

# Bovine Viral Diarrhea Virus

## *in Camelids*

Bovine Viral Diarrhea Virus (BVDV) has existed for decades. It has been particularly devastating to the cattle industry. In recent months BVDV has become a concern for camelids. Unlike cattle, camelids show little or no outward signs of the virus. If camelids show few signs, should BVDV be of concern to the industry? The answer is, "Absolutely!". Current status information and protocols for managing BVDV follow.

### **From the Alpaca Research Foundation**

Bovine Viral Diarrhea Virus (BVDV) has recently been recognized as a source of disease in alpacas. As of February 1, 2006, there are at least 40 persistently infected (PI) alpacas currently identified in North America. One PI alpaca is nearly three years old and appears completely healthy. PI animals propagate further infection. To control the emerging problem, PI animals must first be identified and then permanently removed from the herd. BVDV can be rapidly spread by PIs. The disease could be brought back to the farm by an infected alpaca. If

that animal is pregnant there is a possibility she could then deliver another new PI cria thereby propagating the disease. Testing and eliminating all alpacas for active BVDV before show entry will make a tested show safer for all participants. Testing will therefore significantly decrease both the incidence and spread of BVDV. The combination of show testing, on-farm biosecurity measures, on-farm herd testing and careful attention taking animals from one farm to another should reduce the incidence of BVDV in the North American alpaca herd.

### **BVDV - Overview, Testing and Recommendations**

#### ***Overview:***

Bovine Viral Diarrhea Virus (BVDV) is currently a rare alpaca disease. There is clear evidence indicating that the disease has been present in the North American alpaca herd since at least 2001 and likely originated from BVDV infected cows. Recent testing of a small fraction of the national herd has revealed at least 40 persistently infected (PI) alpacas.

Ongoing research and testing is being funded by the Alpaca Research Foundation (ARF) to determine the prevalence of BVDV in our national herd. This virus has potential to spread within the alpaca industry under specific circumstances. The Alpaca Research Foundation, together with the Alpaca Owner Breeders Association (AOBA), is providing this information to alpaca breeders so that informed decisions can be made in consultation with their veterinarian. These include recommendations on education, testing, bio-security and management. We feel certain these are the keys to prevent and then to control this disease in our alpaca industry.

#### ***Clinical Information:***

Healthy alpacas can get the virus from infected alpacas and cows. This virus is fought off by that alpaca's immune system, is acute (short lived), and results in an increase in the ability of that alpaca's antibodies to neutralize the virus. Potential symptoms in an alpaca infected with BVDV include, but are not limited to, a mild fever, decreased appetite and rarely diarrhea, but animals may show no symptoms.

The BVDV infection in a pregnant alpaca can be a completely different matter. The infected pregnant dam acts just as described above – virtually no symptoms and no consequences to her. However, the consequences of infection on the developing fetus can be serious. These range from no effect to abortion to birth of a persistently infected (PI) cria. This PI state results because the fetal immune system accepts the virus as its own. The fetus is unable to fight the virus and never develops antibodies to that strain of virus for its entire life.

However, some PI crias grow to adulthood with no signs of any clinical disease. Note that a PI animal does not get the infection outside the womb. The only way to become a PI alpaca is viral exposure in utero (before birth). Not every “poor doer”, small or aborted fetus has BVDV infection or is a PI. The reason PI animals are such a problem is that they shed huge quantities of infectious virus through respiration and all body fluids into the environment every day with some of them looking and acting perfectly normal.

The reason for these drastic measures is that the unrecognized PI alpaca can spread this disease in the North American alpaca herd. Since there is no treatment for BVDV infection, the PI animal must be identified and then euthanized or completely quarantined.

To protect our alpaca population from PI animals, we recommend the following testing protocols.

## Testing Protocols

### Introduction:

The following testing protocols are recommended to identify Bovine Viral Diarrhea Virus (BVDV) infection in alpacas. BVDV is a newly recognized disease in alpacas that can cause abortions and viral persistent infections (PI). It has the potential to spread from farm to farm via poor bio-security, commingling of animals at sales or

shows and through transport of PI animals for breeding, sales or other reasons. Therefore, education, bio-security and early detection are critically important.

