Fatal BVD Virus-induced Disease: Role of Persistently Infected Animals

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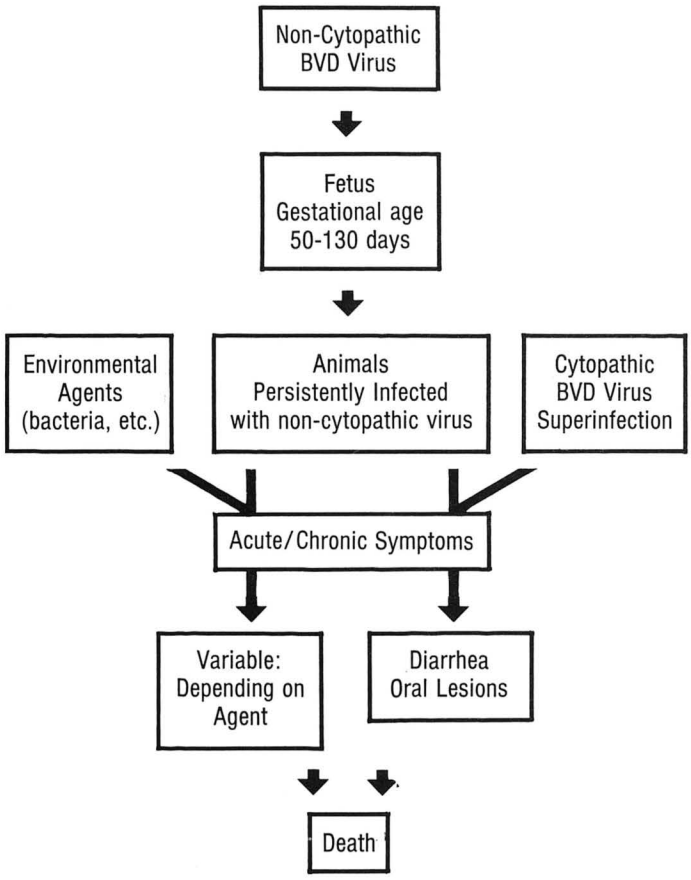
Nearly 40 years have passed since the initial recognition of an acute diarrheal disease of cattle which caused death in a high proportion of affected animals (1). The cause of this epizootic of diarrheal disease was subsequently ascribed to a virus which is now commonly referred to as bovine virus diarrhea (BVD) virus. A second clinical entity known as mucosal disease was also associated with infection by BVD virus (2). However, the diagnosis of mucosal disease is reserved for a more chronic or wasting syndrome in which there may be extensive erosion of the oral mucosa, intermittent diarrhea, anorexia, dehydration and eventual death. Attempts to experimentally replicate these field cases of disease were uniformly unsuccessful even though viral isolates from fatal cases were used. Over the intervening years, a few pieces of the BVD virus puzzle were identified, but no clear picture emerged until very recently. I will briefly review these recent findings and indicate areas of research which are needed in order to develop effective strategies for the prevention of BVD virus-induced disease.

The information which is presented in Figure 1 is a summary of the data which was generated at the National Animal Disease Center, Ames, Iowa, (3) and the ARC Institute for Research on Animal Diseases, Compton, England (4). The Figure indicates that four elements are necessary for the development of acute/chronic symptoms associated with BVD virus infection in which the eventual outcome is death of the animal. The first element which is necessary is the availability of a fetus which is developmentally unable to mount an immune response to a foreign antigen. The exact age at which the fetus becomes immunologically competent is not clearly defined, but it appears to be after 130 days. The second element which is necessary is the infection of the fetus with a noncytopathic biotype (NCPB) of BVD virus. This can result in the persistent infection of the fetus without the development of an immune response to the virus (5). The animal which develops from this fetus may appear normal, but it chronically sheds virus and remains negative for antibodies to BVD virus. At some point in its life, this persistently infected animal (third element) becomes superinfected with a cytopathic biotype (CPB) of BVD virus (fourth element). This superinfection with a second BVD virus by a mechanism which is as yet unknown triggers the disease process which sooner or later leads to the death of the animal. With these four elements in place, it is now possible to produce fatal BVD virus-induced disease under experimental conditions. Given the complexity of this disease process, it is now clear why most experimental infections failed to reproduce the field experiences, and also why deaths associated with BVD virus infections are so sporatic.

