Cow - Calf and Feedlot Session

Dr. Ed Johnson, *Presiding*

Interpretation of Serologic Tests for Bovine Viral Diseases

Robert F. Kahrs, D.V.M., Ph.D,
Department of Preventive Medicine, College of Veterinary Medicine, University of Florida, Gainesville, Florida.
and
Jan Thorsen, D.V.M., Ph.D
Department of Veterinary Microbiology and Immunology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

The value of serology in diagnosis, export authorization, vaccine, evaluation, or epidemiologic studies depends on test sensitivity and specificity and the accuracy of interpretation of results. Unless combined with accurate history, complete physical examination, careful necropsy, and viral isolation procedures, serology has serious diagnostic limitations particularly when infertility, abortion or congenital abnormalities are concerned.

Realistic expectations from serology require appreciating it as a retrospective educational tool rather than as an immediate aid to decision making in specific outbreaks. Most serologic diagnoses require two specimens from the same cow and a wait for reports. Interpretation of reports requires knowledge of the specific situation and an understanding of immunology.

After infection or inoculation with viruses, cattle undergo a complex chain of cellular events resulting in production of specific immunoglobulins (antibodies). Antibodies are a heterogenous group of macromolecules varying in structure, function and time of appearance.

In the cow, antibodies react specifically with viruses rendering then incapable of infecting cells or aiding in destruction of virus-infected cells. Following antibody development, a virus may be eliminated from an animal, but frequently it lingers as an active or latent persistent infection.

In the laboratory, the reaction between viruses and antibodies can be detected by neutralization, complement fixation (CF), hemagglutination inhibition (HI) or agar gel immunodiffusion (AGID) tests and other direct or indirect methods. The test used to detect specific antibodies to each virus is usually chosen because of its simplicity and specificity. Once adopted, a serodiagnostic test is replaced only when another procedure is demonstrated convincingly to be more sensitive, specific, or easier to perform.

Several types of immunoglobulins are detected in serodiagnosis. About 12% of serum immunoglobulins consists of a large molecule (macroglobulin) known as immunoglobulin-M (IgM) which appears in serum shortly after infection (Butler, 1973). Two major subclasses of immunoglobulin G(IgG & IgG) appear later in infection and constitute the majority (about 86%) of serum antibody. Immunoglobulin-A (IgA) is present in various exocrine secretions and functions locally in tissues and on mucuous membranes. It comprises a small proportion of immunoglobulins present in serum (about 2%). Most primary exposures to antigen elicit an early IgM response followed by production of IgG and IgA.

Tests vary in ability to detect the various classes of antibody. Thus results and interpretation of a serologic test differ with each virus. Also variations between laboratories and within a laboratory are inevitable.

Although naturally occurring antibodies to some bacterial and viruses have been found in bovine serum (Gibson, 1930; Meloen, 1978), detection of serum antibodies usually indicates previous exposure, vaccination or colostrum acquisition and implies some degree of partial

protection against subsequent infections.

Serum antibodies can be acquired actively or passively. Actively acquired antibodies (active immunity) are produced by an animal in response to natural infection or vaccination with inactivated or modified live virus (MLV) vaccines. Passively acquired antibodies are synthesized by one animal (donor) and transferred to a second (recipient) animal by blood transfusion, inoculation of antiserum or concentrated globulin or by ingestion of colostrum.

Human infants acquire transplacentally the humoral antibody spectrum of the mother. However, a calf is born without serum antibodies unless it experienced a prenatal infection. Maternal antibodies, predominately IgG (81%), IgM (7%) and IgA (7%) are concentrated in the colostrum (Butler, 1973). If the calf nurses immediately after birth, these are quickly absorbed by the intestine and reach the bloodstream, giving the calf a humoral immune status similar to the dam and causing reaction to serologic tests despite lack of previous infection of vaccination.

Colostrum and milk antibodies in the gut may provide local protection against rotavirus (McNulty) *et al.,* 1976), coronavirus (Mebus, 1978), bovine viral diarrhea (BVD) (Coria and McClurkin, 1978) and bovine leukemia virus (Van Der Maaten *et al.,* 1981). Antibodies of colostral origin (IgG and IgM) have also been found in the epithelium of the respiratory tract (Morgan and Bourne, 1978) where they may have a protective effect (Davidson *et al.,* 1981).

