Enzootic Bovine Leukosis

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Abstract

Bovine leukosis is a cattle disease caused by the retrovirus, bovine leukaemia virus (BLV). Although the natural host of BLV is domestic cattle, it is also considered a model for understanding pathogenesis and development of novel therapies for Human T-cell lymphotropic virus. Cattle infected do not usually present clinical signs, however a persistent lymphocytosis and development of tumour lesions may occur. Tumour lesions have been described in the abomasum, central nervous system, eye, heart, lymph nodes, liver, spleen, spine, uterus and other structures. EBL is responsible for high economic losses derived mainly from trade restrictions and decreased milk yield. Many countries have implemented specific control and surveillance programs for their eradication. These programs are based test-and-slaughter and consist in blood sampling of cattle periodically and compulsory slaughter of those positives. According to the legal criteria in Europe, official country or regional freedom from EBL require that at least 99.8% of herds are free from BLV infection. Besides some findings indicated the presence of infected viral particles in foodstuffs, further studies are necessary to confirm the chance of BLV transmission to humans through foodstuff consumption.

Keywords: Bovine Leukosis; Lymphoma; Leukaemia; Tumours; Cattle.

1. Introduction

Bovine leucosis, also known as lymphoma or malignant lymphoma, is a cattle disease caused by the retrovirus, bovine leukaemia virus (BLV) [1]. Although the natural host of BLV is domestic cattle, it is also considered a model for understanding pathogenesis and development of novel therapies for Human T-cell lymphotropic virus [2]. Bovine leukosis was first reported in 1871 in cattle with nodules in an enlarged spleen [3] and can occur in two forms: enzootic bovine leucosis and sporadic leucosis [4]. Enzootic bovine leucosis occurs in adult cattle caused by the BLV. Regarding sporadic bovine leucosis, it can be present by three forms: calf, tymic and skin, affecting mainly calves and young cattle [5]. Cattle infected do not usually present clinical signs, however a persistent lymphocytosis and development of tumour lesions may occur [6]. The route of transmission to new cattle implies the transfer of BLV-positive cells through blood or milk. Thus, insects may play an important role in the transmission of the disease [7]. However, other routes such as natural breeding and veterinary exams such as rectal palpation [8], use of contaminates needles or contaminated dehorning tools [9] may contribute to the transmission of the disease. Congenital transmission have been reported but with less importance [10]. Bovine enzootic leucosis causes heavy economic impact in cattle farms due to commercial restrictions of live animals, embryos or semen [11], decreased milk yield [12], as well by the increased susceptibility to a wide variety of opportunistic infections due to the compromised immune function. Also, some studies indicated the potential of bovine leucosis as a zoonotic disease [13].

2. Aetiology and Pathogenesis of Disease

Bovine leucosis is RNA virus caused by the bovine leukaemia virus (BLV), which belongs to the *Retroviridae* family and *Deltaretrovirus* genus [14]. Naturally affects cattle and buffaloes; however experimental infection in other species has been studied **(Table 1)**. Also, no reservoirs are described in other species including wildlife [15]. The pathogenesis of BLV induces an infection with an absence of chronic viraemia and a long latency period. The infection is transmitted mainly by horizontal route, via iatrogenic through the transfer of blood, by exposure of susceptible cattle lymphocytes B carrying the virus. Thus, blood and milk are the main vehicles for transmission and the transplacental route may be responsible for approximately 5% of infected calves born with enzootic characteristics [10].

Other vehicles such us flies, nasal secretions, saliva or semen have been described but with scarce relevance [16, 17, 18]. Also, BLV has not been found in faeces or urine [19]. Moreover, Benitez *et al*. [20] recently reported an absence of BLV transmission from infected bulls to health heifers in natural breeding.

