Bovine leukemia virus

Bovine leukemia virus (BLV) is a retrovirus and the natural host of BLV is cattle. In general, BLV causes only a benign mononucleosis-like disease in cattle, and only some animals later develop a B-cell leukemia called enzootic bovine leukosis. Lymphosarcoma in cattle may be the result of an infection with BLV and is often referred to as enzootic bovine leukosis.

Etiology bovine leukemia / enzootic bovine leukosis

BLV or Enzootic Bovine Leukosis is caused by BLV, an exogenous C-type oncogenic retrovirus of the BLV-human T-lymphotropic virus group. The causative agent of this malignant disease was isolated in culture in 1969 for the first time.

BLV integrates into the genomic DNA of B-lymphocytes as a DNA intermediate (the provirus). In its natural host (the cattle) leukemia is rare (about 5% of infected animals) but lymphoproliferation is rather frequent (30%). Because the oncogenic properties of the virus were discovered early, a search for evidence of pathogenicity in humans started soon after discovery. Mostly farm workers drinking raw milk were tested for disease, especially for leukemia. It was discovered in 2003 that some humans carry antibodies reactive to BLV. In 2014, researchers discovered the presence of BLV positive cells in the human breast tissue. A case-controlled study was published in 2015, which suggests a possible association between breast cancer and BLV. However, more recently, another case-control study conducted on patients did not find any association between BLV and breast cancer. In addition an exhaustive analysis of 51 whole genomes of breast cancers do not show any trace of BLV DNA and thus excludes a clonal insertion of BLV in breast tumor cells and strongly argues against an association between BLV and breast cancer.

Economic losses

BLV is a major animal health problem worldwide causing important economic losses. Direct BLV losses to the dairy producer include increased heifer replacement costs, loss of income from condemned carcasses of cull cows, decreased milk production, and the inability to export cattle, semen and embryos to countries that maintain BLV control programs, such as the European Union. Further losses may include reduced reproductive efficiency and decreased milk production. Larger herds are more likely to test positive for BLV. Common causes include shared syringes and, to a lesser extent, rectal palpation.
The NAHMS study determined that herds with BLV produced $59 less in annual production per cow, or 3% less milk, than non-BLV herds. However, this figure can vary depending on the prevalence of infection within a herd, and herds with a higher prevalence of infection are likely to sustain greater economic losses. In a Virginia study, the average cost of a case of lymphosarcoma was over $400, and in a herd with 50% of cows seropositive, the rate of lymphosarcoma was about 2 cases per 300 milking cows. The average annual cost in a 50% prevalence herd was nearly $6,400 per 100 milking cows.

Epidemiology

Sero-epidemiological surveys have shown that BLV infection is widespread in all continents except in Europe. In many countries, high prevalences of virus were found from testing by screening of dairy farms in which bulk tank milk samples were collected and tested with an Enzyme Linked-Immunosorbent Assay (ELISA) for the presence of antibodies against BLV. Results of some studies showed that 83.9 percent of the dairy operations were positive for BLV.

Efforts in the implementation of control measures and campaigns to eradicate BLV infection in Western Europe have been successful. Recently, the European Economic Community (EEC) declared most of its member states as officially free of EBL. In contrast, the situation is different in Eastern Europe where the disease is still present in several countries (Bulgaria, Croatia, Estonia, Latvia, Poland, Romania, and Ukraine).

Transmission of the virus

Cattle are infected with BLV through the transfer of blood and blood products that contain infected lymphocytes. Once infected, cattle develop a lifelong antibody response, primarily to the gp51 envelope protein and the p24 capsid protein.