The concentration of antibodies in cows' milk declines rapidly following parturition and, in addition, the calf loses its ability to absorb antibodies shortly after birth. Therefore, the calf acquires all its maternally bestowed serum antibodies during the first day of life. Rotavirus antibodies in cows' milk decrease markedly by 48 hours after parturition (Woode *et al.,* 1975) and onset of rotaviral diahrrhea in calves seems to be related to this (Acres and Baiuk, 1978).

If a calf fails to nurse and is not fed colostrum immediately after birth, it will be vulnerable to numerous infections. Colostrally acquired antibodies are catabolized at a fairly uniform rate. Therefore, the time interval between ingestion of colostrum and the loss of passively-acquired serum antibodies is determined largely by the quantity of antibodies the calf absorbed on the day of birth. Calves with higher initial titers retain passive antibodies longer than calves with lower titers.

It is incorrect to assume a calf is immune only as long as it nurses or that colostrally acquired serum antibodies are lost at weaning. Some calves are weaned about the time their colostrally acquired serum antibodies are depleted, but this is coincidence. The increased incidence of disease associated with weaning of beef cattle is related to stress, dietary changes and infections transmitted when susceptible calves are aggregated or shipped. The fact that weaned calves are no longer nursing has no effect on their serum antibody status (Kahrs, 1971). Thus, it cannot be assumed that serum antibodies in nursing calves are colostrally acquired or that serum antibodies in weaned calves are not. The age of the tested calf has more serious implications than weaning in serologic interpretations.

Effect of Age on Serology

Antibodies in serums of cattle under one year old may result from previous exposure, vaccination or colostrum ingestion. Routine tests do not distinguish actively induced antibodies from colostrally acquired antibodies.

Colostrally acquired antibodies against most viruses disappear by 8 months of age, but there are exceptions. Caution should be used in making assumptions about antibodies detected in young calves. Paired serums are diagnostically important in young calves.

Importance of Paired Serums

When history, clinical signs and lesions suggest a specific disease, a positive serologic diagnosis can be made using 2 serum samples from the same animal. The first or acute sample must be collected at the first sign of infection (before the animal develops antibodies). The second or convalescent sample should be collected 2 or more weeks after the first. A negative acute sample, followed by a positive convalescent sample indicates seroconversion and recent infection. Negative paired samples usually eliminate the diagnosis in question, but there are exceptions. Cattle chronically infected with BVD (Johnson and Muscoplat, 1973; Malmquist, 1968) may have no measurable neutralizing antibodies possibly due to immune tolerance (Lambert *et al.,* 1974; Coria and McLurkin, 1978). Occasionally cattle with clinical bovine respiratory syncytial virus (BRSV) infection may not seroconvert until 5-6 weeks (Lehmkuhl *et al.,* 1979) When BRSV is suspected, a 3rd sample may be required.

If acute and convalescent samples both have antibodies, they should be titrated simultaneously seeking evidence of a 4-fold titer increment (significant rise in titer). Efforts to demonstrate a significant rise in titer are expensive and sometimes frustrating and rising titers are less convincing diagnostically than seroconversion. In addition to occurring late in primary infections, four-fold titer increments can result from laboratory variations, cross reactions, reinfection of partially immune cattle, or stress induced reactivation of latent infections such as infectious bovine rhinotracheitis (IBR) (Sheffy and Rodman, 1973). Lack of titer rise after experimental reinfection of cattle possessing serum antibodies has been demonstrated with IBR (Frank et al., 1977; LeJan and Asso, 1981) BVD, (Nuttal et al., 1980), and BRSV (Elazhary et al., 1981). Seroconversion coupled with virus isolation represents reasonable evidence of a primary infection.

Diagnostic Utility of Single Serums

It is not always possible to collect paired serum samples. Single samples can be of value in the diagnostic process of

elimination. It is often difficult to get useful information from a single sample because the time of collection is critical and serum antibodies can result from inapparent infection, vaccination or passive immunity as well as clinical infection. Most tests don't distinguish between vaccination titers and infection titers.

A negative serum collected two or more weeks after disease usually indicates lack of recent infection with the virus in question. If the single negative serum was collected less that two weeks following disease, a subsequent sample is required.

