Serologic Testing in the Diagnosis of Virus Diseases of Cattle

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Introduction

Great expectations are seldom realized by "sending a blood sample to the lab." This statement is particularly appropriate for diagnosis of illness or abortion in cattle. Serologic tests for infectious bovine rhinotracheitis (IBR), bovine virus diarrhea-mucosal disease (BVD-MD) and bovine myxovirus parainfluenza-3 (BPI-3) are performed throughout the U.S.A. These services are valuable only to those veterinarians who are able to place virus serology in its proper perspective with thorough clinical observation, good history-taking and careful necropsy examination and who appreciate the significance and the limitations of serology.

The significance of virus serology lies in its retrospective character. Not only do the laboratory procedures take time, but the development of detectable antibody occurs *after* infection.

Lacking useful treatments, the control of bovine virus diseases must be based on preventive measures and these must precede infection. Therefore, the practitioner who submits blood samples expecting to use the results in making decisions about handling a specific episode is doomed to disappointment. On the other hand, if he submits samples because he wants to know what is (or is not) causing a specific problem so that he can recognize similar conditions *in the future* and plan measures for preventing similar episodes, he may get useful information from serologic tests. If you regard laboratory tests as an *educational tool* and are willing to collect *two specimens* from the same cow, are happy to *wait* several weeks for laboratory reports and if you are eager to provide yourself *with knowledge* needed to intelligently *interpret* the results of these tests, then this paper was written for you.

Serum Antibody and Bovine Viral Diseases

In response to virus infection or parenteral inoculation of viruses, cattle usually produce specific immune globulins called antibodies which render the inducing virus ineffective in infecting cells.

1. These antibodies generally afford the animal some degree of protection against reinfection with the homologous virus.
2. These antibodies can be detected by a variety of tests on serums in which their presence can have considerable diagnostic value *if* the test results are properly interpreted.

There are several things about serum antibodies which must be recognized for interpretation of laboratory reports.

1. Presence of serum antibodies can mean one of several things:
   a. the animal may have been *actively* immunized by:
      1. recent or long-past *natural infection* (with or without manifest disease)
      2. *artificial immunization* with modified live virus (MLV) vaccines or inactivated vaccines
   b. the animal may have been passively (and temporarily) immunized by:
      1. ingestion of colostrum from an immune cow
      2. injection of antiserums or globulin concentrates
2. Variation accompanies any effort to measure serum antibody.
   a. tremendous variation occurs *between individual cattle* in:
      1. the time interval between infection and the appearance of detectable serum antibodies
      2. the concentration of antibodies (antibody titer) in the serum
   b. some fluctuation in serum antibody titer (both upward and downward) is observed if the *same cow* is tested repeatedly at weekly or monthly intervals
   c. the laboratory procedures for the detection of serum antibodies are subject to many sources of variation. These variables are controlled as much as possible but nevertheless we frequently find differing results (particularly in titers reported)
      1. between laboratories
      2. between technicians within laboratories

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3. between tests on the same serum

3. The length of time a cow carries a titer following initial acquisition of serum antibodies (duration of antibody persistance) is a significant consideration in the interpretation of serologic tests.
   a. antibody persistance from natural infection or vaccination is probably *lifelong* for *IBR* and *BVD-MD.*
   b. the duration of antibody persistance to BPI-3 is unknown but is probably less than one year.
   c. the duration of persistance of colostrally-acquired passive antibodies varies tremendously between individual calves and there is considerable controversy as to when a hypothetical "average calf" loses the passive immunity acquired by nursing an immune cow.* The best answer is that *every calf is different.* Here are some estimates of the age of loss of maternal antibodies:

      IBR — 1-8 months; average 4-6 months
      BVD-MD — 1-12 months; average 6-8 months
      BPI-3 — 1-6 months

      The tremendous variability indicated above emphasizes that when evidence of antibodies are found in serums collected from cattle under one year of age, the possibility of long-lasting passive antibodies must be considered before concluding this represents evidence of previous active infection. The tests routinely used cannot distinguish antibodies induced by natural infection from colostrally acquired maternal antibodies.

4. The relationship between serum antibodies and resistance to infection should be understood. Cattle with BVD-MD and IBR serum antibodies are usually resistant to infection. (An exception is that low levels of maternal antibodies can sometimes be overwhelmed by severe exposure to virus.) Therefore, presence of BVD-MD or IBR antibody in serums of unvaccinated cattle over one year of age means that the animal *has* had the disease (or at least the infection) and also means that the animal is unlikely to get it again. (IBR immune cattle may experience stress-induced recrudescenses in which local lesions appear and a brief period of virus shedding occurs.)
   a. serum antibody against BPI-3 has *not* been shown to indicate immunity to infection. Therefore, cattle with BPI-3 antibodies are not necessarily resistant to infection.