The pathogenesis of BLV is variable and depends on the differences in the host-virus relationship, which includes: number of infected cells or number of copies of the provirus integrated to the infected cells, the expression of viral antigens and the induction of immune response antiviral and polyclonal or monoclonal proliferation of lymphocytes [1]. The initial infection by the virus is not currently known. Thus, the virus' arrival in the direction of a susceptible individual is done by cells from an infected individual, which contain the viral genome. These allogeneic cells contained in blood, semen or raw milk infect the cells of the new host. Once enter the host, the target of the virus are lymphocytes B that express IgM. The virus is carried to the local lymph node where primary replication occurs before dissemination of virus throughout the body [21]. Then, viral replication continues in the spleen, lungs, bone marrow and other lymph nodes. Then, the viral infection is followed by a polyclonal expansion of a large and diverse population of carrier lymphocytes from one to five integrated proviruses [22].

Table 1: Uncommon species infected by bovine leukosis virus

Species	**References**
Buffaloes	Feliziani *et al*., 2017
Goat	Kettmann *et al*., 1984
Pigs	Anderson and Jarrett, 1968
Rabbits	Wyatt *et al*., (1989)
Sheep	Kettmann *et al*., 1984
	Djilali and Parodi, 1989
	Mammerickx *et al*., 1976
	Rogers *et al*., 1984
Yak	Ma *et al*., 2016

3. Clinical Signs and Lesions

Bovine leukosis is lifelong infection characterised by the development of circulating antibody, which is also present throughout life [23]. Affected animals usually develop antibodies within 2-3 months of infection. However, less than 3% of infected animals develop malignant disease. The peak incidence of disease is observed in cattle among 6-8 years old. However, some cases have been reported in young animals [24, 25]. The clinical signs vary according to the location of the tumour lesion and the organ or organs affected. Thus, presence of one or more superficial enlarged lymph nodes, as lumps by skin palpation, mainly in the neck and hind flank areas may be compatible with bovine leukosis. However, the diagnosis could be difficult when only internal lymph nodes are affected. In some cases, an accidental diagnosis is achieved by rectal palpation or by ultrasonography during routine pregnancy diagnose [26].

Tumour lesions have been described in the abomasum, central nervous system, eye, heart, lymph nodes, liver, spleen, spine, or uterus. Although unspecific, other clinical signs such us chronic bloat, decreased appetite, decreases milk yield, depression, diarrhoea, displaced abomasum, dyspnoea, eye protrusion, fever, increased somatic cell score, indigestion, infertility, lameness, pale mucous membrane, paralysis, posterior paresis, tibiotarsus joint swelling among others may be present [27, 28, 29, 30, 31, 32]. Although bovine leucosis is a chronic and insidious condition, spontaneous regression of cutaneous leucosis in a 2-year-old heifer was reported [33].

Haematological and biochemistry parameters are variable. Thus, up to 50% of cases are leukemic, with high numbers of circulating immature lymphocytes. Anaemia is usually present although its characteristic may vary. Thus, non-regenerative, and macrocytic anaemia or normocytic and normochromic anaemia have been described [27, 30, 34]. Moreover, hypoproteinaemia and hypoalbuminemia are often presented [27, 35].

At necropsy, lesions involved many organs. Multiple peripheral lymph nodes (e.g., mandibular, superficial, subiliac (Fig. 1), mammary, or iliac lymph nodes were swollen. The cut surface of affected lymph nodes presented a firm consistence, white to grey coloured with a homogenous texture. Several masses can be found in other organs such us brain, frontal sinus, kidneys, abomasum, omasum (Fig. 2), liver (Fig. 3), lungs, prerenal tissue, pleura, thymus, ureters or uterus. The bone marrow may present a gelatinous consistence with a yellow to white colour [25].

