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T. E. Wittum
Ohio State University, wittum.1@osu.edu

Dale M. Grotelueschen
Panhandle Research and Extension Center, University of Nebraska–Lincoln, dgrotelueschen@unl.edu

K. V. Brock
Auburn University, brockkv@auburn.edu

William G. Kvasnicka
University of Nevada-Reno, bkvasnicka@cabnr.unr.edu

J. G. Floyd
Auburn University

See next page for additional authors

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Persistent Bovine Viral Diarrhea Virus Infection in US Beef Herds

T. E. Wittum,[a] D. M. Grotelueschen,[b] K. V. Brock,[c] W. G. Kvasnicka,[d] J. G. Floyd,[e] C. L. Kelling,[f] and K. G. Odde[g]

[a] Department of Veterinary Preventive Medicine, The Ohio State University, 1900 Coffey Road, Columbus, OH 43210, USA
[b] Department of Veterinary and Biomedical Sciences, Panhandle Research and Extension Center, University of Nebraska–Lincoln, Scottsbluff, NE 69361, USA
[c] Department of Pathobiology, Auburn University, Auburn, AL 36849, USA
[d] Cooperative Extension Service, University of Nevada–Reno, Reno, NV 89557, USA
[e] Cooperative Extension Service, Auburn University, Auburn, AL 36849, USA
[f] Department of Veterinary and Biomedical Sciences, University of Nebraska–Lincoln, Lincoln, NE 68583, USA
[g] Pfizer Animal Health, Pollock, SD 57648, USA

Corresponding author— T. E. Wittum, tel 614 292-1206, fax 614 292-4142, email wittum.1@osu.edu

Abstract

In the summer of 1996, we screened 18,931 calves in 128 beef herds located in five US states for persistent bovine viral diarrhea virus (BVDV) infection. Of these, 76 herds were randomly selected from the client database of collaborating veterinary practices, and 52 herds were suspected by the collaborating veterinarians to have BVDV infection based on history or clinical signs. Serum was obtained from each calf in the cooperating herds prior to 4 months of age and tested for the presence of BVDV by microtiter virus isolation. Information about each of the herds (including management practices, vaccination history, and breeding- and calving-season production measures) were collected by the collaborating veterinarians using standardized questionnaires. A total of 56 BVDV-positive calves in 13 herds were identified on initial screening. Ten (19%) of the BVDV-suspect herds and three (4%) of the randomly selected herds had ≥1 BVDV-positive calf at initial screening. Multiple BVDV-positive calves were identified in 10 of those 13 herds. Follow-up information was obtained for 54 of the 56 positive calves. Ten out of 54 (18%) died prior to weaning, and 1 (2%) was sold because of unusually poor growth. Thirty-three out of 54 (61%) of the initially positive calves remained BVDV positive at 6 months of age—confirming persistent-infection (PI) status. Dams of 45 of the 56 positive calves were tested, with 3 (7%) identified as positive—indicating most PI calves were products of acute dam infection during gestation. The proportion of cows that

were pregnant at the fall 1995 pregnancy examination was 5% lower in herds with PI calves born during the 1996 calving season than in randomly selected herds without PI calves. Most of the calves we identified with persistent BVDV infections survived to weaning, and could provide a constant source of virus to the herd throughout the breeding season and early gestation.

Keywords: cattle, microbiological disease, bovine viral diarrhea virus, persistent infection

1. Introduction

Bovine viral diarrhea virus (BVDV) infection can produce a variety of economically important clinical manifestations in beef herds. One potential outcome is the creation of calves that are viremic but immunotolerant to the virus. These persistently infected (PI) calves are the result of *in utero* exposure to BVDV prior to the development of a competent fetal immune system (McClurkin *et al.*, 1984). Calves which are persistently infected with BVDV continuously shed large amounts of virus (McClurkin *et al.*, 1984). These PI calves can have high mortality rates (Taylor *et al.*, 1997b) and poor growth rates (Taylor and Kelling) although they can appear normal and survive to maturity (Bolin *et al.*, 1985). Because it is common for calves to be maintained with breeding females throughout the breeding season, PI calves are a potentially important means by which BVDV infection can be maintained in beef herds.

