

# The detection of neonatal calves persistently infected (PI) with bovine viral diarrhoea virus (BVDV) by common antigen-detection tests is negatively affected by colostrum immunity

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## Introduction

Reducing exposure of pregnant cattle to bovine viral diarrhoea virus (BVDV) by early detection and elimination of persistently infected (PI) calves is critical for control and eradication programs. Common test for detection of PI calves such as antigen-capture ELISA (ACE), reverse transcription polymerase chain reaction (RT-PCR), immunohistochemistry (IHC) and the calf-side ELISA snap test (i.e., IDEXX Snap® BVDV antigen test, IDEXX, Fort Collins, CO) are commonly used by producers and veterinarians for the detection of PI cattle. Although most BVDV PI antigen tests are highly sensitive, colostrum immunity may interfere with test performance leading to false negative results. The objective of this study was to identify the most accurate diagnostic test, reliable sample type and ideal time for detecting neonatal PI calves following intake of maternal colostrum.

## Materials and methods

Twelve 18-month-old pregnant Angus-cross heifers were inoculated intranasally with the BVDV 1b strain AU526 between 70 and 89 days of gestation to experimentally induce offspring PI with BVDV. The heifers were monitored daily for abortion until calving. After calving, serum, nasal swabs (NS) and skin (ear notch; EN) samples were collected from newborn calves before colostrum intake (birth), as well as at 12 h, 24 h, 7 d, 14 d and 28 d of life. Two age-matched Angus-cross calves born to BVDV-naïve cows were used as negative controls. At each time point, serum and EN samples were tested for BVDV PI by ACE, RT-PCR, virus isolation (VI) and IDEXX Snap BVDV antigen test. NS samples were tested by ACE, RT-PCR and VI. Additionally, IgG and colostrum derived BVDV neutralizing antibody titers (VNT) were measured in serum. Logistic mixed-effects modeling was used to evaluate the effect of time point, sample type, BVDV antibody titers and their interactions on test positivity. Statistical significance was set to  $P$ -value < 0.05.

## Results

Following abortion in 2 heifers, 10 singleton clinically normal calves were born and confirmed to be BVDV positive on serum and EN samples by ACE, RT-PCR and IDEXX Snap BVDV antigen test. All newborn calves stood within 20 minutes of birth and nursed maternal colostrum within the first hour of life. For all models, the likelihood of finding false negative results was greater within 24 h compared with  $\geq 14$  d, and EN samples were more reliable than serum to detect PI with BVDV at any time point and with any test. A significant interaction between sample type and colostrum-derived VNTs on test positivity was

detected ( $P < 0.05$ ). False negative results in serum, ear notch and nasal swab samples tested by ACE, RT-PCR, IDEXX Snap BVDV antigen test and VI within 24 h ranged from 0% (0/10) to 100% (10/10) depending on the test used. However, at 28 d, serum samples (1/10 tested by IDEXX Snap BVDV antigen test and 2/10 tested by VI) and ear notch samples (1/10 tested by VI) continued to produce false negative results. The probability of detecting false negative results in serum, EN and NS samples by ACE, IDEXX Snap BVDV antigen test and VI was significantly greater in PIs with high levels of colostrum-derived VNTs compared with PIs with decreasing colostrum-derived VNTs ( $P < 0.05$ ). EN and NS samples tested by RT-PCR were more consistent detecting PI with BVDV irrespective of time point and colostrum-derived VNTs. Serum, ear notch and nasal swab samples from negative control calves tested negative for BVDV in every test at every time point.

## Significance

Based on results from this study, screening serum or ear notch samples from individual calves for BVDV PI during the first 24 hours of life through VI, IDEXX Snap BVDV antigen test, ACE or RT-PCR can result in false negative results, and it is not recommended. The interference of colostrum-derived BVDV antibodies in the detection of BVDV PI can be decreased by testing EN from individual calves  $\geq 14$  d with IDEXX Snap BVDV antigen test, ACE or RT-PCR or NS with RT-PCR.

