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Enzootic bovine leukosis

EFSA Panel on Animal Health and Welfare (AHAW)

Abstract

Enzootic bovine leukosis is a disease of cattle caused by bovine leukemia virus (BLV). The virus causes a persistent, life-long infection in a subset of B cells. Malignant tumours (lymphomas) ultimately develop in 2–5% of infected animals, predominantly in adult cattle older than 3–5 years. Lymphomas invariably lead to death of the animal within months. Before 1960, BLV was endemic in dairy herds in Northern/Eastern Europe and North America. Since then it has spread to all continents. The disease has been successfully controlled and eliminated from many countries in Europe. There is no evidence to suggest that any significant reservoir of BLV exists among other species, nor for any role of BLV in human disease or cancers. BLV exhibits a slow, progressive spread within a herd and it is likely to persist if control measures are not applied. The main modes of transmission are perinatal from cow to calf, via colostrum and milk, and close contact that allows transfer of infected lymphocytes, such as dehorning and injections using non-sterile utensils. Transfer between herds is almost entirely by movement of infected animals. Suitable methods have been developed for diagnosis of BLV infection in specimens of blood, milk and lymphomas. EBL has a negative impact on milk yield and leads to increased premature culling. The welfare consequences of lymphomas vary according to the location and magnitude of organ involvement. Criteria for maintaining country freedom from EBL differ substantially between OIE and EU. Surveillance for freedom should be based on a combination of serological testing of adult animals and identification of lymphomas at slaughter.

Keywords: Bovine leukemia virus, leukosis, lymphoma, impact, control

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Summary

Following a request from the European Commission, the EFSA Panel on Animal Health and Welfare (AHAW) was asked to deliver a scientific opinion on enzootic bovine leucosis (EBL). The Panel was asked to consider the disease profile and distribution, modes of transmission and persistence and risk of introduction, diagnostics, impact and control.

The response to the terms of reference has been based on a review of the scientific literature and analysis of historic and contemporary epidemiological data from Europe and other continents. The assessment on the impact of the disease included a systematic literature review.

EBL is a disease of cattle, caused by bovine leukemia virus (BLV). The virus causes a persistent, life-long infection in a subset of B cells and induces immune dysregulation with increased susceptibility to other infections. Ultimately, malignant tumours (lymphomas) develop in up to 5% of infected animals, predominantly in adult cattle older than 3–5 years. Once developed, lymphomas invariably lead to death of the animal within months. In addition, reduced milk yield, subclinical mastitis and premature culling may occur in later stages of infection with BLV.

Before 1960, EBL was an endemic disease in dairy herds in Northern/Eastern Europe and North America, characterised by a very slow spread and late appearance of lymphomas. Since then, BLV continued to spread to all continents by trade in breeding animals. As of 2012, 51 countries world-wide have reported the presence of the disease. In countries where no control programs are implemented, the herd prevalence has continued to increase with most herds being infected today. Following the implementation of eradication programs in the 1960s the disease has been successfully controlled and eliminated from many countries and regions in Europe.

The limited evidence on BLV infections in species other than cattle is most likely the result of spill-over from bovines or unverified false-positive testing results. There is no evidence to suggest that any significant reservoir of BLV exists among other species, including wildlife.

Concerns over a potential human risk were raised in the early considerations to control the disease; however, there is no unequivocal evidence for an etiological role of BLV in human disease or cancers.

BLV exhibits a slow, progressive spread within a herd, and it is likely to persist if control measures are not applied. Main modes of transmission are perinatal from cow to calf, via colostrum and milk, and close contact that allows transfer of infected lymphocytes, such as dehorning and injections using non-sterile utensils. Transfer between herds is almost entirely by movement of infected animals.

The morbidity and mortality due to EBL in the EU is currently negligible. During the last 6 years only 35 lymphoma cases have been reported from all Member States (MS). In 2013, 14 MS reported the presence of BLV-infected herds; however, very few suspect cases and no confirmed slaughterhouse cases of lymphoma were reported. It is highly unlikely that no lymphoma cases have developed, the current surveillance system therefore probably suffers under-reporting. Nevertheless, the current impact of the disease on agricultural production in MS is considered negligible due to the very low herd prevalence.

In countries endemically affected by EBL, the impact is determined by trade restrictions on breeding animals, production losses and carcass condemnation at slaughter. In long-time infected herds the annual lymphoma incidence among dairy cows may reach 1–2%, corresponding to a cumulative incidence of 5% of BLV-infected animals. In modern dairy systems, a minor, but statistically significant reduction in milk yield is observed in BLV-

infected animals. In these systems, longevity is also reduced, expressed as premature culling.

Suitable and sensitive methods have been developed and made commercially available for diagnosis of BLV infection. Sensitive indirect ELISAs have greatly facilitated screening of dairy herds via bulk milk testing. Histological examination can support the diagnosis of malignant lymphomas, but is not able to distinguish between sporadic lymphomas and those induced by BLV. A BLV specific PCR or antibody test must be performed to confirm that lymphomas have been induced by BLV.

Lymphomas are not likely to be detected *in vivo* until they cause conspicuous clinical and pathophysiological manifestation. Overall, animals will suffer when lymphomas have progressed beyond early stages; the welfare consequences in terms of duration and severity may vary according to the location of lymphomas and magnitude of organ involvement.

Applying stringent management tools in dairy herds may lead to a certain reduction of within-herd prevalence but will not be able to eliminate the infection. Hence, the only sustainable, long-term method to obtain freedom from BLV is by eliminating infected animals. This has now been accomplished in most European countries after 40–50 years of control efforts. Beef herds can remain a significant reservoir for BLV.

