ENZOOTIC BOVINE LEUKOSIS

INTRODUCTION

Bovine leukaemia virus (BLV) belongs to the ‘BLV-HTLV’ group of the family Retroviridae. Infection with BLV may result in enzootic bovine leukosis (EBL) which is distinct from sporadic bovine leukosis (SBL). SBL is not associated with BLV infection and presumed to be non-contagious.

Clinical disease in cattle caused by BLV may present as three main syndromes:
- infection characterized by seroconversion but without any clinical signs.
- persistent B cell lymphocytosis.
- the development of solid lymphoid tumours.

Both EBL and SBL occur in southern Africa and in the Southern African context it will therefore be very important to accurately differentiate between EBL and SBL. The epidemiology, direct and indirect economic impact, diagnosis and most importantly control and eradication of EBL is markedly different to that of SBL. Globally EBL infection may also place severe restrictions on the import and export of cattle and semen.

Some clinical findings such as splenic enlargement, rupture and haemorrhage with acute death as consequence are considered one of the more spectacular manifestations of EBL in cattle by one author. Such a finding may raise the suspicion of, and indicate the need, to investigate possible viral involvement in some of the many cases of splenic rupture which have been reported at Vetdiagnostix over the last few years.

Human T-cell leukaemia virus (HTLV) also belongs to the ‘BLV-HTLV’ group of the Retroviridae family but human disease seems attributable mostly to HTLV-1 and not associated with BLV exposure.
EPIDEMIOLOGY

Bovine leukemia virus has a worldwide distribution, with the prevalence and incidence rates differing between geographic regions with rates from 1 to 100 per cent reported. In a limited serological survey in South Africa it was seen that up to 10 per cent of animals, in Jersey herds, had antibodies to BLV, but in beef cattle and especially the indigenous breeds, the antibody prevalence was much lower.

In serological surveys conducted in 132 herds of dairy cattle, by Moola et al, in six magisterial districts of Kwa Zulu-Natal, they found that 91 (69%) herds showed seropositive titres. A total number of 2818 animals were tested and 502 of these (17.8%) animals had positive antibody titers against EBL. The percentages of positive herds varied from 51.5% to 100%, and the percentage of positive animals in positive herds from 7.15 to 38.8%, in the 6 different districts.

The virus is strictly cell-associated and direct exchange of blood cells, usually between an infected animal with persistent lymphocytosis and an uninfected animal, is required for transmission. For successful transmission less than 1% of blood is all that is required. Transmission may be iatrogenic (syringes, needles and any other instruments contaminated with blood), or through direct contact between animals, or mechanical by blood-sucking insects such as Tabanus fusiostatus or by means of milk and colostrum.

The efficacy of costral transmission seems to depend on the titer levels of maternal antibodies. In a fairly recent study published in 2007 by Nagy et al they concluded that calves which are born to BLV positive cows are exposed at parturition and that a proportion of these calves will become infected, but that administration of costrum from BLV infected cows will greatly reduce the risk of infection.

In another recently published (2007) study Kohara et al attempted to experimentally determine if virus could be transmitted via rectal palpation. It was found that BLV negative animals developed antibodies against BLV between 7 to 10 weeks (AGID test) following rectal palpation. Proviral DNA could be demonstrated 1 to 5 weeks earlier by means of PCR compared to the AGID test.

They therefore suggest that this may be a potential route of transmission in infected herds. Other sources report that body fluids and excretions such as saliva and faeces do not seem to play a role in transmission. Bovine leukemia virus has not as yet been isolated from oocytes and spermatozoa or embryos of infected animals, but pre- and peri-parturum infections have been reported.

PATHOGENESIS

Little is known regarding the pathogenesis of BLV infection. Infected animals usually seroconvert, remain infected for life, and develop persistent antibody responses to various BLV antigens. However, only a third of these animals may develop persistent lymphocytosis, and only another third of these usually develop tumours. BLV-infected animals, and more specifically animals in the persistent lymphocytosis stage, may show a general suppressed immune response. These animals may have a higher incidence of secondary infections (eg. Trichophyton verrucosum and some concurrent Theileria sp infections have been reported), a decreased productivity and reproductive rates, and a shorter life span.

The main target cells for BLV in vivo are the B cells, however, other immune cells such as T cells, monocytes and macrophages may possibly also be infected. Persistent lymphocytosis is attributed to polyclonal B-cell expansion, but tumours are monoclonal in origin and most tumour cells seem to be B cells (CD5+). Several articles were published, and very recent review articles by Gillet et al, and Florins et al (listed below), makes for excellent reading, but unfortunately it is far beyond the scope of this article to attempt to discuss their findings in depth.

