# Persistent Infection of Cattle with BVD Virus: Recent Findings on Origin, Prevalence and Relationship to Disease

**Steven R. Bolin**
*NADC, USDA/ARS*
*Ames, IA 50010*

© Copyright American Association of Bovine Practitioners; open access distribution.

## Introduction

Bovine viral diarrhea (BVD) occurs in 2 clinically recognizable forms: an acute disease characterized by fever, anorexia, leukopenia, and diarrhea, and a chronic disease characterized by wasting, salivation, lameness, and intermittent diarrhea. The acute form of the disease frequently escapes detection and has a high morbidity and low mortality. The chronic form of the disease is also known as mucosal disease and has a low morbidity and high mortality. The causative agent, bovine viral diarrhea virus, is a togavirus and a member of the genus Pestivirus.

In the laboratory, isolates of BVD virus are classified as non-cytopathic or cytopathic according to their ability to destroy certain cells grown in culture. Most cytopathic BVD viruses destroy a cell monolayer within 2 days. Noncytopathic BVD viruses replicate without destroying the cell. Either type of BVD virus can be isolated from fetal calf serum and from tissues of cattle that have died from BVD. Antiserum raised against one BVD virus will neutralize all other BVD viruses and, under experimental conditions, neither type of BVD virus produces a severe disease in normal cattle.

**Recently, there have been several reports of a third form of BVD virus infection: an inapparent, persistent infection[1,2,3]. This occurs when the bovine fetus becomes infected with noncytopathic BVD virus before day 125 of gestation[4] .At birth, persistently infected calves lack neutralizing antibodies to BVD virus and have readily detectable amounts of infectious BVD virus in their tissues. Although stunting and increased death loss may occur in persistently infected calves, the majority survive to maturity. Cows that are persistently infected pass the persistent infection on to their offspring. Within the past year, several investigators have linked mucosal disease to persistent infection with BVD virus[5,6,7]. Experimental Reproduction of Mucosal Disease**

Eight cattle persistently infected with 1 of 3 isolates of noncytopathic BVD virus were superinfected with cytopathic BVD virus[7]. All of the cattle developed clinical signs of mucosal disease within 2 to 4 weeks. All, but 1, of the cattle became moribund within 1 week of developing signs of BVD. At necropsy, there were erosions and ulcerations of the mucosa of the alimentary tract, especially over the lymphoid follicles of the ileum. The histopathic lesions were consistent with those typically found with mucosal disease.

Before superinfection with cytopathic BVD virus, 3 of the persistently infected cattle had been superinfected at monthly intervals with 4 different isolates of noncytopathic BVD virus. No signs of disease were seen. Similarly, there were no signs of disease after inoculation of the cytopathic BVD virus into normal cattle without neutralizing antibodies to BVD virus. Simultaneous or sequential inoculation of various combinations of noncytopathic and cytopathic BVD viruses into normal cattle did not produce signs of disease. Hence, mucosal disease was produced only when persistently infected cattle were superinfected with cytopathic BVD virus.

## Isolation of Noncytopathic and Cytopathic BVD Viruses from Field Cases of Mucosal Disease

The investigation described above supports the hypothesis that mucosal disease is due to cattle persistently infected with one BVD virus (probably noncytopathic) becoming superinfected with a second, but in some way different, BVD virus (probably cytopathic). If this hypothesis is correct, it would be possible to isolate the 2 types of BVD virus from field cases of mucosal disease. This was tested by doing virus isolation using spleens from 39 field cases of mucosal disease[8]. The spleens came from 13 herds. In 8 of the herds, cases of mucosal disease had occurred after vaccination for BVD. Both noncytopathic and cytopathic BVD viruses were isolated from individual spleens from all herds and from 29 of the 39 spleens. Cytopathic BVD virus was isolated from all of the spleens. Separation of noncytopathic from cytopathic BVD virus can be difficult and this probably accounts for the failure to isolate both types of BVD virus from 10 of the spleens.