These alpaca BVDV testing recommendations are based on comparative medicine, are derived from those currently used for cattle, but with regard to alpacas are currently incomplete. The ELISA using serum or skin biopsies for use in alpacas appear to be unreliable at this time. Research is ongoing. Consult with your veterinarian and note that updates will be appearing on the ARF and AOBA websites.

Because every state laboratory has different sample requirements, it is critical that you work with your local veterinarian to insure that samples are submitted properly.

1) Appropriate on going farm bio-security has been and will continue to be an important tool that owner’s can use to prevent BVDV from getting onto the farm. Quarantine all alpacas that come to the farm or return from another untested location (shows, breedings, etc.) for a minimum of thirty days. Test all existing and new alpacas before they come to the farm for BVDV with a viral whole blood test. Add a clause to all contracts that provides for testing and full refund if the alpaca is PI.

2) There are three excellent and widely accepted BVDV tests: the polymerase chain reaction (PCR) test, the virus isolation/identification (VI) test and the IHC (immunohistochemistry) test. Blood for either the PCR or VI test can be collected in an EDTA (purple/lavender top) tube. A negative blood viral test would indicate that the alpaca is not PI for the life of that animal when run at a laboratory experienced with alpaca BVDV testing.

A negative IHC test also rules out a PI status.

3) All aborted and stillborn fetuses, crias that die, and any unexplained deaths should be necropsied by a veterinarian or sent intact to your state veterinary diagnostic laboratory. Tissue samples and blood can be sent to this diagnostic lab and tested for disease agents, including BVDV. Low birth weight crias, poor doing crias, “failure to thrive”, very premature crias, and alpacas with unexplained illness should be tested for BVDV.

4) If BVDV is identified from any blood/tissue submission, then the herd could be strategically tested (BVDV herd screening) to see if there is a persistently infected (PI) animal present in the herd as the source of the infection.

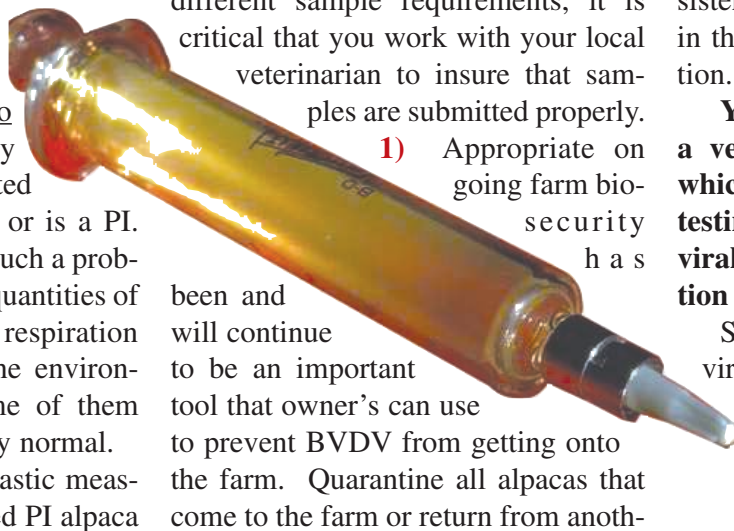
**Your veterinarian should contact a veterinary diagnostic laboratory which is experienced with BVDV testing in alpacas to find out which viral tests are offered for the detection of BVDV.**

Some diagnostic laboratories have viral testing preferences using PCR vs. VI (virus isolation) testing on whole blood (buffy coat).

With proper blood submission, both tests are valid for the detection of active viral infection in alpacas.