Vaccination is currently not an option. Experimental vaccines are currently being tested for efficacy and might become an alternative option to reduce within-herd prevalence in the future.

In regions free of EBL continued surveillance is based on a combination of serological testing of adult animals and identification of lymphomas at slaughter. For dairy herds an active surveillance based on bulk milk testing is the method of choice.

Due to the food safety requirement for identification of tumours at post-mortem meat inspection, testing for EBL lymphomas at slaughter is a feasible and inexpensive, additional surveillance system component.

Criteria for maintaining country freedom from EBL differ substantially between OIE and EU. OIE prescribes only serological testing whereas EU rules allow for surveillance for lymphomas only.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

Enzootic bovine leukosis (EBL) is a disease caused by the bovine leukaemia virus, a member of the family Retroviridae; it mainly affects cattle although natural infection has also been recorded in water buffaloes. Most infections appear to be subclinical, but a proportion of cattle (~30%) over 3 years old develop persistent lymphocytosis, and a smaller proportion develop lymphosarcomas in various internal organs.

While the infection appears to be widespread globally, in the EU there are many Member States that are officially free as per Commission Decision 2003/467/EC[1]: Belgium, Czech Republic, Denmark, Germany, Ireland, Spain, France, Cyprus, Latvia, Lithuania, Luxembourg, Netherlands, Austria, Slovenia, Slovakia, Finland, Sweden and the United Kingdom.

In addition, the following Member States have one or more regions recognised as officially free: Italy, Poland and Portugal.

EBL is a disease included within the category of cattle diseases on the OIE list of diseases in Article 1.2.3. of the Terrestrial Animal Health Code (the Code) of the World Organisation for Animal Health (OIE). This consequently entails notification obligations to the OIE for the EU Member States and its trading partners. Specific international trade standards for EBL are provided for in Chapter 11.9. of the Code as well as in Chapter 2.4.11. of the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.

There are several legislative acts in the EU that pertain to EBL, of which the most relevant ones are:

- Council Directive 64/432/EEC[2] of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine. The Directive specifies, inter alia, that the transport of bovine animals to another Member State is authorised if the animals are accompanied by a health certificate attesting compliance with certain requirements for EBL. For herds with an official EBL free status, it is required that newly introduced animals come from herds with the same health status in order to maintain the official EBL free status. In addition, the Directive lays down the obligation to notify the suspected presence of EBL to the competent authority. Furthermore, Member States are obliged to report annually to the Commission details of the occurrence of EBL on their territory.
- Council Directive 82/894/EEC[3] of 21 December 1982 on the notification of animal diseases within the Community. Its latest amendment introduced the obligation for Member States to notify the Commission of the confirmation of any outbreak, infection or presence of EBL in a herd or the withdrawal of the officially free status in a Member State or region thereof officially free from EBL.
- Council Directive 88/407/EEC[4] of 14 June 1988 laying down the animal health requirements applicable to intra-Community trade in and imports of deep-frozen semen of domestic animals of the bovine species and Council Directive 89/556/EEC of

[1] 2003/467/EC: Commission Decision of 23 June 2003 establishing the official tuberculosis, brucellosis, and enzootic-bovine-leukosis-free status of certain Member States and regions of Member States as regards bovine herds (Text with EEA relevance) (notified under document number C(2003) 1925). Official Journal L 156 , 25/06/2003 P. 0074 – 0078.
[2] Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine. OJ 121, 29.7.1964, p. 1977–2012.
[3] Council Directive 82/894/EEC of 21 December 1982 on the notification of animal diseases within the Community. OJ L 378, 31.12.1982, p. 58–62.
[4] Council Directive 88/407/EEC of 14 June 1988 laying down the animal health requirements applicable to intra- Community trade in and imports of deep-frozen semen of domestic animals of the bovine species. OJ L 194, 22.7.1988, p. 10–23.

25 September 1989 on animal health conditions governing intra-Community trade in and importation from third countries of embryos of domestic animals of the bovine species. The animal health conditions for trade and imports into the Union of those germinal products pertain, inter alia, to EBL.

- Commission Decision 2003/467/EC[5] of 23 June 2003 establishing the official tuberculosis, brucellosis, and enzootic-bovine-leukosis-free status of certain Member States and regions of Member States as regards bovine herds. This Decision provides a list of Member States and regions officially free from EBL and is regularly updated with information provided by Member States based on the principles laid down in Directive 64/432/EEC.
- Council Directive 77/391/EEC[6] of 17 May 1977 introducing Community measures for the eradication of brucellosis, tuberculosis and leucosis in cattle. This Directive specifies that Member States which have established the presence of EBL among cattle in their territories shall draw up plans for the eradication of this disease.
- Council Directive 78/52/EEC[7] of 13 December 1977 establishing the Community criteria for national plans for the accelerated eradication of brucellosis, tuberculosis and enzootic leukosis in cattle. This Directive includes additional provisions for national plans for the eradication of EBL.

The current knowledge of EBL, its distribution and impact deserve special consideration as regards:

i) The severity of the disease, and its morbidity and impact on cattle production;

ii) The epidemiology of EBL and its capacity to spread subclinically;

iii) The proportionality and effectiveness of EBL control measures;

iv) The appropriate surveillance and monitoring activities in Member States, depending on the risk;

And

v) The fact that some Member States are free from the disease.

