Onset and Duration of Transient Infections Among Antibody- Diverse Beef Calves Exposed to a Bovine Viral Diarrhea Virus Persistently Infected Calf

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ABSTRACT

Persistently infected (PI) cattle are the reservoir of bovine viral diarrhea virus (BVDV), yet data describing BVDV transient infections (TI) among non-PI populations are minimal. Study objectives consisted of: 1. Estimating the onset and duration of TI based on serum VI and rRT-PCR, and 2. Determination of the potential of TI cattle to shed BVDV. Two 21-day studies were performed where one PI calf was commingled with a confirmed non-PI cattle population with heterogeneous BVDV antibody status (n=12 and n=15, respectively). After PI exposure, virus isolation on serum and nasal swabs failed to detect BVDV among non-PI cattle. Despite minimal disease (n=1), BVDV transmission occurred as 78% (n=21) of non-PI calves displayed a four-fold rise in BVDV antibody titers, 81.5% (n=22) displayed a transient positive serum BVDV rRT-PCR outcome, and 74.1% (n=20) displayed a transient positive rRT-PCR result on nasal swabs. Median days of positive serum rRT-PCR onset and duration were 10.0 (range: 6-21) and 3.0 (range: 1-9) days, respectively. These data suggest that non-PI cattle can become TI with minimal clinical disease while possessing the potential to transmit BVDV. The speed with which exposed cattle become transiently infected and their potential ability to shed the virus may

impact design and implementation of BVDV control programs.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) possesses world-wide prevalence and is a significant source of production loss in beef and dairy production systems.[1-9] The viral reservoir is cattle persistently infected (PI) with BVDV, which are lifelong shedders of virus, thereby increasing the efficiency of viral transmission compared to non-PI cattle whom are transiently infected (TI) with the virus.[2-4, 10] Cattle PI with BVDV have previously been observed to possess serum viremias,[2,3,11] which have been observed to approximate the viral concentrations in nasal secretions, urine, feces, and uterine secretions.[2,12] Primary BVDV prevention and control strategies are targeted toward either increasing population immunity through vaccination or decreasing pathogen exposure by removing PI cattle and prohibiting their entry into the herd.[13] Consequently, several diagnostic testing strategies,[14-16] vaccination programs,[17-19] and biosecurity protocols[7,20,21] have been suggested to minimize the impact of BVDV.

The onset, duration, and clinical effects among antibody-negative non-PI cattle when experimentally (intranasal or intramuscular inoculation) or naturally infected (by means of PI exposure) with BVDV have been studied.[22-26] These findings suggest that BVDV antibody-negative cattle transiently infected (TI) with BVDV are observed to be viremic (buffy coat and serum) between days 3-15 post-exposure,[23,24] display widespread viral distribution to organ systems,[23] may shed virus in nasal secretions,[26] and display variable clinical outcomes.[22-24]

Despite the knowledge gained from the aforementioned research, few studies have implemented models utilizing PI cattle as the mode of infection among clusters of non-PI cattle possessing diverse levels of BVDV antibody titers typical of cattle in commercial production operations.[27-32] Determining the time necessary for a non-PI calf to develop BVDV infection post-exposure to a PI calf is important, as this may influence the design and implementation of BVDV control programs (eg, length of quarantine periods in cow-calf settings, and predicting morbidity in newly arrived feedlot cattle). Additionally, the duration of the transient infection and the capability of these TI animals to shed BVDV may also influence the magnitude of detrimental health impacts of BVDV in the population.

The over-arching goal of this study was to better characterize the BVDV TI among antibody diverse non-PI cattle (commonly housed in typical beef production settings). Specific objectives of this study were subsequently two-fold: 1. To describe the time to onset and duration of transient infection in a non-PI study population based on serum VI and serum rRT-PCR, and 2. To determine potential for shedding the virus in nasal secretions (by VI and rRT-PCR). A greater understanding of viral transmission in a beef cattle production setting facilitates knowledge-based modifications to current BVDV preventative and control programs.

MATERIALS AND METHODS

All animals were handled in accordance with a protocol approved by the Kansas State University Animal Care and Use Committee. The two studies were performed by introducing a known PI calf into a confirmed BVDV antigen-negative population of cattle. The exposure period in each study lasted 21 days and multiple samples were collected throughout the studies.

