

# Bovine Viral Diarrhea Virus: A Continuing Enigma

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Bovine viral diarrhea virus (BVDV) is considered one of, if not the most important viral pathogen of cattle in the United States. Since it was first recognized in 1946, BVDV has been demonstrated worldwide and has been associated with a broad range of disease manifestations. The broad spectrum of disease reported with BVDV infections of cattle includes subclinical infections, bovine viral diarrhea, acute and chronic mucosal disease, abortion, congenital defects, immunosuppression, immunotolerance, and persistent infections.

Recent advances in our knowledge regarding BVDV have come, for the most part, from an increased understanding of the complex pathogenesis and epidemiology of BVDV infections in cattle. Most recently, new research into the vastly unknown area of BVDV molecular biology has been reported although much is yet to be learned. This report will be a mini-review of the current knowledge and understanding of BVDV (the reader is directed to a number of excellent, more detailed reviews, references 1-3) and will conclude with a short discussion of the current research efforts into this important cattle pathogen in the virology section of the Department of Veterinary Science at NDSU.

## VIRUS

Bovine viral diarrhea virus is presently classified as a member of the genus *Pestivirus* in the family *Togaviridae* (4). Other members of this genus, hog cholera virus and border disease virus, are serologically related to BVDV (4). Numerous antigenically distinct strains of BVDV and border disease virus have been identified, although hog cholera virus does not exhibit the same degree of antigenic variation. Further BVDV strain variation is found in cell cultures where two "biotypes" are observed, cytopathic (CP) and noncytopathic (NCP). The NCP isolates do not cause easily detected changes in cells during viral replication, although both types of virus are capable of infection and disease production in cattle. The NCP strains are found in cattle persistently infected and immunotolerant to BVDV, whereas the CP strains are usually associated with acute infections (5).

BVDV is a small, spherical, lipid-enveloped, positive-strand RNA virus. The genome of BVDV is a single piece of RNA that has a molecular weight of about  $4.4 \times 10^6$  and is about 12,500 bases in length (6,7). In BVDV infected cells there appears to be a single genomic-sized RNA species. The virus has been reported to synthesize between three (8) and eight (9) virus-specific proteins. A biochemical difference in the proteins synthesized in BVDV-infected cells

has been detected by a number of investigators: the NCP strains are missing a protein of 80,000 molecular weight (3,9). It is not known what the function of this protein is, or the functions of any BVDV proteins, although it has been speculated that the protein may play a role in RNA transcription (9). Using a series of monoclonal antibodies, Donis and Dubovi (see review in ref. 3) have determined that there are no antigenic markers of biotype (CP or NCP), so it is necessary to actually observe the virus in cell culture to determine the biotype.

## CLINICAL DISEASE

BVDV is a causative agent of and/or associated with a number of clinically distinct and important disease syndromes that have been well documented over the last 40 years (1-3). There are two major factors involved in the determination of BVDV infections in cattle: the status of the animal and the biotype of the virus. The age and the immune status of the host animal is of major importance to the outcome of the infection. Infections of immunocompetent, nonpregnant cattle are most likely to be subclinical, especially in adult animals, followed by the production of serum-neutralizing antibodies. An important aspect of this form of BVD virus infection is the possibility of an associated immunosuppression, which increases the host's susceptibility to secondary infections. Among those pathogens associated with BVDV infections are bovine herpesvirus type 1 (infectious bovine rhinotracheitis [IBR]) and *Pasteurella hemolytica*.

Bovine viral diarrhea is an acute infection usually seen in cattle from six months to two years of age. This disease may have high morbidity but normally no mortality. The clinical symptoms are a mild diarrhea possibly associated with erosions of the mouth and throat, usually followed by the development of neutralizing antibody and rapid recovery.

BVDV is also associated with bovine respiratory disease, although to what extent is not yet clearly defined. Because of the immunosuppressive properties of the virus it is most likely to play a synergistic role in the development of respiratory disease in cattle. Because of the high frequency of BVDV isolation from respiratory tracts, it is difficult to determine the true role of this virus in the important bovine respiratory disease complex, although there is no doubt BVDV is an important respiratory pathogen of cattle.

BVDV infections of pregnant cows can result in any number of potential outcomes. BVDV is the most important viral pathogen of the bovine fetus and the results of an *in utero* infection can be devastating (1-3). Infection of the fetus has been shown to result in death and resorption,

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mummification, abortion, malformations, and persistently infected calves that may result in chronic "poor doers." The main determining factor in the outcome of fetal infection is the age of the fetus at the time of infection. Infection between about 50 and 100 days of gestation will most likely result in fetal death and between 100 and 150 days will often result in congenital defects (10,11). Infection of the fetus at an early stage (up to about 120 days) may result in an immunotolerant, persistently infected calf, normally associated with NCP strains (12). At this time no animals have been reported persistently infected with CP BVDV. Immunotolerance is a state induced when the fetus is infected prior to becoming immunocompetent (approximately 150 days of gestation). The immunotolerant animal is specifically tolerant to the particular strain of BVDV infecting it, however is still capable of responding to other antigens including antigenically distinct strains of BVDV (12).

Persistently infected cattle resulting from the *in utero* infection present a larger problem for the control and prevention of BVDV infections. Animals persistently infected readily transmit the virus and appear to be a major mechanism for maintaining the virus within a population (10). The persistently infected animal also appears to be the only animal capable of getting mucosal disease, a serious clinical syndrome with low morbidity and high mortality (13,14). Within the past five years the understanding of the pathogenesis of mucosal disease has been greatly increased, and it centers around the immunotolerant, persistently infected animal. The current evidence suggests that mucosal disease is the result of a dual infection with different strains of BVDV. This unique pathogenesis appears to develop only in animals persistently infected with NCP virus (*in utero*) followed by superinfection with a CP strain of BVDV (13,14). There seems to be a specific antigenic relationship that must exist between the two strains for disease production to occur, although this relationship is not yet understood.

