

Economic, Reproductive, and Performance Effects of PI BVD in Commercial Cattle Operations: Managing to Minimize Losses

Robert L. Larson, DVM, PhD, ACT, ACAN, ACVPM
Kansas State University, Manhattan, KS 66506

Abstract

Bovine viral diarrhea virus (BVDV) infection can cause a range of reproductive, performance, and health problems in commercial cow herds. The primary reservoir of BVDV is cattle persistently infected (PI) with the virus, and BVDV is most commonly introduced into a herd through contact with a PI animal. The economic loss in a given herd varies due to factors such as herd immunity and the timing of PI introduction into the herd. Losses due to BVDV are minimized by initiating a biocontrol strategy that includes testing for PI cattle and vaccinating to enhance herd immunity.

Résumé

L'infection causée par le virus de la diarrhée virale bovine (BVDV) peut causer plusieurs problèmes au niveau de la reproduction, de la performance et de la santé dans les fermes commerciales bovines. Les bovins immunotolérants représentent le réservoir principal du BVDV et le virus est le plus communément introduit dans un troupeau par le contact avec un individu immunotolérant. Les pertes économiques dans un troupeau en particulier varient entre autres en fonction de l'immunité de troupeau et du moment où l'immunotolérance s'installe dans le troupeau. Les pertes causées par le BVDV sont minimisées par l'instauration d'une stratégie de biocontrôle incluant la détection des bovins immunotolérants et la vaccination pour accroître l'immunité de troupeau.

Introduction

Bovine viral diarrhea virus (BVDV) infection is responsible for a variety of economically important syndromes in beef herds. Quantifying the economic benefit of removing BVDV persistently infected animals from a herd, and hence the value of diagnostic testing to achieve that goal, is an important consideration for veterinarians.

BVDV infection can cause a complex of disease problems, including respiratory disease, infertility and

fetal infection.² Fetal infection can lead to early embryonic death, abortion, congenital defects, stunting, or the birth of persistently infected (PI) calves.² Persistently infected cattle are the result of *in utero* exposure to the noncytopathic biotype of BVDV prior to the development of a competent fetal immune system by about 125 days of gestation.^{14,36,44} Transplacental infection occurs with high efficiency during pregnancy, and if PI fetuses survive to term, they are continually viremic, but immunotolerant to homologous BVD virus.^{19,59}

The prevalence of PI animals in the general cattle population has been estimated to range between 0.13 and 2.0%.^{9,31,34,67} Differences in reported prevalence may be due to the population tested, the country/continent where the population was located and/or the diagnostic tests utilized. Persistent infection has a clustered distribution, which means a few herds may contain several PI cattle but most herds contain only non-PI cattle.^{8,35} Clustering of multiple PI animals in a herd is due to exposure of numerous susceptible dams to a PI or transiently infected (TI) source of noncytopathic BVDV prior to day 125 of gestation.

The primary reservoir for and source of BVDV are cattle PI with BVDV, with TI cattle considered a less important source. Persistently infected animals are a much more efficient transmitter of BVDV than TI animals because they secrete much higher concentrations of virus for a much longer period of time. After a short latent period, TI animals experience a short period of viremia and virus is shed in body secretions and excretions from days four to 15 post-infection.^{12,19} In contrast, PI animals usually have a very high and persistent viremia, and BVDV is shed throughout life from virtually all secretions and excretions including nasal discharge, saliva, semen, urine, tears, milk, and to a lesser extent, feces.^{5,10,11,56} Horizontal transmission of the virus from either persistently or transiently BVDV-infected animals to susceptible cattle in direct contact may be via inhalation or ingestion of virus-containing body fluid.¹⁹ In addition, air transmission from PI animals over short distances seems likely; however, when cattle are housed at greater distances from PI animals, the spread of infection is slow or absent.⁶⁶ Horizontal transmission of

BVDV to seronegative cattle has been shown to occur after only one hour direct contact with a single PI animal.⁶⁵ Over-the-fence contact with a PI from neighboring cattle can also introduce BVDV into a susceptible herd.^{47,59}

Suckling calves are commonly in contact with the breeding herd during early gestation, prior to the time the bovine fetus develops a competent immune system. As a result, PI suckling calves are considered to be the primary source of BVDV infection in breeding herds causing pregnancy loss, pre-weaning mortality and the induction of PI calves in the next generation.^{19,44}

Although PI suckling calves are considered to be the primary reservoir for BVDV in a herd, PI adults can also be present in a herd. Adult PI animals are not as common because mortality of PI calves prior to and after weaning has been reported to be very high due to fatal congenital defects and secondary infections that cause enteritis, pneumonia, and arthritis.^{44,45} However, 17 to 50% of PI calves may reach breeding age in some situations.^{3,7,33} PI breeding females not only are a source of horizontal transfer of BVDV, but will always produce a PI calf themselves.^{31,44} Wittum *et al* showed that 7% of PI calves were born to dams that were PI,⁶⁷ meaning that while PI dams can be a direct cause of a small percentage of PI calves, a vast majority (93%) of PI calves are born to non-PI dams due to transient infection of the dam during gestation.⁶⁷

Male PI calves will occasionally be selected for use as breeding bulls. The amount of BVDV excreted in the semen of persistently infected bulls is very high (10^4 - 10^6 TCID₅₀/ml).⁵⁷ BVDV-contaminated semen is an efficient horizontal transmitter of disease from bull to seronegative females.⁵⁵ If PI bulls are used for natural service, PI calves are not common, but seronegative cows may not be able to conceive. Once immunity has developed, cows can conceive and give birth to normal (non-PI) calves.^{3,44} If PI bulls are used for AI, all or most seronegative females bred with the semen will become infected although most will not produce a PI calf.⁴⁹

Circulating virus may exist in herds following removal of PI calves although the efficiency of virus transmission via transient infections alone is not high.³⁰ Limited data in dairies suggest BVDV may circulate in an unvaccinated herd for 2-3 years following removal of all PI animals.^{3,30,48} The length of time for BVDV circulation in vaccinated beef herds without PI animals has not been reported.