## Prevalence of Persistent BVD Virus Infection

Mucosal disease occurs sporadically in cattle. Hence, it would be anticipated that the prevalence of persistent infection is low overall but certain herds may contain several persistently infected cattle. As a test of this 3157 serum and

blood buffy coat samples from 66 herds were tested for antibodies to BVD virus and for the presence of BVD virus[9]. Antibodies to BVD virus were detected in 89% of the serum samples. Noncytopathic or cytopathic BVD viruses were isolated from 60 blood buffy coat samples from 6 herds. This was approximately 2% of the cattle and 9% of the herds tested. Large groups of 23 and 31 persistently infected cattle were found in 2 herds. Subsequently, all of the group of 31 cattle died of mucosal disease.


### Summary

The results of laboratory and field investigations indicate a link between persistent infection with BVD virus and mucosal disease. Both noncytopathic and cytopathic BVD viruses have been isolated from field cases of mucosal disease and mucosal disease has been experimentally produced in cattle persistently infected with noncytopathic BVD virus by superinfection with cytopathic BVD virus. The prevalence of persistent BVD virus infection is unknown but is probably high enough to be of concern. The persistently infected cow is a source of infection for other cattle, passes the persistent infection on to her offspring, and is at risk of developing mucosal disease.



### References

1. Coria, M.F. and McClurkin, A.W.: Specific immune tolerance in an apparently healthy bull persistently infected with bovine viral diarrhea virus. J. Am. Vet. Med. Assoc. 172:449-451, 1978. 2. Cutlip, R.C., McClurkin, A.W. and Coria, M.F.: Lesions in clinically healthy cattle persistently infected with the virus of bovine viral diarrhea - glomerulonephritis and encephalitis. Am. J. Vet. Res. 41:1938-1941, 1980. 3. Straver, P.J., Journee, L.H. and Binkhorst, G.J.: Neurological disorders, virus persistence and hypomyelination in calves due to intrauterine infections with bovine virus diarrhea virus. II. Virology and epizootiology. The Vet. Quarterly 5:156-164, 1983. 4. McClurkin, A.W., Littledike, E.T., Cutlip, R.C., Frank, G.H., Coria, M.F., and Bolin, S.R.: Production of cattle immunotolerant to bovine viral diarrhea virus. Can. J. Comp. Med. 48:156-161, 1984. 5. Roeder, P.L. and Drew, T.W.: Mucosal disease of cattle: A late sequel to fetal infection. Vet. Rec. 114:309-313, 1984. 6. Brownlie, J., Clarke, M.C. and Howard, C.J.: Experimental production of fatal mucosal disease in cattle. Vet. Rec. 114:535-536, 1984. 7. Bolin S.R., McClurkin, A.W., Cutlip, R.C. and Coria, M.F.: Severe clinical disease produced in cattle persistently infected with noncytopathic bovine viral diarrhea virus by superinfection with cytopathic bovine viral diarrhea virus. Am J. Vet. Res., In Press. 8. McClurkin, A.W., Bolin, S.R. and Coria, M.F.: Isolation of cytopathic and noncytopathic bovine viral diarrhea virus from the spleens of acute and chronic cases of bovine viral diarrhea. Manuscript in preparation. 9. Bolin, S.R., McClurkin, A.W. and Coria, M.F.: Prevalence of persistent bovine viral diarrhea virus infection in cattle. Manuscript in preparation.


## Questions & Answers:

*Question:* We have had dairy herds, small herds, big herds, that we have epidemics of high fevers through the herds, or we will have what appears to an epidemic of a respiratory cough or sometimes an outbreak of a half a dozen cows with diarrhea, or whatever, and have over the years been using flourescent antibody testing and doing it on buffy coats, leucocytes, and 75% of the time in these kinds of herds we get positives for BVD . . . I've been freezing up those buffy coats, hoping some day for somebody to tell me what to do with them. At any rate, some of these herds are vaccinated, some of them are not. I've been a big proponent of vaccinating with modified live virus, especially Singer strain, and this sort of thing. What should we do about vaccinating when we have all of these kinds of situations?