Collection of Blood Samples for Viral Serology

Virus-neutralization tests in tissue cultures require that the test serum be incubated at body temperature for several days. If the specimen is contaminated with bacteria or molds, these proliferate and the test must be discarded. Therefore, blood specimens for virus serology should be collected in sterile, sealed units. The recommended unit is the B-D Vacutainer** specially prepared for serologic studies. The specimens should be allowed to clot and stored at room temperature until the clot contracts exuding the serum. Usually maximum serum yield occurs within 24 hours at room temperature. Avoid temperatures over 80°F. When clot retraction is completed, the specimen should be refrigerated. *Cattle* serums should be submitted with the clot because they do not hemolyze rapidly and the risk of contamination involved in decanting the serum or removing the clot usually outweighs the hazard of hemolysis.

When paired specimens are needed, the practitioner has several options. He can 1) draw two vials from the cow, submit one and retain the other. When the serum has completely separated from the clot, retained serum should be carefully decanted into a sterile vial, centrifuged, frozen in a home freezer and then sent to the laboratory along with the second (convalescent) specimen; 2) hold the first specimen until the second has been collected and then submit them together; 3) submit the first as soon as it is collected and then *submit* the second when it is collected. The trouble with the last procedure is that most laboratories discard serums after testing so unless specifically requested to hold the test pending arrival of the second serum, the first serum is frequently unavailable when needed for comparative titrations. Work this out with your own laboratory.

Interpretation of Serological Test Results

1. Diagnosis of Clinical Disease

When history, clinical signs and lesions suggest a specific disease, a positive serologic diagnosis can be made using *two* specimens from the same animal. The first or "acute sample" must be collected at the first sign of disease (before the animal develops antibodies). The second (convalescent stage) sample should be collected three or

*See Appendix A.

**Becton-Dickenson Company, Rutherford, New Jersey. B-D Vacutainer; pink stopper; additive none: for TC and Sera Study.

more weeks after the first. Only a negative acute sample, followed by a positive convalescent sample, is considered diagnostic. Two negative samples collected in this fashion eliminate the virus in question. Two positive samples collected in this fashion are meaningless for diagnostic purposes unless they are titrated simultaneously and the second specimen has a titer *at least* four times higher than the first (4-fold titer increment or "a significant rise in titer"). Efforts to demonstrate a significant rise in titer using two positive specimens are expensive and frequently frustrating and a diagnosis obtained this way is less convincing than a diagnosis based on paired serums in which the first was completely negative and the second positive (regardless of titer).

Often it is not possible to collect paired serum samples. Single samples are of value only as a part of the diagnostic *process of elimination* and an accurate interpretation of a single sample is often difficult because the time of collection is critical.

a. A negative serum collected three or more weeks after a suspicious disease indicates that the animal was still susceptible at the time the sample was drawn and the clinical finding was probably not related to the virus in question. If the single negative serum was collected less than three weeks following the event, the possibility that the animal was infected at the time but had not yet produced detectable antibodies must be considered. (In this case, a subsequent sample would be positive).

Without information about the time relationship between the clinical disease and the collection of the serum specimen, a single positive test has *very limited diagnositic value* because serum antibodies can result from clinical disease, inapparent infection, vaccination or passive immunity. Contrary to popular opinion, the usual serologic tests cannot distinguish between vaccination titers and infection titers.*

2. Diagnosis of Abortion or Congenital Anomalies with Dams Serum

The diagnostic value of serum collected from the *cow* which has aborted is limited because:

a. there are many causes of bovine abortion
b. serum antibodies against viruses are common in cattle
c. abortion and/or birth of calves with congenital anomalies due to viruses can occur many months after the inciting infection. Frequently by the time fetal damage is evident, the serum titer of the dam has stabilized and demonstration of a significant rise in titer is not possible.
   1. abortions (particularly due to IBR) sometimes occur during active infection of the dam
d. usually when a blood specimen is taken around the time of abortion, the time the animal was infected is unknown.

Thus the interpretation of tests on serums collected on the day of abortion is as follows:

a. A negative specimen usually eliminates the virus as a cause of the abortion. However, a second negative specimen taken three weeks later makes this interpretation more convincing.
b. A positive specimen means that the abortion *may be*, but *is not necessarily* due to the virus in question.

3. Diagnosis of Abortions or Congenital Anomalies With Fetal Serum or Serum Collected from Abnormal Calves Before Nursing:

It has been found that calves with BVD-MD induced congenital cerebellar hypoplasia have serum antibody before nursing. This is interpreted as evidence of an immunologic response to an intrauterine infection. The diagnostic implications of testing serums of aborted fetuses for evidence of prenatal infection have not yet been studied but they look very encouraging.

