Epizootic hemorrhagic disease (EHD) is a Culicoides-borne viral disease caused by the
EHD virus (EHDV), associated with clinical manifestations in domestic and wild ruminants, primarily white-tailed deer and cattle. Along with BlueTongue Virus (BTV), EHDV is classified within the genus Orbivirus. EHDV has a 10-segmented RNA genome encoding for seven structural proteins (VP1-VP7) and five nonstructural proteins (NS1-NS4, NS3a).
In late September 2021, EHDV was reported in cattle farms in central/western Tunisia. It rapidly spread throughout the country with more than 200 confirmed outbreaks. For the first time in 2022, EHDV was detected in continental Europe.
Innovative Diagnostics has developed a qualitative duplex real-time PCR (RT-PCR) for a
pan EHDV detection. The test can be used on whole blood, spleen or organs from bovine or small ruminants species. It simultaneously amplifies a specific target from the EHDV genome as well as an endogenous non-target positive (ruminant housekeeping gene).

Material and Methods.

After RNA denaturation (heating at 95°C for 3 minutes), 5 ?l of denatured RNA was added to the ready-to-use mastermix and the amplifications were carried out using a rapid amplification program (65 min).
Specific synthetic RNA was designed to estimate the limit of detection of PCR (LDPCR) and to determine the RT-PCR efficiency. RNAs panels (provided by ANSES, Maisons-Alfort, France) from the 7 EHDV known serotypes, and 27 BTV serotypes or from other pathogens affecting cattle were tested to assess test inclusivity and exclusivity, respectively. Samples were also tested in parallel on a commercially available PCR kit.

Results.

The LDPCR (95%) was estimated around 10 copies/PCR and PCR efficiency was determined above 98% with the synthetic RNA. The analytical specificity of RT-PCR assay was evaluated in silico by aligning the target PCR systems with the databases available on NCBI (National Center for Biotechnology Information). The alignments show 100% in silico specificity for the EHDV targeted region, and no homology with other pathogens.

This was confirmed experimentally, as the panEHDV RT-PCR successfully detected all 7 EHDV serotypes, including Tunisia-2021 strains which emerged in Europe, without showing any cross-reactions with neither BTV nor other pathogens.

Compared to a commercially available ELISA, IDvet's RT-PCR gave earlier Cq values with a mean difference gain of 2,8 Cq.

Conclusion.

Along with the ID Screen® EHDV Competition ELISA, which is available for specific EHDV antibody detection, the new panEHDV RT-PCR allows for rapid and accurate detection of EHDV, regardless of the serotype.