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Testing and Management Strategies for Effective Beef and Dairy Herd BVDV Biosecurity Programs

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Abstract

Elements of a bovine viral diarrhea virus (BVDV) biosecurity program, associated costs and benefits, and criteria veterinarians and producers can use in considering whether to adopt a program are described in this report. Protection against BVDV infections includes proper immunization, identification and elimination of persistently infected (PI) carrier cattle, and prevention of exposure of cattle at risk to BVDV.

The decision to implement a BVDV biosecurity program must be based on a herd-by-herd cost-benefit analysis. Costs include diagnostic testing as well as costs imposed by constraints on production practices, which are necessary to implement the program. Benefits include reduced disease loss, potential added value of replacement breeding animals which can be marketed as test-negative for PI infection, and pregnant females at low risk of carrying a PI fetus. Various herd-level diagnostic strategies have been devised to screen herds for the presence of BVDV, identify and eliminate PI carrier cattle, or help prevent new exposure of the herd to BVDV.

No universally applicable protocol exists for herd-level BVDV biosecurity. The specific biosecurity methods used in each herd depend on the type of livestock production system involved. Important variables to consider are the number of animals in the herd, livestock concentration, distribution of animal groups, and desired level of biosecurity assurance.

When BVDV virus is known to exist in a herd, control of new infections must take into account the possible presence of PI animals. A single PI animal can serve as the source of virus for a herd, and a stringent herd-testing program may be required to identify and eliminate PI animals. Producers must understand the risks of re-exposure occurring in a BVDV-free herd and the importance of continuous management to prevent contact with cattle from other herds.

In BVDV-free herds, it is essential to annually test and confirm the BVDV PI-free status of all calves born. Purchased animals should be isolated and tested before being added to the herd to avoid introduction of transiently infected animals. It is strongly recommended that bred heifers from outside sources not be added to the BVDV-free herd. Purchasing tested, non-pregnant replacement heifers is less risky than buying tested pregnant females. If tested, pregnant cattle are purchased, then the offspring must also be tested to confirm their BVDV PI-free status before introduction into the herd.

Impact of BVDV Infections on Production in Cattle Herds

Since bovine viral diarrhea virus (BVDV) infections are economically important to the cattle industry,[26] effective BVDV control programs can increase opportunity for herd profitability. Productivity losses caused by BVDV stem from reproductive losses, clinical diseases caused by acute infections, losses associated with persistent infections, and immunosuppression, which is a component of multifactorial diseases such as respiratory and enteric diseases.[10] Control of BVDV infection is achieved with biosecurity measures that limit exposure to infected animals[30] and by boosting herd immunity.

Persistent BVDV infections may occur if the fetus is infected during the first trimester of gestation before it becomes immunocompetent.[31] The fetus generally begins to develop immunocompetence during the third or fourth month of gestation.[32] The source of the virus that infects the fetus is viremia of the pregnant dam stemming from either persistent or acute infection. Persistently infected (PI) calves are persistently viremic and shed virus continuously. Persistent viremia develops due to fetal immunotolerance and, thus, failure to develop antibodies against the persisting virus.[14] Viremia is long-term. However, its level may decline with development of neutralizing antibody and become undetectable as the animal ages.[7] Decline of viremia may be due to deterioration of highly specific immunotolerance to the persisting virus, which results in antibody formation and viral clearance.[13] This is likely due to generation of antigenically variant viruses within the PI animal which, over time, stimulate an immune response in the formerly immunotolerant animal.[13] Persistently infected animals are carriers, and they may spread virus throughout the herd.[32]

Persistently infected calves are also at risk of developing mucosal disease.[6,12] Mucosal disease usually occurs in cattle between 6 months and 2 years old, but may occur at any age. Characteristic clinical signs include anorexia, pyrexia, diarrhea, loss of condition and death.[2,3] Gross pathologic lesions may include erosive/ulcerative lesions on the muzzle and lips, buccal mucosa and tongue. Elongated erosive lesions are common in the esophagus. Erosions may also be found on the rumen pillars or reticulum. Erosive edematous and hemorrhagic lesions may occur in the abomasum. Enteritis may be evident, and may vary from cattarhal to hemorrhagic, fibrinonecrotic or erosive/ulcerative. Peyer's patches and lymphoid tissue in the proximal colon may be hemorrhagic. Thymus atrophy and enlarged peripheral lymph nodes may also be noted.[5]