The risk manager is in need of updated scientific advice in order to assess if EBL is a disease for which control measures are still justified. This is linked to the existence of free areas within the EU and in some of its trading partners and the possible risk of reintroduction of the disease in these currently free areas. Another important aspect is related to the determination of the morbidity rate and if it can be considered significant at country or regional level; this consequently needs to be assessed against the control measures and their impact on cattle production.

Therefore, the Commission is in need of scientific advice on the assessment of the significance of the risk posed by EBL, its morbidity and the relevance of control measures and surveillance.

[5] 2003/467/EC: Commission Decision of 23 June 2003 establishing the official tuberculosis, brucellosis, and enzootic-bovine-leukosis-free status of certain Member States and regions of Member States as regards bovine herds (Text with EEA relevance) (notified under document number C(2003) 1925). OJ L 156, 25.6.2003, p. 74–78.

[6] Council Directive 77/391/EEC of 17 May 1977 introducing Community measures for the eradication of brucellosis, tuberculosis and leucosis in cattle. OJ L 145, 13.6.1977, p. 44–47.

[7] Council Directive 78/52/EEC of 13 December 1977 establishing the Community criteria for national plans for the accelerated eradication of brucellosis, tuberculosis and enzootic leukosis in cattle. OJ L 15, 19.1.1978, p. 34–41.

Terms of reference as provided by the European Commission

In view of the above, and in accordance with Article 29 of Regulation (EC) No 178/2002, the Commission asks EFSA for a scientific opinion on the following aspects of EBL:

1) the disease profile and significance comprising the morbidity and mortality rates (both quantitative and qualitative) of the disease in animal populations at country or regional level;
2) the assessment of the persistence of the disease in an animal population or in the environment and the routes and speed of transmission of the diseases between animals, the distribution of the disease in the EU, and, the risk of its introduction;
3) the impact of the disease on agricultural production considering the level of presence of the disease in the Union, the loss of production due to the disease and its impact on animal welfare, the biodiversity and environment;
4) the existence of suitable diagnostic methods and disease control tools;
5) the feasibility, availability, proportionality, effectiveness and impact of the disease prevention and control measures.

1.2. Interpretation of the Terms of Reference

TOR1: the disease profile and significance comprising the morbidity and mortality rates (both quantitative and qualitative) of the disease in animal populations at country or regional level.

The disease profile has been described in terms of clinical features, agent and pathogenesis covering the different stages of disease, host range and zoonotic aspects.

TOR2: the assessment of the persistence of the disease in an animal population or in the environment and the routes and speed of transmission of the diseases between animals, the distribution of the disease in the EU, and, the risk of its introduction.

The response to TOR2 summarizes the epidemiology of EBL. It has been divided into 5 subheadings for clarity, mainly because of different data and methodologies applied:

a) history of epidemiology in EU
b) Current prevalence in EU and other parts of the world
c) routes and speed of transmission
d) persistence in an animal population
e) risk of introduction

TOR3: the impact of the disease on agricultural production considering the level of presence of the disease in the Union, the loss of production due to the disease and its impact on animal welfare, the biodiversity and environment.

The impact of EBL has been considered separately for a) lymphoma frequency, b) impact of BLV infection on various production parameters and c) impact on welfare. The impact of the disease on biodiversity and the environment has been addressed by evaluating evidence, if any, for the presence of BLV in species other than ruminants and wildlife.

TOR4: the existence of suitable diagnostic methods and disease control tools.

The response to this TOR covers the description of diagnostic methods for identification of infection, persistent lymphocytosis and lymphomas. Vaccination can be considered a potential control tool and has been dealt with under control measures.

TOR5: the feasibility, availability, proportionality, effectiveness and impact of the disease prevention and control measures.

Control measures (a) are understood as measures that limit or eliminate disease and/or infection. Disease prevention (b) includes measures to prevent introduction of infection and documentation of freedom from infection. Feasibility is understood as the practicality of implementation whereas proportionality is assessing the added value of allocating resources for that measure. Availability can refer to the measure itself or other conditions needed for the measure to take effect. Effectiveness is understood as the ability to reduce incidence and prevalence, preferably measured on a quantitative scale.

2. Data and methodologies

This scientific opinion has been prepared using published scientific literature and epidemiological data from different regions of the world, published in various governmental reports. Scientific articles in English, German or Spanish have been identified using EBL and bovine leukemia virus (BLV) keywords in these languages using Google scholar and ISI Web of Knowledge. Governmental reports and data derived from meat inspection at slaughterhouses have been identified by searching Google using the same terms.

A technical hearing on enzootic bovine leukosis was held on March 11–12, 2014 with experts from EU, US, Argentina and Japan, to provide an update of the global epidemiological situation and the most recent data and expert knowledge on the impact of EBL. A summary of the hearing, agenda and participants has been included in the minutes of the 5th meeting of the Working Group on enzootic bovine leukosis, Technical hearing on Enzootic bovine leukosis, Parma, 11–12 March 2014.[8]

A basic age-structured model was used to estimate the time it takes before BLV infection will be detected by lymphoma development, if it is introduced in a free herd. The following parameters were included in the modelling: annual cull rate, age at infection, case age distribution and basic reproduction ratio.

A systematic literature review covering the period from 1972 onwards has been performed to support the assessment of impact of EBL on production. The full protocol of the systematic review is given in Annex A (http://www.efsa.europa.eu/en/efsajournal/doc/4188ax1.pdf).