Many potential routes of BLV transmission exist. BLV is mostly transmitted horizontally, essentially through the transfer of infected cells. Since free virus is very unstable, BLV-infected cells (B-lymphocytes, monocytes/macrophages, etc.) present in blood or milk appear to be the best vehicles of natural transmission. Transmission through procedures that transmit blood between animals such as gouge dehorning, vaccination and ear tagging with instruments or needles that are not changed or disinfected between animals is a significant means of BLV spread. Rectal palpation with common sleeves poses a risk that is increased by inexperience and increased frequency of palpation. Transmission via colostrum, milk, and in utero exposure is generally considered to account for a relatively small proportion of infections. Embryo transfer and artificial insemination also account for a small number of new infections as long as common equipment and/or palpation sleeves are not used. While transmission has been documented via blood feeding insects, the significance of this risk is unclear. The bottom line appears to be that transmission relies primarily on the transfer of infected lymphocytes from one animal to the next and that BLV positive animals with lymphocytosis are more likely to provide a source for infection.

Cattle management procedures which involve transfer of infected blood (*i.e.*, dehorning, ear tattooing, rectal palpation, and essentially, the use of infected needles) were postulated as a common mode of transmission. In addition, prolonged direct contact between infected and healthy animals has also been considered as a risk factor for BLV transmission. Transfer of infected blood might also occur in regions with high density of hematophagous insects.

Vertical transmission may occur transplacentally from an infected dam to the fetus, intrapartum by contact with infected blood, or postpartum from the dam to the calf through ingestion of infected colostrum. Perinatal or postnatal transmission of BLV frequently happens in herd conditions. The rate of transmission *in utero* varies between 4 and 18%, with highest risk in calves born from cows with persistent lymphocytosis (PL). Under natural conditions the disease is transmitted mainly by milk to the calf. Infected lymphocytes transmit the disease from the cow to the calf. Although BLV transmission has been postulated to occur from cow to calves via milk, this route of infection was only demonstrated experimentally. Despite the presence of BLV proviral DNA in colostrum and milk from infected cows, calves can remain uninfected over extended periods of time likely due to the protective role of maternal antibodies present in the colostrum.

Spreading of BLV to other species

Natural infection of animals other than cattle and buffalo are rare, although many animals are susceptible to artificial infection. After artificial infection of sheep most animals succumb to leukemia. Although several species can be infected by inoculation of the virus, natural infection occurs only in cattle (*Bos taurus* and *Bos indicus*), water buffaloes, and capybaras. Sheep are very susceptible to experimental inoculation and develop tumours more often and at a younger age than cattle. A persistent antibody response can also be detected after experimental infection in deer, antelopes, goats and buffaloes.

Clinical signs of bovine leukosis and diagnosis

BLV is an oncogenic B-lymphocytotropic retrovirus that infects cattle inducing a persistent infection with diverse outcomes. The great majority of BLV-infected animals (around 70%) are asymptomatic carriers of the virus. In these animals, neither clinical symptoms nor alteration of the total lymphocyte count are evidenced. Persistent lymphocytosis is considered a benign condition associated with BLV infection. For this reason, it is often overlooked. However, these cows may serve as a reservoir of infection.

These animals can only be identified by the presence of anti-BLV antibodies and/or of proviral DNA. Infection with the virus most often does not cause any clinical signs, however, about 30% of the infected animals develop a lymphocytosis, or abnormal increase in lymphocytes in the blood. The

BLV-infected bovines that develop a benign polyclonal proliferation of B cells are called persistent lymphocytosis (PL) affected cattle. This clinical condition is characterized by an increase in the absolute number of peripheral blood circulating B-lymphocytes associated with an inversion of the B/T lymphocyte ratio.

The leukemia does not cause any clinically apparent change in most cows. It is estimated, however, that 1 to 5% of all infected cattle, not just leukemic cattle, develop malignant tumors know as "lymphosarcomas". Typically, this is a disease of adult cattle, although a juvenile form of lymphosarcoma can occur in younger animals. Cattle showing these signs may display protruding eyeballs, weight loss, enlarged lymph nodes, gastrointestinal obstructions, paralysis in the hind limbs, and/or infertility because of tumors in the uterus.