In study one, 12 mixed-breed beef steer calves, averaging 184.2 kg, were procured through a livestock auction market in northeast Kansas. The health history of all calves was unknown at the time of purchase. Fifteen days prior to PI calf introduction (day -15), all calves were delivered to a Kansas State University (KSU) facility consisting of an open-air dirt-floor pen with a total area of 544 m2 (5859 ft2) that was isolated from other cattle populations. Twenty-four hours after arrival (day -14), all calves received a two milliliter (mL) multi-valent clostridial vaccine[a], an injectable dewormer[b], and a

topical insecticide[c]. Additionally, a commercial ear notch device[d] was used to collect a standard triangular ear biopsy, measuring approximately one cm2, from each individual calf. Blood was collected in one 6 mL red top clot tube and one 4 mL EDTA tube from each individual by jugular venipuncture.

A second blood sample collected in the same manner was attained on day -4. In study two, 15 mixed breed beef bull calves, averaging 241.8 kg, were procured through the same industry channels and housed in the same pen as calves in study one. On day -16, all bulls were surgically dehorned and castrated by standard industry approved methods and received a two mL multivalent clostridial vaccine[a], an injectable dewormer[b], and a topical insecticide[c]. Additionally, a commercial ear notch device[d] was used to collect a standard triangular ear biopsy, measuring approximately one cm2, from each individual calf. Blood was collected in the same manner as in study one. A second blood sample was collected on day -6.

In both studies, the inclusion criteria for the BVDV-negative population was a negative antigen capture ELISA (ACE) test on ear tissue (days -14 and -16 in studies one and two, respectively) and two negative serum VI tests (days -14 and -4, and days -16 and -6, in studies one and two, respectively). All tests were performed at the Kansas State Veterinary Diagnostic Laboratory (KSVDL).

In both studies, the inclusion criteria for the BVDV PI animal included two positive ACE (skin) and VI tests (serum) two weeks apart by the KSVDL. In study one, one mixed breed beef steer calf was identified as PI during a previous KSU research study. This calf weighed approximately 181.4 kg on day 0 of study one and had been isolated in an off-site facility prior to introduction. In study two, the PI calf had been identified as potentially PI by a commercial feedlot in central Kansas. This calf weighed approximately 363.6 kg on day 0 of study 2 and had remained at the aforementioned feedlot prior to introduction to the non-PI study population. Genotyping and subtyping analysis of both PI calves was completed by the KSVDL.

On day 0 of both studies, the PI calf was commingled with the non-PI population. Blood was collected in two 5 mL red top clot tubes and two 4 mL EDTA tubes from each individual calf by jugular venipuncture. One ear biopsy measuring approximately one cm^2 was collected from each individual calf and placed in sterile plastic tubes. One sterile nasal swabe was inserted approximately 10 cm (4 inches) into the nostril, rested against the mucosal surface, and gently rubbed against the nasal mucosa in a circumferential pattern five times. Blood was collected in the same manner every other day for the duration of both studies. Due to the frequency of jugular venipuncture, both jugular grooves were clipped prior to day 0, the side of blood collection was alternated between each timepoint, and the site of injection was cleansed with chlorhexidine and alcohol prior to needle insertion to minimize the risk of phlebitis. Ear biopsies and nasal swabs were collected every 4 days for the duration of both studies. Each PI calf remained with its respective population for 21 days after introduction.

Within 1 hour of each sample collection, blood tubes were centrifuged at 2350 rcf (g) for 10 minutes, and serum, plasma, and buffy coat were harvested in a traditional manner. Buffy coat samples in study one were not collected until day 12 of the study; however, buffy coat was collected for the entirety of study two. All specimens (serum, plasma, buffy coat, skin biopsies, and nasal swab samples) were stored at -80°C until further diagnostics were performed.

Throughout both studies, animal caretak-

ers offered a total mixed ration equating to 1.5% body weight (DMI) twice daily while grass hay and fresh water was offered *ad libitum*. All calves were observed twice daily by a veterinarian to monitor individual health status. Rectal temperatures and further examination of individuals suspected of clinical disease were attained prior to treatment. Animals displaying clinical signs of respiratory disease and rectal temperatures ≥ 40° C were classified as individuals experiencing bovine respiratory disease (BRD) and were treated following a predesigned treatment protocol. Cattle meeting the treatment criteria for BRD were administered tulathromycin[e] (2.5 mg/kg SC), florfenicol[f] (40mg/kg, SC), and oxytetracycline[g] (20mg/kg, SC) for the first, second, and third treatments, respectively. The pen was idle for approximately 2 months between the two studies, and during this time, the watering devices and feed bunks were cleansed and disinfected, and the pen floor was scraped and all organic material was removed.