Table 1: Overview of data and methodology applied in response to TORs

TOR	Data and methodology
TOR1	description based on peer-reviewed original articles and reviews.
TOR2 a	peer-reviewed original articles and reviews; EU scientific conference proceedings.
TOR2 b	EU animal health reports; OIE WAHID; peer-reviewed original articles; technical hearing.
TOR2 c,d	description based on peer-reviewed original articles and reviews.
TOR2 e	peer-reviewed original articles and reviews; model simulation.
TOR3 a	EU animal health reports; peer-reviewed original articles; technical hearing.
TOR3 b	Systematic review of impact on milk yield, culling, calving interval, mastitis.
TOR3 c	description based on peer-reviewed original articles and reviews.
TOR4	peer-reviewed original articles and reviews incl. OIE diagnostic manual and Dir. 64/432/EEC
TOR5 a,b	Reviews; review of current legislation

[8] http://www.efsa.europa.eu/en/ahawwgs/docs/enzooticbovleukosis.pdf

3. Assessment

This scientific opinion has been structured according to the terms of reference. One main chapter is dealing with the disease profile, significance and diagnosis, answering ToR 1 and ToR 4. This chapter attempts to describe the intrinsic potential nuisance value, based on general data/knowledge concerning the disease. A second chapter expands on the epidemiology of EBL and its capacity to spread subclinically answering ToR 2. A third chapter is dealing with the impact of the infection and disease referring to ToR 3. The last chapter describes control options and preventive measures to maintain freedom from BLV infection.

3.1. Disease profile and significance (TOR 1)

Enzootic bovine leukosis is a disease of cattle caused by bovine leukemia virus (BLV), a member of the family Retroviridae. The term leukosis was initially used to describe the neoplastic manifestations of transmissible leukaemia/leukosis in chicken (Ellermann and Bang, 1908). Of the diverse bovine neoplasms encountered at post-mortem examination of slaughter animals, lymphomas (often termed lymphosarcoma or malignant lymphoma) have been one of the most frequently identified types (Dukes et al., 1982; Vernau et al., 1992). Early studies suggested that juvenile lymphosarcomas (< 2 years of age) and thymic, and skin leukosis were sporadic and separate entities from EBL. This has subsequently been confirmed by numerous studies showing that only EBL is associated with and indeed induced by bovine leukemia virus.

Before 1960, EBL was a widespread disease in dairy herds in Northern/Eastern Europe and North America with high herd prevalence. In 1964, OIE recommended all countries to set up control programs for EBL (see Appendix A). The main recommendation was to avoid trade of breeding animals from EBL affected herds. In 1967, OIE stated that EBL was the most common tumour in cattle, causing considerable economic losses in Europe and North America, and recommended that countries should set up diagnostic capabilities based on haematological examination. In countries where no control measures were implemented, the disease tended to become endemic.

The main characteristic of EBL from an epidemiological viewpoint is its very slow spread and manifestation. The first lymphomas may only appear 5 years after introduction of infected animals and, if introduced into a free population, it may take decades before a high prevalence and impact is reached. Lymphomas induced by BLV invariably leads to death within months. Ante-mortem, lymphomas may induce a range of clinical manifestations depending on organ involvement and degree of dysfunction. The most frequent are adenopathy, opportunistic infections, weight loss, and a decrease in milk production (Marshak et al., 1962). The development of lymphomas occurs from around 2 years of age and onwards (Bendixen, 1960b).

EBL has been successfully controlled and eradicated from many countries in Europe as a result of decades of systematic control and eradication programmes (Batho et al., 2008). An important consideration, when deciding to invest in eradication of a disease, is whether appropriate measures are in place to minimise the risk of reintroducing the disease, and to have efficient means at hand (notification and control) if it happens. In this respect, EBL can be considered a particularly long-term investment, where the benefits and returns may only come decades later, while critically relying on continuous enforcement of measures to prevent reintroduction. This also implies that measures to prevent reintroduction should remain in place after the disease-free status has been obtained in order not to lose the resource invested in eradication.

The impact of EBL obviously differs between countries where the disease is endemically present, and countries, where eradication programs have led to its elimination, or to a very

low prevalence as it is the case in the EU. In the first scenario the impact is derived from disease manifestations, whereas in the second scenario the impact is related to maintaining a disease-free status and potential loss of investment in eradication. The description of these two scenarios is conceptually different, the first depending on the disease profile, whereas the second is better described in administrative and economic terms. The two descriptions together may provide necessary elements for prioritising between diseases being present or absent, in particular where a policy of 'prevention is better than cure' is applied.

Several studies have exploited a possible link between occupational or dietary exposure to BLV and human cancer (Miller and Van der Maaten, 1982; Matsumoto et al., 2007; Baltzell et al., 2009; Buehring et al., 2014); however, to date there is no firm evidence that BLV constitutes a human health risk.

3.1.1. Agent and host species

An infectious aetiology of EBL was strongly suggested on the basis of early epidemiological studies (Bendixen, 1963), but it was not until 1969 that retrovirus-like particles were demonstrated in cattle with lymphosarcoma (Miller et al., 1969).

Retroviruses infect a wide range of animals, including cattle, sheep, and goats (Burmeister, 2001). They are associated with important diseases in veterinary medicine, e.g. leukaemia/lymphoma (chicken, cat, and cow), pulmonary carcinoma (sheep), anaemia (horse) and immunodeficiency (cattle, sheep, goat, and cat). In the different host species, the different members of the family Retroviridae cause diseases generally associated with either immunosuppression and/or lymphoma formation.

