

# Case report: Detection and management of bovine viral diarrhoea virus type 1b in a large dairy herd

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## Abstract

Bovine viral diarrhoea virus (BVDV) continues to be a challenge for the cattle industry despite routine vaccination that includes killed or modified-live BVDV antigens. At a commercial dairy, 1,081 newborn calves were tested for BVDV antigen by pooled reverse transcription polymerase chain reaction as part of a screening program. Immunohistochemistry confirmed persistent BVDV infections in 13 calves. Ten of the PI calves were available for BVD typing. Both cytopathic and non-cytopathic BVD viruses were isolated. The non-CPE viruses typed as BVD type 1b with a total of 3 different strains found; an identical strain found in 2 calves, another identical strain found in 2 other calves, and a third identical strain found in 3 other calves. A BVDV control program relying solely on BVD vaccination using a modified-live vaccine did not protect calves in this herd from persistent infection with type 1b BVDV. These results demonstrate that control programs that rely only on vaccination to control BVDV without testing for the presence of PI animals are not adequate to control BVD.

**Key words:** bovine viral diarrhoea virus, persistent infection, biosecurity

## Résumé

Le virus de la diarrhée virale bovine (VDVB) demeure toujours un défi pour l'industrie bovine en dépit de la vaccination routinière incluant des antigènes du VDVB inactivés ou atténués. Dans une ferme laitière commerciale, on a testé 1081 veaux nouveau-nés pour des antigènes du VDVB par transcription inverse suivie d'une réaction en chaîne de la polymérase dans le cadre d'un programme de dépistage. L'immunohistochimie a confirmé la présence d'immunotolérance au VDVB chez 13 veaux. Le typage viral a été possible chez dix de ces veaux immunotolérants. On a isolé des virus de la DVB à la fois cytopathogènes et non-cytopathogènes. Les virus non-cytopathogènes typés étaient du type DVB 1b et on a trouvé 3 souches distinctes : une

souche identique trouvée chez 2 veaux, une autre souche identique trouvée chez deux autres veaux et une troisième souche identique trouvée chez 3 autres veaux. Un programme de contrôle du VDVB s'appuyant seulement sur la vaccination contre la DVB avec des vaccins à virus vivants modifiés n'a pas empêché les veaux dans ce troupeau d'acquérir l'immunotolérance contre le type 1b du VDVB. Ces résultats indiquent que des programmes de contrôle qui ne s'appuient seulement que sur la vaccination pour contrôler le VDVB sans dépister les individus immunotolérants ne sont pas adéquats pour contrôler la DVB.

## Introduction

Bovine viral diarrhoea virus (BVDV) is an RNA virus in the *Pestivirus* genera and classified in vitro as cytopathic (CPE) or non-cytopathic (non-CPE) biotypes of BVDV. This classification is important because it is the non-CPE strains that cause persistent infection (PI) in calves born to dams exposed to the virus during the first 125 days of gestation to the pregnant dam.<sup>1,7</sup> A PI calf will have a BVDV infection that their immune system will not recognize as foreign, and therefore will not mount an immune response against the virus. This results in a cycle of BVDV exposure in a herd as a PI calf sheds millions of viral particles in nasal and ocular secretions, urine, and milk.<sup>1</sup> Continual exposure of healthy animals to large amounts of BVDV from a PI animal can cause herd infertility, general immunosuppression resulting in increased risk for secondary diseases, and if the BVDV mutates to a CPE form of the virus, an outbreak of mucosal disease can occur. Vaccination with a CPE strain of BVDV including the 2 genotypes of BVDV, type 1 and 2, has become a standard practice across the cattle industry, and is most often administered as a viral combination product also containing bovine herpes virus-1, bovine respiratory syncytial virus, and parainfluenza-3 virus. Vaccination is only 1 of the 3 parts of a BVD control program that has previously been suggested to include disease surveillance and biosecurity precautions for all introduced cattle.<sup>8</sup>

## Case History

Newborn calves were sampled for BVDV testing as part of the enrollment process for a separate on-farm clinical trial. Jersey (904 calves), Holstein (50 calves), Jersey X Holstein cross (42 calves), and Angus (85 calves) breeds were represented in the calf population. Angus calves were present in this study because Angus embryos were transferred into animals with a low genomic ranking. The Angus calves were raised under the same management program as the dairy-breed calves. On the day of birth a tissue sample was taken, calves were fed colostrum, and then moved into individual hutches where they were maintained for the duration of the BVDV testing procedures. Calves were sampled as they were born over a 4-wk period in November and December 2015.