### Evaluation of tests:

- PI animals can live, reproduce and appear healthy in every way.
  - Any pregnant females who may have been exposed to BVDV during their pregnancy should have their crias tested for BVDV soon after birth using a whole blood viral test or IHC test. Until results are known, these animals should be quarantined. The only valid tests for these crias (up to 3 months) is a viral blood test done on the blood “buffy coat” (PCR or VI submitted in an EDTA tube) or IHC on a biopsy.
- 5) If the test in a live animal is positive for BVDV, this may only represent an acute (short lived) viral



infection. A second sample must be collected three to four weeks later and tested to confirm that the animal is or is not persistently infected. A negative viral result on the second test shows the animal was simply exposed to BVDV and fought the infection. Quarantine after a second positive whole blood PCR or VI (virus isolation), or IHC, is very important. This requires veterinary consultation to determine the need for further testing or possible euthanasia.

The serum neutralization test may be used to demonstrate antibodies to the virus, indicating exposure to the virus. After doing viral testing, use the serum neutralization (SN) test as a subsequent screening tool to evaluate if any alpaca may have ever been exposed to BVDV. Note that this test is NOT useful to detect PI animals.

Submit blood for SN analysis (red topped clot tube) to test for antibodies to BVDV. If the SN test for antibody is negative or low, further testing is required and a veterinarian should be consulted. Recall that the PI alpaca may have no or few antibodies.

6) If you receive any positive BVDV report (viral or SN test), it is essential that you discuss this with your veterinarian. Determine the necessary follow-up testing and contact any farms that may have had contact with your animals so that they can take action to limit the spread of BVDV.

### **Conclusion:**

In summary, although presently rare, BVDV is a potential threat to the alpaca industry. It is imperative that all alpaca owners become familiar with BVDV terminology/science, the potential for animal losses, prevention of viral contamination and testing procedures to identify infected carrier animals (PI) as well as identification of exposed, but non-infectious alpacas.

### **NOTE:**

Testing for PI status need only be done once in the life of the animal as

PI animals can only develop from fetal infection.

### **DISCUSSANT:**

Edward Dubovi, Cornell University

1) PCRs differ from lab to lab; sensitivity and detection limits vary; must ask each lab what test they are most comfortable with in terms of determining PI status; must know in advance if the individual lab can do pooled samples; always send at least 2cc of whole blood.

**“To control the emerging problem, PI animals must first be identified and then permanently removed from the herd.”**

2) VI is always a viable alternative to PCR but VI should not be done on pooled samples of serum—it can be done on pooled samples of mononuclear cells; one of the documented PI crias who is still alive has a viral load of only about 100/cc while the average PI has greater than 1000/cc; this animal would probably have tested negative in a mixed sample in which he was the only positive.

3) Crias less than two months old who have successfully nursed their mother's colostrum or have been given bovine colostrum will have antibodies in the serum which will bind the virus

and cause it to be significantly cleared from the circulation and possibly result in a false-negative test; in this age group PCR should be done on the buffy coat; it is best done on the buffy coat in any case and is therefore not a problem.

4) IHC (immunohistochemistry) seems to be working out ok now.

5) ELISA for antigen-capture is not working out well at all and is not recommended.

6) Can an acutely infected alpaca infect another alpaca? No consensus; however, in the long term the problem is maintained by PI's, not by acutely infected animals.

7) One negative PCR or VI is lifetime evidence against PI.

8) Herdsire breeding farm – best idea is to test every cria coming by the side of mom to be bred; if less than 2 months of age should have buffy coat tested by VI or PCR, but not serum or plasma.

9) Sanitation - remove as much organic material as possible – sanitize with Virkon; without a host virus probably hangs around for a week; feces is less infective than saliva and urine; semen is a definite carrier;

10) There have been breeding-quality bulls who were PI's.

11) BVDV can be spread by white-tail deer.

12) In a quarantine situation visit the quarantine area last on your rounds of the farm; virus will cling to clothing and alpaca wool but it is not clear how long it survives in that situation.

13) Vaccination does not prevent PIs

14) This is not an acute epidemic; it is an ongoing infectious process; it must be treated as a chronic problem with some acute situations within.

15) Dr. Dubovi is sequencing all 15 PIs at Cornell to see if they have a common ancestor; all the PIs at Cornell have been type 1b; the most distant alpaca PI was a cria seen in Minnesota in 2001 and it was type 1b. The most prevalent strain of BVDV in cattle at this time is type 1b (60%).