Vaccines to protect against BVDV infection are extensively used in preventive health programs for beef breeding herds and feedlots (USDA, 1994; USDA, 1995 and USDA, 1998). However, the risk of an acute BVDV outbreak remains an important health concern of beef producers. It is likely that PI cattle are important in the transmission of BVDV infection from infected to susceptible herds (Bolin; Holland and Taylor). The best methods to protect against the production of PI calves when gestating cows are exposed to BVDV are not known. Vaccination of the dam provides partial (but not complete) protection of the fetus (Cortese *et al.*, 1998), and PI calves have been reported in vaccinated herds (Kelling *et al.*, 1990). Little is known about the prevalence of cattle persistently infected with BVDV, how long they remain in infected herds, or the effects of their presence on herd productivity. In addition, risk factors that might predispose herds to persistent BVDV infections have not been reported. The objectives of this study were to estimate the prevalence of beef herds with persistent BVDV infection, to describe the disposition of calves with naturally occurring persistent BVDV infections, to identify herd-level risk factors for persistent BVDV infection in beef herds, and to quantify the effects of persistent BVDV infection on herd measures of health and production.

2. Methods

2.1. Study population

We initially identified veterinarians (in five geographically diverse states) who routinely provided veterinary services to commercial beef herds. Forty-eight

collaborating veterinary practices were identified in the states of Alabama, Nebraska, Nevada, North Dakota, and Ohio. These states were selected to provide both geographical and herd-management diversity. Each of the collaborating veterinary practices served as a sampling cluster for the random selection of 1–5 participating beef herds. Herds were randomly selected from a list of all beef herds that routinely received service from each collaborating veterinarian. Herds were selected from the list frame using a random-numbers table.

Randomly selected herds were contacted by the veterinarian and asked to participate in the study. Interested producers were then screened to ensure that their herds had a minimum of 20 and a maximum of 500 breeding females, that they used a limited breeding season with primarily spring calving, and that they were willing to provide the required information and access to the herd for sampling as required for the study. Randomly selected herds which met these criteria were enrolled in the study as participating herds.

In addition to randomly selected herds, the cooperating veterinarians were asked to identify client beef herds in which they suspected BVDV infection was present based on history and observed clinical signs. Herds which met these criteria were screened as described above and enrolled in the study as participating herds. Some herds were enrolled that calved in both the spring and fall. For these herds, only the portion of the herd that calved in the spring was included in the study population. In addition, three herds with >500 breeding females in which the collaborating veterinarian strongly suspected BVDV infection were also included at the request of the veterinarian. It is likely that this resulted in a biased difference in herd size between BVDV-positive herds and randomly selected negative herds.

2.2. *Sample and information collection*

Blood samples were obtained by the collaborating veterinarians from all calves in the participating herds in the spring and summer of 1996. The samples for a herd were generally collected on 1 day when all calves were processed after the end of the calving season. This is the first point in the beef-herd production cycle when blood samples can be obtained efficiently from all calves in most herds. In addition, PI calves identified at this time are likely to be present in the herd during subsequent breeding and gestation. All blood samples were collected before calves were 4 months old. For each calf, approximately 10 ml of blood was collected into a sterile evacuated serum-separator tube. The blood samples were centrifuged by the collaborating veterinarians, then shipped in the original tube by overnight delivery to The Ohio State University. The samples were centrifuged again if needed and 1 ml of serum harvested. The serum samples were stored at −80°C until testing was conducted.

At the same time that blood samples were collected, the cooperating veterinarians also collected general information regarding herd-management practices as well as breeding- and calving-season information using a standardized questionnaire. Management information was obtained regarding the use of BVD vaccinations on breeding females, recent introductions of new cattle into the herd, exposure to other herds, and information about management of breeding groups. Information was also collected regarding the 1995 breeding season (including the numbers of cows and replacement heifers exposed to breeding, the numbers of cows and

replacement heifers examined for pregnancy in the fall of 1995, and the numbers that were found to be pregnant). Calving-season information for 1996 included the number of calves born, the distribution of calving by 3-week increments, and the number of calf deaths which occurred during the neonatal period and early postnatal period. Further information was collected by the cooperating veterinarians in the fall of 1996 following weaning. Specific information was collected regarding weaning dates, the number of calves weaned, and average weaning weight.

A second serum sample was obtained and tested for BVD viral antigen at approximately 6 months of age from calves that were positive to the initial screening. In addition, a single serum sample was obtained from the dams of calves positive to the initial screening. Sample collection, shipping, and testing procedures were identical to the initial sampling.