It does seem that shortly after infection a very active humoral and cytotoxic immune response is stimulated. This immune response is permanently stimulated throughout the animals life, and seems to result in very efficient suppression of the viral replicative cycle, which then only allows for mitotic expansion of provirus carrying cells resulting in lymphocytosis. It seems that
in the chronic stages of disease the virus is permanently in transition between a latent and a transcriptional active phase. Somatic mutations associated with genetic instability in the proviral clones, which otherwise seem generally stable clones, may then result in leukaemia developing.

The onset of the humoral anti-viral response is seen at about 1-8 weeks post-infection, with antibodies against structural and regulatory protein epitopes produced at high titers. Some of these antibodies are directly lytic for BLV-producing cells and antibody-mediated cytolytic activity increases with progression of the disease towards the acute phase. During the early seroconversion period, cytotoxic T-lymphocytes specific for Tax and envelope epitopes appear in the peripheral blood. Both a virus-dependent and a virus-independent CD4 helper T cell response develop but these cytotoxic and helper associated functions seems to weaken in BLV-infected animals, as the disease progresses, which may explain the lower spontaneous recovery from infections such as Trichophyton verrucosum.

**CLINICAL SIGNS**
Infection usually results in a short-term viraemia and this phase is usually clinically silent. Incubation periods are usually long and may be in excess of three years.

- The usual outcome would be that an infected animal will seroconvert, remains infected lifelong, without any clinical signs developing and remaining aleukaemic.

- 30 to 70 per cent of infected animals may develop persistent lymphocytosis due to polyclonal B cell expansion. This stage may take 3 – 6 years and is typically not associated with clinical disease.

- Only 0.1 to 10 per cent of infected animals develops lymphoma, usually at five to eight years of age. Approximately half of these animals have persistent lymphocytosis before progressing to the lymphoma. Enlargement of one or more lymph nodes may be seen first, but in the later stages of the disease lymph node and lymphoid tissue involvement may be more widespread. Tumours may then be seen in all lymphoid tissue containing organs (bone marrow, spleen, lungs, liver and uterus). These animals usually show inappetence, emaciation, and a loss of performance. The organ or number of organs involved, the rate of tumour growth, and the extent and distribution of tumour metastases and the degree of functional disturbances of organs may greatly influence any possible clinical manifestations, as well as the course of the disease.

The clinical signs may therefore be varied, non specific and in many cases very misleading from a diagnostic point of view. Clinical signs such as exophthalmus, posterior paralyse as a result of tumour invasion of the lumbar epidural space, cardiac failure and sudden death due to involvement of the myocardium, dysphagia or dyspnoea when lymph nodes in the pharyngeal or mediastinal regions are affected, or melaena as a result of ulceration of the intestinal mucosa caused by infiltrating neoplasmas may be seen.

Death may occur several weeks or months after the onset of clinical signs, and usually follows on the development of cachexia, secondary infections, anaemia and architectural and functional disturbances in vital organs. In a small proportion of affected animals clinical disease and death may be peracute or acute, particularly if the spleen ruptures, or the heart or adrenals are affected.

**CLINICAL AND MACROSCOPIC PATHOLOGY**
Leukaemia may be present and is usually a terminal event in most cases. Anaemia (generally normocytic and normochronic), may develop in up to 55 per cent of infected animals. Haemoglobin values may drop steadily during the course of the disease, with values below 5 g/100 ml commonly reported. The haematocrit values may parallel the haemoglobin levels. Increased serum levels of uric acid, aspartate transminase (AST) and lactate dehydrogenase (LDH) may be present, but generally other chemical pathological findings are not remarkable, or may appear only in the terminal stages. In healthy animals during the pre-leukaemic
stage abnormalities may only be seen in the blood. During the tumour-developing stage circumscribed solid tumours or more diffuse tumour cell infiltration in the lymphoreticular and other tissues will be seen. Lymph nodes are usually the most frequently affected, varying from a few to many, to virtually all developing tumours. Organs or tissues such as the abomasum, intestine, myocardium, kidneys liver, epidural tissue or uterus may be involved.