Natural BLV infection has only been confirmed in three species: Bos Taurus (domestic cattle), Bos indicus (zebu) and Bubalus bubalis (water buffalo). However, BLV can be transmitted experimentally to a number of species. Inoculation of sheep and goats with BLV leads to leukemia/lymphoma after a shorter latency periods (~1–3 years) than in cattle. A case of lymphoma was recently reported in alpaca. Infection of BLV in rabbits leads to a fatal acquired immunodeficiency syndrome (AIDS)-like disease similar to rabbit snuffles. Infection with BLV has been suspected, but not confirmed, in some species (e.g. capybara, rhesus monkeys, chimpanzees, antelopes). Under natural conditions, no evidence of BLV infection has been found in other wildlife species (e.g. deer, llama).

BLV belongs to the genus Deltaretrovirus within the family Retroviridae and is structurally and functionally related to other primate and human retroviruses (primate T-lymphotropic virus 1, 2 and 3 (PTLV-1, -2, -3)). Other retroviruses in cattle belonging to different genera are the bovine immunodeficiency virus (BIV) (Gonda, 1992; Gene, 1994), and the bovine Foamy virus (BFoV) (Meiering and Linial, 2001). All retroviruses in cattle cause a persistent, life-long infection. After infection of the cell, the viral genome is reverse transcribed into a DNA copy, which integrates into the genome of the host cell. Using this replication strategy retroviruses become part of the host cell genome and are passed on to all progeny of the infected cell during mitosis. The integrated viral DNA is called provirus. Whether the proviral DNA is transcriptionally active to produce infectious viruses in the infected cell, or remains transcriptionally silent, depends on many cellular and immunological conditions of the host.

BLV replicates mainly in B-lymphocytes. The BLV proviral genome contains regulatory elements (encoding regulatory proteins Tax and Rex) which can activate cellular oncogenes. This activation causes deregulation of the immune system and a chronic, progressive lymphoproliferative disease (Rodriguez et al., 2011). In total, eight different BLV genotypes have been described worldwide with regional clusters (Rola-Łuszczak et al., 2013). No clinical differences have been observed among the different genotypes. Despite sharing particular features of pathogenesis with HTLV and high exposure through milk and meat, all studies have consistently failed to show infection and replication of BLV in humans.

3.1.2. Disease stages

Morbidity in relation to infection with BLV is either directly or indirectly linked to the persistent infection with BLV, or due to the invasive growth of lymphomas in various organs, which ultimately leads to organ dysfunction and general emaciation.

Following infection, various stages in the progression of BLV infection can be identified. These are summarized in the points below and also outlined in Figure 1.

- **Primary infection**: An infected cell (red in Figure 1) with a copy of BLV integrated into the host genome (blue provirus) is transmitted into a susceptible animal. During primary infection, the BLV provirus is expressed into viral particles (blue hexagon), that further infect B cells (yellow). Active BLV replication and initiation of the immune response is responsible for a 'flu-like' syndrome.
- During **persistent infection**, provirus-carrying cells (red) expand mainly by mitosis because of a proliferation of B cells. This phase extends for several months/years and is characterized by an immune dysregulation (e.g. overexpression of cytokines).
- **Persistent lymphocytosis:** In about 30–50% of animals, the number of lymphocytes in blood increases above physiological levels. During this persistent lymphocytosis phase, morbidity is characterized by weakness and opportunistic infections, e.g. mastitis leading to a loss in milk production.
- **Lymphoma phase**: A single infected cell undergoes genetic mutations (black) and forms a lymphoma inside or outside of a lymph node. Lymphomas can occur in infected animals with or without persistent lymphocytosis. A common finding in the field is sudden death due to rupture and hemorrhage of the spleen. The frequency of lymphomas depends on the herd's BLV prevalence.

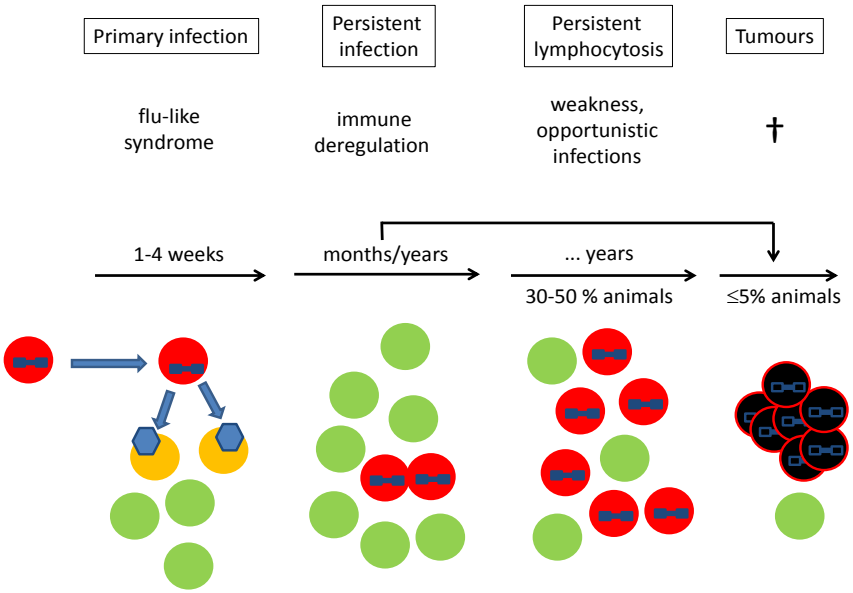


Figure 1: Schematic representation of the different phases of BLV infection. Green cells: Non-infected B-cells; Yellow cells: Cells undergoing infection. Red cells: Infected B-cells harbouring BLV provirus; Black cells: BLV-transformed lymphoma cells.