2.3. BVD virus isolation

Sera from all calves were tested for the presence of BVD viral antigen using the microtiter immunoperoxidase plate assay (Afshar and Saliki) with D89 and C17 monoclonal anti-BVDV antibodies. Isolation was accomplished by inoculation of wells containing monolayers of passaged primary bovine-turbinate (BTU) cells with 50 μl of serum. Plates were incubated for 3–4 days and 50 μl of culture supernatant was passaged for an additional incubation period. Plates were fixed and viral antigen was detected by immunoperoxidase staining using anti-mouse peroxidase conjugate. A positive control consisting of 50 μl of serum from a known persistently infected animal, and a negative control were placed on each microtiter plate. Serum samples which were positive by virus isolation were passaged to prepare virus stocks.

2.4. Herd-production measures

For each herd with adequate information, we calculated the following measures of production:

Proportion of mature cows pregnant = mature cows pregnant fall 1995 ÷ mature cows examined for pregnancy fall 1995

Proportion of replacement heifers pregnant = replacement heifers pregnant fall 1995 ÷ replacement heifers examined for pregnancy fall 1995

Proportion of females that calved = total calves born 1996 ÷ females exposed to breeding 1995

Proportion of females that calved early in the calving season = calves born in the first 6 weeks of the 1996 calving season ÷ total calves born 1996

Neonatal mortality = calves born dead or died within 12 h ÷ total calves born 1996

Postnatal mortality = calves died > 12 h ÷ total calves born 1996

Average weaning weight = actual reported weaning weight 1996 calf crop adjusted for time of weaning

Information was not available to calculate all production measures for all herds. Some information was not recorded or reported by the producers, and some procedures (such as pregnancy examinations and weighing calves at weaning) were not used by all producers. This resulted in sparse data for some of the herd-production measures.

2.5. Data analysis

Because the study population was selected based on herds, the unit of analysis was also the herd. The prevalence of herds with PI calves and 95% confidence intervals (Fleiss, 1981) were calculated separately for randomly selected and suspect herds. Suspect herds in which BVDV-positive calves were not identified were excluded from all subsequent analysis.

Differences in herd characteristics and management practices between positive and randomly selected negative herds were screened for univariable association using ordinary logistic-regression procedures of SAS PROC GENMOD (SAS, 1997). Variables associated with the presence of a PI calf in the herd on screening were used to create a multivariable logistic-regression model. The model was reduced using a backward-selection procedure with the criterion of $P<0.05$ to remain in the model. Final logistic-regression models were evaluated for goodness-of-fit using the Hosmer–Lemeshow statistic (Hosmer and Lemeshow, 1989).

Herd-production measures were compared between herds with PI calves and randomly selected herds without PI calves. All production measures, including herd percentages, were assessed as continuous data using multivariable analysis-of-variance procedures of SAS PROC MIXED (SAS, 1997). When evaluation of the data for violations of model assumptions indicated that the assumption of normality was violated, the data were transformed using the log or arcsin transformation. For each of these outcomes, a model was specified which included variables representing BVDV PI status of the herd, the state in which the herd was located, and the number of calves born in 1996 (as an indicator of herd size). Weaning weight was assessed using a similar model which was extended to include a variable to account for the number of days from the beginning of the calving season until the first weaning date. When calves were weaned on multiple dates, weights of calves weaned after the first weaning date were adjusted, on the basis of weight per day of age, to the first weaning date. Final multivariable analysis-of-variance model goodness-of-fit was evaluated by graphing the predicted versus the residual values of the dependent variables (Schlotzhauer and Littell, 1987).

3. Results

Sera were obtained from 19,636 calves in 133 beef herds in the spring and summer of 1996. Samples collected from five of the herds were not usable because of improper storage and handling prior to and during shipment, leaving a total of 128 herds containing 18,931 calves as the study population. Of these herds, 76 were randomly selected herds and 52 were herds in which the veterinarians suspected BVDV infection. We identified a total of 56 BVDV-positive calves in 13 herds on the initial screening (Table 1). At least one herd with BVDV-positive calves was identified in each of the five states. Ten (19%; 95% CI 10–33%) of the suspect herds, and three (4%; 95% CI 1–12%) of the randomly selected herds had BVDV-positive calves. Multiple BVDV-positive calves were identified in 10 (77%) of these 13 herds.