Discrete nodular lesions may be seen which may be varying sized, greyish-white to yellowish or pinkish in colour, and soft to moderately firm in consistency. If becoming large, some may have areas of haemorrhage and/or necrosis. Diffuse, infiltrative lesions into organs may result in greatly enlarged, pale, soft and friable organs but many times without marked changes in shape. Spinal cord tumours are most frequently seen in the lumbar region, located in the epidural space as small lesions varying from a few millimeters in diameter, to larger masses that may completely encircle the spinal cord.

Myocardial tumours occur particularly in the right atrium often resulting in central venous congestion. Abomasal tumours may result in diffuse thickening of the mucosa and submucosa, with associated ulceration and hemorrhage.

**DIAGNOSIS, CONTROL AND ERADICATION**

Enzootic bovine leukemia may often be diagnosed, or suspected based on the clinical signs and postmortem findings, as described above, may be very varied, non specific and misleading. In many cases certain findings will only give a clue as to the involvement of EBL, and the need to actively diagnostically investigate BLV infection.

An increased incidence of ruptured spleens, chronic recurrent and persistent infections by parasites such as *Babesia* sp, *Anaplasma* sp and *Theileria* sp, and dermatophytes may potentially, and speculatively, all be examples which may alert the clinician to possible concurrent BLV infection.

A diagnosis may also have to be confirmed on a herd basis in many instances, and probably more so in herds where only a few animals are infected, or clinical signs are generally absent or subclinical. In such scenarios the identification of animals with persistent lymphocytosis may be essential. Before the advent of many of the newer diagnostic tests the identification of such animals by means of full blood counts was very valuable. A recent article highlighted the value of full blood counts in the control of EBL in Denmark a few decades ago, where the Bendixen index was developed to identify cattle with lymphocytosis according to age of the animal, and the number of lymphocytes per microliter.

The choice of laboratory tests will largely be determined by the need to diagnose individual infected animals, or to identify infected herds or to institute control, eradication, or disease surveillance programs. Routinely used techniques would be those aimed at detection of antibodies to BLV by various serological tests such as radio-immunoassay, ELISA, neutralization tests, immunodiffusion, immunofluorescence, and complement fixation. These tests mostly detect antibodies to the gp51 and gp30 proteins, but also the p24 protein. Proviral DNA can also be detected in peripheral lymphocytes by polymerase chain reaction (PCR) tests.

PCR tests have the added advantage of being able to be used on a large variety of different fresh specimens, and the fact that it may be used to determine the viral load under certain circumstances. Other diagnostic tests includes transmission electron microscopic, detection of BLV particles in cell cultures of susceptible cells following co-cultivation with cells form theuffy coat of suspected BLV-infected animals. However, these are time-consuming, technologically demanding, and expensive methods, and therefore not routinely used.

In certain countries a lot of emphasis is placed on control and eradication of EBL and various different models have been investigated, evaluated and published but it will not be possible to discuss the detail of these articles here. In the European Union, the disease has been eradicated in infected herds by testing of all animals for antibodies against gp51 at intervals of
3 to 6 months, followed by elimination of seropositive animals until the herd was free of seropositive animals (test and culling programme). Another strategy in BLV-endemic areas, was to completely isolate BLV infected animals (test and isolate programme).

Results of a study by Juliarena et al suggests the importance of also considering nonlymphocytotic BLV-infected cattle during eradication programs as the risk of transmitting BLV infection from nonlymphocytotic cattle may depend on the proviral load. The authors suggests that nonlymphocytotic cattle with high proviral load could be as efficient transmitters as lymphocytotic cattle, in contrast to nonlymphocytotic cattle with low proviral load which could be inefficient transmitters. It seems that most cattle with low proviral load do not develop anti-BLVp24 antibodies, and a lack of an anti-BLVp24 antibody response may potentially be a good marker of this condition.

Over the past years, several attempts have been made to produce a vaccine against BLV, but no effective vaccine is available yet and efforts are continuing. Effective treatment protocols do not exist and all attempts will only be directed at supportive and symptomatic treatment. Although losses due to BLV associated lymphoma can sometimes be high, the major reason for eradication is the restriction that EBL places on the export of animals to countries free of the disease.

**EBL IN OTHER SPECIES**

Cattle are the natural hosts for BLV. There seems to be evidence that BLV persists in water buffaloes but the existence of wildlife reservoirs remains controversial. Experimentally BLV infection has been described in many species such as rabbits, rats, chickens, pigs, goats and sheep. Sheep seems to be the only species consistently developing leukemia, making it a very useful study model, compared to rabbits which only present with immune dysfunctions but not any tumors. Persistent BLV infection has not been seen in the cat, dog, monkey or human although viral-specific seroconversion may occur in these species. Epidemiological studies have revealed that the consumption of raw milk originating from BLV-infected cattle does not seem to increase the frequency of leukemia in man, and that human disease is attributable to HTLV-1, a finding of great epidemiological significance.

