was hauled in a truck (Sept. '78); truck had to be used to haul sheep and goats; cow became ill December 5; nasal discharge, corneal opacity, blindness, anorexia, febrile for 11 days until death.

Post-Mortem: muzzle and tongue erosions and necrosis; lung - emphysematous; lymphadenopathy; keratitis.

Histopath: perivascular cuffing in cerebrum, cerebellum, and spinal cord. lymph nodes - hyperemia and hyperplasia.

No other cases have occurred in this herd.

*Farm B:* Report by Pierson et. al., JAVMA, 8/15/73 Colorado feedlot; of 231 steer and heifers, 87 died within 68 days; 100% mortality of visibly sick cows; panophthalmitis, diarrhea, leukopenia, fever; sheep on same farm but not in direct contact.

*Farm C:* Western Pennsylvania dairy herd; 55 head, Holsteins and Guernseys; May and June 1976-8 cows died; 1 cow died in each of the months of July, October, November and December.

Signs: high fever, lacrimation, keratoconjunctivitis, blindness, incoordination, some cows aborted; diarrhea, some cows showed hemorrhagic cystitis.

Autopsy: two cows posted at P.S.U.; one cow posted at Ohio State University; 1 head examined at P.S.U.: pneumonia, emphysema; hemorrhagic cystitis, hemorrhagic areas of small intestine, non-suppurative meningoencephalitis, CNS perivascular cuffing; vasculitis; 1 cow-hepatitis and nephritis. One or more cows recovered. 17 serum samples submitted to Plum Island - negative for African-strain MCF virus neutralizing antibodies; sheep contact on farm; a new ram was purchased from West Virginia shortly before outbreak; no young stock were affected.

*Farm D:* 120 cow dairy herd; northeastern New York; high fever; Keratitis; ("looked like peracute **IBR**"); depression, incoordination; lymph node hypertrophy; 2 sheep on farm had free access to adult cattle; several pigs had contact with cattle; sheep and swine were asymtomatic; mortality - 1/3 of adult cattle; some recoveries; Plum Island - several positive titers to African-Strain MCF.

# Bovine Leukemia Virus: Transmission and Diagnostic Tests

**Simon J. Kenyon,** *Ph.D., MRCVS*
*Assistant Professor of Medicine*
*New Bolton Center*
*University of Pennsylvania*
*Kennett Square, PA 19348*



## I. Expression Of BLV In The Individual Animal

BLV infection in cattle takes the form of a persistent infection of lymphoid cells. Infection of cells other than lymphocytes or lymphoid tumor cells has not been demonstrated. Although the viral genome is integrated into the DNA of many host lymphocytes, actual expression of the virus or its antigens is rare. BLV has not been observed budding from *fresh* lymphocytes or tumor cells of BLV-infected animals, nor have BLV virion antigens been detected in the same fresh tissues. Thus in BLV-infected cattle, expression of the virus is repressed. This is in striking contrast to FeLV infection in the cat where plentiful expression of FeLV occurs and FeLV antigens are easily detected by a fluorescent antibody test performed on fresh blood smears.

Although direct detection of BLV or its antigens in freshly-harvested bovine cells is not possible, all cows infected with BLV have persistent antibody titers to a number of BLV antigens, the most important of which, from the point of view of current diagnostic tests, are the envelope glycoprotein (gp70) and the major internal virion antigen (p25). Detection of these antibodies forms the basis of the most widely applied diagnostic tests for the presence of BLV infection in cattle.

Another consequence of BLV infection is the development in some cows of a persistently elevated peripheral blood lymphocyte count. This persistent lymphocytosis (PL) is a genetically determined response to BLV infection, and before the development of serological tests for BLV, it was widely used to indicate the presence of lymphosarcoma risk in individual animals or within herds.

## II. Diagnostic Tests For BLV Infection

### *A. Hematologic Keys*

These are based on the presence of PL. Normograms for the expected maximum lymphocyte counts in different

breeds of cattle in different localities have been produced by various investigators, using data from lymphosarcoma-free herds. In general, cattle over 54 months of age with a lymphocyte count of greater than 7,500/mm$^3$ are considered to have PL. Since younger cattle normally have higher lymphocyte counts than mature cattle, they must have correspondingly higher counts to be considered to have PL.