Primary and persistent infection

BLV is a retrovirus that infects B-lymphocytes and induces a persistent infection in cattle with diverse clinical outcomes (Gillet et al., 2007). Viral transmission occurs through the transfer

of BLV-positive cells from an infected animal to a new host. The BLV virus then actively replicates and infects a population of new target cells (the so-called replicative cycle). After a few weeks, the host's immune response strongly limits infection of new target cells. The infected lymphocytes will then proliferate and expand (i.e. clonal expansion by mitosis). The typical proviral load in the peripheral blood (number of cells with integrated proviruses / total number of B cells) is about 1% at this stage. The majority of BLV-infected animals (around 70%) remain asymptomatic carriers of the virus. In these animals, neither clinical symptoms nor alteration of the total lymphocyte counts are clearly evidenced. Thus, they can only be identified by the presence of anti-BLV antibodies and/or viral nucleic acid.

Persistent lymphocytosis

After a latency that extends from a few months to several years, 30–50% of BLV-infected bovines develop a polyclonal proliferation of B cells called persistent lymphocytosis (PL) (Bendixen, 1960a). Polyclonal expansion means that different B-cell clones carrying a BLV virus integrated in the genome proliferate during persistent lymphocytosis. This condition is characterized by an increase in the absolute number of peripheral blood circulating B lymphocytes (above 10,000/mm^3). B-lymphocytes also become more abundant than T lymphocytes causing an inversion of the B/T ratio (Orlik and Splitter, 1996). Persistent lymphocytosis itself is a subclinical feature but these animals may suffer from disturbances of the immune system. Persistent lymphocytosis is usually stable for several years but can also progress to the lymphoma phase. Since the probability of lymphoma development is greater in animals harbouring increased levels of circulating lymphocytes, persistent lymphocytosis in cattle can be considered as a pre-tumour stage (Kettmann et al., 1980b).

Lymphoma development

The most conspicuous clinical manifestation of BLV infection is the development of malignant lymphomas in lymphoid organs (lymph nodes), a condition called lymphoma (often termed lymphosarcoma in USA). Fatal lymphomas are observed in up to 5% of infected animals, predominantly in adult cattle older than 3–5 years. Given that most cattle become infected before or around the first lactation, it can be assumed that cellular transformation and lymphoma formation requires several years to develop.

The clinical signs accompanying development of lymphomas depend on the organ involvement and the stage of progression. The most commonly encountered clinical signs are lymphadenopathy, asthenia, weight loss, constipation, tachycardia, posterior paresis, exophthalmos and fever (Bendixen, 1960b; Marshak et al., 1962).

The development of lymphomas is not necessarily preceded by a phase of persistent lymphocytosis, but this is the case in two-thirds of the animals under classical herd settings (Bendixen, 1963). Unlike persistent lymphocytosis, expansion of lymphoma cells is of mono- or oligoclonal origin meaning that only a single or a few infected cells generate the lymphoma after multiple divisions (Kettmann et al., 1980a).

A particular feature of EBL is the long time period between introduction of BLV into a herd until appearance of lymphomas. Bendixen (1963) describes one such well-documented herd, where the first lymphomas appeared 5 years after movement of breeding animals from a multiple-case leukosis herd. Another well-documented example is given by Zaghawa et al. (2002) where a closed dairy herd was established in Egypt in 1989 by imported breeding animals from US. Clinical signs started to appear in 1996, including decreased milk yield, loss of body weight, and enlargement of superficial lymph nodes. At this time the within-herd seroprevalence in animals above 2 years of age was 72%.

When considering the age distribution of lymphoma development it should be kept in mind that the presence of seropositive animals in the age group > 5 years is not in itself an

indication of the duration of BLV circulating within the herd. A latency period is needed for lymphomas to develop, and therefore the full manifestations of BLV infection within a herd cannot be expected to occur until all cows in the herd have been at risk of becoming infected at a young age, i.e. 5–10 years after introduction of BLV.

Immune dysregulation

B lymphocytes infected by BLV have a peculiar phenotype because they typically express two unusual markers at their external membrane: CD5 (an attenuator of cell signalling) and CD11 (an integrin). These BLV-infected cells persist indefinitely in their host and it is not possible to clear infection. BLV-infected cell clones do not express significant levels of viral proteins but transcribe very large amounts of viral microRNAs. Although the mechanisms of pathogenesis by BLV are not clearly understood, it is expected that these viral microRNAs play a significant role. Another major player in pathogenesis is a viral protein called Tax that acts as an oncogene by stimulating cell proliferation. Tax also activates expression of all other viral structural proteins. Cells expressing viral antigens are eliminated from peripheral blood by an effective cytotoxic and humoral immune response, whereas the immune response is unable to target cells in which viral expression is silenced. Hence, it is impossible to detect significant levels of viral proteins in B cells from peripheral blood.

In BLV-infected animals, there is thus a continuous turnover of infected cells that are spurred to proliferate, but that are almost simultaneously destroyed by the immune response. This process is associated with abnormal expression of a series of cytokines (e.g. IL-2, IL-6, IL-10, IL-12, TNFα and IFNγ) having opposite effects in immune regulation. During this virus-host interaction, lymphoid organs, such as the spleen, exert a major role in controlling infection. After a latency period of several months to years, cytotoxic and helper-associated functions weaken in BLV-infected animals. Therefore, BLV-infected animals exhibit a lower spontaneous recovery from other diseases, such as mastitis (Brenner et al., 1989; Trainin et al., 1996).