Athough the elements as outlined in Figure 1 for the production of acute/chronic BVD disease are known, there are a number of unknown factors. A major deficit in our knowledge concerning BVD virus is the number of unique biotypes of the virus which exist in nature. For years, it has been recognized that on the basis of in vitro growth characteristics all isolates of BVD virus could be separated into two groups, cytopathic (CPB) and non-cytopathic (NCPB) strains. The data represented by Figure 1 is the first evidence that what is seen in vitro has relevance in vivo, i.e., noncytopathic strains are in vivo biotypically different from cytopathic strains. The ability to produce persistently infected animals appears to be a property of noncytopathic strains of BVD virus while the induction of mucosal disease seems to be a property of cytopathic strains. The generalizations expressed in the preceding statement still must be regarded with some caution because only a few strains of BVD virus have been used in controlled experiments. We cannot as yet rule out the possibility that certain NCPB of BVD virus are able to trigger the fatal disease syndrome. Are there other biotypic differences between cytopathic and noncytopathic strains of BVD virus or between different isolates of cytopathic virus? There is some suggestion in the literature that NCPB virus has a tropism for lymphocytes while CPB virus may prefer epithelial cells (6). Experimental evidence also exists to show that certain strains of BVD virus produce more damage to the respiratory tract defenses than do other strains (7). NCPB virus produces persistent infections following in utero infection, but which biotypes are responsible for the production of congenital defects and fetal death? Clearly, a major effort must be made to determine the spectrum of biotypic differences which exist in the population of BVD viruses.

At this point, one might ask whether past experiments are helpful in defining the biological properties of each of the strains of BVD virus. The answer to a large extent is no, and

FIGURE 1. Elements involved in the induction of disease by BVD virus.



the reason for this is that many experiments were done with mixed populations of virus. The source of the mixed populations were several. Firstly, most experiments have been conducted with isolates made from animals dying of a BVD virus infection. As will be discussed later, it appears highly likely that these were persistently infected animals dying as a result of a BVD virus superinfection as outlined in Figure 1. Therefore, the isolate was actually a mixture of CPB and NCPB viruses. Very few researchers made the effort to clone the virus to insure that a single biotype of virus was present in the inoculum which was administered to their test animals. A second source for the production of a mixed population of BVD virus was/is the in vitro culture system for the virus. Anyone who used fetal bovine serum in his test system ran a very high risk of introducing BVD virus. Even today, as many as 50% of lots of fetal bovine serum are contaminated with BVD virus. The researcher who was careful in his research design and used cloned virus may have been defeated by a contaminated lot of fetal bovine serum because it is extremely difficult to detect NCPB virus in a population of CPB virus. Therefore, the reports which show the production of persistently infected animals following exposure to CPB virus either experimentally (8) or through vaccination with modified live vaccines (9) must be viewed with some reservation until properly designed and conducted experiments can be done.

Another major area of uncertainty with regard to BVD virus is the extent of antigenic diversity which exists within the virus population. For years, it has been recognized that hog cholera, border disease and BVD virus share antigenic determinants. Studies have also detected significant differences among various isolates of cytopathic strains of BVD virus (10). However, because of the difficulty in working with the noncytopathic strains, there is a dearth of information concerning the antigenic diversity of these strains. Are there CPB viruses which are antigenically identical to NCPB viruses? The answer to this question may be significant in determining the mechanism for the development of fatal BVD virus-induced disease as depicted in Figure 1. Studies on the viral induced proteins found in BVD infected cells clearly demonstrate major differences in the proteins of CPB viruses and NCPB viruses (Donis and Dubovi, in preparation). Do these differences reflect differences in antigenicity or other biological properties? Until these questions are answered, the development of effective vaccines will be a hit-or-miss proposition.