On the last day of both studies (day 21), the PI calf was removed from the pen. The remaining non-PI calves were maintained at the study site until negative VI tests (on serum) were confirmed on all non-PI cattle. Upon a cumulative VI negative status, all non-PI animals left the facility on the same day.

Serology

Serum neutralization assays for type one, type two, and the homologous PI BVDV were performed at the KSVDL using previously described assay.[33] Antibody titers were quantified from serum samples from all calves from both studies on days 0 and 21.

Virus Isolation

All quantitative VI attempts from both studies were conducted at the KSVDL. Samples for quantitative VI were subjected to four ten-fold serial dilutions, and two hundred µLs of each respective sample/dilution were then added to freshly seeded embryonic bovine lung (EBL) cell cultures in 96 well micro-titer plates. The assay plates were then incubated for 48 hours at 37°C in 4.5% CO_2. After incubation, the media in each plate was discarded, and the cells were washed with phosphate buffered saline (PBS) and fixed in an 80% aqueous acetone solution for 10 minutes. After discarding the acetone, the plates were allowed to dry at room temperature. An indirect fluorescent antibody test was used to detect BVD infected cells. Fifty µLs of the primary anti-BVDV monoclonal antibody (D89)34 wereas added to each well and allowed to incubate for 30 minutes. The primary antibody[h] was then discarded, and the plate washed with PBS and 50 µLs of the FITC labeled secondary antibody[i] were added to each well, and allowed to incubate for 30 minutes.

After incubation, the secondary antibody was then discarded and the plate was washed with PBS and fifty µLs of 50% glycerol were added to each well prior to reading the plates. The CCID-50 was calculated by the method of Spearman and Karber as previously described method.[35] In both studies, if virus could not be isolated from serum of non-PI cattle, VI was performed on available buffy coat samples from one randomly selected non-PI calf (of positive PCR status) in order to isolate virus for genotyping and sequencing. Subsequent comparison between viral strains determined if BVDV isolated from non-PI cattle originated from

the respective PI calf in each study (data not shown).

Real-time Reverse Transcriptase Polymerase Chain Reaction

RNA extraction of serum samples

One hundred µLs of serum from all calves were utilized for extraction of viral RNA for use in quantitative real-time RT-PCR (rRT-PCR) analysis. Magnetic bead-based nucleic acid isolation method was used to extract viral RNA using a commercial isolation kit[j] by following the manufacturer's instructions. A commercial processor and protocol[k,l] was utilized for RNA extraction with an elution volume of 90 µL. The RNA from the samples was stored at -80° C until use.

RNA extraction of nasal swab samples

The nasal swab samples were vortexed at low speed for 10 seconds. One hundred µLs of the sample was then utilized for RNA extraction by following the procedure as described above.

Quantitative real-time RT-PCR (rRT-PCR) analysis

The rRT-PCR assay was performed using a commercial test kit[m] to identify BVDV RNA in the serum samples. The qRT-PCR analysis was performed in a 25 µL reaction volume containing 2X RT-PCR buffer (12.5 µL), 25X BVDV primer probe mix (1 µL), 25X RT-PCR Enzyme Mix (1 µL), sample template (8 µL), and nuclease free water (2.5 µL). The PCR analysis also included appropriate positive and negative controls. Commercial templates[n] served as positive controls for the PCR reaction. A reaction containing all the reagents but no template was also included to serve as a negative control. The assay was performed in a commercial system[o] under standard run mode. Thermal profile for the PCR reaction included an initial cDNA synthesis step of 10 min at 45 °C followed by RT inactivation and initial denaturation step for 10 min at 95 °C, then 40 cycles of 95 °C for 15 sec, and 60 °C for 45 seconds. The rRT-PCR data analysis was performed with the help of commercial software[p]. Prior to the data analysis, the rRT-PCR run was validated by verifying the cycle threshold (Ct) values of positive and negative controls reactions (BVDV RNA target, $Ct \leq 28$; Xeno RNA control target, $Ct \leq 31$; and no-template control reaction, no signal or $Ct = 40$). The results were then interpreted based on Ct distributions per the manufacturer's recommendations[l], where $Ct \leq 38$ were considered positive, Ct values between 38-40 were considered suspect results, and $Ct > 40$ were considered negative.