Fig 1: Gross features of bovine leukosis: subiliac lymph node enlarged and irregular

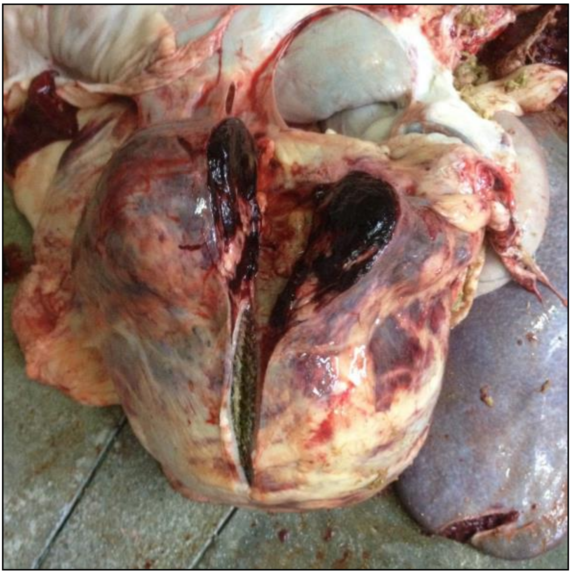

Fig 2: Gross features of bovine leukosis: omasum with increased wall thickness, ulcers and bleeding

Fig 3: Gross features of bovine leukosis: Liver with nodular whitish yellow lesions

4. Diagnosis

Diagnosis of BLV in live cattle based on clinical finding is difficult due to the unspecific symptoms as previously discussed. Although the presence of enlarged lymph nodes in adult cattle may be compatible with BLV, diagnosis must be confirmed by laboratory assays. In live animal, traditional diagnostics methodologies include direct identification as well as the use of molecular methodologies such as agar gel immuno-diffusion (AGID), polymerase-chain-reaction (PCR) or Enzyme-linked immunosorbent assay (ELISA). Regarding the direct identification of BLV, in vitro culture of blood leucocytes [1] and further observation by electron microscopy is required. However, the lack of specificity as well as the cost and expensive equipment required make this technique not appropriate for diagnostic purposes.

Today, serological methods for the detection of specific BLV-antibodies in serum or milk are usually used in cattle older than 6-9 months. The AGID is used worldwide due to its simplicity and specificity although false-negative results in samples from cows in the periparturient period or in those cattle with low titles of antibodies, may occur [14]. Regarding ELISA, several commercial kits are available. Usually, ELISA detects antibodies against gp51 or p24, although higher titles are observed against the first one [36]. However, recent research indicated that differences in the test sensitivity are scarce [37]. Also, no differences were observed regarding sensitivity and specificity values among several ELISA commercial kits [38], even when used to test field samples of p24 antibodies in raw milk and whey [36]. The ELISA has the additional advantage, compared to AGID that can be used to detect antibodies in milk samples and also displayed higher sensitivity than AGID. Diagnose of BLV by PCR is usually intended to confirm previous serological tests (ELISA and/or AGID). Other uses of PCR are diagnose of BLV in calves below 6–9 months to avoid false-negative results due to existence of colostrum antibodies, early diagnose before specific antibodies are developed, confirmation of doubtful results from ELISA/AGID test, differentiating between clinical cases of sporadic and enzootic lymphomas or confirming the diagnose from sampled obtained at slaughterhouses.

Different PCR assays have been reported in the literature such as simple PCR [39], real-time PCR [40], real-time quantitative reverse transcription[41], real-time PCR using coordination of common motifs [42], loop-mediated isothermal amplification assay [43, 44] or field-deployable automatic nucleic acid-extraction/insulated isothermal PCR [45].

Other approaches to the diagnosis of BLV have been suggested. Thus, a combination of blood cell counts and lymphocyte counts analyzed by principal component analysis has been proposed by [46]. Similar approach was reported by Nakada *et al*. [47] where the use of generalized linear models with quasi-Poisson errors based on the study of white blood cell and lymphocyte counts plus proviral load using PCR, provides a cost-effective and more rapid alternative detection of BLV-suspected cows. Recently, use of strip-dried format combined with ELISA and PCR for BLV diagnose have been studied by Saushkin *et al* [48]. Results showed similar results as classical ELISA and PCR analysis with the advantages of sampling, storage, transportation, and more antibodies stability at high temperatures than serum samples. In case of death cattle, at necropsy gross features of bovine leukosis can be observed, namely in lymph nodes (Fig. 4A). Histopathological analysis together PCR or ELISA can be considered the gold standard of BLV diagnose. Gross features of bovine leukosis Histopathological, neoplastic cells diffusely proliferated in the affected lymph nodes (Fig. 4B) without the lost of normal structure of the lymph nodes. The neoplastic cells were large and round with a small amount of cytoplasm, showed irregular thickening of nuclear membranes, crafted nuclei with nuclear atypia and granular-patterned chromatin. Presence of mitotic figures is common [50]. Neoplastic cells can infiltrate several structures such as abomasal wall, adrenal gland, bladder, bone marrow, diaphragm, heart, lamina propria of the intestines, liver, lungs, pancreas, spleen, Peyer´s patches, thymus, tonsil or uterus [25, 27, 28, 49]. Neoplastic cells characterized by a variable size, irregular nuclei, variable amount of citoplams with scant characteristics citoplams [50].