Newborn dairy calves were tested for BVDV using an ear tissue sample taken at birth with a tissue punch gun,<sup>a</sup> and submitted to a veterinary diagnostic lab<sup>b</sup> for pooled reverse transcription polymerase chain reaction (RT-PCR) BVD testing. When these calves were born, the dairy was utilizing a vaccine protocol that included vaccination with a modified-live bovine viral diarrhea virus vaccine; 2 doses as a calf and another dose pre-breeding, following by annual revaccination when 3 to 4 weeks in milk. The BVDV from the PI calves was typed to better understand the epidemiology of the unexpected finding of PI calves. The dairy described in this case report was an open herd relying only on vaccination for BVDV control, leaving them at a higher risk to develop PI calves and to continue a cycle of BVDV within the population.

## Clinical Findings and Diagnostics

Of the 1,081 samples submitted, 26 tested positive for BVDV using RT-PCR testing. As the dairy did not have a recognized history of PI calves and no observed clinical BVDV, these results were unexpected. Ear tissue from 26 calves initially tested positive, and 14 of 26 were selected for additional testing and were confirmed positive using antigen capture enzyme-linked immunosorbent assay (ELISA) 2 wk later (1.3%). The BVDV PI status was confirmed by a third sample collected from 13 of the 14 animals 2 wks later utilizing immunohistochemistry (IHC) testing.<sup>c</sup> All 13 samples tested by IHC were positive for BVDV.

Three weeks after receiving IHC results, blood samples from 10 PI calves were collected and submitted for viral isolation.<sup>b</sup> Non-CPE BVD was isolated from the buffy coat of all 10 samples, and CPE BVD was additionally isolated from 3 of the buffy coat samples. The isolated BVD viruses were submitted for typing<sup>d</sup> based on phylogenetic analysis. Typing was based on comparison of sequences amplified by PCR from the 5' untranslated region as described previously.<sup>1</sup>

Samples from all of the 14 selected PI calves were not submitted for BVDV typing because 4 calves died while waiting for preliminary test results. Three of the calves that died were submitted to the veterinary diagnostic lab,<sup>b</sup> and BVDV

was detected via PCR in lung tissue. The fourth calf was not submitted for examination, but was positive on 3 previous BVDV tests, thereby confirming that it was PI with the virus. The death of the 4 PI calves was not unexpected, as McDaniel and co-workers reported earlier that 7 of 15 PI dairy bull calves died within 2 wks after initial testing using IHC assay.<sup>6</sup>

The 14 confirmed PI calves were all Jersey calves from Jersey dams. The Jersey breed was the most common breed on the dairy, and in the calves tested for BVDV as part of the pre-trial screening procedures. Dams of the 14 PI calves were all first-lactation heifers, and a tissue sample was collected from each of these dams for BVD testing via antigen capture ELISA. All 14 dams were negative for BVDV.

## Outcome

The PI calves were removed from this herd after the blood sample was taken for BVDV typing. The calves were 6 to 8 weeks of age at the time of removal from the herd, and had been housed in individual hutches at the calf-raising operation throughout the confirmatory testing period. With the confirmation of PI status and results of BVDV typing, dairy management decided to reevaluate their BVDV control program to include vaccination, surveillance, and biosecurity. The dairy historically used a modified-live 5-way viral vaccine containing BVD type 1a and type 2 antigens, and heifers received 3 doses of vaccine before entering the breeding pen. Cows were vaccinated annually in subsequent lactations. Due to the finding of BVDV type 1b in all PI calves, the management team decided to maintain the same vaccination schedule, but switched to a modified-live vaccine with published results showing protection from BVDV1b.<sup>5,11,12</sup> Testing of newborn heifer calves for PI status was also added to the herd protocol, which was a convenient change because the same tissue sample could be used for both BVD PI testing and genomic testing.