Diagnostic Laboratory Tests to Identify PI Cattle

Cattle PI with BVDV can be identified by virus isolation from whole blood (buffy coat) or other tissue, immunohistochemistry (IHC) staining of viral antigen

in skin biopsies, antigen-capture enzyme-linked immunosorbent assay (ACE), and polymerase chain reaction (PCR) methods.^{18,39} Persistently infected animals produce an exceptionally large number of BVDV particles that can be isolated from virtually any tissue sample. Virus isolation is considered to be very specific for BVDV infection; however, colostrum antibodies may temporarily reduce the amount of free virus in the serum of young calves, making the test less sensitive in young calves.^{11,54} In the presence of passively acquired BVDV antibodies, virus from PI calves cannot be detected in serum or whole blood by virus isolation. However, once maternal antibodies have disappeared, BVDV can be demonstrated repeatedly. Maternal antibodies had disappeared and BVDV could be isolated by six weeks of age in all four PI calves in one study,¹¹ and by eight weeks of age in all eleven PI calves in another study.⁵⁴ A few PI calves will develop neutralizing antibody and can clear the virus from serum. Therefore, virus isolation will only be possible from WBC samples, not serum in some PI cattle.¹¹ Virus isolation methods are labor intensive and take several days to complete. An additional shortcoming is that virus isolation will not differentiate between TI animals and PI animals, unless positive cattle are re-tested and remain positive at a later date (i.e. three weeks later).

An immunohistochemical (IHC) test for BVDV infection using skin biopsy samples, such as ear notches, is available that differentiates between PI animals and transient BVDV infections.⁵² Transiently infected animals may have internal organ tissue samples that are IHC positive. However, when skin samples were evaluated, transiently infected animals either had no staining, or staining was confined to the epidermal keratinocytes and follicular ostia, in contrast to PI cattle with antigen-positive staining cells in all layers of the epidermis, all levels of hair follicles, and the hair bulb.⁵² This test is suitable for herd screening because samples can be taken from cattle of any age, sample collection is simple, the samples are stable for transport and handling, and the test is both sensitive and specific for BVDV PI cattle.^{4,20,52} In addition, use of modified-live vaccine does not appear to cause false positive IHC results when testing for PI animals.¹⁷

The antigen-capture ELISA (ACE) test can be done on serum or skin samples and can be done more rapidly than IHC or virus isolation. When performed on skin samples (most commonly ear notch), the sample is sent individually in tubes containing phosphate buffered saline. BVD antigen transfers from the hair follicle in the skin sample to the fluid in the tube and the saline is used in a microwell plate for the ELISA test. The ACE test will pick up some transient infections and although the sensitivity is high, it may not be quite as sensitive as IHC.

PCR testing for BVDV infection is more rapid than virus isolation and can detect virus in antigen-antibody complexes. PCR tests are sensitive and have been shown to differentiate between BVDV genotypes. However, a single BVDV positive blood (buffy coat) or serum sample tested by PCR does not allow the diagnostician to differentiate between viremia from a postnatal acquired infection (and possibly MLV vaccination) and viremia due to being persistently infected. Because PCR tests can identify minute amounts of virus, this test can be used in pooled samples of serum or milk (and possibly phosphate buffered saline transport fluid) in surveillance programs.

Economic Cost of the Presence of PI Cattle in a Herd

The presence of PI animals in a breeding herd can result in decreased pregnancy percentage compared to herds with no PI calves.⁶⁷ This decreased pregnancy percentage could be due to ovarian dysfunction,^{26,35} failure of fertilization,²⁴ early embryonic death,^{1,33,46} and/or mid-gestation fetal loss⁶⁰ in cattle acutely infected with BVDV. Grooms *et al* found that BVDV could be isolated on days six and eight following infection with noncytopathic BVDV in ovarian stromal and macrophage-like cells, and oophoritis was evident from six to 60 days post infection.²⁶ McGowan *et al* reported that conception percentage, determined 20 days after insemination, was lower in heifers intranasally-infected with BVDV nine days before insemination compared to controls (44 vs 70%; $P=0.055$).²⁶ In addition, conception percentage was numerically lower in heifers exposed to a PI cow-calf pair four days after insemination than in unexposed controls (60 vs 79%; $P=0.255$).⁴⁶ The intranasally-exposed heifers also experienced significant embryo-fetal loss, resulting in a pregnancy percentage, determined 77 days after insemination, significantly lower than controls (33 vs. 79%; $P=0.018$).⁴⁶ Rufenacht *et al* found that dairy cows infected with BVD during the first 45 days of gestation (as indicated by seroconversion to BVD during that time frame) had the same conception percentage as cows that either had previous exposure to BVD (seropositive for BVD by start of the trial) or that were not exposed to BVD during gestation (no seroconversion during trial). Similarly, BVD-exposure status did not influence late gestation pregnancy loss (>210 days). However, cows infected with BVD during mid-gestation (days 46-210) had greater pregnancy loss compared to those that were seropositive prior to breeding or that were not exposed to BVD during mid-gestation (pregnancy loss of 15.8 vs 6.1%; $OR=3.1$; $P<0.02$).⁶⁰

In addition to decreased pregnancy percentage, reproductive efficiency can be decreased due to fatal

congenital defects following fetal infection of BVDV between 100 and 150 days of gestation.¹⁹ The teratogenic lesions associated with fetal infection with BVDV include microencephaly, cerebellar hypoplasia, hydranencephaly, hydrocephalus, defective myelination of the spinal cord, cataracts, retinal degeneration, optic neuritis, microphthalmia, thymic aplasia, hypotrachosis, alopecia, brachygnathism, growth retardation and pulmonary hypoplasia.²

Studies have reported a pre-weaning mortality proportion for PI calves of 20 to 83%, which can result in substantial loss in herds with a high prevalence of PI calves. However, overall mortality percentages between herds with or without PI calves were not statistically significantly different because of the low prevalence of PI calves within most PI-positive herds.⁶⁷ In herds with PI animals present, Larson *et al* modeled scenarios both with and without a 10% negative effect on pre-weaning mortality percentage, and found that the greatest economic loss due to the presence of PI animals was decreased calving proportion, with a lesser loss due to pre-weaning mortality.⁴⁰

The effect of introducing a PI animal into a beef herd (confined breeding and calving seasons) depends on the timing of the introduction relative to the breeding season and the resulting immunologic status of the herd during early gestation. Even in the absence of vaccination, the number of PI animals and the amount of BVDV infection in a herd seems to be self-limiting.²⁹ A likely scenario for a BVDV-exposed herd is to experience an initial peak of disease and then in subsequent months and years, to experience low-level chronic reproductive losses. If a PI animal enters the herd either by birth or by purchase near the start of the breeding season, a high percentage of the herd may not be immunologically protected to the degree necessary to prevent viremia, conception failure, abortion, or fetal infection. Once the PI animal is in contact with the breeding herd for a long enough period of time, the majority of the herd should become infected and seroconvert. Seropositive animals are less likely to have conception failures, abortions, or infected fetuses compared to seronegative animals. If no intervention is applied to the herd, the number of susceptible females the following year should be greatly decreased and the number of abortions and infected fetuses (both persistently infected and immunocompetent) should decrease. A model developed by Cherry *et al* indicates that in continuous calving dairy situations, the proportion of PI animals in the herd will reach an equilibrium of about 0.9 to 1.2% in herds with no BVDV control procedures.¹⁵

Testing Strategies to Identify PI Cattle

Because the persistently infected animal is an im-

portant reservoir and transmitter of BVDV, control programs must first identify and remove these animals from the breeding herd. Because of vertical transmission of the virus from viremic dams to their fetuses, PI animals should be removed prior to the start of the breeding season in beef herds with a controlled breeding season. In order to find and remove PI cattle prior to the start of the breeding season, all calves, all replacement heifers, all bulls, and all non-pregnant dams without calves due to not becoming pregnant, aborting, or calf mortality must be tested for PI status.³⁸ Any female that is still pregnant at the time the herd is tested should be isolated from the breeding herd and kept isolated until her calf is tested and found to be negative.