As disease progresses, a major proportion of B cells in persistent lymphocytosis tends to reduce proliferation, whereas another subpopulation continues to proliferate and stimulate the immune response. Ultimately, genetic modifications (such as p53 mutations and chromosomal aberrations) occur in an infected cell, triggering the onset of lymphomas (Dequiedt et al., 1995; Zhuang et al., 1997; Tajima et al., 1998; Florins et al., 2008; Frie and Coussens, 2015).

3.1.3. Zoonotic aspects

BLV sequences have been found in human breast cancer tissue (Buehring et al., 2001; Baltzell et al., 2009) but no evidence has yet indicated an etiological role of BLV in human disease.

Studies have exploited a possible link between dietary exposure to BLV and human cancer (Miller and Van der Maaten, 1982; Matsumoto et al., 2007). Farm workers drinking raw milk were tested for disease, especially for leukemia, but neither leukemia nor other signs of infection could be detected. Erythroleukemia was observed in two infant chimpanzees fed unpasteurized milk from a cow naturally infected with BLV (McClure et al., 1974).

It should be noted that a potential human risk was included in the early considerations to control EBL (Theilen et al., 1968); however, there is no unequivocal evidence for an etiological role of BLV in human disease.

3.2. Diagnosis (TOR 4)

Historically, a herd diagnosis of EBL relied upon clinical observations, detection of elevated levels of lymphocytes and lymphomas supported by post-mortem examination. Hematologic keys were developed for the determination of persistent lymphocytosis, and lymphocyte counting became the main diagnostic tools for a number of years. In 1969, BLV was detected in mitogen-stimulated lymphocyte cultures from a cow with lymphoma (Miller et al., 1969) and two years later antibodies to BLV were demonstrated in the blood of infected animals by serological tests (Miller and Olson, 1972).

Comparative studies showed that the haematological tests detected less than half of the animals infected with BLV (Straub, 1978) and serological tests therefore gradually replaced haematology. The serological methods are suitable for large scale screenings but unable to identify early infected cows before the onset of antibody formation, and unable to distinguish between colostral antibodies and antibodies generated in response to BLV infection.

Techniques based on DNA amplification methods, namely polymerase chain reaction (PCR), allow direct detection of BLV proviral DNA. PCR tests can be applied to detect early infections, to verify inconclusive serological test results, and to determine if lymphomas are induced by BLV.

The applicability of the various diagnostic methods is illustrated in Figure 2.

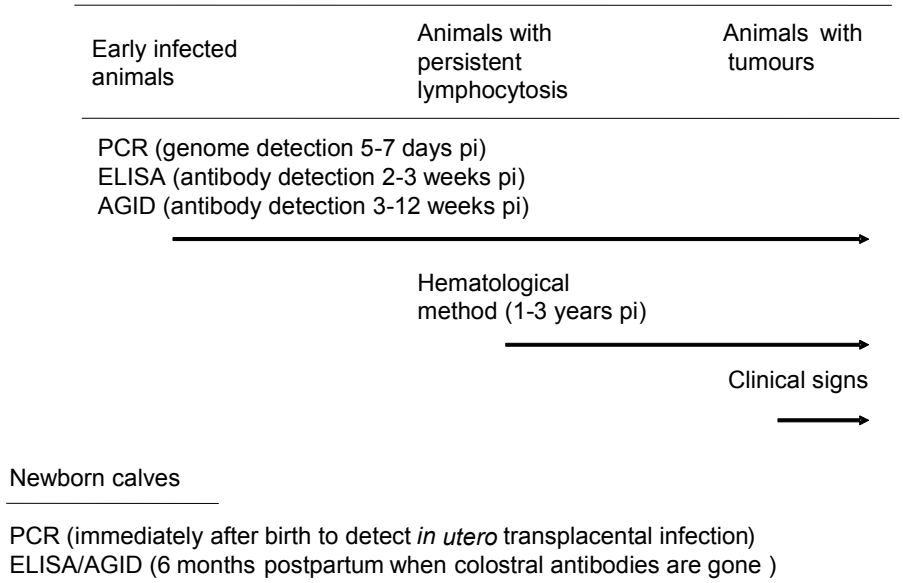


Figure 2: Diagnostic methods applicable during various stages of BLV infection

3.2.1. Haematology

Persistent lymphocytosis, which is characterized by an increase in the absolute lymphocyte count, develops in 30-50% of BLV-infected cattle. This feature was exploited in haematological testing to establish a herd diagnosis in control and eradication programs. Various leukosis keys were developed relating lymphocyte counts to respective breed and age group of animals, presenting maximum values above which an animal was classified as having persistent lymphocytosis (Götze et al., 1954; Bendixen, 1960a).

Some countries established control or eradication programs based on the use of haematological tests for identification of infected animals. These programs had varying

degrees of success, depending on whether the tests were applied to individual animals only or were used at a herd level (Flensburg and Streyffert, 1977).

3.2.2. Post-mortem examination

BLV infection should always be suspected when neoplastic proliferation of lymphoid tissue with either solid lymphomas in multiple sites or diffuse infiltration of organs are present (Marshak et al., 1962). These include peripheral and visceral lymph nodes, uterus, abomasum, heart (especially right atrium), liver, spleen and kidneys. Spleen lymphomas may lead to rupture and sudden death. Lymphomas are usually soft, grey-white, and can include areas of necrosis. Enlargement of palpable lymph nodes is the most typical clinical manifestation of BLV-induced lymphoma. In the lymph nodes lesions comprise hyperplastic follicles and infiltration of lymphoid cells in the medullary sinuses and their focal accumulation in the cortical region (Ishino et al., 1990).