Acute BVDV infections can also cause reproductive failure in cattle.[41] The outcome of intrauterine infections with BVDV is primarily determined by gestational stage and by virulence and biotype of the virus.[32] Acute BVDV infection of the female can alter ovarian function, which can lead to reduced fertility.[23] BVDV infection of fetuses during the first and second trimester may cause fetal death and abortion. Third-trimester abortions have also been attributed to BVDV infection.[32] Congenital defects, such as cerebellar hypoplasia, may result if fetal infection with BVDV occurs between the fourth and sixth months of gestation.[11]

Immunosuppression from acute BVDV infection plays a role in clinical respiratory tract or enteric diseases in calves.[10] Cattle acutely infected with BVDV also shed virus, but for a relatively limited time period as compared to PI cattle.[33] The BVDV probably persists in the environment for no more than a couple of weeks, so transmission of virus is primarily direct. Virus may be transmitted two ways: vertically from dam to offspring, or horizontally through inhalation or ingestion of material contaminated with body secretions and excretions from infected animals.

Herd-specific Factors to Consider Before Implementing a BVDV Biosecurity Program

The decision to implement a BVDV biosecurity program in a particular herd should be based on a cost-benefit analysis.[26] Costs of a BVDV biosecurity program include the costs of diagnostic testing and the costs imposed by constraints on management practices necessary to implement the program.[37] For example, commingling of cattle or other movement of animals may be restricted by the design of a BVDV biosecurity program.

The benefits of a BVDV biosecurity program are numerous and include reduced disease loss from such postnatal acute infections as neonatal calf diarrhea.[17] Fewer prenatal infections, leading to reproductive failures, should occur. Moreover, such a program has the potential to add value to breeding animals. Bulls or replacement heifers marketed as test-negative for PI infection tend to bring higher prices, as do pregnant females at low risk of carrying a PI fetus.

The goal of an effective BVDV biosecurity program is to reduce the risk of BVDV entry into a cattle herd, especially at a time of the production cycle when the consequences of infection are greatest.[30] Cattle at highest risk for losses from BVDV infection are pregnant females (which may suffer reproductive losses), and calves less than 6 months of age. Cattle that are less than 6 months old and immunosuppressed by acute BVDV infections are susceptible to respiratory tract disease.[10] This problem is especially important in this age group if they have undergone some common stressors (eg. reduced feed and water intake, fatigue, and exposure to numerous respiratory pathogens) typically associated with weaning, transport and concentration in feedyards.

Principles of BVDV Biosecurity

An effective BVDV biosecurity program involves three principles: 1) enhancement of immunity, 2) prevention of exposure of cattle at risk to BVDV infections, and 3) elimination of PI carriers (which are reservoirs of infection) from the herd.

Enhancement of immunity to BVDV is accomplished by vaccination. Cattle immunized with commercially available vaccines may be less likely to exhibit clinical signs of acute BVDV infection.[16] However, the ability of vaccination to reliably protect the fetus against naturally occurring BVDV infection has been questioned,[20,39] and published field observations[29] support this

concern. Experimental challenge-exposure studies have demonstrated a reasonable degree of protection against fetal infection using a modified live virus vaccine[15] (MLV), and partial protection using an inactivated vaccine.[21] In one study, 10 of 12 dams were vaccinated prebreeding with MLV vaccine and experimentally-challenge exposed between 70 and 75 days of gestation. These were successfully protected against giving birth to PI calves.[15] In an earlier study of dams vaccinated prebreeding with inactivated vaccine and experimentally-challenge exposed between 80 and 90 days of gestation, 36% of fetuses were protected against infection.[21] In both studies, fetuses of all the non-vaccinated control dams were infected with BVDV following experimental-challenge exposure.[15,21]

Since no BVDV vaccine has been shown to give complete fetal protection,[40] producers are well-advised against relying solely on vaccination to protect their herds from losses due to BVDV. They should also implement management practices to eliminate PI carrier cattle and avoid exposure to BVDV infection.

The second principle of BVDV biosecurity, prevention of exposure of at-risk cattle to BVDV infection, is achieved by managing animal movement.[30] Exposure that results in BVDV infection occurs primarily from direct contact with infected cattle. Transmission of BVDV can therefore be minimized by physically separating groups of cattle. Exposure resulting in infection is reduced in beef herds maintained on range under low-animal-density conditions. Dairies are better suited to be managed by segregating cattle by age groups to minimize exposure that results in infections.

While strict physical separation may minimize the rate of BVDV transmission, contact with PI animals within groups is still likely to result in transmission, and some movement of cattle between groups is inevitable in most production systems. Obviously, commingling groups of cattle from different sources appreciably increases the risk of BVDV transmission.