## Discussion

It is a common misunderstanding among producers that the use of BVDV vaccine and the absence of clinical disease is evidence that a farm is free of BVDV. As this case illustrates, just over 1% of all newborn calves tested were persistently infected with BVDV, despite vaccination and no recognized disease. As PI cattle are extremely efficient vectors for spreading BVDV, the presence of this number of PI cattle insures that BVDV will remain in circulation if they are not removed from the herd. BVDV shed into the environment by PI cattle will infect healthy calves and increase the risk for respiratory disease, as well as cause overall immunosuppression.<sup>1,7</sup> Further, exposure to a PI animal elicits an immune response that has a physiological cost; constant exposure also impacts well-being. This justifies testing calves for BVDV that are enrolled in clinical trials to reduce the risk of confounding BVDV and immunosuppression. Infection

with BVDV reduces circulating lymphocytes and depletes lymphoid tissue,<sup>1,2</sup> which can have detrimental outcomes in a young calf population when pathogens and environmental and nutritional stressors are challenging the calves' immune system. Therefore, it is in the best interest of calf raisers to remove PIs from the population early in the calf's life.

Persistently infected calves had not been identified on the study farm previously, and the results were unexpected. This was the reason for the multiple confirmatory tests, including antigen-capture ELISA and IHC. Calves positive on the RT-PCR test were not removed from the population while waiting for confirmatory results, exposing neighboring calves to the virus. In the future, all calves on the farm with an initial positive BVDV test will be physically removed from the healthy population while waiting for a confirmatory test. It was not in the best interest of the farm's calf population to keep PI calves for such a long period of time, but in this case it did provide the opportunity for BVDV typing.

BVD type 1b virus has previously been reported as the most common strain found in the field, and is the most prevalent subtype found in PI calves.<sup>4,9,10</sup> This was true for the BVDV typed in the 10 calves sampled on the dairy in the current case report. A recent case report from a semi-closed beef herd diagnosed 2 PI animals that typed with the same strain of BVD type 1b.<sup>3</sup> Three different BVDV type 1b strains were isolated among the 10 calves on the current farm, which indicates that there were multiple introductions of different BVDV1b strains into the operation. There was also CPE BVDV isolated in 3 of the calves, which was most likely from a recent vaccination. According to the dairy's vaccine protocol, all of the calves would have received their first parental modified-live BVD vaccine before the final blood sample was taken for BVDV typing. The CPE BVDV found as a mixed infection with non-CPE virus in the PI calves was not genotyped because our interest was only in the non-CPE virus causing persistent infection.

The PI calves from this dairy were born to dams that had been vaccinated a minimum of 3 times with a modified-live BVDV type 1a and type 2 vaccine before breeding. This did not protect pregnant heifers from in utero infection with BVDV type 1b that was circulating in the herd, and subsequent development of PI in their offspring. There are multiple reasons for this failure: 1) the vaccine might not have been handled or administered correctly; 2) the animals did not respond to the vaccine; 3) the strains of BVD in the vaccine did not offer cross protection; or 4) there was an overwhelming BVD challenge. The reproductive rate ( $R$ ) of a pathogen is a calculated number describing the number of animals 1 infected animal can transmit disease to, and  $R$  for BVD will change depending on the number of PI animals in a herd.<sup>8</sup> A highly contagious disease with greater pathogen challenge, for example when PIs are present in a population, will have a high  $R$  value, meaning 1 infected animal can infect a large number of herdmates. In cattle practice we rely on herd immunity for population medicine protection because

not all animals will respond to a vaccine. As the number of PI animals in a herd increases,  $R$  will increase and reliance on herd immunity will become more of a challenge because 100% of the animals will need to have a protective immune response to vaccine. When PI animals are present in a herd, the viral challenge is too large and overwhelms the strategy to control disease solely with vaccination. To reduce  $R$  for BVDV in a herd, reducing exposure by removing PI animals will decrease the disease challenge, reduce  $R$ , and improve disease control through vaccination.