A second serum sample was obtained at approximately 6 months of age from 43 (77%) of the 56 calves initially positive (Table 2). Thirty-three (77%) of these second samples were BVDV positive—confirming PI status of the calf. The remaining 10 (23%) of the second samples were negative—suggesting that the positive initial sample represented an acute BVDV infection. Among the 13 calves that were positive at initial screening but from which we did not obtain a second serum sample, 10 were reported to be dead by the owners before 6 months of age. One additional calf was sold prior to the follow-up testing because of exceptionally poor growth. These 11 calves with one BVDV-positive serum sample and clinical signs consistent with persistent BVDV infection were considered to be probable, unconfirmed PI calves. The final two calves that were positive initially were lost to follow-up. One of these calves was not adequately identified and the other was from a herd where all calves were sold at a young age because of market conditions. A serum sample was obtained from the dam of 45 of the initially positive calves (Table 2). Three of these dams (6.7%) located on two (15%) farms (both of which were suspect herds) were found to be BVDV positive. All three were dams of confirmed PI calves. One additional dam of a confirmed PI calf located at a third farm was found dead prior to follow-up testing. However, no attempt was made to determine the cause of death of this cow.

Table 1. Frequency and prevalence of beef herds identified with calves persistently infected with BVDV (confirmed and probable) in five US states in 1996

	Average calves per herd	Total herds tested		Herds with PI calves		Prevalence	
		R [a]	*S* [b]	*R*	*S*	*R* (%)	*S* (%)
Alabama	31	13	2	0	1	0	50
Nebraska	171	27	27	2	4	7	15
Nevada	259	10	7	1	2	10	29
North Dakota	158	14	12	0	2	0	17
Ohio	54	12	4	0	1	0	25

[a] Randomly selected herd.
[b] Suspected BVDV infected herd.

Table 2. Summary of BVDV testing of 56 BVDV infected calves and their dams identified in 13 beef herds located in five US states in 1996

Classification	No. of calves/dams	%
Calves positive to initial screening	56	
Lost to follow-up [a]	2	
Calves with follow-up information	54	
Survived to weaning negative on retest	10	18.5
Survived to weaning positive on retest 33 61		
Died prior to weaning	10	18.5
Sold for poor growth prior to retest	1	2
Dams of calves positive to initial screening	56	
Dams not tested (one cow died during summer)	11	
Dams tested	45	
BVDV negative	42	93
BVDV positive	3	7

[a] One positive calf at initial screening was not adequately identified, and one positive calf was sold without follow-up.

Table 3. Descriptive statistics for categorical variables representing current practices at the time of the study among beef herds with PI BVDV calves and randomly selected beef herds without PI BVDV calves in five US states in 1996

	Positive herds (%)	Negative herds (%)
Any use of BVD vaccination	92	82
Any use of BVD vaccination of cows	67	62
Any use of BVD vaccination of heifers	92	79
Any use of KV BVD vaccination of cows	50	39
Any use of KV BVD vaccination of heifers	58	35
Any use of MLV BVD vaccination of cows	25	30
Any use of MLV BVD vaccination of heifers	58	62
Introduction of animals into the herd – 1995 breeding season through 1996 calving season		
Any cattle	83	77
Calves for grafting	17	14
Yearling bulls	75	39
Mature bulls	42	33
Yearling heifers	17	8
Open cows	0	3
Pregnant heifers	0	11
Pregnant cows	17	20
Other cattle	17	5
Herd used multiple bulls per breeding group 1995 breeding season	83	65
Herd commingled with other herds 1995 breeding season	17	9
Replacement heifers reared off-farm	0	9
Replacement heifers bred to begin calving prior to cows	58	56

Table 4. Descriptive statistics for continuous variables among beef herds with calves persistently infected with BVDV and randomly selected beef herds without PI BVDV calves in five US states in 1996

	Positive herds			Negative herds		
	n	Median	Range	*n*	Median	Range
No. of calves born during 1996 calving season	12	245	31–1650	65	89	10–603
No. of breeding groups utilized 1995 breeding season	11	3	2–9	64	3	1–12
Total BVDV vaccinations applied to replacement heifers	12	1	0–3	66	1	0–4
Total BVD MLV[a] vaccinations applied to replacement heifers	12	1	0–2	66	1	0–3
Total BVD KV[b] vaccinations applied to replacement heifers	12	1	0–1	66	0	0–2
Total BVD vaccinations applied to cows	12	1	0–2	66	1	0–2
Total BVD MLV vaccinations applied to cows	12	0	0–1	66	0	0–1
Total BVD KV vaccinations applied to cows	12	0.5	0–1	66	0	0–1

[a] Modified live virus.
[b] Killed virus.