The third principle of BVDV biosecurity, elimination of PI carriers from the herd, is achieved by testing the herd and closing the herd to incoming animals which are potentially PI or acutely infected carriers. Persistently infected cattle are the primary reservoirs of viral exposure within herds. Therefore, removal of PI carrier cattle from herds and preventing introduction of PI cattle are important BVDV biosecurity practices.[17,30]

Identification and removal of PI cattle requires accurate herd-based laboratory diagnostic testing.[36] Before implementing a large-scale herd-based testing program for BVDV in a herd with promise for success, the attending veterinarian must consider the type of production system involved, the objectives of testing and the expected returns from the testing strategy for that herd. The objectives of herd-based testing for BVDV are usually: 1) to screen herds for evidence of cattle with acute or persistent BVDV infection,[33] 2) to identify and remove PI carrier animals from the herd,[30] or 3) to prevent new BVDV exposure in the BVDV-free herd[17] (Figure 1).

Screening the Herd for Evidence of Cattle with Acute or Persistent BVDV Infection

The objective of screening for evidence of cattle with acute or persistent BVDV infections is to determine the BVDV status of herds that do not have a history of clinical BVDV infection. Determining if BVDV infection is active in the herd (i.e. animals with either acute or persistent infections are present) may be most effectively achieved by testing for the presence of antibodies to BVDV in non-vaccinated, sentinel animals.[27,28] This testing strategy is based on use of a subset of unvaccinated calves from each group or herd of cattle that serve as contact control animals. These animals are tested at 8 months of age or later for BVDV antibodies.[30] Absence of BVDV antibodies in the sentinel animals indicates an absence of cattle that shed virus—both acutely infected and PI animals—and the herd may be considered BVDV-free. Conversely, presence of BVDV antibodies[22] in this non-vaccinated sub-group of young animals indicates current or recent exposure to BVDV,[30] signifying the presence of acutely infected and/or PI animal(s) in the herd.[26] Additional testing is then indicated to determine if PI carrier cattle actually are present and to identify and eliminate them.

Use of inactivated BVDV vaccine may be conservatively recommended for this application if there is a concern for the possibility of MLV virus being shed from vaccinated animals to sentinel contact control animals, resulting in development of BVDV antibody titers in those animals (an implication based on label precaution statements for modified live vaccine). This inadvertent scenario would result in the erroneous conclusion that a virulent field strain of BVDV was circulating in the herd, resulting in a false positive test result. In practice, this precautionary step may be unnecessary. It has been shown possible to identify herds containing PI cattle, in which all animals are vaccinated, on the basis of distribution of BVDV antibody titers.[39]

The optimal number of animals to use as non-vaccinated sentinels depends upon stocking density, and varies among herds. Obviously, the probability of BVDV transmission increases directly with increased livestock population density. It is arbitrarily recommended that 10 percent or ten animals of a group of cattle, whichever is greater, be reserved as sentinels. This number is subject to adjustment and may be reduced for herds maintained in confinement or increased for herds maintained at a low stocking density on rangeland.

Dairy herds have been screened for evidence of BVDV PI carriers in the lactating herd by polymerase