According to tumoral lymphocytes type (B or T), immunohistochemical examination showed that neoplastic cells were positive for BLA-36 and negative for CD3 [25], CD79a and CD5 [50]. Although other research reported negative results against BLA-36 antibodies and positive results against CD3 [49]. Recently, bovine leukemia has recently been classified according to the cell markers by immunophenotypic analysis [51] in five groups: classical enzootic bovine leukosis (cEBL), polyclonal EBL (pEBL), B-cell-type SBL (B-SBL), T-cell-type SBL and non-typeable cases.

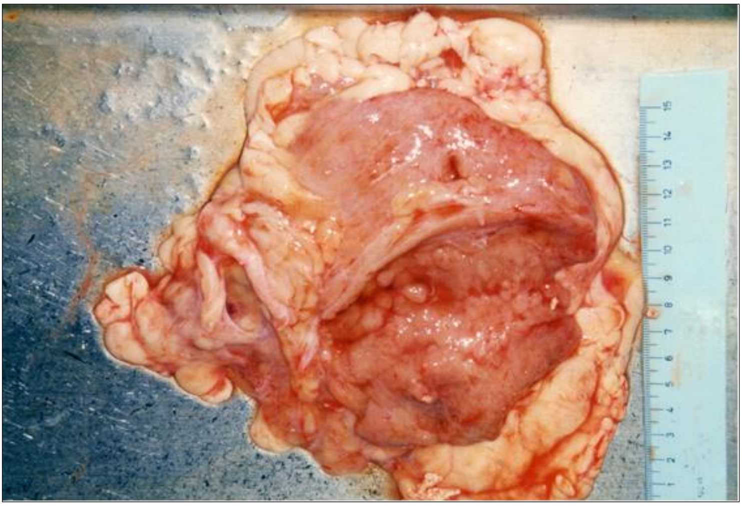

Fig 4A: Gross features of bovine leukosis: iliac lymph node enlarged and irregular

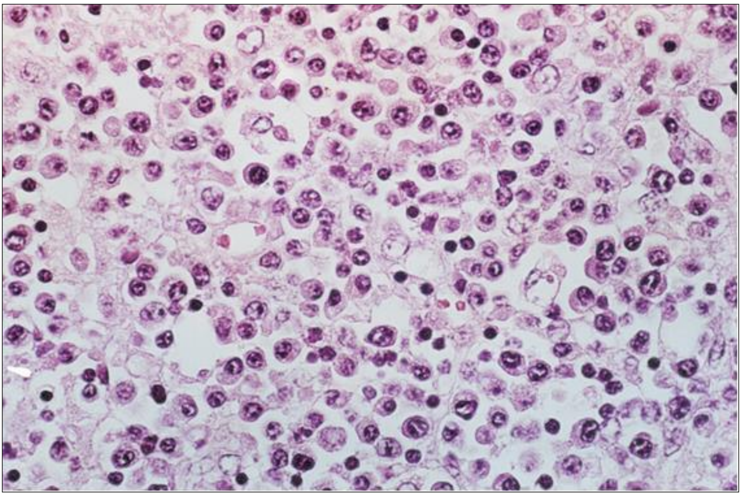

Fig 4B: Microscopic features of bovine lymphoma: iliac lymph node (H&E, 400x)