In both studies, the case definition for an animal at risk for becoming rRT-PCR positive included the timeframe from when the PI calf was introduced to the BVDV negative subpopulation (day 0) up until a positive test result was first confirmed among individual cattle. Therefore, when/if the calf displayed a positive PCR outcome, that respective calf was no longer at risk for positive PCR status. Given that serum was collected every other day, the duration of positive PCR status was defined from the initial day of positive PCR status to the day prior to the first negative PCR test. For example, if a calf had positive serum PCR results on days 8 and 10 but tested negative on day 12, it would be classified as PCR positive for a duration of three days (days 8 – 11).

Statistical Analysis

The individual calf was the experimental unit throughout the data analysis. Descriptive statistics and graphical procedures for all measured variables were obtained by a commercial software package[q]. Assessment for normality among continuous distributions (day of TI onset and duration of TI)

m. BVD RNA Test kit, Applied Biosystems, Austin, TX
n. 25X BVDV Control RNA and Xeno™ RNA control, Applied Biosystems, Austin, TX
o. 7500 Fast Real-Time System, Applied Biosystems, Austin, TX
p. 7500 SDS (v1.4) software, Applied Biosystems, Austin, TX
q. Microsoft Excel®, 2003, Redmond, WA
r. Proc Univariate, SAS (version 9.1), Cary, NC

Table 1: *Individual animal outcomes among the non-persistently infected calf population across studies one (n=12) and two (n=15). Serum and nasal swabs were collected every other day and every fourth day (including day 21), respectively. Cumulative nasal swab PCR status reflects the individual animal status at day 21 (post PI introduction).*

Calf	Study	Antibody titer to homologous PI BVDV strain (Day 0)	Antibody titer to homologous PI BVDV strain (Day 21)	Day of positive PCR Onset	Duration of positive PCR status (days)	Cumulative Nasal Swab PCR status
1	1	0	1024	8	2	+
2	1	0	2048	10	3	-
3	1	0	2048	8	7	-
4	1	8	512	10	2	+
5	1	8	512	10	3	-
6	1	16	2048	8	2	+
7	1	16	1024	16	2	+
8	1	16	512	6	3	-
9	1	16	512	NA*	NA	+
10	1	64	5000	6	5	+
11	1	64	5000	NA	NA	+
12	1	128	2048	6	3	+
1	2	0	2	14	2	+
2	2	0	4	12	3	+
3	2	0	8	10	3	+
4	2	0	16	6	3	+
5	2	0	32	8	3	+
6	2	0	32	8	7	+
7	2	0	32	8	9	-
8	2	0	128	10	9	+
9	2	0	128	10	9	+
10	2	8	128	14	2	-
11	2	8	256	NA	NA	+
12	2	8	256	NA	NA	+
13	2	16	256	12	2	-
14	2	32	512	21	1	+
15	2	256	1024	NA	NA	+

was performed by visual assessment as well as by formal statistical methods[r]. As per standard practice, if continuous data were determined not to be normally distributed, the median value and range of data (not the average and standard error) were implemented as the necessary point estimate and description of data variation, respectively.

RESULTS

All pre-day 0 ear biopsies and serum samples from the non-PI calves in both studies were negative for BVDV. The status of both PI animals was confirmed through ACE (on ear tissue) and VI (on serum). In study one, a cumulative BRD morbidity risk of 8.3% (1/12) was observed among the non-PI population while no morbidity (0%) was observed in study two. On day 0 25% (n=3) of non-PI calves enrolled in study one were negative for type one and type two BVDV antibodies as well as antibodies to the homologous strain infecting the PI calf. The remaining non-PI population (n=9) possessed antibodies to both BVDV genotypes as well as the homologous PI strain (Table 1). On day 21 post-exposure, 41.8% (n=5), 16.7% (n=2), and 100% (n=12) of the non-PI calves displayed a four-fold rise in serum antibodies to BVDV type one, type two, and the homologous PI strain, respectively.