Thus, the impact of BVDV on individual cattle as well as populations is variable dependent on a number of factors. It is clear that while BVDV is the causative agent in a number of overt disease entities, the major impact of BVDV infection is most likely associated with the persistently infected, immunotolerant animals and those animals immunosuppressed by BVDV infection.

## PREVENTION AND CONTROL

Prevention and control of BVDV infection and disease is a major problem facing the veterinary profession. Persistently infected animals, BVDV contamination of vaccines to other pathogens, virus in semen, and the ubiquitous presence of BVDV as well as any number of other factors belie the difficulties in control of this virus. Difficulty with identification of persistently infected animals is a major problem. However, the identification and removal of those animals may be the best way of trying to rid a herd of BVDV.

The effectiveness of BVDV vaccines has been less than satisfactory and on occasion detrimental. The use of modified live virus vaccines (MLV) has been associated with a number of problems, including: mucosal disease outbreaks as a result of MLV administration, immunosuppression, increased mortality in feeder calves following MLV vaccination, and the immunity induced may not be protective or long lived against heterologous strains of BVDV (15-17). Killed virus vaccines to BVDV do not cause disease, immunosuppression, or congenital infections. However, killed virus vaccines do require the use of adjuvants and multiple

doses, they do not induce good cellular immune responses, and the induced immunity may not be long-lived (1,3,15). Consequently, killed virus vaccines are safer than MLV vaccines, but they are more expensive to use and are not necessarily efficacious. The use of either MLV or killed vaccines can help to control the problem, but neither will completely eliminate, control, or prevent BVDV infections.

The outlook for BVDV control is not too promising with the currently available products. The hope is that with a better understanding of the molecular biology of BVDV and through biotechnology (genetic engineering) an effective, safe, and economical vaccine will become available.

## MOLECULAR BIOLOGY

There is currently little knowledge of the molecular biology and replication of BVDV. This is the second main area of current research emphasis, but there have been few reports with regard to BVDV genome structure and function. Very recently, research was reported on the organization and sequence of the RNA genome of the NADL strain of BVDV (6,7). From that work and an earlier report, the size of the BVDV genome has been determined and the basic structure of the RNA elucidated. A major implication of these findings is that the genetic organization of the BVDV genome more closely resembles that of another virus family, the Flaviviridae, and that BVDV, hog cholera, and border disease should be reclassified as a new genus of flaviviruses (6). This has major implications as to the strategy of BVDV replication and possibly its eventual prevention and control.

Therefore, the current outlook for BVDV research revolves around the continued problems of pathogenesis, replication strategy, and molecular biology. A main theme that runs throughout current BVDV research is the examination and comparison of cytopathic and noncytopathic strains. Our research will approach these problems by making use of BVDV isolates, both cytopathic and noncytopathic, obtained by the North Dakota State University Veterinary Diagnostic Laboratory.

## BVDV RESEARCH AT NDSU

Our research on BVDV is designed to obtain information about the basic strategy for replication and the molecular biology of paired cytopathic and noncytopathic BVDV isolates from North Dakota. The viruses to be used in this research project have already been isolated by the Veterinary Diagnostic Laboratory. A number of paired, i.e. isolated from the same animal, cytopathic and noncytopathic BVDV strains have been obtained, separated, and plaque-purified by standard methods (16). In addition, the well characterized Singer (cytopathic) and NY-1 (noncytopathic) are currently being used in our laboratory as model systems. The main idea behind the research is to determine as much basic information as possible with regard to BVDV and to then begin to apply this information for improving diagnostic procedures as well as methods of control. Most of the information we seek will be obtained by utilizing modern molecular biological approaches and ultimately applied with biotechnology.

Our approach is to examine the basic differences between the two biotypes of BVDV, cytopathic and noncytopathic, by studying the mechanism of replication in cell cultures and the basic molecular biology by constructing molecular clones of copy DNA (cDNA) made to the genomic RNA of BVDV. Since there appears to be a specific relationship required in the pathogenesis of mucosal disease, a better understanding



of the relationship of such pairs of virus will provide improved chances for the development of efficacious diagnostic tools and vaccines.

We are studying the proteins synthesized by BVDV in an effort to better understand the replication mechanism as well as define those antigens most important to the immune response of infected animals. This information will allow us to characterize the immune response of animals, help to clear up the confusion of classification of this group of viruses, and add valuable information as to which of the viral proteins should be utilized in vaccine production and diagnostic probes.

Molecular cloning of the BVDV genome of paired CP and NCP isolates of BVDV will provide information regarding the organization of the virus, the strategy the virus uses to replicate itself and to cause changes in the host, the function of the genes of the RNA and products, and the location of genes that may be useful for vaccine and diagnostic applications. We have already cloned the cytopathic Singer strain and are currently in the process of analyzing the clones to determine the similarities of the molecular structure to the two previously reported strains. We are also presently trying to utilize our clones of Singer to construct a potential diagnostic probe that would be capable of detecting small amounts of BVDV in clinical samples. We are hopeful of finding a sequence of Singer strain that will detect many, ideally all, strains of BVDV. Ultimately, the development of a genetically engineered vaccine could come from this type of research effort. The advantages of such a vaccine would be the elimination of the safety problems of the modified-live vaccines, enhanced efficacy, and improved cost over the killed products now available. Although those are the ultimate goals of this type of research, it is important to stress the difficulty in working with such an elusive agent as BVDV and the importance for patience in the application of biotechnology to this particular problem. Numerous academic and industrial groups are working on just these types of research projects, so there is reason to be hopeful that eventually some very positive answers will be provided to this important problem.

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