One or more confirmed or probable PI calf was identified in 12 of the 13 herds with BVDV-positive calves on the initial screening. In one of the randomly selected herds, the only BVDV-positive calf on initial screening was lost to follow-up when all calves on the farm were sold. As a result, the herd PI BVDV status could not be determined, and this herd was excluded from further analysis. Therefore, 2.7% (95% CI 0.5–10%) of the randomly selected herds had confirmed or probable PI BVDV calves.

Differences in herd characteristics and management practices between randomly selected herds without PI calves and herds with PI calves are presented in Table 3 and Table 4. Herds with positive calves were larger ($P<0.01$) than random herds

Table 5. Comparison of estimated[a] herd measures of production among beef herds with PI BVDV calves and randomly selected beef herds without PI BVDV calves in five US states in 1996

Herd-production measure	Positive herds		Negative herds	
	n	%	*n*	%
Fall 1995				
Proportion of mature cows pregnant	12	89	39	94[b]
Proportion of replacement heifers pregnant	12	93	27	85
Spring 1996				
Proportion of females that calved	12	86	62	88
Proportion of females that calved early in the calving season	11	64	55	68
Perinatal mortality	11	4.6	65	4.3
Postnatal mortality	11	2.6	65	2.7

[a] All estimates are predicted values (least-square means) from multivariable ANOVA adjusted for the state in which the herd was located and number of calves born in 1996. Average weaning weight was also adjusted for days from the beginning of the calving season until weaning.
[b] Percentages differ ($P < 0.05$) between BVDV-positive and -negative herds.

without positive calves. The introduction of yearling bulls was more common among the positive herds than the random negative herds on univariable analysis, but this difference was not present when adjusted for herd size. Other differences in herd characteristics and management practices (including the use of BVDV vaccinations of breeding females) were not detected.

Differences in herd measures of production between randomly selected herds without PI calves and herds with PI calves are presented in Table 5. The random negative herds had a 5% higher ($P < 0.05$) expected proportion of cows pregnant than did herds with PI calves. However, expected proportions of replacement heifers pregnant were similar between positive and negative herds. Other measures of herd production (including calving proportions, mortality, and average weaning weight) were also similar between positive and negative herds.

4. Discussion

We found that 3% of randomly selected herds in this population had calves with confirmed or probable persistent BVDV infections. One additional herd may have contained a calf with persistent BVDV infection, but the calf was lost to follow-up. If this calf had been confirmed as PI, our resulting estimate would be 4%. Bolin *et al.* (1985) reported that 9% of 66 herds contained cattle with persistent BVDV infections. However, their study herds were not randomly selected, and contained herds both with and without histories of BVDV infection. As a result, our results may be a better representation of expected occurrence of PI BVDV infection in beef herds. However, our study population was not selected to represent all beef herds in the states included in the study. Unfortunately, better estimates of the prevalence of herds with persistent BVDV infections are not available.

We found that 19% of herds in which the herd veterinarian suspected BVDV infection based on history or clinical signs contained calves with confirmed or probable persistent BVDV infections. Many of these herds in which we could not identify PI calves may have experienced acute outbreaks of BVD, but PI calves were no longer present or had not yet been produced. In addition, some of these herds might not have had BVD infections at all. Given the wide variety of clinical manifestations of BVDV infection, veterinarians may commonly include it in differential diagnosis listings for enteric, respiratory, or reproductive disease problems in beef herds. As a result, BVDV infection in beef herds may be suspected by veterinarians when it is not present. However, the higher risk of PI calves in BVDV suspect herds compared to randomly selected herds indicates that history is useful in identifying herds at higher risk of containing PI animals.