At arrival, 6.7% (n=1) of non-PI calves enrolled in study two were negative for both type one and type two BVDV antibodies as well as antibodies to the homologous strain possessed by the PI calf (Table 1). Of the remaining non-PI population in study two, 40% (n=6) possessed antibodies to type one, type two, and the homologous PI virus, 20% (n=3) possessed antibodies to both BVDV genotypes but not the homologous PI virus, and 33% (n=5) possessed only BVDV type two titers. By day 21 of study two, 26.7% (n=4), 26.7% (n=4), and 60% (n=9) of the non-PI calves displayed a four-fold rise in serum antibodies to BVDV type one, type two, and the homologous PI strain, respectively.

All serum VI tests for BVDV from non-PI calves were negative at all sampling points on both studies. Conversely, serum VI tests from both PI calves in each respective study were positive on every day of sample collection with a range of serum BVDV concentrations of $10^{1.70}$ – $10^{3.70}$ CCID-50/mL of serum. Similarly, VI performed on nasal swabs did not identify BVDV among the non-PI population; however, nasal swabs collected from the PI calves were positive by VI on days 16 and 20 in study one and days 0, 4, 8, 12, 16, and 21 in study two.

Due to the inability of VI to isolate BVDV in serum in either study from the non-PI population, BVDV was successfully isolated from one buffy coat sample (the classical gold standard) of one randomly selected TI calf for genotyping and sequencing. As expected, virus was detected and genetic sequencing determined that the homologous BVDV from each respective PI calf were genetically different; however, each was classified as BVDV type 1b. Additionally, isolated BVDV from the aforementioned TI cattle was homogenous with virus isolated from each respective PI calf in both studies (data not shown).

Given the PCR manufacturer's recommendation of Ct values indicating positive test results (Ct ≤ 38), 83.3% (n=10) and 80% (n=12) of the non-PI calves were serum PCR positive for BVDV nucleic acid at least once during studies one and two, respectively (Table I). The remaining calves in studies one (n=2) and two (n=3) were found to be PCR negative (Ct > 40) at all collection points. However, 80% (n=4) of these PCR negative non-PI calves displayed a four-fold increase in serum antibodies to the homologous BVDV PI strain in their respective study indicating that they were indeed TI.

Cumulatively, across both studies, 77.8% (14/18) and 88.9% (8/9) of non-PI calves with and without antibodies to the homologous PI strain of BVDV prior to exposure to the PI calf, respectively, became PCR positive to BVDV at some point during their respective study (Table 1). The day of initial positive PCR status and subsequent duration

were both determined not to be normally distributed. Therefore, across both studies, the median day of the initial positive BVDV PCR diagnosis was day 10.0 (range: 6 – 21 days) post-exposure to the PI calf while the median duration of positive BVDV PCR status was 3.0 days (range; 1 – 9 days) (Table 1).

In studies one and two, respectively, nasal swabs from 66.7% (8/12) and 80% (12/15) of non-PI cattle were found to be PCR-positive for BVDV at least once (Table 1). Among the non-PI calves that tested PCR positive on serum for BVDV, 70% (7/10) of calves in study one and 91.7% (11/12) of calves in study two had positive nasal swabs. Due to the infrequency of nasal swab collection, the time of onset and duration of PCR positive status of nasal swabs were not estimated.

DISCUSSION

The results of this study indicate that BVDV is easily and rapidly transmitted from an individual recognized as PI with BVDV to non-PI individuals (demonstrated by rRT-PCR positive tests and a 4-fold rise in BVDV antibodies to the homologous PI virus over a 21-day timeframe). However, in this study, VI on serum (and nasal swabs) failed to detect BVDV among all TI cattle. Results of BVDV rRT-PCR indicates that BVDV nucleic acid can be identified in the serum of commingled cattle beginning approximately 6 days post-exposure to the PI calf and remaining for approximately 3-4 days in duration. These data also indicate that BVDV can be found in nasal discharge of TI cattle. The experimental design demonstrated in this study including the natural method of exposure to BVDV by a PI calf, the procurement methods of the non-PI calves (ie, livestock market derived), and the broad distribution of individual BVDV antibody titers at arrival parallels typical scenarios encountered in stocker, backgrounding, and feedlot production systems throughout the US.[29-32, 36]

Despite the lack of positive serum VI findings among non-PI cattle in the present study, these data suggest that cattle exposed to a PI BVDV individual can rapidly become TI with BVDV (as assessed by rRT-PCR on serum samples and indicated by a four-fold rise in BVDV antibody titers to the homologous PI strain) while lacking positive VI status in serum and discernible clinical disease. These findings are complemented by positive rRT-PCR outcomes on nasal swabs denoting that BVDV nucleic acid was circulating in the population, thereby serving as a potential source for further transmission to co-mingled cohorts. Furthermore, the positive VI on buffy coat samples from selected TI calves matched the BVDV sequenced from the respective PI calves indicating that nucleic acid identified by rRT-PCR did originate from infectious virus shed by the PI calf in each study.