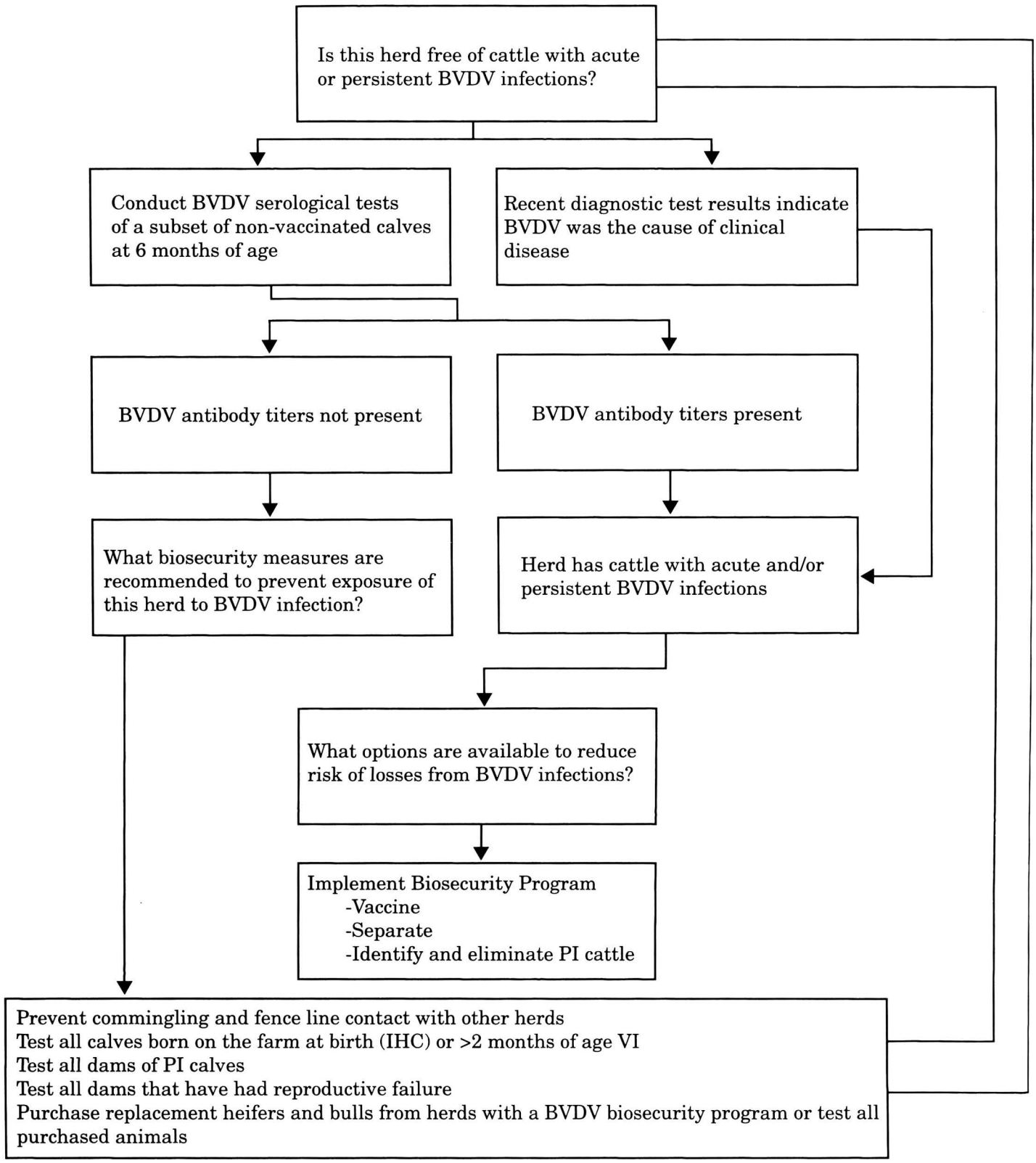

Figure 1. The objectives of herd testing for BVDV.

chain reaction (PCR) assay of the somatic cells of bulk-tank milk.[18,34] This approach may lack sufficient sensitivity for a whole-herd test, because only actively lactating animals are included. Other testing methods must be used to determine whether PI animals are present in the nonlactating group.[18] The PCR assay has also been successfully used to detect acutely infected cattle by testing somatic cells of milk from an experi-

mentally infected cow,[34] and from bulk-tank milk of naturally infected cows.[18] Sensitivity of the bulk-tank milk PCR assay for detecting acutely infected cows is lower than for detecting PI animals.[18] Thus, use of the PCR assay of somatic cells of bulk tank milk to detect acutely infected animals has limitations. Using PCR in an experimental acute infection of a cow, BVDV RNA was only detected from days 6 to 10 post-inoculation.[34]

Identifying and Removing PI Carrier Cattle from the Herd

Diagnostic tests for detection of PI animals

Three laboratory diagnostic tests are typically available to identify and remove PI animals from beef or dairy cattle herds: 1) virus isolation (VI) test, 2) immunohistochemistry (IHC), and 3) PCR assay. Each test has inherent advantages and limitations (Table 1) which help guide selection for a strategic testing program.

The VI test is available in two laboratory formats. The standard (macro) VI test—the most widely used and reliable BVDV diagnostic test[8,29]—is typically used as a standard to compare the sensitivity and specificity of other tests. The procedure is labor-intensive, which is a major drawback. It is impractical for laboratory personnel to efficiently test large numbers of samples, making it less suitable for a large herd. The test can, however, be used for testing a limited number of samples. The standard VI test may be used to test washed mononuclear cell preparations (buffy coats) from blood samples collected in tubes with anticoagulants. Use of that specimen avoids interference from antibodies, which reduce test sensitivity.

An alternative VI test format is the immunoperoxidase microtiter plate VI assay.[1,35] This test, which is relatively sensitive and specific, is designed to efficiently test large numbers of samples. It uses blood serum, and is practical for herd screening programs. Presence of maternal antibodies may affect the ability to isolate BVDV from the serum of younger PI cattle,[8,29] so blood is collected for VI after the calves reach 2 months of age when maternal antibody titers have declined.

