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Quantity of *Bovine viral diarrhea virus* (BVDV) RNA in clinical samples obtained from persistently infected calves using quantitative RT-PCR

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Introduction:

Skin from an ear notch is a convenient tissue for detection of *Bovine viral diarrhea virus* (BVDV) persistently infected (PI) cattle because it is easy to collect and requires minimal supplies and equipment. Pooling ear notch PBS supernatant for RT-PCR is a popular method to screen many animals at a reduced cost. The pooling method usually involves soaking the ear notch skin samples in a small amount (approximately 2 mls) of phosphate-buffered saline (PBS) and pooling a portion of the supernatant for RT-PCR testing.[1] If the pooled supernatant tests positive by RT-PCR, the samples are then tested individually. While ear notches have become the sample of choice for PI testing, there is minimal information on the quantity of viral RNA copies in the PBS supernatant containing the soaking skin and how the skin sample compares to blood, serum, and nasal swabs from the same animal.

The purpose of this study was to develop a quantitative real-time RT-PCR (qRT-PCR) for quantification of BVDV RNA in a variety of clinical samples obtained from PI cattle. Serum, whole blood, nasal swabs and skin samples were collected from live PI cattle over one year, extracted within 2 days of collection, and analyzed by qRT-PCR during one week. The data derived from allowed for an estimation of RNA copes in four clinical specimens obtained from seven PI calves.

Materials and Methods:

A quantitative RT-PCR (qrt-PCR) was developed. Briefly, a DNA product of approximately 199 base pairs was generated from a BVDV type 1a isolate (NADL) from a single-step RT-PCR reaction. The purified RT-PCR product was then cloned to a plasmid vector according to manufacturer specifications. After incubation, propagation, and purification, a copy number of the purified plasmid was calculated from the concentration of extracted DNA by spectrophotometry. A standard curve was calculated from 10-fold serial dilutions of plasmid DNA in an estimated range of 10^{-1} to 10^{6} copies/ml. The minimum detection limit using the plasmid template was defined as the lowest plasmid copy number producing a threshold cycle significantly different from reactions with no template DNA. The minimum detection limit of the qRT-PCR test was 10^{2} copies/ml.

Seven PI dairy calves obtained from a large dairy were sampled 10 times during 2009. The seven calves were born within 2 weeks of each other and the virus shed by all seven calves was identical and similar to the 1b subgenotype. The first sampling date was February 8, 2009 and the last sampling date was October 18, 2009 and samples were collected approximately every 3 to 4 weeks.

Samples included a whole blood sample collected in a EDTA blood tube, a serum sample obtained from a centrifuged blood sample, a triangular ear notch approximately 5 mm from the

base of the haired ear margin to the cut tip obtained from the lower ear margin and a cotton nasal swab collected from one nostril.

Additional validation on pooling was accomplished by collecting PBS supernatant from individually test negative samples and spiking supernatant from PI calves. Twenty five microliters of ear notch supernatant from the PI calves was added to 1.25, 2.50 and 5.0 milliliters of supernatant from test negative calves to represent pools of 1:50, 1:100 and 1:200, respectively.

Results:

All 70 buffy coats, ear notch and nasal swab supernatant samples tested positive by qRT-PCR (100%, 95% confidence interval .95 – 1.0). Sixty nine of the seventy serum samples tested positive by qRT-PCR (99%, 95% confidence interval .93 – .99). The average $\log^{10}$ RNA copy number in the ear notch PBS, nasal swab PBS and buffy coat samples were 5.15, 5.54 and 5.65, respectively. The average $\log^{10}$ RNA copy number in serum was 4.66.

Pools generated with negative ear notch supernatant and spiked samples from the PI calves produced positive results in 209/210 of pools. All seventy pools of 1:100 and 1:200 tested positive and 69/70 of 1:50 pools tested positive by RT-PCR.

Discussion:

Viral quantification often provides valuable information both in viral diagnostics and pathogenesis research. In this study, the goal was to estimate the quantity of BVDV RNA copies in four diagnostic samples collected from seven PI calves shedding an identical virus over one year. Estimating the quantity of RNA copies in clinical samples would be used to determine the limitations of pooling samples.

The quantity of virus in the serum samples from this study are similar to the quantities in other reports that quantified with virus isolation. A previous study following 7 PI calves had serum virus titers that ranged from 5×10^3 to $5 \times 10^{5.2}$ Variation in viral serum concentration has been reported in other studies and concentrations ranged from of $10^4 – 10^6$ $CCID_{50}$/ml.[3,4,5]

In summary, buffy coats, serum, ear notch and nasal swab supernatant contains, on average, 10^4 to 10^5 BVDV RNA copies per ml and was approximately 1000 fold higher than the minimum detection limit of the RT-PCR test. Ear notch PBS supernatant from negative animals tested positive in 209 of 210 pools when spiked with supernatant from a single PI animal.

References:

1. Kennedy JA, Mortimer RG, Powers B: 2006, Reverse transcription-polymerase chain reaction on pooled samples to detect bovine viral diarrhea virus by using fresh ear-notch-sample supernatants. J Vet Diagn Invest 18:89-93.
2. Brock KV, Grooms DL, Ridpath J, Bolin SR: 1998, Changes in levels of viremia in cattle persistently infected with bovine viral diarrhea virus. J Vet Diagn Invest 10:22-26.
3. Brock KV, Redman DR, Vickers ML, Irvine NE: 1990, Quantitation of bovine viral diarrhea virus in embryo transfer flush fluids collected from a persistently infected heifer. J Vet Diagn Invest 3:99–100.
4. Palfi V, Houe H, Philipsen J: 1993, Studies on the decline of bovine virus diarrhoea virus (BVDV) maternal antibodies and detectability of BVDV in persistently infected calves. Acta Vet Scand 34:105–107.
5. Paton DJ, Lowings JP, Ramirez GC: 1994, Stability of the gp53 gene of a bovine viral diarrhea virus isolated at different times from a persistently infected steer. Br Vet J 150:603–607.