If a herd has had confirmed PI calves, or if the history strongly suggests the presence of PI calves, the *a priori* assessment of PI prevalence is fairly high, making the predictive value of a positive test high enough one can conclude that the individual is a PI and the herd has PI animals present and a second confirmatory test may not be justified. In contrast, if the veterinarian has no previous evidence of PI cattle in the herd, confirming the initial test with a second test may be advisable before making conclusions about the individual and herd. Once a calf is identified as PI, it should be euthanized or removed for slaughter and the dam should be tested. Most dams of PI calves are not PI themselves, and if confirmed as non-PI, can re-enter the breeding herd because naturally acquired immunity is considered to prevent future fetal infections.⁵³ Dams identified as a PI should be sold to slaughter immediately.

In most whole-herd testing situations, individual tests (IHC or ACE) of skin samples is preferred because they can be accurately performed on animals of any age and a single sample is all that is usually needed. Other tests such as PCR or virus isolation may be used in some situations.

Economic Value of Diagnostic Testing to Identify and Remove PI Cattle

Determining the value of diagnostic testing depends on identifying the potential performance impact

that a diagnosis and corresponding management intervention would have on a herd compared to an expected economic baseline without testing, given expected prevalence in the herd. The economic assessment is then made by quantifying the performance differences as manifested in enterprise analysis and expected changes in farm profitability.

Larson *et al* used a 10-year (1991 to 2000) dynamic farm profitability simulation model that generates annual cash flow, balance sheet and income statements to compare three production scenarios: 1) herds with no PI calves, 2) herds with at least one PI calf present with a negative effect on pregnancy percentage, but no effect on pre-weaning mortality or weaning weight, and 3) herds with at least one PI calf present with negative effects on pregnancy percentage, pre-weaning mortality and weaning weight. Each scenario incorporated herd performance and economic interactions. Data from Wittum *et al* which estimates the pregnancy percentages and pre-weaning mortality for herds with or without at least one PI calf present was used to model all three scenarios.⁶⁷ Because the Wittum study involved herds from five geographically diverse states and a fairly large number of herds positive for the presence of at least one PI calf (n=13), the authors assumed that the positive herds represented a cross-section of levels of herd immunity, gestational status and virus virulence combinations present in the US. Farm economic activity for each scenario was reported as return to fixed costs as determined by subtracting variable costs from income for each year of the evaluation. Herd size was not varied between the scenarios, therefore more heifers needed to be retained in herds with at least one PI calf identified because of decreased pregnancy percentage.

Using cattle and feed prices for the 10 year period from 1991 to 2000, Larson *et al* estimated that the average cost of having at least one PI animal present in a beef cow herd ranged from about \$15 per cow exposed for breeding if the presence of a PI animal reduced pregnancy percentage from 92.7 to 89.6% (Table 1). And, if the presence of a PI animal increased pre-weaning mortality from 7.2 to 7.9% as well as reducing pregnancy percentage, the average cost of having a PI animal was estimated to be \$20 dollars per cow exposed (Table 1).⁴⁰

Table 1. Average cost over 10 years for the presence of BVDV PI cattle in a herd (adapted from Larson *et al*, 2002).⁴⁰

	10 Year Average	
	Return to fixed cost	Difference from herd with No PI cattle
No PI cattle present	\$78.82	
At least 1 PI – reproductive effect only	\$63.49	\$15.33
At least 1 PI – reproductive and calf mortality effects	\$58.66	\$20.16

The model used by Larson *et al* considers the cost of BVD infection to the cowherd to the point of selling the calves at weaning. One could conjecture that PI BVD calves in a group of stocker or feedlot cattle could increase the morbidity, mortality and potentially decrease the growth and carcass performance of not only the PI calves, but also in-contact pen-mates. Because of the nature of the model used, the entire cost of BVD PI cattle to the beef industry is not addressed, and therefore the values reported as costs to cowherds probably underestimates the cost of BVD PI cattle to the beef industry as a whole.

By doing a whole herd screening the initial year in herds where PI animals are known to be present, and screening all replacement animals (15% annual replacement rate) in subsequent years, the dollars available per test to equal the return to fixed cost of doing nothing is \$61.32 to \$80.64. This level of return indicates that whole herd screening and removal of PI cattle is economically justified if PI presence is known.

In herds where PI presence is not know, the economic benefit of testing to find PI animals must be evaluated in relation to the prevalence of herds with PI cattle present and the cost of whole-herd screening. Practitioners are able to categorize US beef herds as high-risk for the presence of BVDV PI animals compared to randomly selected herds.⁶⁷ Wittum *et al* identified 48 veterinary practices from five geographically diverse states (Alabama, Nebraska, Nevada, North Dakota and Ohio) that routinely provide veterinary services to commercial beef herds to participate in a BVD PI prevalence study.⁶⁷ Using a random-numbers table, 76 herds were randomly selected from client lists for evaluation of BVDV PI prevalence. In addition, these veterinarians were asked to identify client herds in which they suspected BVDV infection based on history and observed clinical signs; these herds were also evaluated for BVDV PI prevalence (52 herds). The prevalence of herds with at least one PI animal in randomly selected herds was 3.9% with a 95% confidence interval (CI) of 1 to 11%, compared to 19.2% of herds with a history of BVDV-compatible syndromes (95% CI of 10 to 33% of herds; Table 2).⁶⁷

One procedure for screening herds for PI cattle prior to the start of the breeding season involves initially testing all replacement heifers and bulls, all calves, and all dams without calves due to calf death or failure to calve.³⁸ In subsequent years, a strategy of vaccination and herd isolation from the start of the breeding season until four months after the end of the breeding season to decrease the risk of exposure to animals acutely infected with BVDV should be implemented. Once the herd is free of PI calves and cows, only replacement breeding animals need to be tested for persistent infection with BVDV. Therefore the cost of a BVD PI screening program is high in the initial one to two years, and then lower in following years.