Table 1. Diagnostic laboratory tests for detection of PI carrier cattle

Test	Specimen	Advantages	Limitations
Serological tests	Serum	Practical method for testing large numbers of of samples.	Requires modification of herd vaccination program
Standard virus isolation (VI)	Serum (2+ months) Blood (Any age)	High specificity	Not practical for testing large numbers of samples. Presence of antibodies may interfere with test sensitivity when testing serum.
Immunoperoxidase microtiter plate assay (Virus isolation)	Serum (2+ months)	Practical for testing large numbers of samples. Herd test.	Presence of maternal antibody may interfere with test sensitivity when testing serum.
Immunohisto-chemistry (IHC) test	Skin biopsy (Ear notch)	Practical for testing large numbers of samples. Test sensitivity not affected by presence of antibody. Useful in any age group of cattle. Biopsy sample may be saved for future testing.	Biopsy requirement.
Polymerase chain reaction (PCR) test	Bulk-tank milk; serum or whole blood.	High sensitivity. Test sensitivity unaltered by presence of antibody.	Limited availability of test. False positive tests may occur.

The second diagnostic test to screen for PI carrier cattle is IHC.[9,38] Skin biopsies (ear notches) are collected for testing from any age animal, fixed in formalin and submitted to the diagnostic laboratory. The fixed skin biopsies are sectioned, stained and examined for BVDV antigen. Preliminary results of comparative evaluations of IHC and VI show good correlation in test sensitivity and specificity between IHC and VI for detection of PI carrier animals.[9] Sensitivity of IHC is unaffected by the presence of maternal antibody so calves of any age, including newborn calves, may be tested. Since IHC testing service is not available from all diagnostic laboratories, availability of this test may be limited in certain areas.

The third diagnostic laboratory test to screen a herd for presence of BVDV PI carrier cattle is the PCR assay for BVDV RNA.[18,34] The PCR may be used to test individual animals using serum or whole blood samples, or to test whole herds by using pooled samples, such as bulk-tank milk or pooled serum samples. The BVDV PCR test is not universally available from all diagnostic laboratories. The test is highly sensitive, which was recently confirmed in a bulk-tank milk assay report in which 1 PI animal was detected in a herd of 162 lactating animals.[18] A potential complication with the PCR assay is lack of test specificity, resulting in false positive test results. This problem may occur sporadically because of non-specific reactions with contaminating viral RNA (unpublished observation). Therefore, it is generally advisable to confirm positive BVDV PCR assay results with VI tests.

Herd testing strategies to identify PI cattle

Comprehensive strategies for testing and eliminating BVDV carrier cattle from beef and dairy herds are outlined in Figures 2 and 3. The greatest proportion of PI animals in BVDV-infected herds are calves less than 6 months of age;[25] therefore, PI carriers in a herd may be more efficiently identified in the early phases of the testing program by targeting calves rather than dams. Testing of young animals also has the advantage of determining if recent infection has occurred in the herd.[30] This strategy allows the testing program to be subdivided into phases rather than simultaneously testing all animals in a herd.

Initially, calves are tested as a follow-up to information about the PI status of the calves and their dams. Since PI dams always give birth to PI calves, testing calves for PI by VI or IHC may provide diagnostic information about that calf's dam without actually sampling and testing the dam (the dam of a PI-negative calf is not PI, while the dam of a PI calf may be PI). Dams of calves identified as PI must then be tested to determine their PI status.

A complication of centering the testing on the calf population in dairy herds is that bull calves may not be available for testing by VI at 2 months of age when colostral antibody titers have declined. Alternatively, skin biopsies for IHC testing, or pre-colostral serum samples obtained at birth, could be used.

Persistently infected cattle may die young or be culled for reduced growth and performance. Because of this attrition, few PI cattle are expected in the breeding herd. Nevertheless, some PI cattle perform normally[14,24,29] or well enough to be retained in the adult breeding herd, so it is important to use laboratory testing to identify PI cattle from all age groups. Brief exposure of the herd to PI animals of any age presents a significant risk of exposure resulting in acute BVDV infection. The presence of even a single PI animal jeopardizes the effectiveness of a BVDV biosecurity program; therefore, a testing strategy to identify all PI carrier cattle in a herd is mandatory. All cattle in the herd are tested (note that unborn PI calves would not be detected; therefore, newborn calves must be tested at birth).[37]

The PCR assay of the somatic cells of bulk-tank milk has been used to screen for evidence of BVDV PI carriers in the lactating herd.[18,34] This diagnostic approach is less expensive than testing individuals, but has limitations as previously discussed.