Retraining of employees and inspection of vaccination equipment was performed on this dairy, as well as ensuring the vaccine was kept at the correct temperature during storage and handling. The choice of the specific vaccine used on the dairy was also evaluated, and a choice was made to use an alternate vaccine that had been shown in published studies to provide 96% protection against BVDV1b fetal infection.<sup>5,11,12</sup>

As illustrated by this case, vaccination alone is inadequate for a BVDV control program. Surveillance testing and biosecurity also need to be a part of the 3-pronged approach to BVD control. Testing of newborn calves will allow producers to find PI calves early and remove them from the herd and calf population. Testing should also be done on all incoming purchased cattle to ensure adult PI cattle are not introduced into the herd. With PCR technology and pooling of tissue samples, diagnostics are very economical, ranging from \$3 to \$10/animal depending on the volume of samples submitted. With the increased adoption of genomic testing, BVDV testing can be done on the same sample to ensure an animal is not PI before investing in genomic testing.

## Conclusion

The finding of BVDV type 1b PI calves in a herd that was well vaccinated with a modified-live BVDV type 1a and type 2 vaccine demonstrates that vaccination is not the only tool in a BVDV control plan. Biosecurity and disease surveillance, in addition to vaccination, are needed to control BVDV on farms.

## Endnotes

<sup>a</sup>Allflex TSU Applicator, ALLFLEX USA, INC, DFW Airport, TX

<sup>b</sup>Oregon State Veterinary Diagnostic Lab, Corvallis, OR

<sup>c</sup>Iowa State Veterinary Diagnostic Lab, Ames, IA

<sup>d</sup>National Animal Disease Center, United States Department of Agriculture, Agricultural Research Service, Ames, IA

## Acknowledgements

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# Vira Shield® Research Brief 1

## Impact of Vaccination with an Inactivated or Modified-Live Viral Vaccine on Reproduction<sup>1</sup>



### Study overview

A study was conducted to determine how vaccination with an inactivated or modified-live viral (MLV) vaccine would impact reproductive parameters in beef cows.

### Key study results

- Treatment of cows and heifers with Bovi-Shield® during pre-breeding decreased pregnancy success compared to treatment with Vira Shield®
- Treatment with Bovi-Shield tended to reduce the percentage of cows that calved in the first 21 days of the calving season compared to Vira Shield
  - This decrease in calving percent remained over the entire calving season
  - Delaying when the animal conceives/calves can have implications on the success of a cow/calf operation, including pounds of calf weaned, rebreeding and longevity in the herd

### Background information

#### TRIAL DESIGN

- Total head — 1,304\*
- Nine herds
  - Blocked by age and calving date in each herd
- Three treatments
  - Control
  - MLV (Bovi-Shield Gold FP 5 L5 HB)
  - Inactivated (Vira Shield 6 L5 HB)

#### STATISTICS

- Data were analyzed using the GLIMMIX procedure in SAS — treatment, day postpartum and the treatment by day postpartum interaction were analyzed
- No treatment by year interaction ( $P > 0.66$ )
  - Herd was included as a random variable to account for unknown differences between herd and years