The variety of organs where white blood cells occur explains the many symptoms: enlargement of superficial lymph nodes, a digestive form, a cardiac form, a nervous form, a respiratory form, and others. Lymph node enlargement is often an early clinical sign.
Clinical signs associated with this form of lymphosarcoma depend heavily on the location and size of the tumor and thus are highly variable, because the affected organ(s) will dictate the predominant clinical signs. Juvenile lymphosarcoma is often characterized by a sudden onset of diffuse lymphoid hyperplasia with or without visceral organ involvement. Weight loss, fever, tachycardia, dyspnea, bloat, and posterior paresis have all been described with this form of lymphosarcoma. Profound lymphocytosis (>50,000/μL) often accompanies this fatal form of bovine lymphosarcoma. Lesions of the spleen are often initially asymptomatic but may result in rupture of the spleen and exsanguination into the peritoneal cavity. Lymphosarcoma of the liver is often asymptomatic but may lead to jaundice and liver failure. Disease of the kidney and ureter can lead to abdominal pain and the subsequent development of hydroureter or hydronephrosis and clinical signs associated with renal failure.

Diagnosis of BLV infections
Lymphosarcoma is often included on the differential diagnosis list for many diseases because of the wide range of clinical findings. Viral infection is diagnosed by serology or virology, persistent lymphocytosis is identified by hematology, and neoplastic tumors are identified by histologic examination of biopsies. Positive serology or virology for BLV confirms viral infection but not the presence of lymphosarcoma.

Serology is the most common and reliable way to diagnose infection with BLV. Agar gel immunodiffusion is still recognized by most countries as the official import/export test, but ELISA is the most common test for routine diagnostic use. Serology is unreliable in calves that have ingested colostrum from BLV-positive cows because of the passive acquisition of maternal antibodies till 4–6 months of age. This means that care should be taken in interpreting positive results in young calves that are under six months as they may have positive antibodies from the dam through colostrum feeding.
It is also difficult to predict which seropositive cows will eventually develop the lymphosarcoma form of the disease, although it is likely that cows that have developed a persistent leukemia are at greater risk. Therefore, testing serum to determine infected cows is not a useful tool in making culling decisions unless the cow is showing clinical signs consistent with lymphosarcoma, and has an elevated lymphocyte count in her blood.

PCR is a sensitive and specific assay for diagnosis of BLV infection in peripheral blood lymphocytes. This test can identify proviral DNA of BLV in the lymphocytes of infected animals and differentiate positive from negative calves in the presence of maternal antibodies.

Post-mortem findings are characteristic and include widespread white tumours in most organs. The diagnosis of lymphosarcoma must be made by cytology or histopathology. Cytologic diagnosis is sometimes difficult because of the frequency of blood contamination of the aspirates.

Treatment

No apparent treatment is available for the disease. Testing and removing positive animals from the herd is one method of control. In herds where the disease is widespread, it is important to limit spread by avoiding contact with blood between animals.

Vaccines against BLV
During the last few decades, a series of attempts were performed to develop a vaccine against BLV, such as inactivated virus vaccines. Protection roughly correlated with the efficiency of this vaccine to induce a strong neutralizing humoral response, but vaccinated animals became infected with high challenge doses. Many other types of vaccines showed rather disappointing results.

Preventing BLV infections
The high individual animal prevalence of BLV reported in the dairy studies suggests that testing and culling of seropositive animals may not be a cost effective method to control the disease. Instead, preventing disease transmission by implementing preventive practices would likely be more cost-effective.

Eliminating the movement of blood from infected animals to naive animals is the cornerstone of prevention protocols. In calves, feeding colostrum from seronegative cows is often advocated. The replacement of whole milk feeding with high-quality milk replacer may also be considered. Bloody milk should never be fed to calves.