Because young calves in the herd are the most likely source of virus exposure for pregnant cattle, timeliness of testing and removal of PI cattle is important to prevent exposure of unborn fetuses to BVDV. Emphasis must be placed on testing and removing PI calves from the breeding herd before they have an opportunity to transmit BVDV to pregnant cattle and expose fetuses to infection. In typical beef herds, testing and removing PI calves must be completed before the breeding season begins to prevent contacts of PI calves with pregnant cows. Testing each calf in the current calf crop does this. Replacement heifers, open cows, bulls, and dams of any positive calves also are tested. Serums from cattle greater than 2 months old or skin biopsies from cattle of any age, including newborn calves, are tested. Cattle in dairies can be segregated by age to prevent contact between pregnant cattle and young calves until PI testing of the calves is completed.

When diagnostic testing for PI cattle is done in a herd to determine the source of herd infections following occurrence of clinical disease caused by BVDV, and PI carriers are not identified after whole-herd testing, then other sources of infection should be considered. Other potential sources of BVDV infections in a herd include the presence of acutely infected cattle[25,33] or exposure to acutely-infected or PI cattle in neighboring herds with fence-line contact. Acute BVDV infections of calves are common when colostral immunity wanes, such as late summer or fall in traditional spring-calving beef herds. Thus, acutely infected calves in contact with susceptible pregnant cows may be sources of fetal infection.