Larson *et al* showed that at the low prevalence of herds with at least one PI animal reported for randomly selected herds, the dollars available to remove PI animals may or may not justify whole-herd diagnostic screening. If the true prevalence of herds with at least one PI animal is 1% (at the low end of the 95% confidence interval),⁶⁷ the average annual dollars available for screening is only \$0.15 (Table 3). Using a ten-year period, if all the calves and dams without calves are tested the initial year, the cost of the initial screening is prorated over 10 years, and only replacements (at the rate of 15% of the mature herd) are screened in subsequent years, \$0.60 would be available for costs associated with each animal tested (Table 4). This indicates that the cost of screening exceeds the risk of economic loss if the herd prevalence for PI presence is 1%. A strategy to implement a BVDV biosecurity program for incoming cattle (including PI screening) and to maintain a BVDV vaccination program appears to be a better economic alternative compared to whole herd screening for PI animals when history does not indicate problems suggestive of BVDV.

If, however, the true prevalence of randomly selected herds with at least one PI animal is 11% (at the high end of the 95% confidence interval),⁶⁷ an average of \$1.69 per cow annually is available for the cost of screening the breeding herd (Table 3). This may justify a strategy where all the calves are screened the initial year, and replacements are screened in subsequent years

Table 2. Prevalence estimate and 95% Confidence Interval for the presence of at least one PI animal in both randomly selected and BVDV-suspected herds.⁶⁷

	No. herds tested	No. positive herds	Prevalence estimate (%)	95% CI (%)
Randomly selected (Low Risk)	76	3	3.9	1-11
BVDV suspect (High Risk)	52	10	19.2	10-33
Total herds	128	13		

Multiple positive calves in 10 (77%) of 13 herds
Range of 1 to 13 positive calves in a herd

Table 3. Average annual value of testing to remove PI cattle from the herd (per cow exposed for breeding) (adapted from Larson *et al*, 2002).

	Herd prevalence of at least one PI animal	10 year average value of testing to remove PI cattle per cow exposed for breeding
Reproductive effect only		
Randomly selected herds	1%	\$0.15
	11%	\$1.69
BVDV PI suspect herds	10%	\$1.53
	30%	\$4.60
Reproductive and calf mortality effects		
Randomly selected herds	1%	\$0.20
	11%	\$2.22
BVDV PI suspect herds	10%	\$2.02
	30%	\$6.05

Table 4. Dollars available for testing if whole herd screening is done for one year and in subsequent years only replacement animals are screened (per animal tested per year) over a 10-year horizon

	Herd prevalence of at least one PI animal	Dollars available for testing per animal tested per year
Reproductive effect only		
Randomly selected herds	1%	\$0.60
	11%	\$6.76
BVDV PI suspect herds	10%	\$6.12
	30%	\$18.40
Reproductive and calf mortality effects		
Randomly selected herds	1%	\$0.80
	11%	\$8.88
BVDV PI suspect herds	10%	\$8.08
	30%	\$24.20

in random herds. In such a scenario, if the cost of the initial screening is prorated over 10 years and the herd has a 15% replacement rate, \$6.76 would be available for each animal tested (Table 4). This amount may cover the labor, diagnostic laboratory and consulting fees required to initiate a screening protocol.

The economic conclusion is the same if BVDV also affects pre-weaning mortality and weaning weight to a similar extent as modeled, assuming that the cost of initial screening is prorated over 10 years. The dollars available to screen herds for the presence of PI cattle only increases to \$0.80 per test if the prevalence of herds with at least one PI calf is 1%. If the prevalence is 11%, the dollars available per animal tested is \$8.88 (Table 4).

By pre-screening herds based on a history of BVDV-compatible problems or positive laboratory tests so that the prevalence of herds tested with at least one PI calf increases to 10% to 30%, the economic reward from identifying and removing PI animals is likely to exceed the cost of the presence of PI animals. If the true prevalence of herds with at least one PI animal is 10%, at the low end of the 95% confidence interval reported by Wittum *et al*,⁶⁷ the average annual dollars available for screening is \$1.53 (Table 3). If all the calves and dams without calves are tested the initial year, the cost of the initial screening is prorated over 10 years, and replacements (at the rate of 15% of the mature herd) are screened in subsequent years, \$6.12 would be available for each animal tested (Table 4).

If, however, the true prevalence of herds with at least one PI animal is 30% (at the high end of the 95% confidence interval),⁶⁷ an average of \$4.60 per cow is available annually to screen the breeding herd (Table 3). This would probably justify a strategy whereby all calves are screened the initial year and replacements are screened in subsequent years. In such a scenario, if the cost of the initial screening is prorated over 10 years and the herd has a 15% replacement rate, \$18.40 would be available for each animal tested (Table 4).

If BVDV also affects pre-weaning mortality and weaning weight to a similar extent as was modeled, and the same proportion of diagnostic test costs is applied, the dollars available to screen herds with a history of BVDV-compatible problems for the presence of PI cattle increases to \$8.08 per test if the prevalence of herds with at least one PI calf is 10% (Table 4). If the prevalence is 30%, the dollars available per test is \$24.20 (Table 4).

Monitoring Herds for BVDV PI Risk

The cost of initiating a BVDV PI whole-herd testing protocol on a farm or ranch is significant. Because of the relatively low prevalence of herds with at least one PI animal, veterinary practitioners may not be economically justified to initiate whole-herd screening protocols to find PI BVDV beef cattle for herds at low risk for the presence of PI cattle or herds that cannot gain significant market price advantage for selling groups of cattle that have been tested and determined to be free of PI individuals.^{40,67} However, if a ranch has significant exposure risk to BVDV PI cattle, or if significant marketing advantages exist, a protocol to screen the herd annually can be defended based on its likelihood to improve or protect economic return.⁴⁰ Several strategies can be employed to monitor herds for their risk of having PI cattle present. The interpretation of results from these strategies would be different if the goal were to monitor for the presence of BVDV rather than PI animals. If complete eradication of BVDV is desired, the effort and cost of monitoring is much greater than for monitoring for the presence of PI cattle.

Screening for PI cattle should take place prior to the start of the breeding season so that PIs can be identified and removed before contact with pregnant females, thereby eliminating the opportunity for a PI to cause reproductive failure and to create more PI animals in the next calf crop. Screening for PI animals at a later time, such as weaning, is discouraged. If samples are taken at weaning, although PI cattle can be removed from the herd, those continuously viremic animals were in contact with pregnant females throughout much of gestation and can cause reproduction and production losses, including the creation of PI cattle in the next calf crop.