### Study results

Chart 1. Pregnancy success

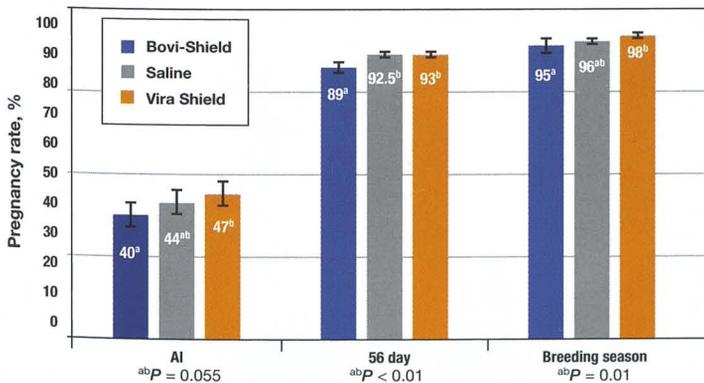


Chart 2. Calving by group

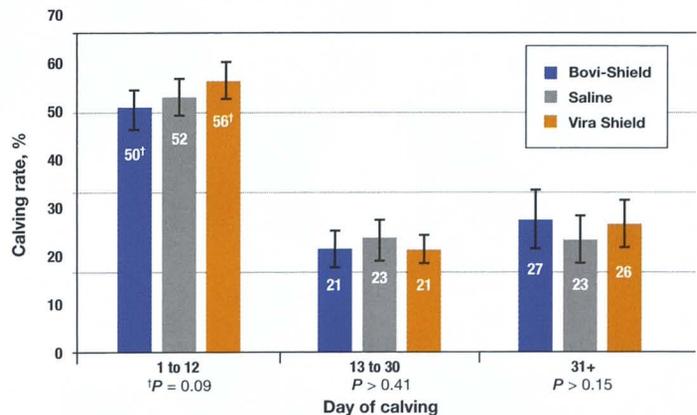
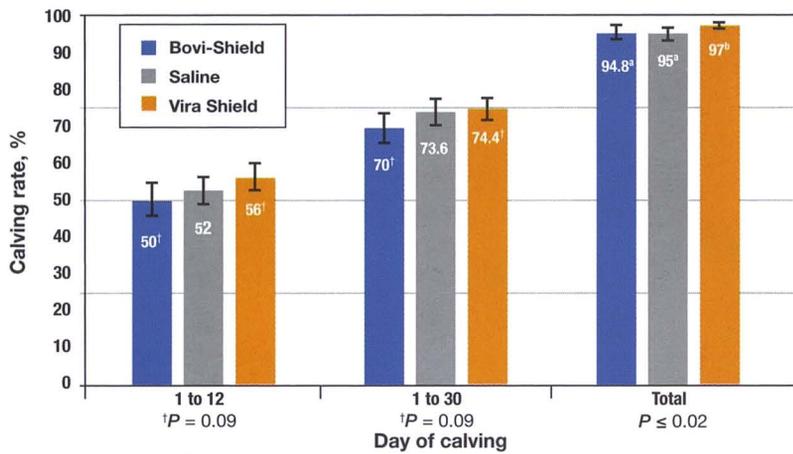


Chart 3. Cumulative calving data



## Conclusions

- When evaluating reproductive vaccines, it's critical to consider the impact of decreased calving on the success of the cow/calf herd
- Your vaccine program may have impacts on estrus synchronization (ES) and timed artificial insemination (TAI), which in turn impact the economic efficiency of your operation
  - Potential impacts of ES and TAI include shortened calving season, increased calf uniformity, more calves born earlier in the season, enhanced preweaning growth and heavier calves at weaning<sup>2</sup>
  - There is a nearly \$50/hd advantage for managing ES and TAI on your operation<sup>2</sup>
- Ensure that you're getting the most out of your breeding program by maximizing your reproductive vaccine program. To learn more about evaluating your vaccine program and how Vira Shield can help improve reproductive parameters, reach out to your veterinarian or Elanco sales representative

The label contains complete use information, including cautions and warnings. Always read, understand and follow the label and use directions.

\*1,436 animals entered the initial study, but 132 were sold prior to calving for non-reproductive purposes.

<sup>1</sup>Perry, G., Larimore, E., et al. 2016. "Influence of vaccination with an inactivated or modified-live viral reproductive vaccine on reproductive parameters in beef cows." South Dakota State University.

<sup>2</sup>Rodgers, J.L., Bird, S.L., et al. 2012. "An economic evaluation of estrus synchronization and timed artificial insemination in suckled beef cows." J Anim Sci. Vol 90(11):4055-62.

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