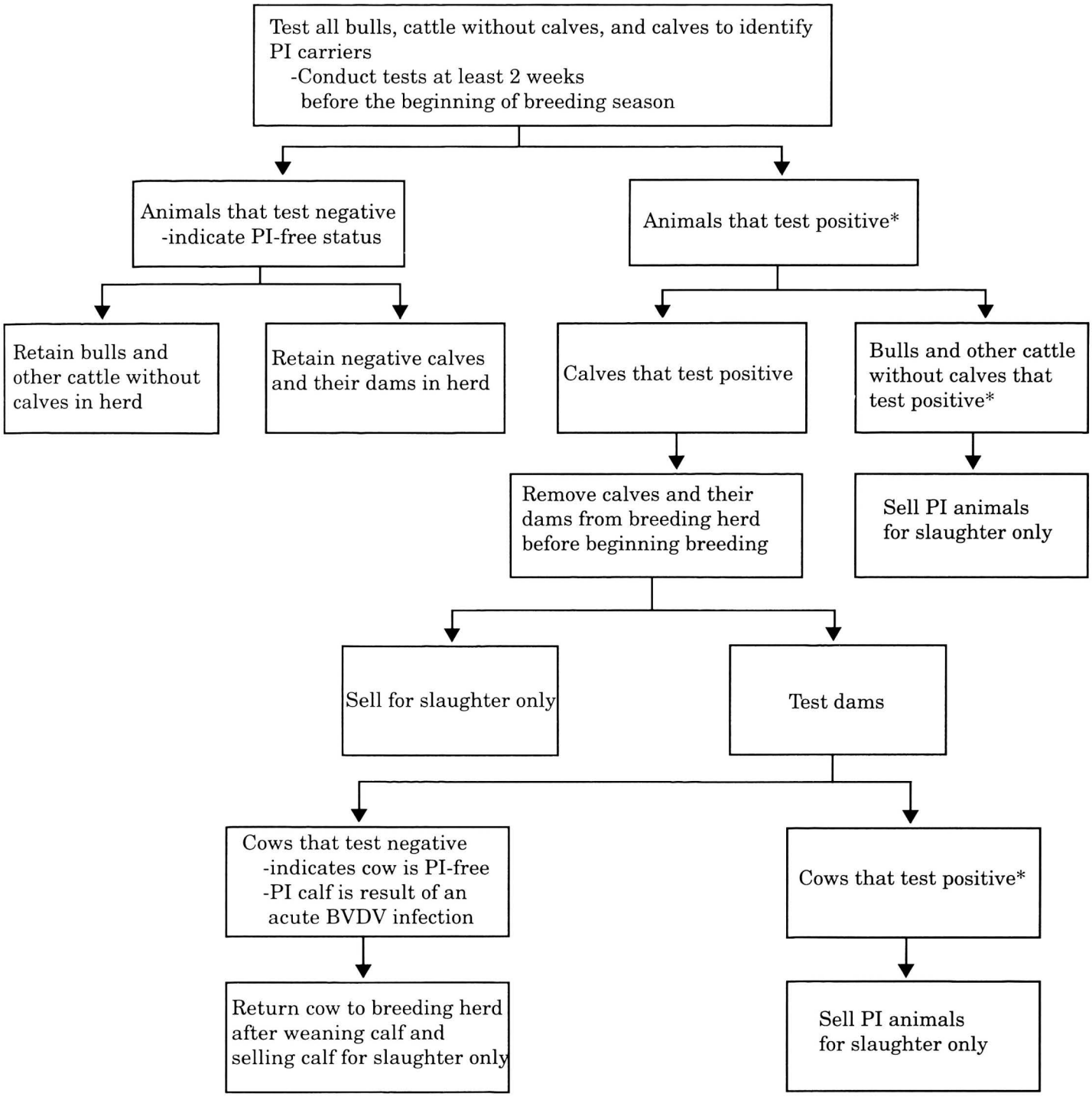

Figure 2. Flow chart for testing a beef herd, prebreeding, to detect and eliminate BVDV PI carrier cattle. *After 30 days retest animals that test positive to confirm PI status. Animals that test negative in second test indicates that they were transiently infected when initially tested and therefore are non-PI.

Preventing New BVDV Exposure from Occurring in the BVDV-free Herd

Probability of re-exposure of a BVDV-free herd to BVDV infection after completion of a PI carrier testing and removal program must be considered and carefully weighed before committing to the financial and management challenges imposed by an extensive BVDV testing program. BVDV infection may be introduced into a herd through contact with cattle from other herds (*e.g.* commingling on range, fence-line contact, purchased additions), or from herd additions born PI because of acute BVDV infection during gestation.[37]