Use of Production Records and Laboratory Tests of Sick and Dead Calves

The minimal level of surveillance for every herd should include monitoring of herd fertility (early breeding season pregnancy proportion, pregnancy per insemination proportion, and total pregnancy proportion), neonatal calf morbidity and mortality proportions, and weaning proportions. Because of the negative effect of the presence of PI calves in a breeding herd on measures of reproductive efficiency, the presence of physical abnormalities at birth, and calf survivability to weaning, an unacceptable level of these symptoms increases the risk that BVDV is a problem in the herd and increases the likelihood that whole-herd screening for PI cattle will be economically rewarding.³² Although, in many situations, pregnancy rate drops significantly at the time of conception of the oldest PI animal, and about six months later, calf mortality increases; using production records alone lacks sensitivity for identifying herds with PI animals because the clinical indications of PI presence may be less noticeable in some outbreaks.³² The clinical signs and time sequence following introduction of BVDV infection into different herds varies considerably due to the different proportions of seronegative animals in the critical period of pregnancy and different virulence among BVDV strains.²⁹

In addition to monitoring production records, minimal surveillance should include the necropsy examination of as many aborted fetuses, stillborn calves, and calves that die pre-weaning as possible, with whole blood submitted for determination of BVD viremia, and serum submitted for serologic evidence of infection. In addition, moribund calves from clusters of pneumonia, neonatal scours, or septicemia outbreaks that are not easily explained by sanitation or other problems should also be tested for BVDV exposure and PI status. If most perinatal and pre-weaning mortalities are examined for BVDV antigen and found to be negative, it is not likely that PI animals are present in the herd. The presence of PI animals in the herd will be established by a single confirmed test. The presence of PI animals is not ruled out and may be considered likely if few moribund or dead cattle are tested and found to be PI negative, but other tests indicate the presence of viremia or serology indicates recent BVDV infection and the possibility of PI animals being in contact with the moribund or dead sample animals.

The advantage of utilizing production measures and necropsies to determine if herds have either a high or low risk for the presence of BVDV PI animals is that minimal expense is involved, and these management tactics are inclusive for the monitoring of other disease and production problems. This level of monitoring is

probably appropriate in herds with no evidence for the presence of PI animals and that are at low risk of PI introduction.⁴⁰ The disadvantage is that at least one PI animal is allowed into the herd before production losses are identified, and production losses will continue for at least one year after intervention is initiated.

Use of Pooled Samples of Blood or Serum for PCR Testing

Herd monitoring for the introduction of PI animals can also be accomplished with pooled blood or serum samples (and possibly saline in which skin samples have been soaked) for PCR testing. By pooling samples, the expense of screening herds with a low prevalence of PI animals is minimized. Polymerase chain reaction is well suited to pooled-sample testing for the presence of BVDV PI animals in that it is sensitive enough to detect minute amounts of virus. A single PI animal was detectable in pools of 200 to 250 negative whole blood samples.⁵⁰ Animals contributing to negative samples are all assumed to be non-PI, whereas positive pools may contain samples from PI animals or transiently viremic animals. If the initial pool is PCR-positive, it must be split and retested to differentiate viremic and non-viremic animals. Once the viremic animals are identified, they must be classified as transiently infected or PI with a subsequent test in three weeks. The best size of the initial pool is determined by the balance between the cost savings of having large numbers of individuals represented in negative pools and few individuals represented in positive pools that require further diagnostics. If pool size is too large, there is an increased chance that any single pool will test positive, requiring additional testing to identify the few truly viremic individuals in the pool. If the samples are grouped in unnecessarily small pools, the cost benefit of pooling samples is lost to the large number of negative pools tested for each positive pool identified.⁵⁰ Muñoz-Zanzi *et al* developed a simulation model to determine that the economically optimum sample size depends on prevalence of true positives in the population. For a PI prevalence of 0.5 to 1.0%, the optimum number of samples in an initial pool is 20 to 30, using a described re-pooling strategy for test-positive initial pools.⁵⁰ As prevalence increases the least-cost initial pool size decreases.⁵⁰

Use of Annual Whole-Herd Individual Animal Testing

Certain high biosecurity herds, such as herds selling or developing replacement breeding animals, may elect to undergo a high level of surveillance even in the absence of evidence that PI animals are present. This high level of biosecurity may be important to their mar-

keting plan or may indicate a high value placed on avoiding the small, but real risk of introducing BVDV virus into the herd with subsequent negative reproductive, health and marketing consequences. The first year that a beef herd adopts this strategy, all suckling calves, all females that were bred that failed to present a calf on test-day, all replacement heifers and all bulls should be tested. If any calf is confirmed as a PI animal, his dam should be tested as well. In subsequent years, only suckling calves and any purchased animals need to be tested. If pregnant animals are purchased, the dam should be tested prior to or at arrival and the calf should be tested immediately after birth. Heifer development operations should test every heifer prior to or at arrival at the facility.

Other Sources of BVDV to Consider

Embryo Transfer

Embryo transfer is a potential route of transmission of BVDV. If the embryo recipient is PI, vertical transmission to the transferred embryo will occur with the creation of a PI fetus.¹⁰ Although there is no evidence to suggest that BVDV is present inside the embryos of viremic females, the virus can be present on the intact zona pellucida of PI and transiently infected females and the virus is present at high levels in the uterine environment of PI donors.⁶² Established washing procedures will remove contaminating virus, but if these procedures are not followed, BVDV from the collection fluids or virus present on the zona pellucida can be horizontally transferred to a susceptible recipient cow.^{61,62} Vertical transmission from the recipient cow to the fetus can occur resulting in fetal death or the birth of a PI calf. BVDV infection of the recipient cow and fetus can also occur when both the donor and recipient are free of BVDV if BVDV-contaminated fetal serum is used in the embryo transfer process or if contaminated liquid nitrogen is in direct contact with embryos.^{6,61}

Other ungulate species (domestic and wildlife)

Other ungulate species may be potential sources of BVDV to susceptible cattle herds. Transmission of BVDV between sheep and cattle has been demonstrated, but the importance of this transmission has not been established.¹³ BVDV has also been isolated from pigs, but again, the importance of pigs as a source of the virus to susceptible herds is not established.^{41,64} Deer seropositive to BVDV have been identified in North America and Europe.^{16,21,51} And at least one case of a captive deer in Europe being persistently infected with BVDV has been documented.²⁵