Most herds in which we identified a calf with a BVDV infection had multiple confirmed or probable PI calves. The presence of multiple PI cattle in beef herds has been reported previously (Taylor; Kelling; Bolin and Holland). This suggests that the production of PI calves is not a rare clinical manifestation of BVDV infection in a herd. In addition, it is likely that we did not identify all PI BVDV calves in the study herds. Because neonatal mortality is a potential clinical outcome, some PI calves born in these herds might have died prior to testing. Also, the sensitivity of

the immunoperoxidase assay has been reported to be slightly lower than standard virus isolation (Saliki *et al.*, 1997)—which could result in a small number of false-negative results at both testings. The presence of maternal antibody may also have prevented the isolation of BVDV from some PI calves. However, because calves in the study herds generally were tested first following the completion of the calving season, most were at least 2 months old at the initial screening.

The presence of multiple PI calves in most affected herds indicates the presence of multiple susceptible females in the breeding herd that experienced acute BVDV infections at the appropriate stage of gestation. Effective BVDV vaccination programs may alleviate this susceptibility and reduce the risk of dam infection during gestation. Reducing the exposure of susceptible animals to BVDV through herd-management practices (such as maintenance of a closed herd) also may prevent the production of PI calves. Beef herds might be commonly exposed to multiple strains of BVDV through the introduction of new animals, mixing with other herds, or transport of animals away from and back to the farm. Antigenic diversity of BVDV recovered from a beef herd has been reported (Bolin *et al.*, 1991).

Few of the dams of calves with persistent BVDV infections in our study population were infected with the virus. Beef females with persistent BVDV infection can appear normal and survive to breeding age. These PI females can become pregnant and will produce PI offspring. Our results suggest that this is an uncommon event. It appears that most calves with persistent BVDV infections are the product of acute dam infection during gestation. This suggests that the importance of PI calves is their presence in the herd during the breeding season immediately subsequent to their birth, rather than their long-term presence in the herd.

We could not identify herd-level risk factors for the presence of calves persistently infected with BVDV in our study population. Herds with PI calves were larger than randomly selected herds without PI calves. This result is likely due to sampling bias resulting from our inclusion of suspect herds much larger than 500 breeding females (at the request of the collaborating veterinarians). This request was not made for random herds. As a result, we cannot determine if herd size is a risk factor for the presence of PI BVDV calves in beef herds.

Herds with PI BVDV calves appeared to be similar to randomly selected herds without PI calves in all other herd characteristics and management practices that we measured—including the use of BVDV vaccines. This might indicate that these factors are not associated with the risk of PI BVDV calves in beef herds, and that effective vaccination programs and management procedures are needed. However, many of the herds with PI calves were suspect herds with histories of BVDV infection. It is likely that control measures such as vaccination and biosecurity programs had been implemented in these herds prior to our study. We collected information about herd characteristics and management practices in place at the beginning of the study. This may have prevented our detection of differences which might have existed prior to the introduction of BVDV into these herds.

Herds in which PI BVDV calves were identified had 5% lower adjusted proportion of mature cows pregnant in the fall of 1995 than randomly selected herds without PI calves. This is likely the result of conception failure and pregnancy

wastage which can result from BVDV infection (McGowan *et al.*, 1993). Proportion of replacement heifers pregnant were similar between herds with and without PI calves. This finding is consistent with a common beef herd-management practice where yearling replacement heifers are maintained separately from mature cows until after calving. Under this system, yearling replacement heifers would not be exposed chronically during gestation to PI calves present in the herd. We did not find differences in other measures of herd production (including perinatal or postnatal calf mortality) between herds with PI BVDV calves and randomly selected herds without PI calves. These results suggest that BVDV infection can be present in a beef herd without large effects on production. However, many of our herds with PI BVDV calves had histories of BVDV infection. As a result, we may have attempted to measure differences at a time when many of the positive herds were in the process of controlling the infection. Greater differences in production might have been observed immediately following introduction of the virus into these herds, or might be observed later if the infection is not controlled. It should therefore not be assumed based on these data that outbreaks with important effects on production cannot occur.

Our data suggest that persistently infected BVDV calves can be an important means of maintaining BVDV infection in beef herds. Most of the calves we identified as BVDV positive at the initial screening survived to weaning, and could provide a constant source of virus to the herd throughout the breeding season. This situation could result in PI calves born in a herd each year without appropriate intervention. Our data also suggest that PI females returning to the breeding herd are not a common means of maintaining BVDV infection in beef herds. Efforts to eradicate PI animals in infected beef herds should therefore emphasize testing of calves prior to the beginning of the breeding season, with follow-up testing of dams of positive calves and animals without calves available for testing.

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