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Diagnostic Testing and Strategies for BVDV

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Introduction

Clinical diseases in cattle resulting from infection with bovine viral diarrhea virus (BVDV) are recognized as being responsible for economic losses throughout the world. Economic losses are caused by decreased performance, loss of milk production, reproductive wastage, and increased risk of morbidity and mortality. Because of increased awareness of the significant impact that BVDV can have, efforts to control this virus are increasing. As we learn more about BVDV, there also is an increasing realization that successful control of this virus requires a strategy that involves multiple components and is customized to fit the goals and capabilities of each producer. By developing a complete program, the risk of BVDV associated losses can be reduced significantly. The tools available for controlling BVDV include 1) a multitude of diagnostic tests and strategies for detecting both acute and persistent infections, 2) vaccines available in a variety of combinations with other important disease-causing pathogens, and 3) biosecurity practices. Although this article focuses on diagnostic tests and strategies, it should be noted that using one tool (e.g., diagnostics) without using others could result in an inefficient BVDV control program.

Table 1: Reasons for initiating diagnostic tests.

Reason For BVDV Testing	Suggested Diagnostic Test
Diagnosis of acute infection including: • Sick animals • Dead animal • Abortion	• Virus isolation from tissues, serum or whole blood, preferably tissues that have high concentrations of lymphoid cells including Peyer's patches, ileum, spleen, thymus (fetus), lung, liver. • PCR from tissue, serum or whole blood.
Detection of persistently infected (PIs) calves younger than 4 months of age.	• PCR on pooled skin samples • Skin IHC • Skin ELISA • SNAP® BVD test.
Detection of PIs calves older than 4 months of age.	• PCR on pooled skin samples • Skin IHC • Skin ELISA • Blood (serum) ELISA • SNAP® BVD test.

BVDV Diagnosis Assays and Strategies

Choosing and applying the appropriate BVDV diagnostic test and strategy requires a firm understanding of the disease pathogenesis. Without this understanding, proper interpretation of test results is difficult. It is important to either develop a good understanding of BVDV or work with an animal health professional who understands the complexities of the virus.

BVDV diagnostics are used for essentially two reasons (Table 1). The first is to identify if BVDV is the cause of or part of a clinical problem that has been identified. A variety of diagnostic assays are available for identifying virus in blood or nasal swab samples taken from sick animals or tissue samples taken at necropsy. In addition, detection of an immune response to BVDV (antibody titers) can be useful in situations where previous information about an animal's immune status is available.

The second use of BVDV diagnostic assays, and the most important use in a BVDV control program, is for the identification of cattle persistently infected with BVDV, or PIs. Cattle that are persistently infected with BVDV continuously shed large amounts of virus and serve as the major mechanism to spread the virus in the cattle population. PIs are infected with and shed BVDV for their entire life. By identifying and eliminating PIs, the risk of BVDV transmission within and between farms is reduced significantly. Persistently infected cattle can be identified by detecting virus in either blood or tissue samples. Again, a variety of assays have been developed that can be used to detect PIs.

Currently, the most commonly used sample for identifying PIs is skin. A small notch of skin, often taken from the ear (but can be taken from anywhere), can be submitted to diagnostic labs where different tests can be used to detect virus. Blood also is commonly used to identify PIs. Any animal testing positive should be isolated and retested within 3 weeks before being classified as a PI. With the development and refinement of new technologies, such as pooled PCR, the cost of screening large numbers of animals has been reduced significantly, making it increasingly practical for producers to routinely include PI testing in their BVDV control program. A summary of currently available tests can be found in Table 2.

Real Life Scenarios

There are a variety of different BVDV testing strategies that have been developed and promoted. In the end, the strategy that you use depends on your goal, your management capabilities, economic considerations specific to your operation, and which tests are available. A good way to look at different BVDV testing strategies is to start by defining what it is you are trying to accomplish. Let's look at several "scenarios" that are common to US dairies and discuss some possible solutions.

Scenario 1: Is BVDV causing a clinical problem? In this scenario, we are interested to know if BVDV is causing an animal or animals to be sick or is involved in the death of an animal. In this case, we are interested in identifying BVDV during an acute infection in a live animal or at necropsy. We want to submit samples to a diagnostic lab that is capable of detecting virus using virus isolation or PCR. Whole blood keep are the samples of choice for detecting acute infections in live animals. Other appropriate samples include nasal swabs and blood serum. At necropsy, lymphoid tissues are the first choice for detecting BVDV. These would include Peyer's patches and/or ileum in the small intestine, regional lymph nodes, and spleen. Other useful tissues depending on the clinical presentation include lung, liver and kidney. If you are dealing with an abortion, include thymus tissue.