*Answer:* That's a good question, and it is a question that if anybody has access to *The Veterinary Record* and you've been watching the letters to the editor for the last four or five months, they have been kicking this around simply for the legal ramifications. The English are aware of what I have just presented. In fact two groups in England working at the same time we were working have come up with almost identical results and identical hypotheses. They are able to get their material published far quicker than we can, so therefore their practitioners are aware of it and they are already into the legal ramifications of it. I don't want to make any recommendation to you for that reason. My tendency would be to stick with the killed virus vaccine. I don't know of any post vaccinational mucosal disease deaths following the killed virus vaccine. We certainly know now that if there are persistently infected animals in the herd, there is a chance you will get a post vaccinational death if you use a modified live virus vaccine.

*From the floor:* We haven't had any post vaccination deaths that I know of, at any rate, but we've had 200 cow herds that a couple of times a year will have a dozen or so fresh cows get thin. Post mortem will reveal abomasal ulcers or the coughing business and occasionally high fevers and the like, and do very poorly on production and never peak. Do we probably have a lot of persistent shedders in those herds.

*Answer:* In that survey I showed slides of, about half the herds we looked at, veterinarians thought they had this problem. And in not one of those herds that they thought they had BVD did they have BVD. It was in those herds that were going along just smoothly that we found the persistently infected animals. Your question is very difficult for me to answer, as we are all aware that there is a limited number of signs that you can see and a multitude of agents that can cause them.

*Question:* I was thinking in terms of when we have already got positive FAs on buffy coats.

*Answer:* I don't like to do FAs on buffy coats. I feel like I get too much non specific or false positive stuff. I would far prefer if you think you've got BVD in a buffy coat, freeze and thaw that buffy coat and put it on cell culture and then do your FA on those cells. You get a lot of non specific sticking of antibodies to white blood cells and you also get some background flourescence, autofluorscence in white blood cells.

*Question:* Would this also be true even if these are from cows with clinical signs that would fit BVD? You can see these false postitives.

*Answer:* Bluetongue can give you clinical signs that will fit BVD. That Johne's cow that was scouring sure looked

© Copyright American Association of Bovine Practitioners; open access distribution.

like chronic BVD. BVD is not the only disease that will give you those signs, or the only condition.

*Question:* Do you have an estimate on the time the maternal antibodies run out in these calves and is there any possibility that despite any maternal antibody, if vaccinated with another strain they might still get immunity, like you talk about distemper and measles vaccines in pets?

*Answer:* On some animals, yes. In some animals with a titer of 1:32 even you'll get an anamnestic response following vaccination. Maternal immunity will last 4-6 months. An interesting thing in these persistently infected calves, if the cow is not persistently infected, if she's a normal animal that doesn't have antibodies when she's infected, when the calf's in utero, the calf will pick up those colostral antibodies to BVD and it will have an antibody titer for about 2-3 months. It will lose that antibody titer quicker than a normal animal will. But a normal animal will carry that passive protection for 4-6 months.

*Question:* Let me be among the first to congratulate you and the British workers for shedding some light on this various intriguing disease, BVD. The work has been excellent. In Canada we have wondered for many years why some calves developed fatal BVD disease when vaccinated and I think we may have a good explanation now. If we follow your work and the British work, can you see anything wrong with the hypothesis that the way to control this disease, even in the weaned beef calf, is to vaccinate the pregnant female before breeding to insure a high level of antibody production in the pregnant females and to forget about vaccinating weaned calves, but perhaps vaccinate heifer replacements again before breeding? That is what we're currently recommending in western Canada and I'll tell you better in 5 years whether it works!

*Answer:* Unfortunately it always takes that long.