5. Risk Factors of Blv and Preventive Measures

Enzootic Bovine leukosis causes heavy economic impact in cattle farms due to commercial restrictions of live animals, embryos, or semen [11], increased susceptibility to a wide variety of opportunistic infections due to the compromised immune function. Also, countries where exist national control programs based on test-and-slaughter control; positive animal were compulsory slaughtered. This measure also implies the lost of the genetic performance. Risk factors of BLV should be related to the pathogenesis and mode of transmission of the virus. Thus, practices that imply potential transmission of contaminated blood cells by BLV throughout tools such us gouge dehorning, use of sleeve for more than one cow or sharing needles have been reported [13, 52, 53, 54].

Transmission of contaminated B-cell by arthropods have been suggested by Foil and Issel [55], although its real role in the transmission of BLV is currently unknown. However, Kobayashi *et al.* [53] indicated that large number of horseflies in summer are risk factors for BLV. The potential transmission of BLV by ticks have been referred in some literature but its real impact on cattle health is still unknown [56]. This fact may probably associate with the life cycle of ticks, since, unlike mosquitoes, it stays in the same animal once attached. However, implementation of correct farm practices including fly control measures [57] and prophylactic internal and external parasite treatments should be carried out by farmers as preventive measure of BLV infection.

Deficient housing conditions have been referred by Kobayashi *et al.* [53]. Although this characteristic is difficult to explain, it could be related to high farm flies density associated to lack of farm hygiene (i.e. deficient cleaning and disinfection, deficient manure removal) as reported by Sun *et al.* [58].

Regarding animal management, infection rates are associated with the proviral load. Entrance or purchase cattle from livestock with unknown sanitary status or from herds with historic of BLV positive, even over 5 years [52] may increase the odd of infection. Other factors such as large sized herd, use of well water, elderly cattle, multiparous dairy, high lymphocytosis' values or lack of veterinary support have been referred as risk factors for BLV infection [58, 59]. Thus, self-replacement, good farm practices and biosecurity measures represent the most important preventing measures.

6. Epidemiology

Enzootic Bovine Leukosis is a notifiable disease by the OIE and described worldwide. According to the WAHIS report of OIE **(Table 2)**, EBL still represents a livestock concern. Prevalence values of BLV vary across countries and are based on reports that estimate the BLV prevalence throughout large blood sampling schemes to detect antibodies in serum and/or milk.

Table 2: Worldwide report on enzootic bovine leukosis, according to

Infection present (with no clinical disease)		**Demonstrated clinical disease**	
Country	***Reporting year***	***Country***	***Reporting year***
Croatia	Jul - Dec, 2017	Brazil	Jan - Jun, 2018
French Polynesia	Jan - Jun, 2018	Canada	Jan - Jun, 2018
Panama	Jan - Jun, 2018	Colombia	Jan - Jun, 2018
Poland	Jan - Jun, 2018	Costa Rica	Jan - Jun, 2018
Reunion (France)	Jul - Dec, 2017	Dominican Republic	Jan - Jun, 2018
Serbia and Montenegro	Jul - Dec, 2006	Ecuador	Jan - Jun, 2018
Venezuela	Jul - Dec, 2017	El Salvador	Jan - Jun, 2017
		Former Yug. Rep. of Macedonia	Jul - Dec, 2017
Infection/infestation limited to one or more zones		Greece	Jan - Jun, 2016
Country	***Reporting year***	Guatemala	Jul - Dec, 2017
Italy	Jan - Jun, 2018	Japan	Jan - Jun, 2017
Latvia	Jan - Jun, 2018	Kazakhstan	Jan - Jun, 2018
Lithuania	Jan - Jun, 2018	Korea (Rep. of)	Jul - Dec, 2016
		Kyrgyzstan	Jul - Dec, 2017

