BVD: GETTING A POSITIVE DIAGNOSIS

Mary Lynne Vickers
Diagnostic Virologist
Livestock Disease Diagnostic Center
University of Kentucky
Lexington, KY 40511

Introduction

Bovine viral diarrhea virus (BVDV) is a ubiquitous virus that is the most frequently isolated viral pathogen of cattle in some areas. BVDV virus infection is often suspected because of the wide range of disease problems it causes. This variety of clinical signs has complicated the diagnosis of this viral infection because there are no simple answers. It is, however, essential that a definitive diagnosis be made before costly control measures are implemented.

Bovine Viral Diarrhea Virus

There are two biotypes of BVDV, cytopathic and noncytopathic. The cytopathic virus produces visible changes in cell cultures which will eventually destroy the monolayer of cells. The noncytopathic virus grows and reproduces in cell cultures but there is no visible change to indicate its presence. Detection of this noncytopathic virus is achieved by immunofluorescent or immunoperoxidase staining of the cells with BVDV specific reagents. This cytopathic effect is a phenomenon of in vitro cell culture and not related to the disease-causing properties of the virus. Noncytopathic should not be interpreted as nonpathogenic because this is, in fact, the biotype of the virus most frequently isolated from clinical specimens.

Laboratory Procedures for BVDV Diagnosis

Virus isolation is still the most definitive demonstration that a viral agent is involved with a disease syndrome. Cell cultures of bovine origin are susceptible to infection with this virus. Cells that have been used for isolation most frequently are of kidney, spleen, testicle or tubrinate origin. Continuous cell lines will support the growth of BVDV, however, they may be less sensitive for virus isolation. Low passage or primary cultures may be more sensitive for isolation from clinical specimens but these are not readily available to many diagnostic laboratories.

The efficiency with which BVDV replicates in cell culture is one of the major problems complicating diagnosis of this virus. Virus isolation for BVDV is usually conducted in cells of bovine origin grown in media supplemented with fetal calf serum. Fetal calf serum from commercial sources is frequently contaminated with BVDV or antibodies to the virus. BVDV laboratory contamination of cells or media used for bovine virus isolation has prevented efficient isolation and identification of the virus leading to inconsistent results and confusion.

Good reagents for confirming BVDV in cell culture are necessary in obtaining a diagnosis of BVDV or in eliminating laboratory contamination. Good reagents start with monospecific BVD antiserum. This antiserum can be conjugated with a fluorescent tag and used for the fluorescent antibody (FA) test to identify BVDV. Specificity and sensitivity of the conjugate is dependent on the quality of the antiserum and the efficiency of the conjugation process. The supply of quality reagents may vary.

The most rapid viral diagnostic technique commonly used is the detection of virus antigen in tissue samples using the fluorescent antibody (FA) test. Fresh tissue is frozen and sectioned in a cryostat, fixed in acetone and stained using a fluorescene conjugated antibody specific for BVDV. A fluorescent microscope is used to observe cellular fluoresence typical of the virus. The realiability of this technique depends on the quality of the reagents and the ability of the technician. Virus

isolation should always be used when the fluorescent antibody test results are suspicious. In addition to the FA test, antigen in cell culture can be detected by enzyme immunoassay (EIA). BVD antiserum and anti-species enzyme conjugated antibody are used to detect the virus antigen in the cells. The method can be adapted for processing large number of samples in a short time period and at a reasonable cost. EIA can be used to screen herds for persistently infected animals.

The recent availability of monoclonal antibodies (1,2) to BVDV may improve the specificity of reagents available for diagnostic purposes. Use of monoclonal antibodies to compare different strains has revealed considerable antigenic variation between BVDV strains (3,4). Use of monoclonal antibody reagents against antigens that are highly conserved should provide reliable diagnostic reagents.

Fluorescent antibody techniques cannot be used on tissues fixed for standard histologic procedures. There are techniques for detecting antigen in fixed tissues (5). The technique requires additional labor in preparing the specimens and is probably not available as a routine procedure in most diagnostic laboratories.

Newer tests that are being developed in research laboratories for detection of BVDV include the use of PCR (polymerase chain reaction) and DNA probes (6,7). These may become available in the future for diagnosis of BVDV but currently are hampered by the inability to find sequences that are conserved in the genome of all BVD strains.

Serologic Techniques

BVDV antibodies can be detected by the serum neutralization test. Because of the widespread presence of BVDV antibodies in the cattle population, an acute and convalescent serum sample is necessary for serologic diagnosis of infection. Antibody response to BVDV infection in a normal immunocompentent animal is usually indicated by a titer of 1:256 or greater. There is no standardization among diagnostic laboratories as to what BVDV strains are used in the neutralization test. Most laboratories are probably using one of the type strains, i.e. Singer or NADL, which are cytopathic strains. Paired serum samples should always be tested in the same laboratory at the same time. Although it is possible to set up a neutralization test using a noncytopathic strain this is not routinely done in diagnostic laboratories.

