Considerations for Bovine Viral Diarrhea (BVD) Testing

Academy of Veterinary Consultants (AVC) *ad hoc* BVD Committee

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Abstract

Available diagnostic tests for bovine viral diarrhea virus (BVDV) and the persistently infected (PI) BVDV carrier state are reviewed and presented in table format. The test of choice will depend on the age of animal being tested, whether the animal is alive or dead and whether the veterinarian is only interested in identifying PI animals or if transiently infected (TI) animals are also of interest. Economic considerations, including the likelihood of finding a PI animal in a given population (expected prevalence), cost of disease due to the presence of a PI animal and the economic risk of selling a PI animal to a customer, will impact the choice of BVD testing strategy. Potential advantages and disadvantages for the available laboratory tests and suggested tests for particular situations are presented in table format.

Résumé

Les tests diagnostics disponibles pour la détection du virus de la diarrhée virale bovine (BVDV) et de l'état d'immunotolérance envers le BVDV sont revus et présentés sous forme de tableau. Le choix d'un test dépendra de l'âge de l'animal testé et selon que l'animal soit vivant ou mort. De plus, il doit être ajusté selon l'intérêt du vétérinaire à identifier les animaux immunotolérants ou ceux qui sont infectés temporairement. La stratégie de détection sera aussi influencée par des considérations économiques incluant les chances de détecter un individu immunotolérant dans une population (prévalence attendue), le coût entraîné par la présence d'un individu immunotolérant et le risque de vendre un individu immunotolérant à un client. Les avantages et les inconvénients possibles reliés à chacun des tests disponibles de même que les choix suggérés dans des situations particulières sont présentés sous forme de tableau.

Introduction

Infection with bovine viral diarrhea virus (BVDV) contributes to a variety of economically important disease syndromes in beef cattle.[13,16,17] In response, the Academy of Veterinary Consultants (AVC) adopted a position statement in November of 2001 stating, "It is the resolve of the Academy of Veterinary Consultants that the beef and dairy industries adopt measures to control and target eventual eradication of BVDV from North America." To support the position statement, the AVC formed an *ad hoc* committee that produced and published a peer-reviewed literature review and a BVD

decision and management guidelines document.[14,15] To further support the aims of the AVC Position Statement, the *ad hoc* committee prepared this review of current BVDV testing methodologies.

In addition, Tables 1 and 2 provide current recommendations from the AVC *ad hoc* BVD Committee when considering testing strategies for BVD.

Bovine Viral Diarrhea Virus Ecology

The primary reservoir for and source of BVDV are cattle persistently infected (PI) with BVDV, with transiently (acutely) infected (TI) cattle considered a less important source. Cattle become PI as a result of exposure *in utero* to non-cytopathic BVDV prior to development of a competent immune system, which occurs by about 125 days of gestation.[7,18] Persistently infected animals are generally much more efficient transmitters of BVDV than transiently (acutely) infected animals because they secrete much higher levels of virus for a much longer period of time. After a short incubation period, transiently infected animals become viremic, and virus may be shed in body secretions and excretions from days four to 15 post-infection.[5,11] In contrast, PI animals usually have a very high and persistent viremia, and BVDV is shed throughout life.[1,3,4,28] In relatively uncommon outbreaks of severe acute BVD, the spread following acute, transient infection is described as significant and is similar to that found with PI cattle.[2,6,9] Horizontal transmission of BVDV to seronegative cattle has been shown to occur after only one hour of direct contact with a single PI animal.[31] Over-the-fence contact with a PI animal from a neighboring herd can also introduce BVDV into a susceptible herd.[22,29] Transiently infected cattle are considered to be much less efficient at transmitting the virus to susceptible animals.[20,25,26] However, seroconversion among cattle without PI animals present indicates that transmission from transiently infected animals does occur, although spread is slower.[19,23]

Bovine Viral Diarrhea Virus Diagnostic Testing

Various methods have been developed to identify cattle PI with BVDV, including virus isolation from serum, blood, and other tissues (VI); fluorescent antibody staining (FA) of tissues; immunohistochemical (IHC) staining of skin biopsy specimens for viral antigen; antigen-capture ELISAs (AC-ELISA) applied to serum, plasma or phosphate buffered saline-incubated skin samples; and reverse-transcriptase polymerase chain reaction (RT-PCR) assays.[10,30] Virus isolation from buffy coat or serum samples, RT-PCR assays and AC-ELISA applied to serum or plasma detect viremia, but are not able to differentiate between transient and persistent infection. Thus, for cattle with positive test results, a

second sample must be obtained three to four weeks later and tested to differentiate transient from persistent infection. The specificity for IHC staining of skin biopsy specimens (and probably AC-ELISA) appears to vary by PI prevalence as an indication of BVDV exposure level, with good specificity in herds with low BVDV exposure, and poorer specificity in herds with high PI prevalence.[8,27]

The best test method to use in a particular situation will depend on the age of animal being tested, whether the animal is alive or dead, and whether the veterinarian is only interested in identifying PI animals or if TI animals are also of interest. In addition, economic considerations, such as the likelihood of finding a PI animal in a given population (expected prevalence), cost of the presence of a PI animal and the economic risk of selling a PI animal to a customer, will impact the BVD testing strategy.