Fomites

Fomites may serve in the transmission of BVDV

from PI cattle to susceptible animals. A 19-gauge needle was able to infect susceptible cattle with BVDV when used IV within three minutes of drawing blood from a PI animal.²⁷ Nose tongs were able to infect susceptible cattle with BVDV when used for 90 seconds within three minutes of being used in a PI animal.²⁷

No evidence has been presented that insects are a source of BVDV transmission in field outbreaks. However, a role is possible in that BVDV was isolated from non-biting flies (*Musca autumnalis*) collected from the face of a PI animal, and experimental BVDV transmission between a PI animal and susceptible animals occurred when 50 biting flies were fed on the PI animal for five minutes, and 15 minutes later fed on susceptible animals.^{27,64}

Control Strategy to Limit Losses due to BVDV in Beef Cowherds

The primary goals of BVDV control in breeding herds are to prevent fetal infection in order to eliminate BVDV-associated reproductive losses (thereby preventing the birth of PI calves) and to reduce losses from transient BVDV infections.²⁸ Cattle that have been infected with BVDV after birth and recovered are considered to be protected from clinical disease following subsequent exposure to the virus, even if they are seronegative.⁵⁸ Seropositive animals due to natural exposure are also considered to be protected from future fetal transmission of the virus so that an immunocompetent dam that is not PI BVDV could have at most one PI calf. While vaccination does provide some protection from fetal infection, the herd level protection is not equal to that generated by natural exposure. As a result, BVDV control is generally achieved by a combination of removal of PI cattle, vaccination and a biosecurity system that prevents the introduction of PI animals into the herd and minimizes the contact with potentially viremic cattle or wildlife.³⁷

Removal of PI animals

Herds should be monitored to determine the risk that one or more PI cattle are present. If the presence of PI cattle is confirmed or strongly suspected, a whole-herd screening protocol as described earlier, should be undertaken to identify and remove PI individuals.

Biosecurity to prevent herd exposure to PI animals

Biosecurity to prevent herd exposure to PI or transiently infected animals is important, especially after the removal of PI cattle, because with the removal of PI BVDV shedders, the percentage of naturally protected seropositive animals in a herd decreases.³⁷ All replacement heifers and bulls that enter the breeding herd, whether raised or purchased, should be tested and confirmed to

not be PI prior to the start of breeding. If a pregnant animal is purchased, it should be segregated from the breeding herd until both the dam and the calf is confirmed to not be PI. Fence line contact with neighboring cattle should be managed so that stocker cattle are not adjacent to the breeding herd during early gestation, and other cowherds are not adjacent unless they also have a strict biosecurity and vaccination program in place.

Vaccination as a component of biosecurity

Biosecurity also involves application of a vaccination protocol to reduce the risk of fetal infection in the event of cowherd exposure to a viremic and shedding animal. To date, using *in vitro* information and limited field trials, one can only make empirical recommendations regarding what constitutes an effective vaccination program to limit postnatal and gestational BVDV transmission. Live, replicating vaccines (MLV) have inherent properties that may enable them to stimulate more complete protection against transplacental infection.³⁷ For that reason, one recommendation is to vaccinate unstressed, healthy heifers with MLV vaccine. Vaccine administration should be timed so that a protective immune response coincides with the first four months of gestation. This is done to maximize the potential for adequate immunity to protect against fetal infection and reproductive failure or the birth of persistently infected calves. In heifers not previously vaccinated, the primary series should consist of two administrations. The first dose should be given when the heifers are six months of age or older, and the second dose should be given two months before breeding. Beef cows should be revaccinated annually before breeding according to label directions.³⁷

Control Program for BVDV in Stocker/Feedlot Operations

Because pregnancy is not a common or desirable component of stocker and feedlot operations, vertical transmission and reproductive losses due to BVDV is not a concern. However, BVDV viremia or seroconversion has been associated with respiratory disease outbreaks in feedlot situations.^{22,23,42,43} Persistently infected cattle are a primary source of BVDV transmission to in-contact susceptible cattle during marketing, trucking, and while in feeding pens and pastures. Vaccination is currently the primary control intervention for BVDV in stocker and feedlot operations. Screening cattle for the presence of PI individuals prior to purchase or at arrival is currently being evaluated for economic return. The economic return will depend on the prevalence of PI cattle, the cost of the testing strategy, the sensitivity and specificity of the test used, and the economic cost of the disease to the operation.

Conclusions

The cost of initiating a BVD PI whole-herd screening protocol on a farm or ranch is significant. Because of the low prevalence of herds with at least one PI animal, veterinary practitioners may not be economically justified to initiate diagnostic whole-herd screening protocols for PI BVDV cattle for all their clients. Wittum *et al* found that among herds where practitioners suspected BVDV-induced syndromes, 19.2% were found to have at least one PI animal present upon screening (95% CI, 10-30%).⁶⁷ By using herd history to pre-select herds that are more likely to benefit from a diagnostic screening protocol for BVDV PI animals, veterinarians can provide a diagnostic and consulting service to their clients that is justified economically. In pre-selected herds, the cost of diagnostic testing is less than the risk of BVDV PI cattle and their cost to herd profitability.