The problem with the use of hematologic keys in the diagnosis of BLV infection is that only about 30% of the cows positive for BLV in serologic tests have PL. Thus, tests which rely on the presence of PL to indicate the presence of BLV infection are particularly insensitive. Additionally, in our study herds, 35% of cows which die of lymphosarcoma have never had PL. Thus, the hematologic keys identify neither most BLV-infected animals nor even the BLV-infected animals which will die of lymphosarcoma.

Recognition of PL is of some value, however, as an aid in the diagnosis of clinical cases of lymphosarcoma. Since 65% of the cases of lymphosarcoma come from the 30% BLV-infected cattle that have PL, it is clear that BLV-infected cows with PL have a higher risk of developing lymphosarcoma than BLV-infected cows without PL. Thus the presence of PL in a sick cow raises the clinician's index of suspicion for lymphosarcoma. However, the presence of PL is not diagnostic for lymphosarcoma. PL is a benign proliferative response to BLV infection, and most cows with PL are perfectly healthy and never develop lymphosarcoma.

### B. Serologic Tests

*1. Agar Gel Immunodiffusion (AGID) Test*

This test is a double diffusion test similar to the Coggins test for the detection of antibodies to the EIA virus in horses. The AGID test for BLV, using the BLV envelope glycoprotein as antigen, is now the most widely used test for BLV infection in cattle. An AGID test, using an envelope glycoprotein antigen prepared by Pitman-Moore from tissue culture-grown fetal lamb kidney cells, has been licensed by the USDA for performance by laboratories that are officially recognized for EIA testing. A slightly more sensitive AGID test, using a different preparation of the envelope glycoprotein antigen, is available at the Bovine Leukemia Research Unit of the University of Pennsylvania, School of Veterinary Medicine, New Bolton Center.

Less sensitive AGID tests using the p25 internal virion antigen are no longer routinely used.

*2. Fluorescent Antibody Test*

This test used long term cultured BLV-infected bovine cells as target cells in a fixed cell immunofluorescence assay. It detected antibodies to the p25 antigen and is no longer offered since it is less sensitive than the glycoprotein AGID test.

*3. Radioimmunoassay.*

The RIA for antibodies to the BLV p25 antigen is the most sensitive serologic test for the diagnosis of BLV infection in cattle. This test is available at the University of Pennsylvania, New Bolton Center.

For most purposes, the AGID test for BLV infection is adequate. It is sufficiently sensitive for most herd studies and surveys, is simple to perform, and is widely available. However, in any eradication scheme or other situation in which it is essential to identify all infected animals, the greater sensitivity of the RIA makes it the test of choice.

*4. Virus Neutralization Assay*

This test depends on the ability of BLV antibodies to prevent a cytopathic effect (syncytia formation), induced by BLV from occurring when tissue culture-grown BLV is inoculated on to monolayer cultures of e.g. bovine embryo spleen cells. This test is almost as sensitive as the RIA, but is time consuming and expensive.

### Interpretation Of The Serological Tests For BLV

None of the tests for BLV infection can be used to make or confirm a diagnosis of lymphosarcoma in an individual animal. They simply indicate whether or not the cow is infected with BLV. However, the absence of BLV antibodies in a sick cow would tend to rule out lymphosarcoma from a list of differential diagnoses, since BLV antibodies can be detected in approximately 95% of clinical cases.

Studies on well-characterized herds of cattle have shown that in lymphosarcoma-free herds, BLV infection is infrequent or absent. In herds into which cattle from lymphosarcoma herds have been introduced, but in which clinical cases have not occured, about 20% of cows are infected. In multiple-case lymphosarcoma herds, the frequency of BLV infection is frequently greater than 50%, and in some herds may approach 100% in the adult cattle. It is estimated that frequency of lymphosarcoma among BLV-infected cattle is no greater than 5%.

Since BLV antibodies are present in the colostrum of BLV-infected dams, the serological tests are of no value in determining whether young calves are infected with BLV unless a serum sample is taken before the calf suckles. Passively acquired BLV antibodies may persist for 7-10 months in calves receiving colostrum from BLV-infected cows. In calves of this age group, other direct tests of BLV infection must be made. These tests involve growing the calf's lymphocytes in tissue culture and then testing them for the presence of BLV or its antigens.