Techniques for detecting BVDV antibody by ELISA have been reported (8,9). These techniques are not routinely used in most diagnostic laboratories. Purification of sufficient virus for this procedure is laborious and time consuming.

Collecting and Submitting Samples for Diagnosis

The probability of obtaining an accurate diagnosis is directly related to the quality of the samples submitted. This is especially true for viral diseases. Samples for virus isolation must be taken during the acute phase of the disease. For many viral diseases, virus is shed for only a few days after signs of infection become apparent. Waiting to observe what effects antibiotic treatment has on the infection will result in eliminating the chance to detect the virus. This is true in the case of BVDV infection except for the persistently infected animal. In addition to samples for virus isolation, acute and convalescent serum samples should be taken. A single serum sample particularly one taken at the time of onset of signs of disease is of little diagnostic value.

Samples properly collected but not properly shipped, also decrease the probably of detecting the virus. Swabs, blood, and tissue samples for virus isolation and/or fluorescent antibody technique should be shipped cold, not frozen, and as soon as possible to the laboratory.

Testing for Persistently Infected Animals

Congenital BVDV infection before the onset of immunological competence of the

fetus results in immune tolerence to the BVDV infecting the fetus (10). This calf will shed BVDV but not be capable of producing antibodies against this strain of BVDV. These calves may be "poor doers" or appear pefectly normal. Many of these calves do not survive the first months of life, and most are thought to die before two years. Some will reach reproductive age and, if bred, will produce a persistently infected calf. Control of a BVDV problem in a herd necessitates the identification and removal of persistently infected animals.

Identificiation of these animals is relatively easy. They literally shed virus from every secretion and excretion. The blood is an excellent sample for virus isolation, using either serum or the buffy coat fraction. Nasal, vaginal or prepuceal swabs are also suitable. The virus can be isolated from almost any tissue sample taken from the animal. Probably the most frequently submitted and the most unsuitable sample is feces.

When screening newborn calves for persistent infection, the blood sample 74ould be taken before colostrum is received. Maternal antibody from the infected dam can interfer with virus isolation from the blood of the calf.

Most persistently infected animals lack antibody to BVDV. The exception is an animal that has been vaccinated for BVDV or exposed to a different strain of BVDV. These animals are still persistently infected and shedding virus although they have antibody to BVDV.

Mucosal Disease

The persistently infected animal will eventually succumb to the fatal form of this infection which is mucosal disease (11,12). This may be a relatively acute death or a slow, chronic, debilitating disease. The persistently infected animal is infected with the noncytopathic biotype of BVDV. Superinfection with the cytopathic form of the virus results in mucosal disease. This cytopathic virus can originate from a modified live vaccine, another contact animal or probably more frequently from a spontaneous change in the noncytopathic virus.

Mucosal disease is probably the easiest form of this virus infection for the diagnostic laboratory to identify. BVDV antigen can be readily identified in tissues obtained from these animals. Clinical signs combined with the gross pathology, histopathology and isolation of the virus can confirm the diagnosis.

Abortion and Congenital Defects

Abortion, stillbirth or congenital defects of the fetus occurs when susceptible dams are infected with BVDV during gestation. Submission to the diagnostic laboratory of the whole fetus and placenta when available are recommended. Alternatively, tissues from the major organs particularly the lung, liver, spleen and kidney should be submitted. BVDV antibodies can be detected in the blood of fetuses infected late in gestation. The FA test will detect BVDV antigen in some fetuses, and virus isolation should be performed. Submission of more than one fetus, if available, may increase the probability of detecting the virus. Frequently, there are no microscopic lesions in BVDV infected fetuses. Some diagnostic pathologists argue the significance of BVDV isolation in a fetus with no lesions. However, the virus isolation should at least alert the veterinarian to the presence of the virus in the herd.

Acute and convalescent serum samples are usually not useful because the infection resulting in abortion may have occurred weeks or months previously. Serology can be useful to determine the presence of BVDV in an unvaccinated herd.

Post-natal BVDV Infection

Post-natal infection with BVDV in a normal animal is frequently a mild infection in which the animal responds immunologically to clear the virus and produce protective antibody. This is probably the most difficult for the laboratory to

diagnose. Serum samples from sick and contact animals for serology should be obtained. Virus isolation can be attempted from swabs from mucosal surfaces, tracheal washes or anticoagulated blood samples from which the buffy coat can be separated. Samples should be taken as soon as possible after onset of symptoms. Fecal samples are not suitable for the detection of BVDV.

When virus is isolated, differentiating a postnatal infection from a persistently infected animal can be achieved from a second virus isolation on blood several weeks later. This blood sample should be negative for BVDV.

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Summary

Bovine viral diarrhea infection is frequently suspected because of the wide range of disease problems it has been associated with. The diagnosis and control of bovine viral diarrhea virus remains a challenge that requires the cooperation of the producer, veterinarian and the diagnostic laboratory. This article reviews the procedures laboratories use to diagnosis BVDV and suggests what samples the veterinarian needs to submit to the diagnostic laboratory to increase the probability of obtaining a diagnosis.