*Question:* If you take an antibody titer on a cow that has been vaccinated and it is positive, and it has been some period of time and she's not dead, can you assume that animal is not infected with a non-cytopathic virus?

*Answer:* Some of these animals, a lot of these animals that are persistently infected carry an awful lot of non-cytopathic virus in their serum . . . up to 100,000 infected particles per mill of serum. And if you take an animal that has that much non-cytopathic virus, and you run your standard microtiter SN plate, you'll get a false positive titration that might go 1:64 to 1:32 without any trouble. The reason you get a false positive is the non-cytopathic virus interferes with the cytopathic virus. So if you have cells that are exposed to non-cytopathic virus, that virus gets in there and starts to replicate, cytopathic virus can express itself so you don't get CPE. If you don't see CPE in the microtiter plates you say the animal has a titer. It doesn't have an antibody titer, what you've got is a virus titer. So on some animals, and this is going to make things tough for us down the line, you can't just run a neutralization test, because you will get a false positive because of the amount of non-cytopathic virus in the serum. So, to answer your question, the fact that you get a low titer following vaccination, I can't tell you for sure she is not persistently infected. That low titer may be because she is persistently infected. It may be non-cytopathic virus.

*Question:* What if you get a high titer?

*Answer:* I've never seen a titer from the virus go over 1:64 dilution. If you get a high titer she should be protected. Now, what may happen, what we saw with these vaccinated animals and what Lee saw. In Lee's animals he never challenged them with a second cytopathic virus. He just vaccinated them. They did the same these animals did. They made an antibody titer to cytopathic BVD virus. And they made a good titer. And that titer held as long as they lived, but they ultimately died. But in most of them, it was half a year or longer with the majority being a year to two years, or 48 to 92 weeks before they died. When they die you post them. You don't see the classic gross lesions of mucosal disease. But you sure get that squirting diarrhea and you get severe blunting and fusing of the villi in the intestinal tract. So you can do that post, and if you didn't have the virology to back you up, you'd be hard pressed to make a diagnosis just on gross lesions.

*Question:* If you had a valuable cow, like an ET cow, that you wanted to identify whether or not she was persistently infected with non-cytopathic virus, how would you go about doing it?

*Answer:* I'd contact me and send me a little serum!

*Question:* You mentioned that it takes about 2 months to recover the non-cytopathic BVD virus. All too often diagnostic laboratories will detect a non-CPE BVD virus either from clinically ill animals with BVD signs or otherwise dying from other conditions. Would you tell me how you conciliate the two-month minimum that you are talking about, which is rather quick detection of the virus, and second, what is the significance of the non CPE virus in animals dying from conditions other than clinical BVD?

*Answer:* We do run into this problem. They are not all nice, healthy looking animals. We do have a higher incidence of calf mortality. We do have unexplained deaths in these animals. We will do a post mortem and we can't figure out from a gross post mortem why they died. The animals are more susceptible, or let me put it this way, the animals that I have seen and worked with, I get the impression that they are more susceptible to secondary bacterial invaders, more susceptible to pneumonias, and perhaps more susceptible to bacterial diarrheas. So that might explain why you can get your non-cytopathic virus out, yet you feel they died from something other than BVD. They probably did. They were persistently infected.

*Question:* I don't intend to pin you down at all, but did you or did you not say that you prefer the killed vaccine over the modified live?

*Answer:* The only reason I said that is because we don't know of any instance following use of the killed virus vaccine that anybody has gotten a post-vaccination mucosal disease. We're concerned that it could happen with a modified live virus vaccine. So I'm giving you this bit of information. Being aware that they are concerned with the legal ramifications of this in Europe already, you may want to think about it. But I'm not standing up here and telling you, don't use modified virus live vaccine.

*Question:* Would you use a killed product in your cows?

*Answer:* Yes, I would.

© Copyright American Association of Bovine Practitioners; open access distribution.