Disease restricted to certain zone(s) / region(s) of the country		Malta	Jul - Dec, 2017
Country	***Reporting year***	Nicaragua	Jan - Jun, 2018
Argentina	Jan - Jun, 2018	Russia	Jan - Jun, 2018
Australia	Jan - Jun, 2018	Serbia	Jul - Dec, 2017
Hungary	Jul - Dec, 2017	South Africa	Jan - Jun, 2018
Israel	Jan - Jun, 2018	Ukraine	Jan - Jun, 2018
Moldova	Jan - Jun, 2018	United States of America	Jan - Jun, 2018
Portugal	Jan - Jun, 2018	Uruguay	Jan - Jun, 2018
		Bulgaria	Jul - Dec, 2017
		Honduras	Jul - Dec, 2017
		Montenegro	Jul - Dec, 2017
		Paraguay	Jan - Jun, 2018
		Peru	Jan - Jun, 2018
		Romania	Jan - Jun, 2018
		Sri Lanka	Jul - Dec, 2016

Data obtained from the OIE - WAHIS interface (Accessed on 30-01-2019) http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statuslist/index/newlang/en?header_disease_type_hidden=0&header_disease_id_hidden=35&header_selected_disease_name_hidden=Leucosis+bovina+enzo%F3tica+%28-+-%29+&header_disease_type=0&header_disease_id_terrestrial=35&header_disease_id_aquatic=-999&header_public_country_code=0

These studies give us an overview of the virus spread within and between herds. Thus, seroprevalence EBL values in cattle ranged from about 50% to the total eradication to the disease [52, 60, 61, 62, 63, 64, 65], although epidemiological data of sample such as population size, production (dairy or beef), type of sample (blood or milk) or laboratory technique (ELISA or PCR) should be carefully interpreted. In the EU, official country or regional freedom from EBL require is that at least 99.8% of herds are free from BLV infection according to the criteria in Council Directive 64/432 /EEC [66].

7. Impact on Public Health

Currently, the risk of potential zoonosis has probably been underestimated, particularly in occupational settings. Thus, in the context of one health, identification of emerging hazards should be properly managed to guarantee the public health. Discussion about its zoonotic potential started 30 years ago without epidemiological or serological evidences of transmission to man [58, 67, 68]. However, recent epidemiological studies about incidence of some types of tumours in animal-related professions such farmers or abattoir workers suggest a possible association due to contact to viruses that cause cancer in food animals [69, 70]. Workers who, work in abattoirs over 2 years, having direct contact with animals or animal products or worked as a butcher in an abattoir displayed are important risk factors (OR from 2.9 to 8.2) [71, 72].

In 2001, the zoonotic potential of BLV was discussed again due to the identification of antibodies against BLV in people [73] suggesting the possibility of infection with the virus. Further research carried out by Buehring *et al.* [74] reported that 59% of the cases of women with breast cancer presented positive results by PCR. Thus, current research [75] showed the presence of BLV genes in humans breast tissue with a high homology between amplified gene from human breast tissues and from bovine cattle with leucosis. Although research found BLV in human tissues showed a potential risk for the acquisition and proliferation of this virus in humans, the role of BLV as a aetiological agent is still unknown [76].

Regarding the potential of the pathogenic agent of BLV as a foodborne pathogen, BLV in raw beef and fresh milk for human consumption have been detected by PCR, indicated that viral particles could be present [77]. Further studies are needed to confirm the presence of infected viral particles, even though the present findings could represent a first approach to BLV transmission to humans through foodstuff consumption.

8. Conclusions

EBL is responsible for high economic losses derived mainly from trade restrictions and decreased milk yield as described above. Thus, many countries have implemented specific control and surveillance programs for their eradication. These programs are based test-and-slaughter programs as other diseases such as bovine brucellosis or bovine tuberculosis and consist in blood sampling of cattle periodically and compulsory slaughter of those positives. According to the criteria established in Council Directive 64/432 /EEC[66], official country or regional freedom from EBL require that at least 99.8% of herds are free from BLV infection.

Besides some findings indicated the presence of infected viral particles in foodstuffs, further studies are necessary to confirm the BLV transmission to humans through foodstuff consumption.

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