References

1. Archbald LF, Fulton RW, Seder CL, Al-Bagdadi F, Godke RA: Effect of the bovine viral diarrhoea (BVD) virus on preimplantation bovine embryos: a preliminary study. *Therio* 11:81-89, 1979.
2. Baker JC: Bovine viral diarrhoea virus: a review. *J Am Vet Med Assoc* 190:1449-1458, 1987.
3. Barber DML, Nettleton PF, Herring JA: Disease in a dairy herd associated with the introduction and spread of bovine virus diarrhoea virus. *Vet Rec* 117:459-464, 1985.
4. Baszler TV, Evermann JF, Kaylor PS, Byington TC, Dilbeck PM: Diagnosis of naturally occurring bovine viral diarrhoea virus infections in ruminants using monoclonal antibody-based immunohistochemistry. *Vet Pathol* 32:609-618, 1995.
5. Bezek DM, Stofregen D, Posso M: Effect of cytopathic bovine viral diarrhoea virus (BVDV) superinfection on viral antigen association with platelets, viremia, and specific antibody levels in two heifers persistently infected with BVDV. *J Vet Diagn Invest* 7:395-397, 1995.
6. Bielanski A, Nadin-Davis S, Sapp T, Lutze-Wallace C: Viral contamination of embryos cryopreserved in liquid nitrogen. *Cryobiology* 40:110-116, 2000.
7. Binkhorst GJ, Journee DLH, Wouda W, Straver PJ, Vos JH: Neurological disorders, virus persistence and hypomyelination in calves due to intrauterine infection with bovine virus diarrhoea virus. I. Clinical symptoms and morphological lesions. *Vet Q* 5:145-155, 1983.
8. Bolin SR: The current understanding about the pathogenesis and clinical forms of BVD. *Vet Med* 85:1124-1132, 1990.
9. Bolin SR, McLurkin AW, Cutlip RC, Coria MF: Frequency of persistent bovine viral diarrhoea virus infection in selected cattle herds. *Am J Vet Res* 46:2385-2387, 1985.
10. Brock KV, Redman DR, Vickers ML, Irvine NE: Quantification of bovine viral diarrhoea virus in embryo transfer flush fluids collected from a persistently infected heifer. *J Vet Diagn Invest* 3:99-100, 1991.
11. Brock KV, Grooms DL, Ridpath, J, Bolin SR: Changes in levels of viremia in cattle persistently infected with bovine viral diarrhoea virus. *J Vet Diagn Invest* 10:22-26, 1998.
12. Brownlie J, Clarke MC, Howard CJ, Pocock DH: Pathogenesis and epidemiology of bovine virus diarrhoea virus infection of cattle. *Annals Rech vet* 18:157-166, 1987.
13. Carlsson U, Belak K: Border disease virus transmitted to sheep and cattle by a persistently infected ewe: Epidemiology and control. *Acta Vet Scand* 35:79-88, 1987.
14. Casaro APE, Kendrick JW, Kennedy PC: Response of the bovine fetus to bovine viral diarrhoea-mucosal disease virus. *Am J Vet Res* 32:1543-1562, 1971.

15. Cherry BR, Reeves MJ, Smith G: Evaluation of bovine viral diarrhoea virus control using a mathematical model of infection dynamics. *Prev Vet Med* 33:91-108, 1998.
16. Davidson WR, Crow CB: Parasites, diseases, and health status of sympatric populations of sika deer and white-tailed deer in Maryland and Virginia. *J Wildlife Dis* 19:345-348, 1983.
17. DuBois WR, Cooper VL, Duffy JC, Dean DD, Ball RL, Starr BD, Jr: A preliminary evaluation of the effect of vaccination with modified live bovine viral diarrhoea virus (BVDV) on detection of BVDV antigen in skin biopsies using immunohistochemical methods. *Bov Pract* 34:98-100, 2000.
18. Dubovi EJ: Laboratory diagnosis of bovine viral diarrhoea virus infections. *Vet Med* 91:867-872, 1996.
19. Duffell SJ, Harkness JW: Bovine virus diarrhoea - mucosal disease infection in cattle. *Vet Rec* 117:240-245, 1985.
20. Ellis JA, Martin K, Norman GR, Haines DM: Comparison of detection methods for bovine viral diarrhoea virus in bovine abortions and neonatal death. *J Vet Diagn Invest* 7:433-436, 1995.
21. Frolich K, Thiede S, Kozikowski T, Jakob W: A review of mutual transmission of important infectious diseases between livestock and wildlife in Europe. *Ann New York Acad Sci* 969:4-13, 2002.
22. Fulton RW, Purdy CW, Confer AW, Saliki JT, Loan RW, Briggs RE, Burge LJ: Bovine viral diarrhoea viral infections in feeder calves with respiratory disease: Interactions with *Pasteurella* spp, parainfluenza-2 virus, and bovine respiratory syncytial virus. *Can J Vet Res* 64:151-159, 2000.
23. Fulton RW, Ridpath JF, Saliki JT, Briggs RE, Confer AW, Burge LJ, Purdy CW, Loan RW, Duff GC, Payton ME: Bovine viral diarrhoea virus (BVDV) 1b: predominant BVDV subtype in calves with respiratory disease. *Can J Vet Res* 66:181-190, 2002.
24. Grahn TC, Fahning ML, Zemjanis R: Nature of early reproductive failure caused by bovine viral diarrhoea virus. *J Am Vet Med Assoc* 185:429-432, 1984.
25. Grondahl C, Uttenthal A, Houe H, Rasmussen TB, Hoyer MJ, Larsen LE: Characterisation of a pestivirus isolated from persistently infected mousedeer (*Tragulus javanicus*). *Arch Virol* 148(8):1455-1463, 2003.
26. Grooms DL, Brock KV, Ward LA: Detection of bovine viral diarrhoea virus in the ovaries of cattle acutely infected with bovine viral diarrhoea virus. *J Vet Diagn Invest* 10:125-129, 1998.
27. Gunn HM: Role of fomites and flies in the transmission of bovine viral diarrhoea virus. *Vet Rec* 132:584-585, 1993.
28. Harkness JW: The control of bovine viral diarrhoea virus infection. *Ann Rech Vet* 18:167-174, 1987.
29. Houe H: Epidemiology of bovine viral diarrhoea virus. *Vet Clin North Am Food Anim Pract* 11:521-547, 1995.
30. Houe H: Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. *Vet Micro* 64:89-107, 1999.
31. Houe H, Baker JC, Maes RK, Wuryastuti H, Wasito R, Ruegg PL, Lloyd JW: Prevalence of cattle persistently infected with bovine viral diarrhoea virus in 20 dairy herds in two counties in central Michigan and comparison of prevalence of antibody-positive cattle among herds with different infection and vaccination status. *J Vet Diagn Invest* 7:321-326, 1995.
32. Houe H, Meyling A: Surveillance of cattle herds for bovine virus diarrhoea virus (BVDV) infection using data on reproduction and calf mortality. *Arch Virol Suppl* 3:157-164, 1991.
33. Houe H, Myrup Pedersen K, Meyling A: The effect of bovine virus diarrhoea virus infection on conception rate. *Prev Vet Med* 15:117-123, 1993.
34. Howard CJ, Brownlie J, Thomas LH: Prevalence of bovine virus diarrhoea virus viremia in cattle in the UK. *Vet Rec* 119:628-629, 1986.
35. Kafi M, McGowan MR, Jillella D, Davies F, Johnston S, Kirkland PD: The effect of bovine viral diarrhoea virus (BVDV) during follicular development on the superovulatory response of cattle. *Therio* 41:223, 1994.
36. Kahrs RF: Effects of bovine viral diarrhoea on the developing fetus. *J Am Vet Med Assoc* 163:877-878, 1973.

