DIAGNOSIS OF BOVINE VIRAL DIARRHEA VIRUS: A KEY COMPONENT TO A COMPREHENSIVE BVDV CONTROL PROGRAM

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Bovine viral diarrhea virus (BVDV) is one of the most important viral pathogens of cattle worldwide. Because of the insidious and complex nature of BVDV, laboratory diagnosis is critical in preventing and controlling BVDV infections. These same characteristics often make laboratory diagnosis challenging. A firm understanding of the disease is required to select the appropriate diagnostic strategies and samples for diagnostic submission and then make sound interpretations of the results. What follows is information on the various diagnostic tests currently available for BVDV and then a discussion on how they are used in strategically.

Virus Isolation

Virus isolation has historically been the most common method for identifying cattle infected with BVDV. The virus is relatively easy to isolate from a variety of specimens including serum, buffy coats (white blood cells or WBC's), nasal swabs, and tissue samples.

Several factors must be considered in selecting the appropriate sample and method for virus isolation. Since BVDV appears to replicate best in lymphoid cells, samples that contain these cell types should be considered, especially when attempting to identify acute infections. These samples would include whole blood from which buffy coats can be isolated and lymphoid tissue such as peyer's patches, mesenteric lymph nodes, spleen and thymus from postmortem cattle or aborted fetuses. In cattle persistently infected with BVDV, virus can usually be isolated from serum, buffy coats and a majority of tissues (although lymphoid tissues are preferable). Sample condition is important for successfully isolating BVDV and should be considered when submitting samples. The method of virus isolation may depend on the type of BVDV infection being considered. The immunoperoxidase microtiter plate assay (IPMA) is commonly used for rapidly screening herds to identify cattle persistently infected (PI) with BVDV but is less appropriate for identifying cattle acutely infected with BVDV. Techniques involving multiple cell passage may be necessary to identify low virus titers often associated with acute infections.

Antigen Detection

Antigen detection consists of using labeled antibodies to directly detect the presence of BVDV antigens in submitted samples. The most common application of antigen detection is the enzyme-linked immunosorbent assay (ELISA). The ELISA can be used for detecting virus in blood, nasal swabs and skin samples such as ear notches

Another common use of antigen detection is directly identifying BVDV in tissues. The fluorescent antibody (FA) test is most commonly used because of the rapidity that it

can be performed. This assay is often used with smear preparations made from samples such as nasal swabs, lymph nodes and spleen and is often performed on gross necropsy samples as a first line screening assay for virus presence. The sensitivity and specificity of this test varies widely.

Immunohistochemistry (IHC) identifies BVDV antigen in frozen or formalin fixed tissues. This has clear advantages as tissue morphology is maintained which allows virus to be identified in conjunction with histopathological findings. IHC is useful when investigating disease outbreaks that involve the respiratory, gastrointestinal or reproductive system where BVDV is suspected. Using immunohistochemistry, BVDV can be detected in properly fixed tissues for an extended period of time whereas the ability to isolate virus from fresh tissues can dissipates rapidly with time. This is especially advantageous when field samples cannot immediately be submitted to a diagnostic lab. IHC can also be used to look retrospectively for BVDV or other pathogens of interest in properly fixed tissues.

More recently, immunohistochemistry and fluorescent antibody testing has been used to detect BVDV in skin samples of cattle persistently infected with BVDV. The amount and distribution of antigen in skin biopsies from PI cattle appears to be much different than those undergoing acute infection, making it a useful tool for identifying PI animals

Nucleic Acid Detection

Nucleic acid detection assays involve the direct identification of BVDV viral genomic RNA or a synthesized copy of the RNA called cDNA. Nucleic acid detection systems can be very sensitive and specific. However, technical and costly protocols have limited their use as a common diagnostic tool. The polymerase chain reaction (PCR) is the most commonly used nucleic acid detection assay. It is estimated that PCR is 10 to 1000 times more sensitive than virus isolation. Detection of BVDV by PCR requires that genomic RNA be extracted from the sample of interest. This can be problematic as RNA is rapidly degraded if exposed to Rnases, which are ubiquitous in nature. Careful sample handling is necessary to reduce this pitfall. Samples in which BVDV RNA has been detected include serum, buffy coats, nasal swabs, homogenized tissues, semen, and milk.

Specific PCR primers have been designed to differentiate between the reported genotypes of BVDV. This may be useful in designing vaccine programs aimed at controlling different genotypes of BVDV.

Most recently, PCR has been used in herd screening protocols where samples from multiple animals are pooled together. This strategy takes advantage of the high sensitivity of the assay while reducing the cost per animal tested. PCR has also been used to screen bulk tank milk as a herd screen for BVDV.

Serology

Serological assays have commonly been used to diagnose viral infections. However, the nearly ubiquitous exposure of cattle in the United States to BVDV (naturally or by vaccination) has made serology at times difficult to interpret. Potential

uses of serological assays for diagnosing BVDV include paired acute and convalescent sera in individual cattle thought to have acute exposure to BVDV and screening of unvaccinated animals to determine the prevalence of exposure in a herd. Screening of young, unvaccinated cattle between 6-12 months of age may be useful in determining if BVDV has recently been or is currently circulating in a herd. Serological assays are also available to help differentiate between exposure to type I or type II BVDV.