As indicated in Figure 1, the infection of a fetus with NCPB of BVD virus can result in the production of a persistently infected animal. The unique characteristics of these animals are that they continuously shed virus and they possess no antibodies to the virus. The lack of immunological response to the virus has led some to speculate that these animals may have a generalized immune dysfunction. However, studies have found that these animals respond normally to other antigens and the only dysfunction that can be detected is toward BVD virus antigens (5, 11). Many of these animals are not recognized as being different from their herd mates until they develop a fatal BVD virus-induced disease. Many textbooks indicate that mucosal disease is most frequently seem in animals 6 to 24 months of age. What happens before 6 months of age? It would appear that many persistently infected animals die before 6 months, but the involvement of BVD virus has been unrecognized or considered insignificant. Our experience at the Diagnostic Laboratory at Cornell indicates that animals less than 6 months of age do die of BVD virus-induced disease as outlined in Figure 1. CPB and NCPB virus has been isolated from calves dying of acute diarrhea with and without classic oral lesions. The role of BVD virus in the death of these animals is fairly clear. However, we also find that a number of calves which present with a chronic pneumonia are persistently infected with BVD virus. What role does BVD play in this disease? These animals do not appear to be infected with a second biotype of BVD virus and thus do not fit the superinfection pattern in Figure 1. I believe that the virus does play a role in this disease by reducing the animals inate ability to respond to environmental agents. In my opinion, persistently infected animals are not "normal" even though they grossly appear to function in a normal manner. When confronted with a bacterial pathogen, the persistently infected animal cannot

mount an adequate defense because of the stress of the BVD virus infection whereas the nonpersistently infected animal can. The animal may die of a bacterial pneumonia, but the underlying cause was the BVD virus infection. It is this type of calf mortality that is not being recognized as being due to the persistent BVD virus infection. Thus, persistent infections can have a detrimental impact on the economic potential of these animals even in the absence of superinfection.

As indicated previously, the persistently infected animal continuously sheds virus into the environment. Pregnant susceptible animals which come in contact with these virus shedders can become infected and produce more persistently infected animals. In addition, the persistently infected animal can be bred successfully and produce normal looking persistently infected offspring. Once a persistently infected animal enters a herd containing susceptible animals, one can expect to see other manifestations of BVD virus infections such as abortions. The amount of virus found in the blood of a persistently infected animal can reach a million infectious units per ml. Common use of blood contaminated instruments such as needles could be a factor in the spread of BVD virus in the herd. Abortions following vaccinations may not be related to the product administered, but may be the result of the iatrogenic spread of BVD virus from persistently infected animals.

Are there a sufficient number of persistently infected animals in the bovine population to account for the fatal BVD virus-induced disease? The answer to this question appears to be yes. From a recent study on the prevalence of persistently infected animals in selected herds, over 3,000 animals were examined and 54 (1.7%) were determined to be persistently infected (12). This value agrees well with other herd estimates (13) and with studies examining the number of BVD virus infected fetuses found in slaughterhouse surveys (14, 15). For individual herds, the number may range from 0 to 25%. At the very least, these estimates indicate that the existence of persistently infected animals is not a rare event.

For the development of fatal BVD virus-induced disease, the persistently infected animals must be superinfected with a different biotype of the virus. What is the source of second virus? There are numerous possibilities and I will mention only a few. In many instances, the outbreak of disease can be traced to herd additions. The new animals bring in the second BVD biotype or the new animals are persistently and the second biotype comes from the resident herd. The second biotype may be introduced into the herd through the use of a modified live BVD vaccine. These vaccines are generated from CPB viruses and thus fit the characteristics for a superinfecting virus. From the accounts of fatalities following use of modified live BVD virus vaccines, it is reasonably certain that the animals which died were those which were persistently infected with a NCPB of BVD virus (16, 17). From an immediate economic standpoint, it is not desirable to have animals die following vaccination, but the modified live BVD virus vaccines did identify those animals which were persistently infected and eliminated them from the herd. Over the long haul, this can only be viewed as a positive development. For those herds where there is no obvious source of the second virus, one must ask the question of whether CPB virus can arise from NCPB virus through a mutational event. In this manner, an outside source of the virus may not be necessary. CPB virus arising within the herd by a mutational event would be antigenically identical with the NCPB virus. However, it is not known whether antigenically identical viruses can interact to produce fatal BVD virus induced disease.