Transmission of the virus can be decreased in adult cattle by changing rectal sleeves in between cows. Artificial insemination or embryo transfer (using negative recipients) may limit transmission. In beef herds, the use of a negative bull may limit transmission, but natural service is an uncommon method of viral transmission unless breeding is traumatic.
Additional recommendations include disinfection of equipment that has come in contact with blood or body tissue. Single use, disposable needles should always be used for blood collection and IM injections. It is preferable to use single-use disposable needles for vaccination, but the risk of transmitting BLV virus via SC vaccination is low. Handling facilities that become contaminated with blood should be cleaned between animals. Fly control helps minimize the potential for tabanid-associated transmission. Blood transfusions and vaccines containing blood, such as those used for babesiosis and anaplasmosis, are particularly potent ways to spread the disease, and donors must be carefully screened.

Control and eradication of BLV infections
In Europe attempts were made to eradicate the virus by culling infected animals. The first country considered to be free of infection was Denmark. Thereafter, the Netherlands and the United Kingdom followed. Like the North American states, those of the Eastern countries in Europe did not try to get rid of the virus. But the Eastern Europe states started to monitor and control leukosis after the changes at the end of the last century.

A series of attempts were developed to reduce prevalence, chiefly by **eradication of infected cattle, segregation of BLV-free animals, and/or corrective management strategies**. Although having been instrumental in regions such as the EU, these strategies were unsuccessful elsewhere mainly due to economic costs, management restrictions and lack of an efficient vaccine. "**Test and Eliminate**" approaches require governmental economic compensation policies to be successful. If no official subsidizing action is adopted by the local authorities, the costs of implementation of such a strategy quickly exceeds the potential benefits. Countries such as USA, Canada, Argentina, and Japan lacking financial compensatory policies usually failed to obtain adherence to enrol in these programs
The most commonly recommended eradication protocol is as follows: 1) identify infected animals using a serologic test, 2) cull seropositive animals immediately, 3) retest the herd in 30–60 days, 4) use PCR to test young calves and as a complementary test to clarify test results in herds with a low prevalence of infection, and 5) repeat testing and cull until the entire herd tests negative. Testing is then repeated every 6 mo. The herd is declared free when there have been no positive tests for 2 yr. Additions to the herd should have two negative tests 30 and 60 days before arrival.

When test and cull programs are economically untenable, "**Test and Segregation**" programs have been recommended but are rarely implemented. These programs necessitate running two completely separate operations and require additional resources, including money, time, and available workforce.

Given the high prevalence of BLV infection in most herds, and the relatively benign nature of infection in most cows, it would not be economical to test and cull positive cows. Before starting a BLV "**Corrective Management**" program, a herd should have an estimate of the prevalence of infected cows within the herd. Serum samples from a representative number of cows (25% for small herds, between 5 to 10% for larger herds) should be collected and submitted for testing. Another method that dairy managers can use to estimate the cost of the disease in their herd is the number of cows that are condemned each year because of lymphosarcoma. This information is reported by federal veterinarians to the seller of the cattle, but is easier to attain for herds that take their own cattle directly to market. It is likely that some lymphosarcoma cows go undiagnosed on many farms, presenting as a poorly performing cow, or one that won't get up. A thorough examination by a veterinarian, including a blood count and differential, augmented by a field necropsy, may help identify losses from "unknown" BLV cows.
Dairy producers have to balance efficiency of labor with benefits gained from any management practice. Following are key management practices that are proposed to reduce the prevalence of BLV in a herd:

- Use separate needles and discarding syringes that have been contaminated with blood. This includes maintaining a "clean" needle in a multiuse drug vial.
- Identify BLV positive cows and change palpation sleeves after examining a BLV positive cow and before examining a negative cow.
- Feed colostrums and milk from BLV negative cows only. Alternatively, feed milk replacer rather than milk, and pasteurize colostrum.
- Use electric or gas "burning" dehorners rather than gouging equipment.
- Clean all tattoo and ear tag equipment before each use.
- When practical, separate BLV positive animals from BLV negative animals. This may be difficult on most farms.