The virus neutralization (VN) assay is the most commonly used method for the detection of BVDV antibodies. In this assay, the ability of antibodies in test serum to neutralize a reference BVDV isolate is measured. A lack of assay standardization between diagnostic labs makes VN titer comparisons between labs difficult. Lab-to-lab variation does exist because of the use of different reference strains, cell lines and culture conditions. For this reason, it is difficult to compare titers that originate from different labs. Within lab titer variations may also exist over time. Therefore, it is important to submit paired acute and convalescent sera simultaneously. Titers to type 1 and 2 BVDV can differ substantially depending on the antigenic exposure. It is prudent to request both type 1 and 2 BVDV serology to completely evaluate the serological response to BVDV

Diagnosis of Acute Infections

During disease outbreaks suspected of being caused by BVDV, identifying cattle acutely infected with the virus is often attempted. Virus isolation must be attempted during a narrow window of time following infection. Virus is usually only detected for 1 to 4 days somewhere between 3 and 12 days post infection. In general, BVDV can be isolated from white blood cells obtained from whole blood samples for a longer period of time, making this the sample of choice for ante-mortem isolation of BVDV during acute infection. Nucleic acid methods such as PCR will also detect BVDV specific RNA during a broader period of time following acute infection. Lymphoid organs are the tissue of choice for isolating BVDV post mortem. As with any pathogen, autolysis reduces the chance of isolating BVDV from tissue. Tissue samples should be collected, chilled (not frozen) and shipped to the diagnostic lab as soon as possible. If submission for virus isolation cannot be done immediately, it may be useful to collect formalin fixed samples for immunohistochemistry.

Acute and convalescent serological assays can be used to detect a 4-fold rise in BVDV antibody titers, indicative of acute infection. Because of the relatively slow rise in titers following acute infection with BVDV, paired sera should be obtained 21-30 days apart. In acute herd outbreaks, collection of samples from unaffected herdmates may help in making a more definitive diagnosis.

Diagnosis of Persistent Infections

ELISA, immunohistochemistry or fluorescent antibody testing of skin biopsies (ear notches) is increasingly being used as a tool for identifying cattle persistently infected (PI) with BVDV. This technology appears to be as accurate for detecting PI cattle as virus isolation. Advantages of these methods include ease of sample collection, lack of inhibition by colostral antibodies and cost. Although most animals that are positive by ear notch testing are persistently infected with BVDV, it is possible to

detect antigen following an acute infection. For this reason, it is recommended virus persistence be confirmed by a follow up test 2-3 weeks later.

Virus isolation can also be used for detecting PI's. Most PI cattle have high levels of virus in their blood that can easily be detected in serum samples. In rare PI cattle, the level of virus may drop to an undetectable level using virus isolation from serum but can still be detected in white blood cells. In neonatal PI calves, colostral antibodies will eliminate serum BVDV until the antibodies dissipate at which time viremia becomes detectable again. This usually happens within 4 months after colostrum ingestion. During this time, BVDV can still be detected by virus isolation from white blood cells. As a single positive sample may be the result of a transient acute infection, confirmation of viral persistence should be done by serial virus isolation 3-4 weeks apart. Positive virus isolations on follow-up tests signify persistent infection with BVDV.

Herd Screening

A key to controlling BVDV is detecting and eliminating cattle persistently infected with BVDV. Before attempting to identify individual PI cattle, it is essential that strong evidence exist that PI cattle are likely to be present on the farm. This evidence may include detection of BVDV in necropsy samples, PCR identification of BVDV in bulk tank samples, or seroconversion of immunocompetent cattle unrelated to vaccination. Detection of antibody titers in sentinel animals has also been capitalized on as an indicator of circulating BVDV. High BVDV titers in non-vaccinated calves 6-12 month of age has been shown to be a reliable indicator that cattle persistently infected with BVDV are present in the herd. When evaluating antibody titers, it is important to evaluate both type 1 and type 2 BVDV titers as they may vary significantly depending on the antigenic exposure that has occurred.

Once it is determined that screening of individual animals is to be implemented, several strategies can be considered. The simplest and quickest strategy is to test all cattle on the farm in a narrow time frame. Any positive cattle should be isolated and then retested in 2-3 weeks. To complete herd screening, calves born over the next year should also be tested. Another strategy is to focus on the population that is most likely to contain PI cattle. Most PI's do not survive to adulthood. Therefore most PI's will exist in the replacement population of animals. Strategies may include testing all calves at one time or sequentially testing at a strategic management point such as birth or calf-hood vaccination. In addition, a test result on a calf will give you an indication of the dam's virus status. The dam of a calf that tests negative for BVDV will also be negative. If a calf is found to be PI, its dam could be PI and should be tested. This strategy is extremely useful in beef herds and can be adapted easily to dairy herds. Regardless of the strategy, the proper virus detection test needs to be selected.

Summary

Many diagnostic tests are available for aiding in the control and prevention of BVDV. Development of new technologies and testing strategies are making BVDV testing affordable and effective. Cattle producers are encouraged to consult with their animal health professionals on how best to use BVDV diagnostic tests and test

strategies in their overall herd health program

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