It is quite clear that the persistently infected cow represents the major reservoir for BVD virus in the bovine population. To prevent the development of persistently infected animals, one must prevent the virus from reaching the fetus. Can currently available vaccines do the job? The answer is at best maybe. The reason for the uncertainty has already been discussed. Since we do not know the range of antigenic diversity within the BVD virus population, there is no way to predict whether an animal vaccinated with one antigenic type will respond rapidly enough immunologically to eliminate a virus of a different antigenic type before the virus infects the fetus. Data released by vaccine manufacturers only show how well vaccinated animals respond to virus infection with the production of neutralizing antibodies, but no data is presented which would indicate whether a fetus carried by the vaccinated animal would be protected from infection with the challenge virus. To the contrary, a recent report indicates that animals vaccinated with an experimental inactivated BVD virus vaccine were not able to respond to a heterologous strain challenge in a manner which could protect the fetus from infection (18). Although this type of experiment is helpful in indicating the shortcomings of currently available vaccines, they do little in the way of developing a better product. This can come about only when we have adequate data on the antigenic diversity within the BVD virus population and the distribution of this diversity within the bovine population. A vaccine based on one strain of BVD virus may work well in one region of the country, but be relatively ineffective in another region because the vaccine strain is not the prevalent antigenic type in all regions. It is also possible that one vaccine may be effective in preventing BVD virus associated respiratory disease in feedlots, but be ineffective in protecting the fetus. Which vaccine type, modified live or killed, provides the greatest protection for the fetus? At this point in time, we can only speculate on the effectiveness of the vaccines because we do not have an adequate data base from which to draw reasonable conclusions.

As I have indicated, there are many questions which must be answered before we will have a clear understanding of the pathogenesis of BVD virus. Careful examination of field cases of fatal BVD virus-induced disease must be done to determine the overall validity of the scheme in Figure 1. However, it now appears that most fatalities associated with

BVD infections alone were due to the superinfection of persistently infected animals. This common mechanism for disease production was obscured because of the widely different clinical presentations of the disease. It was difficult to see what an animal which died of acute disease within a few days had in common with an animal which died of a chronic wasting syndrome over many weeks. The reasons for these widely divergent syndromes are still unclear, but I believe that they may be related to the interaction of different combinations of NCPB and CPB of BVD virus, either because of antigenic differences or other biotypic differences. The recognition of a common mechanism for fatal BVD virus-induced disease will be a valuable step in the development of effective measures to prevent losses associated with BVD virus infections.

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Questions & Answers:

Question: Can you discern from the literature whether it is the cytopathic or the non-cytopathic that produces abortions?

Answer: I think looking back through that data one would have to conclude that both of them are involved. It is clear in instances that non-cytopathic virus can induce the abortions. But I think it is also clear that cytopathic biotypes can also do it. So I don't think as far as the abortion is concerned, at this stage we can split the two based on whether it can abort or not. I think both of them are involved.

Question: Can killed vaccine prevent non-cytopathic virus infection in the cow as opposed to worrying about whether it gets to the fetus?