When testing young calves for PI status, maternal antibody interference is a concern. Skin samples assayed for viral antigen by IHC or AC-ELISA, VI from buffy coat lysates (two repeated samples three-four weeks apart), or RT-PCR of whole blood (two repeated samples three-four weeks apart) are the best tests to minimize the risk of false negative results.[12,32] When testing older animals such as replacement heifers, bulls, or feeder calves, cost and other factors may cause one to consider pooled blood samples for PCR testing. 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. Muñoz-Zanzi *et al* developed a simulation model and determined that the economically optimal 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, and as prevalence increases the least-cost initial pool size decreases.[24] If one is interested in identifying transiently (acutely) infected animals, positive test results from FA or IHC of tissue samples other than skin, VI or PCR from serum, whole blood, or tissues, or serology of unvaccinated cattle may provide evidence for transient BVDV infection.

Occasionally, cattle that initially test positive for PI status should be retested at least three to four weeks later to determine viremic status (repeated virus isolations or PCR of serum or blood) to confirm the PI status of the animal. A qualified laboratory experienced with BVDV testing using tests with high specificity for PI status in populations with relatively low PI prevalence will rarely have false-positive results. However, situations may arise when a false-positive is more likely or when a false-positive has a relatively greater cost. False-positive PI tests appear to be more likely if a herd has

Table 1. Suggested diagnostic laboratory tests for given testing situations.

Situation	Test	Rationale
Testing sick suckling calves (scours, pneumonia, septicemia, etc.) for possible BVD involvement	♦ IHC or AC-ELISA from skin sample will identify PI calves and sometimes TI calves ♦ PCR of blood or serum to identify both PI and TI calves – (cost consideration)	Maternal antibody may interfere with microtiter VI and AC-ELISA using serum or plasma, therefore these tests are not recommended for young calves. If a live calf is IHC or AC-ELISA negative from a skin sample, but BVDV positive from a blood or serum sample, transient infection is likely. False positive indication of PI with IHC or AC-ELISA of skin samples from TI cattle can occur in situations with high viral exposure due to the presence of multiple PI cattle.
Testing dead suckling calves (scours, pneumonia, septicemia, etc.) for possible BVD involvement	♦ IHC or AC-ELISA from skin sample will identify PI calves and sometimes TI calves – IHC will work if skin is not desiccated ♦ IHC, FA, or VI from tissues (thymus, Peyer's patches, mesenteric lymph nodes) to identify infected calves (won't differentiate between PI and TI)	Maternal antibody may interfere with microtiter VI and AC-ELISA using serum or plasma, therefore these tests are not recommended for young calves. If a dead calf is IHC or AC-ELISA negative from a skin sample, but positive from a tissue sample, transient infection is likely.
Screening a herd (suckling calves, cows that lost calves, replacement animals) because there is laboratory evidence of BVDV in the herd	♦ IHC or AC-ELISA from skin sample will identify PI cattle and sometimes TI cattle	Maternal antibody may interfere with microtiter VI and AC-ELISA using serum or plasma, therefore these tests are not recommended for young calves. The positive predictive value of the IHC and AC-ELISA tests in herds with confirmed BVD presence is high; therefore any animal that is positive is usually considered PI. However, false positive IHC or AC-ELISA of skin samples from TI cattle can occur in situations with high viral exposure due to the presence of multiple PI cattle.
Screening open replacement heifers (raised or purchased), purchased open cows, or bulls (raised or purchased)	♦ IHC or AC-ELISA from skin sample will identify PI cattle and sometimes TI cattle ♦ PCR – pool serum or whole blood into groups of 30-40 or less. Test individual skin samples of animals in positive pools to identify PIs. Animals in negative pools are considered not-PI	The positive predictive value of any of these tests in animals that don't have any other risk factors for being PI is good, but not perfect. Therefore, any positive test in valuable animals could be confirmed by segregating the animal and using IHC, AC-ELISA, VI, or PCR of serum or blood samples taken not less than 21 days later. This will eliminate TI animals and false-positive animals from being incorrectly called a PI.
Screening purchased pregnant replacement heifers or cows prior to entry into the herd	♦ IHC or AC-ELISA from skin sample will identify PI cattle and sometimes TI cattle ♦ PCR – pool serum or whole blood into groups of 30-40 or less. Test individual skin samples of animals in positive pools to identify PIs. Animals in negative pools are considered not-PI ♦ Isolate pregnant cattle from resident herd until the calf is born and tested for PI status via IHC or AC-ELISA from a skin sample	The positive predictive value of any of these tests in animals that don't have any other risk factors for being PI is good, but not perfect. Therefore, any positive test in valuable animals could be confirmed by segregating the animal and using IHC, AC-ELISA, VI, or PCR of serum or blood samples taken not less than 21 days later. This will eliminate TI animals and false-positive animals from being incorrectly called a PI. A PI test-negative pregnant dam can have a PI fetus. Cattle that conceived off the premises should be isolated from the resident herd until the calf is born and determined to be PI test-negative.
Screening raised replacement heifers and bulls prior to sale by a seedstock supplier	♦ IHC or AC-ELISA from skin sample will identify PI cattle and sometimes TI cattle ♦ PCR – pool serum or whole blood into groups of 30-40 or less. Use skin samples of animals in positive pools to identify PIs. Animals in negative pools are considered not-PI	The positive predictive value of any of these tests in animals that don't have any other risk factors for being PI is good, but not perfect. Therefore, any positive test in valuable animals could be confirmed by segregating the animal and using IHC, AC-ELISA, VI, or PCR of serum or blood samples taken not less than 21 days later. This will eliminate TI animals and false-positive animals from being incorrectly called a PI.
Testing ill or dead stocker or feedlot animals for possible BVD involvement	♦ IHC or AC-ELISA from skin sample will identify PI cattle and sometimes TI cattle – IHC will work if skin is not desiccated	The positive predictive value of any of these tests in animals that have other risk factors for being PI is very high, therefore any test-positive animal is likely a PI. To rule out possible transient BVD infection interfering with identification of PI animals, any positive test can be confirmed in three weeks.