Reviewing the most recent literature it becomes clear that most of the recent, and probably immediate future research, may be done at the molecular level; and that BLV may become a very important pathogen to study, and specifically in sheep as a model, to try and establish the mechanisms of leukogenesis - and in particular as potential model for human disease. From recent research it seems that the majority of target B cells express positive marker staining for MHCII, surface IgM, CD5 and Cd 11b.

Fluctuation in the last three markers may be seen in the later stages of the disease. The regulatory proteins of importance seem to be Tax, Rex, R3 and G4. Tax and Rex proteins are required for the transcription and post - transcriptional activation of viral expression. The G3 and R3 are not required for infectivity but seem to be involved in the maintenance of a high viral load. G4 seem to have oncogenic potential. Other cytokines, probably involved, and under investigation seem to be the interleukins 2, 6 10 and 12, tumour necrosis factor alpha (TNFa) and interferon gamma (INFy).

**CONCLUSION**

The clinical signs and postmortem findings, as described above in EBL infected animals, may be very varied, non specific and in many cases very misleading from a diagnostic point of view necessitating confirmation by means of different laboratory tests, often on a herd basis. For all practical purposes, the laboratory tests of choice in the Southern African context would be the ELISA and PCR, backed by a macroscopic post-mortem and histology, for the diagnosis, control and eradication of EBL in infected herds. The list of more important differential diagnoses may include lymphoid hyperplasia in lymph nodes, spleen and other tissues as may be observed as a result of chronic inflammation in diseases such as East Coast fever, malignant catarrhal fever, mycobacterial infections, actinobacillosis, actinomycosis or hepatic necrofulosis.
The identification of animals with persistent subclinical lymphocytosis may be of great benefit in the identification and control EBL infection in some affected herds. Although the direct and indirect losses due to BLV associated lymphoma, and disease can sometimes be high, the major reason for eradication in many countries abroad may be the restriction that EBL places on the export of animals to countries free of the disease.

REFERENCES
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CDP - QUESTIONAIRE

1. Which one of the following statements is false?
   a. BLV belongs to the family Retroviridae.
   b. BLV causes EBL.
   c. BLV does not cause SBL.
   d. HTLV also belongs to the same group as EBL.
   e. BLV causes human disease.

2. Which one of the following statements is false?
   a. BLV infection has a worldwide distribution.
   b. BLV infection has been confirmed in South Africa.
   c. BLV infection seems to exist in water buffalo.
   d. Sheep are not susceptible and do not develop leukemia.
   e. Persistent BLV infection has not been seen in the cat, dog, monkey and human.

3. Which one of the following statements is not true.
   a. The virus is not strictly cell associated.
   b. Transmission of BLV requires direct exchange of blood cells.
   c. Less than 1 microliter of blood is adequate for transmission.
   d. Mechanical transmission (iatrogenic, blood sucking insects) may occur.
   e. Transmission via milk and colostrums and rectal palpation is possible.

4. Which of the following statements are true?
   a. Infected animals remains infected for life.
   b. All infected animals develop persistent lymphocytosis.
   c. Suppression of the general immune response may be seen in animals with persistent lymphocytosis.
   d. Infected animals do not develop a persistent immune response.
   e. The immune response may be humoral and cytotoxic.

5. Which one of the following statements is correct?
   a. The main target cell is the (CD 5 +) B-cell.
   b. The main target cell is the T-cell.
   c. The main target cell is the macrophage.
   d. Lymphocytosis is mainly monoclonal and of T-cell origin.
   e. Tumours are mainly polyclonal and of B-cell origin.

6. Which of the following statements is not correct regarding the clinical signs which may be observed:
   a. Clinical signs are usually not seen in the majority of infected animals.
   b. Persistent lymphocytosis may be seen in 30-70% of animals after 3-6 years.
   c. Exophthalmus, posterior paresis and melena may be seen in animals in the chronic stages of disease.
   d. Acute deaths may be seen due to splenic rupture in the chronic stages of disease.
   e. Development of tumours occur from 5-8 months of age.

7. The most practical means for the diagnosis in South Africa may be:
   a. Viral isolation.
   b. The complement fixation test and histology.
   c. Histology alone.
   d. PCR test alone.