ARF has funded research to provide new information about BVDV in alpacas and will announce results on their website as they are available. ARF will also be in close contact with AOBA and will be sponsoring the first annual Special Lecture which this year will be presented by Dr. Ed Dubovi on BVDV at the AOBA National Conference (Louisville, KY; May 18, 2006) regarding ongoing research, testing and results.

## **Information from Washington Animal Disease Diagnostic Laboratory**

### ***Testing Alpacas and Llamas***

The following tests are recommended by the Washington Animal Disease Diagnostic Laboratory (WADDL) and Washington State University Veterinary Teaching Hospital (WSU-VTH) for testing alpacas and llamas. These are based on the most current information available and may change as research is completed. Herds should be examined on a case-by-case basis as testing may not be warranted in some situations.

### ***1. Herd Screening***

Submit whole blood samples (purple top tube, (PTT) (whole blood in EDTA), and red top tube, (RTT) (serum) individually marked to WADDL. WADDL will test PTT sample for BVDV by PCR. Up to 10 samples can be pooled and tested, which may reduce testing costs. Individual samples from positive pools would be retested to identify individual positive animals within the pool.

- Negative results (BVDV not detected) = no BVD infection
- Positive results (BVDV detected) = persistent OR transient BVD infection suspected

Definitive diagnosis of persistent infection requires submission of another blood sample (PTT) from an individual positive sample to be collected in 3-4 weeks and re-tested. Serum samples (RTT) can be used to

check for BVDV antibody to determine prior exposure through serologic testing. If unable to test entire herd, test all juveniles less than 2 years old and breeding males and females. Again diagnosis of BVDV persistent infection would require 2 blood samples collected 3-4 weeks apart.

### ***2. New Arrivals to a Herd***

Quarantine for minimum of 30 days. Quarantining is not only important to allow screening for BVDV but also for other diseases. Herd biosecurity is important to protect your herd from diseases new animals may bring with them. For animals that will be remaining on your property a minimum 30-day quarantine is recommended before introducing new animals to your herd.

- Negative results (BVDV not detected) = no BVD infection
- Positive results (BVDV detected) = persistent OR transient BVD infection suspected  
Remain in quarantine until retested in 3-4 weeks
- Negative re-test = most likely a transient infection
- Positive re-test = persistent infection suspected

A negative-tested dam can be returned to the herd, but recommend quarantining just before delivery until newborn cria is tested with PCR and identified as BVD infected or not.

### ***3. Other Recommended Tests***

Necropsy and submit fixed and fresh tissues to test all aborted and stillborn crias and crias or adults with unexplained deaths. Submit whole blood (PTT) and serum (RTT) from the respective dam as well.

In addition to performing an abortion screen it is a good opportunity to evaluate the herd's trace mineral status.

## **Additional Information**

### ***1. What is BVDV?***

Bovine Viral Diarrhea Virus (BVDV) is one of several world-wide pes-

tiviruses known to infect domestic and wild ruminants, camelids, and swine. For cattle producers the virus causes economic losses through decreased weight gains, decreased milk production, reproductive losses, and death. As with most viral infections, there is a wide range of clinical signs from inapparent infections to diarrhea, respiratory tract infections, hemorrhage, abortions, congenital defects, and death.

### ***Acute Infection***

Bovine viral diarrhea refers to a mild disease caused by a BVD virus infection in immunocompetent cattle. In general, animals develop acute BVD 10-12 days after infection. Since BVDV infects white blood cells, whole blood (buffy coat) is the sample of choice for isolation of BVDV from clinically ill animals.

### ***Persistent Infection (PI)***

BVDV can lead to a persistent infection in a calf if it is infected during a certain time in gestation. If infected prior to complete development of the fetal immune system, the virus will not be recognized as a foreign pathogen. After birth, the calf will shed the virus and infect other animals in the herd. Sometimes these calves look sick but they can also look perfectly healthy thereby making it impossible to visually identify these animals.