Scenario 2: Is BVDV circulating in my farm? This is a common question asked when BVDV is thought to cause underlying chronic herd health or reproduction problems. In this case, we want to make a diagnosis at the

herd level. This in turn may lead to the development of a strategy of looking for individual PIs (see scenario 3). There are a couple of strategies that can be used to “screen” herds for BVDV. The first is to screen bulk milk samples for BVDV using PCR. Bulk milk PCR can detect about 1 PI in approximately 300 cows, although there may be some variability in this depending on how much virus is being shed in the milk of a PI. For larger farms, this strategy can be accomplished using inline milk samplers. The problem with bulk milk testing is that it only detects in lactating cows, young stock are not evaluated.

The reality is that most PIs are in the replacement herd, not in the milking herd. Regardless, if the bulk tank PCR is positive, this is a significant finding and virus is likely circulating in the farm. If it is negative, it does not rule out BVDV from still being present. Another strategy is to test sentinel (indicator) animals for the presence of BVDV antibodies. The best sentinel animals are young stock (heifers, bulls, steers) between the ages of 6 months and 1 yr that have NOT been vaccinated for BVDV.

The idea is that if they have high antibodies to BVDV, and they are older than 6 months of age, and they have NOT been vaccinated, then the antibodies are due to natural exposure to circulating BVDV. The accuracy of this method increases if you: 1) use more animals (we typically recommend 5-10); 2) they are closer to 6 months than 12 months (younger animals with titers suggest more recent circulation of virus); and, 3) if they have exposure to lots of animals on the farm (sentinels only reflect the animals they have had contact with). A final way to continuously screen the herd for BVDV is to routinely have dead animals and abortions examined by a veterinarian and have appropriate samples submitted for diagnostic testing.

Scenario 3: We know BVDV is here, now we want to find the PIs. This is the most common BVDV testing that occurs today -- find the PIs so that we can get rid of them. To accomplish this, we must use a test that actually detects the virus in the animal. This can be virus isolation, PCR, antigen capture ELISA, SNAP® BVD test or Immunohistochemistry (IHC). All of these tests have been used successfully. Antigen capture ELISA and PCR are most commonly used today because they are most adaptable in the laboratory for testing large numbers of samples. PCR can be used on a pooled sample which helps to further reduce the cost. However, there is controversy among investigators as to what is the correct pool size. Too many samples and the sensitivity of the assay may go down. Fewer samples means higher per animal costs. At the Michigan State University Diagnostic Center for Population and Animal Health (DCPAH), we use pools of 10. We feel this pool size gives us the best sensitivity while still maintaining a reasonable per animal cost. Samples that can be used include blood or tissue. Skin samples are most commonly used and they are often taken from the ear – thus the “ear notch test” – but please note that skin can actually be taken from anywhere on the body.

Skin can be tested with IHC, PCR or ELISA. Blood can be tested with PCR, ELISA, SNAP® BVD test or virus isolation. One caution with blood, in very young calves (<4 month of age) a whole blood sample must be used instead of serum. The reason for this is that maternal antibodies will neutralize virus in serum. Once maternal antibodies wane (around 4 months of age), virus will begin reappearing in serum. With whole blood, white blood cells can be isolated and tested which eliminates the issues of maternal antibodies.

The next decision is what animals to test. This answer often comes down to how fast you want to screen the herd and what are your management capabilities. The most cost-effective way to screen a herd is to test replacement animals or any adult animal if the farm does not have replacement animals. The idea here is that when you test a young animal, its BVDV status gives you information about its mother. If a calf is negative (i.e., NOT a PI), then its dam CANNOT be a PI. If a calf is positive and determined to be a PI, then its dam MIGHT be a PI and needs to be checked. If a dam does not have a replacement on the farm (e.g., she has given birth to all bulls), then you need to check her as well. This strategy allows you to really get

two animals tested for the price of one. The caveat here is that you need to be able to match calves with their dams – something that is not always easy. If you cannot do this, then you are left with testing all animals on the farm. If you have limited resources, focus on the young stock because that is where PIs are most likely to be.