To our knowledge, this is the first report characterizing this form of TI of BVDV by natural exposure by co-mingling of non-PI individuals with PI individuals. Previous studies depicting transient infections from natural BVDV exposure have isolated BVDV in serum and buffy coat by VI methods.[25-28] However, our findings of a four-fold rise in serum antibody titers to the homologous BVDV strain shed by the PI calf (experienced by a majority of the non-PI sample population) coupled with positive rRT-PCR results among serum samples and nasal swabs is clearly indicative of BVDV circulating within in each group of non-PI calves.

The rRT-PCR results in the current study indicate that BVDV nucleic acid can be identified by rRT-PCR in serum from days 6 – 21 post-PI exposure with subsequent duration of positive rRT-PCR results for approximately 3 – 4 days. Brownlie et al (1987) described that BVDV antibody-negative non-PI cattle were viremic from days 4 – 7 post-BVDV.[22] Unfortunately, this report does not provide the methodology of how BVDV exposure occurred (experimental or natural exposure) or how viremia was diagnosed (compared to antibody production and rRT-PCR findings in the present

study). Nonetheless, it does demonstrate a similar duration of TI (~ 3 – 4 days) with a tighter timeframe of TI onset than what was observed in the present study.

Conversely, previous authors have observed that BVDV could be isolated from the buffy coat of antibody diverse groups of non-PI calves from days 6 – 35 when exposed to PI cattle.[27,28] These previous observations suggest that an immunologically diverse population of cattle (as in the present study) may display a high degree of variability in the timing of onset of BVDV potentially attributed to the pathogenicity of the virus shed by the PI animal, overall herd immunity, or by behavioral dynamics (ie, differences in the rate of intermingling) that differ among populations.

The nasal swab findings from the present study concur with prior research which observed a lack of BVDV detection (by VI) in nasal swab samples among non-PI cattle when exposed to PI calves.[25,27,28] However, a previous study observed the ability of VI to identify BVDV shedding in nasal secretions among antibody-negative TI cattle upon prolonged exposure to a PI calf.[26] Although the ability of TI cattle to infect non-PI cohorts was not measured in the present study, positive rRT-PCR results on nasal swab samples from the non-PI population (with variable levels of BVDV antibodies to the homologous PI strain) suggest that BVDV (or at least viral nucleic acid) was being shed in nasal secretions potentially affording TI cattle the capability of transmitting the virus. Previous studies have observed that TI cattle can potentially transmit BVDV to susceptible populations over long durations with and without the presence of PI cattle.[26,37,38] These data suggest that BVDV transmission may not only be associated with virus excreted from PI cattle, but that TI cattle may contribute to viral transmission. Nonetheless, further research is warranted to determine the role that TI cattle play in the transmission of BVDV within production systems.

Our data indicates that VI performed on serum and nasal swabs may lack the sensitivity for diagnosing many transient BVDV infections. These results also indicate that we as veterinary professionals might need to reassess our timetable for testing to define PI BVDV individuals if the rationale for PI testing is to limit BVDV transmission to a naive or susceptible population. Current testing is often completed after extensive co-mingling of animals of unknown status in stocker, back-grounding, and feedlot production systems. These results would indicate that exposure to PI individuals in these scenarios might be too late to avoid the consequences of BVDV transmission and infection on other co-mingled individuals. This approach might indicate and justify earlier PI-BVDV testing (ie, at the cow-calf production level).

Despite a lack of positive VI findings on serum and nasal swab samples among the non-PI sample population, numerous positive serum and nasal rRT-PCR results strongly supports our conclusions that BVDV infections among non-PI cattle in this study due to co-mingling with a PI individual. Cattle became TI with BVDV soon after exposure to PI and maintained the infection for several days. These transient infections with BVDV may contribute to enhanced BVDV transmission to additional co-mingled animals under these conditions. However, the ability of TI cattle to infect non-TI cattle was not evaluated in the current study.

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