Table 2. Tests currently available for persistent BVDV infection (PI).

Test	Cost	Advantages	Disadvantages	Specimens / Shipping
Virus Isolation ◆ 1-3 week turnaround	Moderate to High cost	◆ The Gold Standard for BVDV diagnosis ◆ High specificity ◆ Virus is available for study at a later date	◆ Slow lab turnaround ◆ Laboratory labor-intensive ◆ Specimens must be shipped on ice to keep virus viable ◆ Potential false negative due to interference by maternal antibodies ◆ To distinguish between PI and TI , must retest positive cattle in 3-4 weeks	◆ Whole blood (10 mL) or serum (2-3 mL) ◆ Send in insulated container with cold packs ◆ Do not freeze samples
Immunohistochemistry (IHC) of skin ◆ 2-5 day turnaround	Low cost	◆ High sensitivity ◆ Usually identifies persistent infections (PI) only – transiently infected animals usually test negative	◆ Laboratory labor-intensive ◆ Formalin usage ◆ Will generally not identify transiently infected animals	◆ Skin samples – usually taken from ear with ear notching pliers ◆ Send fresh on wet ice or stored in 1:10 volume of 10% neutral buffered formalin ◆ Samples can be held in formalin for several weeks (lab dependent) ◆ Work closely with your laboratory to provide their preferred sample
Antigen-capture ELISA of serum ◆ 1-5 day turnaround	Low cost	◆ High sensitivity	◆ Potential false negative due to interference by maternal antibodies ◆ To distinguish between persistent and transient infections, must retest animal in 3 weeks	◆ Serum (2 mL) ◆ Send in insulated container with cold packs
Antigen-capture ELISA of skin ◆ 1-3 day turnaround	Low cost	◆ High sensitivity ◆ Usually identifies persistent infections (PI) only – transiently infected animals usually test negative	◆ Will generally not identify transiently infected animals	◆ Skin samples – usually taken from ear with ear notching pliers ◆ Send in insulated container with cold packs ◆ Do **not** allow to dry out – can hold samples by freezing ◆ Determine laboratory's preferred method of packaging and shipping
Polymerase chain reaction (PCR) ◆ 1-3 day turnaround	Moderate to high cost (can be reduced by pooling 30 or more samples)	◆ High sensitivity	◆ Potential for laboratory contamination (false positive) ◆ To distinguish between PI and TI , must retest positive cattle in 3 weeks	◆ Whole blood (10 mL) or serum (2-3 mL) ◆ Send in insulted container with cold packs

many PI cattle and BVDV exposure is extremely high (which may result in TI cattle testing false-positive for PI status). It may also be desirable to confirm a positive PI test if the PI test-positive animal is valuable, but at very low risk for being PI, such as in a seedstock herd with an aggressive BVD PI control plan.

Skin biopsies for IHC or AC-ELISA can be accurately tested for PI status for several weeks after collection if they are properly collected and stored, depending on the laboratory and the testing procedure.[21] However, in most situations the rapid removal of PI animals is desirable. Therefore, samples should be sent to the laboratory in a timely manner, usually within a week of collection. For cow-calf herds, PI status of the herd should be determined before the breeding season begins.

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