Some laboratories attempt to distinguish between vaccinal responses and natural immunity on the basis of titer levels. Such interpretations are probably unreasonable unless vaccination or exposure status is controlled experimentally and the time frame clearly delineated.

The single serum sample has usefulness in the diagnosis of exotic infections and in retrospective studies on newly isolated viruses.

Antibody Surveys

Single serum specimens from representative individuals in cattle populations help estimate the extent and geographic distribution of infection. When coupled with history, serologic surveys provide estimates of prevalence of inapparent infection. Antibody prevalence rates don't indicate the time antibodies were acquired; however, the ages of seropositive and seronegative cattle provide some suggestion of the time of infection.

Single serum specimens are used for import authorizations. When negative, they indicate the tested animal was free of antibody when the sample was collected, but provide no assurance of freedom from incubating or occult infections. When positive, they indicate passive immunity, previous infection or previous vaccination, but usually provide little insight into the potential of a carrier state.

Diagnosis of Abortion or Congenital Anomalies with Dams Serum

The diagnostic value of serum collected at the time of abortion is minimal because there are many causes of bovine abortion, serum antibodies against endemic viruses are common in cattle, and abortion or birth of calves with congenital anomalies can occur months after the inciting infection. Unless abortion occurs early in the infection, associated changes in serum titers are completed before samples are collected.

Interpretation of test on serums collected on the day of abortion requires caution. A negative speciman usually elimates the virus in question, but a second negative specimen taken 2 weeks later is needed to make this interpretation convincing. If the second speciman contains antibodies, seroconversion has occurred indicating infection. However, caution must be used in attributing abortions to viruses with high antibody prevalence such as IBR, BVD, PI-3 and BRSV, because seroconversion may be unrelated to the abortion.

Diagnosis of Abortion or Congenital Anomalies with Fetal Serum or Serum From Colostrum Deprived Neonates.

Intrauterine fetal infection can be inapparent or can induce death, fetal damage, or a classic immune response (Schultz, 1973). Generally calves don't acquire maternal antibodies until nursing. Thus antibodies in aborted fetuses or presuckle calves indicate response to infection acquired transplacentally.

Serology on aborted fetuses has excellent diagnostic potential (Kahrs et al., 1971; Miller and Wilkie, 1979). This approach is under-utilized because fetal serum is difficult to obtain. It is usually hemolyzed, mixed with other fluids, and contaminated with substances toxic to cell cultures and other substrates used in viral serology. Use of indirect fluorescent antibody assay helps avoid these problems. (Miller and Wilie, 1979).

Fetuses or colostrum-deprived neonates have been reported to have serum antibody against BVD (Brown et al., 1979), IBR, PI-3, adenovirus and coronavirus (Miller and Wilkie, 1979; Sato et al., 1980), bluetongue (Richards et al., 1971), bovine leukemia virus (Van Der Maaten *et al.,* 1981), enteroviruses (Dunne *et al.,* 1973), parovirus (Sato *et al.,* 1980), and Akabane virus (Hartley *et al.,* 1975).

Possible causes of erroneous conclusions about intrauterine fetal infection are test error, unobserved colostrum aquisition, and the remote possibility of antibody leakage across a damaged placenta (Miller and Wilkie, 1979).

Antibody in serum from fetuses or colostrum-deprived neonates indicates fetal infection but not necessarily a cause-and-effect relationship because fetuses, like adult cattle, can experience inapparent infection (Hubbert, 1975). Further, multiple viral antibodies have been detected in aborted fetuses (Dunne *et al.,* 1973; Bergland *et al,m* 1974).

Failure to detect antibody in an aborted fetus is not grounds for eliminating a virus from etiologic consideration because acute fetal infections, as occur with IBR, can cause fetal death before antibody production occurs.

In diagnosing prenatal infection by serology in live calves, it is critical to determine that the specimen was collected prior to nursing. If the calf is standing, it is useful to examine the teats of the cow for evidence of nursing and ascertain at necropsy that the calf had no milk in its stomach. Assurance of colostrum deprivation is no problem with dystocias, abortions, stillbirths and ataxic calves which are unable to stand.

Serological testing of neonates has potential for surveying etiology of various bovine congential disorders as well as the diagnosis of individual cases.

Serologic testing of adult cattle has value in diagnosis, export authorization, vaccine evaluation and epidemologic surveys but credible interpretation requires considerable knowledge and caution.

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