37. Kelling CL: Planning bovine viral diarrhoea virus vaccination programs. *Vet Med* 91:873-877, 1996.
38. Kelling CL, Grotelueschen DM, Smith DR, Brodersen BW: Testing and management strategies for effective beef and dairy herd BVDV biosecurity programs. *Bov Pract* 34(1):13-22, 2000.
39. Larson RL, Brodersen BW, Grotelueschen DM, Hunsaker BD, Burdett W, Brock KV, Fulton RW, Goehl DR, Sprowls RW, Kennedy JA, Loneragan GH, Dargatz DA: Considerations for bovine viral diarrhoea (BVD) testing. *Bov Pract* 39(2):96-100, 2005.
40. Larson RL, Pierce VL, Grotelueschen DM, Wittum TE: Economic evaluation of beef cowherd screening for cattle persistently-infected with bovine viral diarrhoea virus. *Bov Pract* 36:106-112, 2002.
41. Liess B, Moenning V: Ruminant pestivirus infection in pigs. *Rev Sci Tech Off Int Epiz* 9:151-161, 1990.
42. Loneragan GH, Thomson DU, Montgomery DL, Mason GL, Larson RL: Prevalence, outcome, and animal-health consequences of feedlot cattle persistently infected with bovine viral diarrhoea virus. *J Am Vet Med Assoc* 226: 595-601, 2005.
43. Martin SW, Bateman KG, Shewen PE, Rosendal S, Bohac JE: The frequency, distribution and effects of antibodies to seven putative respiratory pathogens on respiratory disease and weight gain in feedlot calves in Ontario. *Can J Vet Res* 53:355-362, 1989.
44. McClurkin AW, Coria MF, Cutlip RC: Reproductive performance of apparently healthy cattle persistently infected with bovine viral diarrhoea virus. *J Am Vet Med Assoc* 174:1116-1119, 1979.
45. McClurkin AW, Littledike ET, Cutlip RC, Frank GH, Coria MF, Bolin SR: Production of cattle immunotolerant to bovine viral diarrhoea virus. *Can J Comp Med* 48:156-161, 1984.
46. McGowan MR, Kirkland PD, Richards SG, Littlejohns IR: Increased reproductive losses in cattle infected with bovine pestivirus around the time of insemination. *Vet Rec* 133:39-43, 1993.
47. Miller MA, Ramos-Vara JA, Kleiboeker SB, Turnquist SE, Rosenkrans KS, Larson RL: Elimination of persistent bovine viral diarrhoea virus infection in a beef cattle herd. 45th Annual Meeting, American Association of Veterinary Laboratory Diagnosticians, St. Louis, MO, Oct 19-21, 2002, pp 69.
48. Moerman A, Straver PJ, de Jong MCM, Quak J, Baanvinger Th, van Oirschot JT: A long term epidemiological study of bovine viral diarrhoea infections in a large herd of dairy cattle. *Vet Rec* 622-626, 1993.
49. Meyling A, Jensen AM: Transmission of bovine virus diarrhoea virus (BVDV) by artificial insemination (AI) with semen from a persistently infected bull. *Vet Micro* 17:97-105, 1988.
50. Muñoz-Zanzi CA, Johnson WO, Thurmon MC, Hietala SK: Pooled-sample testing as a herd-screening tool for detection of bovine viral diarrhoea virus persistently infected cattle. *J Vet Diagn Invest* 12:195-203, 2000.
51. Nielsen SS, Roensholt L, Bitsch V: Bovine virus diarrhoea virus in free-living deer from Denmark. *J Wildlife Dis* 36:584-587, 2000.
52. Njaa BL, Clark EG, Janzen E, Ellis JA, Haines DM: Diagnosis of persistent bovine viral diarrhoea virus infection by immunohistochemical staining of formalin-fixed skin biopsy specimens. *J Vet Diagn Invest* 12:393-399, 2000.
53. Orban S, Liess B, Hafez SM Frey HR, Blindow H, Sasse-Patzer B: Studies on transplacental transmissibility of a bovine virus diarrhoea (BVD) vaccine virus. I. Inoculation of pregnant cows 15 to 90 days before parturition (190th to 265th day of gestation). *Zbl Vet Med B* 30:619-534, 1983.
54. Palfi V, Houe H, Philipsen J: 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, 1993.
55. Paton DJ, Goodey R, Brockman S, Wood L: Evaluation of the quality and virological status of semen from bulls acutely infected with BVDV. *Vet Rec* 124:63, 1989.
56. Rae AG, Sinclair JA, Nettleton PF: Survival of bovine virus diarrhoea virus in blood from persistently infected cattle. *Vet Rec* 120:504, 1987.
57. Revell SG, Chasey D, Drew TW, Edwards S: Some observations on the semen of bulls persistently infected with bovine virus diarrhoea virus. *Vet Rec* 123:122-125, 1988.
58. Ridpath JF, Neill JD, Endsley J, Roth JA: Effect of passive immunity on the development of a protective immune response against bovine viral diarrhoea virus in calves. *Am J Vet Res* 64:65-69, 2003.
59. Roeder PL, Harkness JW: BVD virus infection: prospects for control. *Vet Rec* 118:143-147, 1986.
60. Rufenacht J, Schaller P, Audige L, Knutti B, Kupfer U, Peterhans E: The effect of infection with bovine viral diarrhoea virus on the fertility of Swiss dairy cattle. *Therio* 56:199-210, 2001.
61. Singh EL: Disease control: procedures for handling embryos. *Rev Sci Tech Off Int Epiz* 4:867-872, 1985.
62. Singh EL, Eaglesome MD, Thomas FC, Papp-Vid G, Hare WCD: Embryo transfer as a means of controlling the transmission of viral infections. I. The *in vitro* exposure of preimplantation embryos to akabane, bluetongue and bovine viral diarrhoea viruses. *Therio* 17:437-444, 1982.
63. Tarry DW, Bernal L, Edwards S: Transmission of bovine virus diarrhoea virus by biting flies. *Vet Rec* 128:82-84, 1991.
64. Terpstra C, Wensvoort G: Natural infections of pigs with bovine viral diarrhoea virus associated with signs resembling swine fever. *Res Vet Sci* 45:137-142, 1998.
65. Traven M, Alenius S, Fossum C, Larsson B: Primary bovine viral diarrhoea virus infection in calves following direct contact with a persistently viraemic calf. *J Vet Med B* 38:453-462, 1991.
66. Wentink GH, Van Exsel ACA, Goey I, van Lieshout JA: Spread of bovine virus diarrhoea virus in a herd of heifer calves. *Vet Quart* 13:233-236, 1991.
67. Wittum TE, Grotelueschen DM, Brock KV, Kvasnicka WG, Floyd JG, Kelling CL, Odde KG: Persistent bovine viral diarrhoea virus infection in U.S. beef herds. *Prev Vet Med* 49:83-94, 2001.