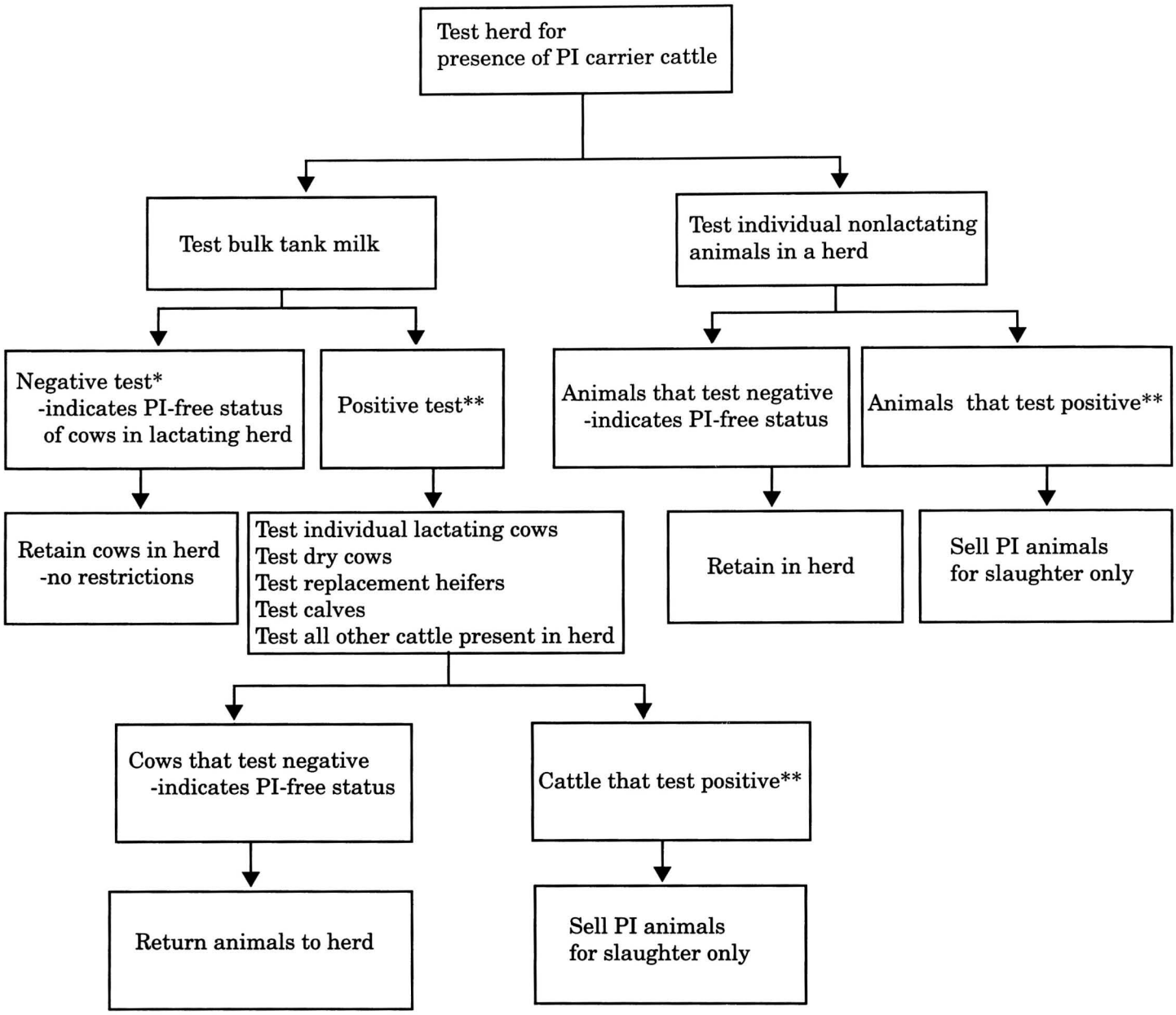

Figure 3. Flow chart for testing a dairy herd to detect and eliminate BVDV PI carrier cattle.
*A bulk tank milk sample may yield false negative results if 1) no shedding in the milk, 2) shedding virus in milk below detectable levels, 3) virus RNA destroyed in milk, or 4) there are PCR inhibitors present.
**After 30 days retest animals that test positive to confirm PI status. Animals that test negative in second test indicates that they were transiently infected when initially tested, and therefore are non-PI.

Biosecurity against BVDV cannot be assured in a herd if cattle are allowed to commingle or have fenceline contact with herds of unknown BVDV status. A BVDV biosecurity program requires that all purchased cattle be tested for PI status by virus isolation, or originate directly from herds that can document extreme unlikelihood of BVDV infection due to a strict biosecurity program. Purchased additions to the herd should be isolated and tested before mixing with the herd to avoid introduction of transiently infected animals.[4,17,37]

It is strongly recommended that bred heifers not be purchased from outside sources and added to the BVDV-free herd. Purchasing tested, nonpregnant replacement heifers presents less risk than buying tested pregnant females.[36] If tested, pregnant cattle are purchased, then the offspring of those animals must also be tested to confirm their BVDV PI-free status before being introduced into the herd.[37] Calves born on the farm also have potential to be PI, and should be tested each year by VI or IHC.

Table 2. BVDV biosecurity program for beef herds

Objective	Approach
Screening the beef herd for cattle with active acute or persistent BVDV infections	BVDV serology of a subset of calves at 6 months of age, non-vaccinated and commingled with herdmates.
Identifying and removing BVDV persistently-infected (PI) cattle from the beef herd	Test most recent calf crop by: 1) Virus isolation (VI) from serum of all calves >2 months old, and/or 2) Immunohistochemistry (IHC) of skin biopsy (ear notch) of all calves, replacement heifers, cows without calves and bulls. Test the dams of all test-positive calves.
Preventing new BVDV exposure in the BVDV-free herd	Prevent commingling with other herds. Prevent fence-line contact with other herds. Test all calves born on the farm, at birth (IHC), or >2 months of age (VI). Test all dams of PI calves. Test all dams that have had reproductive failures. Purchase replacement animals from a herd with a BVDV biosecurity program or test all purchased animals, including bulls, prior to arrival for PI status (VI or IHC). Isolate and acclimate all animals for 30 days prior to introducing them into the herd. Conduct annual herd screening tests as described above.

Table 3. BVDV biosecurity program for dairy herds

Objective	Approach
Screening the dairy herd for cattle with active acute or persistent BVD infections	Check lactating herd by testing for evidence of virus in bulk tank milk (PCR), and/or screen the balance of animals in the herd by conducting BVDV serological tests on a subset of cows or calves 6 months of age, non-vaccinated and commingled with herdmates.
Identifying and removing BVDV persistently-infected (PI) cattle from the dairy herd	Virus isolation from serum or IHC of skin biopsies from all calves, replacement heifers, bulls, and dry cows. If the bulk-tank tests positive with PCR, test individual cows in the lactating herd, dry cows, replacement heifers, and calves by VI or IHC. Retest animals that test positive to confirm PI status. Sell all cattle confirmed PI for slaughter.
Preventing new BVDV exposure from occurring in the BVDV-free dairy herd	Prevent commingling with other herds. Prevent fence-line contact with animals from other herds. Test all calves born on the farm, at birth (IHC), or >2 months of age (VI). Test dams of PI calves. Purchase replacement animals directly from herds with a long-standing active BVDV biosecurity program. Or test all purchased animals prior to arrival for PI (VI or IHC.) Isolate and acclimate all purchased animals for 30 days prior to introducing them into the herd. Conduct annual herd screening tests for PI and/or acutely infected animals.

Purebred breeders must test all animals in their herd, promptly culling PI animals and selling them for slaughter only. Producers of seedstock should offer only test-negative animals for sale. This is essential to protect the reputation of the seedstock producer for future sales, as well as preserve the health status of the buyer's herd. Biosecurity programs for BVDV in beef and dairy herds are summarized in Tables 2 and 3.

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