4. Interpretation of Herd Tests for Serum Antibody

The expense of testing an entire herd is usually prohibitive. Furthermore, many laboratories which don't levy a fee limit the number of samples from a single herd in order to avoid indiscriminate use of facilities. In epidemiologic studies, herd antibody prevalence (the % animals positive at a point in time) is a useful parameter. If one knows the rate of turnover (culling rate), then the percent of immune cattle in the herd provides an estimate of the time of the most recent herd infection with IBR and BVD-MD, viruses with lifelong antibody persistance. A 95% herd antibody prevalence indicates very recent infection while a 20% prevalence in a herd with a 20% annual turnover rate indicates the herd has been free of infection for about four years. This type of estimate is frequently reinforced by the age distribution of positive cattle. Frequently in a herd with 20% antibody prevalence, all positive cattle are greater than four years old. In addition, the time of

**Some laboratories run every serum at two or three dilutions and interpret titers below a certain level as vaccination titers and titers above a certain point as caused by natural disease. Study of the variables in the bovine immune response and the tests measuring it suggest this interpretation is probably unreasonable.*

infection can frequently be closely estimated by learning the date when negative animals were introduced into a herd with high antibody prevalence. (If the only negative animal in a herd was purchased on March 1, then you assume the last herd infection occurred and subsided before that date.) Selecting four or five animals from a herd can frequently give an estimate of herd antibody prevalance, but caution must be used in extrapolation of a small sample to the entire herd.

APPENDIX A

Colostrally-Acquired Passive Immunity

*Unlike human infants which acquire the immune status of the mother by the transplacental route, a calf is born without serum antibody unless it has experienced a prenatal infection. Antibody is concentrated in the colostrum and if the calf nurses immediately after birth it ingests antibodies which reach the bloodstream after absorption through the gastro-intestinal tract. The calf then has an immune status similar to the dam. The concentration of antibody in the cow's milk declines rapidly following parturition and the *calf loses its ability to absorb antibodies shortly after birth.* Therefore, the calf acquires all the maternal antibody it will ever get during the first day of life. If a calf fails to nurse and is not fed colostrum immediately after birth, it will be vulnerable to numerous infections. The colostrally acquired immunity is steadily dissipated by metabolic processes at a rate that is fairly uniform among calves. Therefore, the time interval between ingestion of colostrum and the loss of passive immunity is determined largely by the amount of serum antibody the calf accumulates in that crucial first day of life. Calves with the higher initial titers will retain passive immunity for a longer time than calves with lower initial titers. Some people erroneously believe that the calf is immune only as long as it nurses its dam and that passive immunity is lost at the time of weaning. Some calves are weaned about the time their colostrally-acquired maternal antibody has diminished, but this is a coincidence. The disease implications of weaning of beef cattle are related to stress, dietary change and communicable disease transmitted when many susceptible calves are aggregated or shipped. The fact that weaned calves are no longer nursing has no effect on their serum antibody status.

Summary

Serologic tests for IBR, BVD-MD and BPI-3 sometimes aid in the differential diagnosis and sometimes only mislead the person who submitted the specimens. When negative, these tests can be useful in the diagnostic "process of elimination." Efforts to obtain a *positive* etiologic diagnosis are frequently frustrating. The relationship between the time of infection and the time of serum collection is a critical factor in the interpretation of test results. Because the time of infection is usually unknown, paired samples are essential. The aseptic specimens needed for virus serology should be collected in B-D Vacutainers. Antibodies induced by natural infection cannot be distinguished from antibodies induced by vaccination or from colostrally acquired maternal antibodies.

The Role of the Feedlot Veterinarian

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While the growth of the feeding industry during the past few years has been the most spectacular in all of agriculture, little anticipation has been shown for the complexities of the health problems being generated. Antiquated methods of market assembly, crowding, and casual shipment over long distances to unsanitary and inadequately sheltered quarters have created health hazards peculiarly defiant of rational management by means other than complete reorganization of the system. Faced with the here and now of huge masses of sick cattle, the veterinary medical profession was still in the talking stage about the need for trained technical assistants. The feeding industry therefore settled for availability, and the most available labor source was "the screw worm program eradicated cowboy." Experienced in catching and holding and "doctoring," any who showed a lingering childhood fascination for playing doctor on a grander scale became "feedlot vets" almost overnight. They were quickly dazzled by the huge armamentarium of the modern veterinarian, all eagerly pressed upon them by the zeal of merchandising. Some few veterinarians, many of them representing drug interests, and a newly mobilized army of drug salesmen competed and collaborated in shaping this rabble into the first line of defense against the onslaught of cattle

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