Comparative study of four commercial BVDV Antigen ELISA and five commercial real-time RT-PCR for detection of different European BVDV strains and isolates

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Abstract

The methods used were four commercial BVDV Antigen ELISA (HerdChek BVD Ag/Serum Plus, IDEXX; Procheck BVD Ag Pippus, Prionics; Serelisa® p80 Ag mono indirect, Syntibiotic and Ingezim®) BVD, Ingenasa) and five commercial real-time RT-PCR (BIovi-SL® BVDV TaqMan RT-PCR kit, Adiavet GmbH; cador® BVDV RT-PCR; QIAGEN; Kit TaqMan®; LS; Virotype® BVDV LDL and Adilaset BVD Realtime, Adiagène). Whereas the E2 Ag capture ELISA of IDEXX clearly detected all strains and isolates (n=180), ELISA 1, 2 and 3 (all NS2/3 Ag capture ELISAs) missed 14, 7 and 7 strains and isolates respectively. In addition, ELISA 2 and 3 scored four and one suspect result respectively. The IDEXX ELISA showed a very good analytical sensitivity. All strains and isolates tested except nine scored S/P values which were five times higher than cut-off of IDEXX ELISA (cut-off S/N 0.3). Three commercial real-time RT-PCRs scored all strains and isolates positive, however, RT-PCR 2, 3, 4 and 5 showed Ct-values of greater than 35 with 3, 8, 1 and 12 samples, respectively, indicating a putative lower analytical sensitivity. RT-PCR 4 missed one and RT-PCR 5 missed 18 out of 180 strains and isolates, respectively. The IDEXX ELISA and three commercial RT-PCRs showed 100% sensitivity in this study. Whereas the IDEXX ELISA is used to test individual samples in the field, RT-PCRs normally apply pooling protocols due to economic aspects. The impact of pooling has not been assessed in this study. RT-PCRs especially with high Ct-values of greater than 35 may score different with pooled samples.

Material and methods

ELISA kits

Four commercial ELISA Ag kits were used for this study: HerdChek BVD Ag/Serum Plus, IDEXX; Procheck BVD Ag Pippus, Prionics; Serelisa® p80 Ag mono indirect, Syntibiotic and Ingezim BVD, Ingenasa. The IDEXX HerdChek BVDV Ag/Serum Plus Test Kit can be a highly suitable tool for use in plasma, whole blood or tissue sample preparation. The HerdChek BVDV Ag/Serum Plus Test Kit is fulfilling this requirement.

IDEXX ELISA

The IDEXX ELISA is fulfilling this requirement.

ELISAs

In addition, ELISA 2 and 3 scored four and one suspect result respectively.

RT-PCR kits

Five commercial real-time RT-PCR kits were also tested: BoVir-SL BVDV TaqMan RT-PCR kit, Adiavet GmbH; cador® BVDV RT-PCR; QIAGEN; Kit TaqMan™; LS; Virotype® BVDV LDL and Adilaset BVD Realtime, Adiagène.

Strains and isolates

In total, 180 strains and isolates were used. The following reference strains were kindly supplied by Friedrich-Loeffler Institut (FLI), Germany: a) Type 2 strains: 4204; CS8694; CS8777; M13; 9009; SE6536; SE185; M12; SE1146; SE6444; Bure b) Type 1 strains: Issa/O2/24; P114. c) Other strains: porcine giant cell disease virus 1495.

The following reference strains were kindly supplied by CER Martos®: Oregon (type 1a); NADL (type 1a); Oakland (type 1b), New York (type 1b); SI1239 (type2); UVR420 (type2). 118 BVDV type 1 field isolates from Normandy (France) were tested: 154 tissue culture supernatants (strains A and B) and 4 whole blood isolates. All these strains were identified as BVD-positive after isolation in tissue culture and identification by indirect immunofluorescence test in a field laboratory (Laboratoire Départemental de l’Orne, Alençon, France). The Macon atypical BVDV strain® (type 1I) was also tested (tissue culture supernatant) by all commercial kits.

Tissue culture BVDV-free MDBK cell line was used

All samples were treated with the same RNA extraction protocol (High Pure Viral Nucleic acid kit, Roche). RNA extracts were stored at -70°C and used for RT-PCR.

Results

Whereas the E2 Ag capture ELISA of IDEXX clearly detected all strains and isolates (n=180), ELISA 1 (n=179), 2 (n=175) and 3 (n=176) (all NS2/3 Ag capture ELISAs) missed 14, 7 and 7 strains and isolates respectively (Figure 1). All strains and isolates tested except nine scored S/P values five times higher than cut-off with the IDEXX ELISA. Three commercial real-time RT-PCRs scored all strains and isolates positive. However, RT-PCR 2, 3, 4 and 5 showed Ct-values of greater than 35 with 3, 8, 1 and 12 samples, respectively, indicating a putative lower analytical sensitivity. The RT-PCR 4 missed one and RT-PCR 5 missed 18 out of 180 strains and isolates (Figure 2).

Discussion

The goal of a BVDV eradication program is to detect and eliminate cattle which are persistently infected (PI) with BVDV. Besides excellent diagnostic sensitivity and high diagnostic specificity, detection of all BVDV strains and isolates present (known) in the field is a key factor for successful use of a BVDV diagnostic assay in a BVDV eradication program. The present study shows that the IDEXX ELISA is fulfilling this requirement. However, there are a number of tests available which miss strains and isolates. This can put BVDV programs at risk. New strains, originating from mutation or addition of BVDV should systematically be tested with commercially available diagnostic assays. It is important to use the tests with high analytical sensitivity.

Conclusions

The IDEXX HerdChek BVD Ag/Serum Plus test kit was the only commercial ELISA that detected all strains and isolates tested in this study.

References


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