Even if you test everything that is walking around on the farm today, you must keep in mind there is another susceptible population that has yet to hit the ground –the gestating fetuses. So to be complete, you need to continue to test newborn calves. We recommend continuing to test newborns for a minimum of 9 months to ensure there are no hidden fetal infections that may lead to the birth of a future PI.

Scenario 4: I am expanding and want to reduce the chance of bringing BVDV onto my farm? Here is the basic recommendation: isolate the new animals. Collect a sample (skin, blood) and screen for BVDV as soon as possible. If pregnant, you will need to screen the calf when it is born as well. The alternative is to buy BVDV test-free animals.

Scenario 5: I have an animal that was positive on a virus detection test - now what? If you are searching for PIs and a test comes up positive, then you have a decision to make. 1) Isolate the animal and retest in 2-3 weeks to confirm that the virus is still there, thus confirming a "persistent infection", or 2) assume the animal is a PI based on one test and eliminate it from the herd. Here is the deal – when a virus detection assay turns up positive, the majority of the time, that animal turns out to be a PI. However, if you happen to test an animal that is undergoing a transient infection and you collect the sample at the right time, all of the virus detection tests could potentially detect virus. This is rare, but it does occur. Our recommendation is to isolate and retest 14-21 days after the sample for the first test was taken, especially if it is a valuable animal.

Scenario 6: I want to market cattle as BVDV PI free? In this scenario, we need to use a test that actually detects virus in the animal. This can be virus isolation, PCR, antigen capture ELISA, SNAP® BVD test or IHC. Antigen capture ELISA and PCR are most commonly used today. Blood also can be tested with PCR, ELISA or virus isolation (note the cautions about testing blood from young animals discussed in scenario 3). The IDEXX SNAP® BVD test is useful in that it is a cow-side test and can be used to rapidly screen an animal prior to a sale.

What to Do With PIs

First and foremost, the animal needs to be eliminated from contact with other cattle. Three options are: 1) humanely euthanize, 2) sell to a slaughter only market, or 3) isolate and raise for slaughter. NOTE: There is no evidence that BVDV is a zoonotic disease (transferable to humans). The option obviously depends on the age and size of the animals. Most importantly, do not create a situation where a PI may serve as a reservoir of virus transmission to another farm. It is unethical to sell animals you suspect as being PI's at an auction where the animals may be retained in a breeding herd (e.g., don't take it to your local livestock market).

Diagnostic Test	Relative Cost	Specimen	Used For	Notes
Polymerase chain reaction (PCR)	Moderate to High	Serum, Whole Blood, Tissue	Identifying PI's and acute infections	Rapid and sensitive.
Pooled - Polymerase chain reaction (PCR)	Low	Skin - usually taken from ear	Identifying PI's	Skin samples can be pooled to reduce costs. Number per pool depends on laboratory
Immunohistochemistry (IHC) of skin	Low	Skin - usually taken from ear	Identifying PI's	Fresh or formalin fixed samples. Work closely with your laboratory to provide their preferred sample
Antigen-capture ELISA - ACE	Low	Serum or skin	Identifying PI's	Rapid results. Serum testing may be inhibited by passive immunity; not recommended for young calves < 4 mo
Rapid Immunomigration Assay	Moderate	Serum or skin	Identifying PI's	Animal side assay (IDEXX BVD TEST), rapid results.
Virus Isolation	Moderate to High	Serum, Whole Blood, Tissue samples - spleen, lung, small intestine, thymus	Identifying Acute or PI infections	Gold standard test for detecting BVDV, however expensive, takes a long time to conduct and requires specialized labs
Virus Neutralization or Antibody ELISA on individual animals	Low	Serum	Identification of virus exposure - NOT useful for detecting PI's	Detects immune response (titer) to BVDV
Bulk tank PCR	Low	Milk	Herd screening	Can detect 1 PI in 300 cows. Looks only at lactating cows
Sentinel serology	Low	Serum	Herd Screening	Conducted on unvaccinated young stock between ages of 6-12 months of age

Summary

Research has led to the development of a variety of tools that are useful in the control of BVDV. These tools include multiple different diagnostic tests, vaccines and biosecurity tools. Diagnostic tools can be used to answer a variety of questions related to BVDV. Selecting the correct diagnostic tools requires an understanding of how BVDV works. BVDV diagnostic strategies should not be a stand-alone BVDV control program, rather they should be incorporated with a well-designed BVDV vaccine program and the implementation of strategic biosecurity protocols.

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