Answer: That's one point I forgot to mention. In most instances they are sufficiently related antigenically that you will get a decent immune response with the killed vaccines if you challenge with a non-cytopathic virus. And it may be well that for such things as feedlot situations where you're asking for some protection against shipping fever which might be due to a previous BVD infection that these vaccines are totally adequate. It may be what we need is a different type of vaccine for the prevention of the infection of the fetus. So it seems as if the killed vaccine can give you a good immune response when challenged with a non-cytopathic virus. But the data just are not there. At least they have not been published. I just don't think experiments have been done on fetal protection. It is a case of somatics about protection of infection and also clinical disease. Now if you are worrying about trying to prevent shipping fever and if you think BVD is a predisposing element to it, what you want to do is protect that animal from having a serious lung infection of BVD. I think in that instance you're asking the vaccine to do something a little less stringently than shutting the virus down before it gets to the fetus. I think that there are many instances where that virus is going to replicate in that cow for three days, four days, five days, I don't know, maybe ten days. In that replication phase, which occurs in the lymphocytes, it is a systemic infection, the evidence would suggest that, at least with the experimental work done with these killed vaccines, they cannot prevent the fetus from being infected. The cow herself, as far as predisposing her to other bacterial infections, lung infections, or whatever, may be fine. But if you want to knock out BVD and break this cycle, the fetus has got to be protected. Maybe there's a vaccine out there, but I don't think the study has been done.

Question: Will the persistently infected animal develop an antibody titer to the cytopathic virus in a killed vaccine?

Answer: The general feeling and experience is that it will not. Now a titer of 1:4, 1:8 in these animals is in line with USDA efficacy of vaccines, (they only ask for a 1:8). But if you're talking about protection of animals I like to see something a little higher than that, so there is a moderate antibody response of a very low nature. If the rest of your animals are giving titers of 1:28, 1:56, and you see 1:8 there, you've got to believe that there's something wrong with that animal. Now there's a little fly in the ointment. You can vaccinate with a modified live vaccine and develop very high antibody titers to the modified live vaccine. The antibody that is produced will recognize only the modified live vaccine. It does not recognize the virus that established persistent infection. This is an unusual event that is rare enough but it has been documented in several instances, but curiously enough these animals eventually die of mucosal disease. So we have recommended doing that. In particular herds where you don't have an idea about, you don't want to go through the expense of just randomly bleeding the entire herd to determine the antibody prevalence. The other option is to go through and vaccinate the whole herd and then do an anitbody screen. In our instance we do screen like 1:16, 1:32. If we analyze a titer of up to 1:16 after a killed vaccine, then we just exclude it from viral isolation. Anything below that we'll go back and isolate. Now that's a very crude way of cutting it. But with a monetary restraint you don't do viral isolation on the whole herd and try to eliminate some of them. So we do recommend trying that, looking for the non-responder and that's the one

you go in and culture.

Question: Is there a need, in fact, for a test, for the virus itself to better detect this group?

Answer: The ideal test may come down the road if we can get a battery or a single monochrome antibody that is phenomenally specific for BVD and in that instance what you could do is take buffy coats, the lymphocytes, or perhaps even a blood smear. Our estimates are that 1-5 percent of lymphocytes are infected in these animals. So you should be able to do a blood smear or buffy coat smear with a very specific reagent and determine whether or not they are positive or negative to BVD. Currently available FA regents are absolutely useless to do that. There are a lot of false positives. And BVD is a virus and the reason it has taken so long to deal with it is because it produces very little antigen in the infected cell. So fluorescent antibody staining on tissues is sometimes questionable, particularly on lymphocytes. But that's going to be the ideal test. A good fluorescent reagent that can specifically stain infected lymphocytes and we won't have to go through isolation.

I guess the question I always go back to is that it depends on the management situation on the farm. If you can be absolutely sure that you can segregate out the animals that have been vaccinated with a modified live vaccine and keep those away from your pregnant animals in the herd, then I don't see any difficulty doing that. There are certain farm situations where you can do that six-month-old animals that they vaccinate with a modified live vaccine, if they can keep them from your pregnant heifers. Subsequent boosts can be done in your adult cow herds with the killed vaccine to continue boosting the titer. I would switch back and forth from one product to another to try and get as many antigenic variances I can in the system. Under the right conditions you can develop a very effective vaccination program using modified live vaccine, but it depends on the management.