### Natural Transmission Of BLV

The predominant mode of transmission of BLV is by contact with infected cattle after six months of age, when the protective effect of colostral antibodies has worn off. Even though animals between six months of age and first parturition are susceptible to BLV infection, the incidence of infection in this age group under most dairy management systems remains fairly low because of the lack of contact with other infected animals. Thus, most animals become infected when they join the adult herd after having their first

calf. The fact that transmission of BLV is more likely to occur in summer has led to the speculation that insect vectors may frequently be responsible for BLV transmission. This idea is supported by the fact that BLV-infected lymphocytes have been recovered from the mid-gut of biting flies which have been allowed to feed on BLV-infected cattle, as well as the fact that BLV has been successfully transmitted by intradermal inoculation of as few as 2,500 lymphocytes. That this number of lymphocytes may be contained in 0.005 ml of blood also points up the risk of transfer of BLV on hypodermic needles.

The other major mode of transmission is *in utero* transmission from dam to fetus across the placenta. Although this is not as frequent as contact transmission, it does occur in 20% of the pregnancies of BLV-infected cattle. It does not appear, however, that cows which produce an infected calf at one pregnancy have an increased likelihood of producing an infected calf at the next. Calves which become infected *in utero* have BLV antibodies detectable in pre-suckle serum samples. After nursing, as mentioned above, diagnosis of BLV infection in these calves must rely on direct tests of BLV infection, since all calves nursed on BLV-infected dams will have BLV antibodies of maternal origin.

Although infectious BLV can be demonstrated in the milk of up to 50% of BLV-infected cattle, transmission through ingestion of milk is not an important natural route of transmission. This may be due to the protective effect of colostral antibodies present during the nursing period, or due to the failure of infected lymphocytes to cross into the systemic circulation where they may replicate the virus.

**Although BLV antigens have been detected in the urine of infected cows, infectious BLV has not been detected, and attempts to transmit the virus by inoculation of urine into susceptible recipients have failed. Neither BLV nor its antigens have been detected in saliva or semen, and neither secretion has been implicated in the natural transmission of the virus.**

# Viral Diseases Of The Bovine Eye

**William C. Rebhun,** *D.V.M.*
*Assistant Professor of Medicine & Ophthalmology*
*New York State College of Veterinary Medicine*
*Cornell University, Ithaca, N.Y. 14853*

### Infectious Bovine Rhinotracheitis (IBR)

IBR virus is a Herpes virus which manifests itself in many ways in cattle. These include the respiratory form, infectious pustular vulvovaginitis (IPV) form, abortion form, neonatal septicemic form, and ocular form. These various forms of the disease may be found alone or as any combination within an infected herd. Clinically, the virus seems to appear principally in one form within each affected herd. As with other Herpes viruses, recrudescences have been observed in IBR infected herds during periods of stress for individual or groups of cows.

The ocular form of IBR is a severe conjunctivitis characterized by ocular discharge, chemosis, conjunctival injection, and multiple white foci 1.0-2.5 mm in diameter scattered over the palpebral and bulbar conjunctiva. The lesions may affect one or both eyes. In the most severe cases, peripheral corneal edema and vascularization may be observed due to the tremendous conjunctivitis. This is a non-ulcerative keratitis and leaves the central cornea clear-aiding differentiation between IBR conjunctivitis and infectious bovine keratoconjunctivitis ("pinkeye").

The conjunctival lesions follow a definite pattern as they heal. The first day of infection may only reveal a serous lacrimal discharge. On days 2-5, the typical white focal lesions appear. On days 5-9, the small white focal lesions seem to coalesce and undergo necrosis. During this time, the conjunctiva demonstrates the most severe chemosis and infection. The lesions resolve 10-20 days following the onset of infection.

The cattle may or may not show systemic signs of fever, depression, inappetance, and decreased milk production. These signs seem to result directly from the febrile response when present. The respiratory form or nasal lesions have been described in conjunction with the ocular form, but the ocular form has been seen in several herds as the only lesion. Pregnant cattle may abort following the ocular form of the disease-especially those in the last half of gestation.

Virus can usually be isolated from the affected eyes during the first 7-9 days of infection. After this time, the virus is generally not found on culture. Fluorescent antibody techniques completed on conjunctival scrapings are the simplest and quickest means of positive diagnosis. Acute and convalescent serology may be helpful to confirm diagnosis if viral culture or FA techniques are unavailable.

Treatment should consist of cleansing away of discharges plus broad spectrum topical antobiotics to discourage secondary bacterial conjunctivitis. In addition, idoxyuridine or other anti-Herpes ophthalmic preparations could be utilized to speed healing. However, on a practical basis, most cases resolve fully in 10-20 days without treatment.

The best treatment is prevention by prophylactic vaccination of heifers and cows with IBR vaccines.