### ***2. Why is BVDV important to my alpacas or llamas?***

This question cannot be completely answered at this time. There is much research that needs to be performed to fully understand the implications of BVDV in alpacas and llamas.

Research has shown that llamas and alpacas can be infected with the virus and develop clinical signs. There have also been reports of suspected persistent infections in crias. In cattle, persistent infected calves are the primary source of spreading the infection to other animals. It is not known if

persistently infected crias are the primary source of herd infection in camelids, but it is suspected. Alpacas and llamas are sent all over North America and lapses in biosecurity could permit a persistent infected cria to infect other animals and herds.

### **3. What are some concerns among veterinarians and researchers regarding BVDV in alpacas and llamas?**

A few current questions among veterinarians and scientists requiring investigation: Are there true persistent infections or longer transient infections than seen in cattle? How accurately do the bovine-based tests diagnose infections in camelids? Is there a new pestivirus specific to camelids or a mutation of the BVD virus that appears to “prefer” camelids?

### **4. What are some possible clinical signs seen in alpacas and llamas?**

Typical signs that a client may see include fever, oral ulcers, anorexia, diarrhea, abortion, ill-thrift, and congenital defects.

### **5. How is BVDV transmitted?**

The most efficient method of BVDV transmission in camelids is not known. Transmission in cattle has been primarily by ingestion or inhalation of the virus. The virus can be found in all body fluids (respiratory and oral secretions, urine, milk, and semen) and feces. Transplacental (cow to fetus) transmission also occurs.

Transmission is assumed to be similar in other susceptible species including alpacas and llamas.

### **6. What species can transmit BVDV?**

Virus can potentially spread between domestic ruminants (cattle, sheep, goats), camelids, and wildlife (deer, elk, etc).

### **7. Is there a vaccine available for alpacas and llamas?**

Currently there is no BVDV vaccine licensed for use in camelids. There are several vaccines available for use in cattle. The vaccines do not prevent infection but reduce the clinical disease effects. At this time, it is not recommended to vaccinate camelids until more is understood about the virus. Unwarranted vaccination can interfere with diagnostic testing and identifying truly infected animals.

### **8. Can BVDV infections be prevented?**

No, BVDV infections cannot be prevented but they can be reduced. Maintaining a closed herd, implementing strict biosecurity protocols for all incoming animals (recommended not just for reducing BVDV infections), and periodic screening of open herds can reduce the occurrence.

## **Testing Strategies:**

### **Acute Infection:**

BVDV acute infection can be diagnosed by virus isolation, polymerase chain reaction (PCR) or serology.

Virus detection must be done in the first 3-10 days after infection. A whole blood sample is the best sample for BVDV detection by PCR or virus isolation. Paired acute and convalescent samples collected 3-4 weeks apart are required to identify four fold increase in serum antibody titers following recovery from clinical illness.

### **Persistent Infection:**

Definitive diagnosis of persistent infection in camelids cannot be based upon testing done at a single time point. Detection of BVDV persistent infection requires showing virus is present in a particular animal over time (the infection persists). Although the BVDV antigen ELISA test done at a single time point is used to detect BVDV persistent infection in cattle, whether or not similar interpretation of the test in camelids is accurate is not known. Therefore, persistent infections in camelids should be determined by detecting virus (by PCR or virus isolation) in sequential samples collected 3-4 weeks apart.

The following websites have information on BVDV:

[www.alpacaresearchfoundation.org](http://www.alpacaresearchfoundation.org)  
<http://www.alpacaresearchfoundation.org/minutes/arf2005mar24.html>  
<http://diaglab.vet.cornell.edu/news/alpaca.asp>  
[www.vetmed.wsu.edu/depts\\_waddl/bydcamelids.asp](http://www.vetmed.wsu.edu/depts_waddl/bydcamelids.asp)  
<http://www.ars.usda.gov/News/docs.htm?docid=10851>



**“Currently there is no BVDV vaccine licensed for use in camelids.”**