Bovine lymphomas have been evaluated using the formulation developed for human non-Hodgkin's lymphomas (Vernau et al., 1992), and this classification scheme was readily applicable to the histologic classification of bovine lymphomas. The predominant cell type in EBL-associated lymphomas was the diffuse large cleaved cell type, which occurred in 38% of enzootic lymphomas versus 14% in sporadic lymphomas. The mitotic indexes of EBL-associated lymphomas were significantly greater than mitotic indexes in sporadic lymphomas.

According to Council Directive 64/432/EEC 'all animals slaughtered within the territory of a Member State or region must be submitted to official post-mortem examinations at which all lymphomas which could be due to the EBL virus are sent for laboratory examination' to maintain official freedom from EBL. However, it is not specified what is meant by laboratory examination.

Histological examination can support the diagnosis of malignant lymphomas, but is not able to distinguish between sporadic lymphomas and those induced by BLV. Therefore, surveillance for freedom from EBL based on identification of lymphomas at slaughter is only valid if all suspect lymphomas are tested specifically for the presence of BLV genome by PCR or, if a serum sample from such animals can be obtained, for BLV-antibodies.

3.2.3. Detection of antibodies against BLV

In BLV-infected cattle seroconversion occurs from two weeks to three months after infection. Several methods can be used to detect antibodies against BLV. The agar gel immunodiffusion (AGID) test was the first test used for detection of antibodies to BLV (Miller and Olson, 1972). Since then, a number of different tests have been developed: indirect fluorescent antibody test (Ferrer et al., 1972), indirect immunoperoxidase test (Ressang et al., 1976), early polycaryocytosis inhibition test (Guillemain et al., 1977), complement fixation test (Miller and Maaten, 1974), virus neutralization test (Ferrer and Diglio, 1976), and radioimmunoassay (Devare et al., 1977). The identification of the envelope glycoprotein gp51 (Onuma et al., 1975) and application in the AGID test has provided a more sensitive assay for identification of BLV-infected animals, because antibodies to gp51 glycoprotein appear earlier, more regularly and reach higher titres than those directed against the viral p24 capsid protein.

Enzyme linked immunosorbent assays (ELISA) have gradually replaced AGID for routine diagnosis of BLV infection (Florent et al., 1988; Portetelle et al., 1989; Have and Hoff-Jørgensen, 1991; Klintevall et al., 1991) and several ELISA test kits are commercially available today. Evaluation of the diagnostic performance of five different ELISA kits using field sera showed that the sensitivity was very similar (between 97% and 100%) while the specificity varied from 78% to 100% (Reichel et al., 1998). Both AGID and ELISA are

recommended by the World Organization for Animal Health (OIE, 2013) as prescribed tests for serological diagnosis of BLV infection. Both tests have been validated using an international reference serum standard named E05. E05 defines the lower limit of sensitivity for routine testing in AGID or ELISA for serum and milk.

The OIE reference serum E05 has been produced at the Reference Laboratory for EBL in Germany and was tested according to the OIE Quality Standard and Guidelines for Veterinary Laboratories (OIE, 2008). It consists of an international pool of 24 sera from naturally BLV-infected cattle representing all known subtypes which have been tested for their antibody content individually in four different enzyme-linked immunosorbent assays (ELISA) and agar gel immunodiffusion (AGID) tests. It was tested in ring trials in different commercial ELISA and AGID tests by the OIE Reference Laboratories for EBL in the United Kingdom, Germany and Poland, by 27 national diagnostic laboratories in Germany, and by three test kit manufacturers. The results showed that the current reference serum is comparable with regard to antibody content and reactivity with the former EBL OIE positive reference serum E04. It is subject to a batch control every 6 months in order to confirm antibody content and reactivity.

The AGID standardization method described in the Annex D, Chapter II section B of 64/432/EEC differs from the method recommended in the OIE Manual (OIE, 2013). In the method described in Chapter II section B of the Directive 64/432 the antigen is calibrated against the OIE reference serum E05. The standardization process must include a standard (reference) antigen. However, a standard antigen is currently not available from the OIE reference laboratories for EBL. In the method described in the OIE Manual the antigen is standardized against the OIE reference serum E05 only. This serum is available from the OIE reference laboratory for EBL in Leipzig (Germany).

The main advantage of ELISA over AGID is its higher sensitivity. In large serological study done with sera from dairy cattle in Argentina the sensitivity of ELISA was 97.2%, while AGID test showed a sensitivity of 79.7% (Trono et al., 2001). When pooled sera were used to classify the entire herd's status, the ELISA allowed detection of antibodies in herds with a prevalence lower than 1%, whereas the AGID test detected only 50% of the herds detected by ELISA (Mammerickx et al., 1985). An additional advantage of ELISA is that not only serum but also milk can be tested (De Boer et al., 1989; Have and Hoff-Jørgensen, 1991; Klintevall et al., 1991). This is particularly useful for bulk milk investigation (Portetelle et al., 1989). The sensitivity of indirect ELISA will determine the number of animals that can be included in a bulk milk sample (OIE, 2013). Currently available kits allow for pooling of up to 100 cows, and bulk milk testing is now universally applied as a primary monitoring test for dairy herds. However, false positive reactions are occasionally encountered in ELISA. This can be attributed to unusually high immunoglobulin content in the sera of some animals. Non-specific reactions have also been noted in sera from cattle vaccinated against BVD or piroplasmosis. The presence of heterophilic antibodies in cattle sera, which bind non-specifically to mouse immunoglobulin, can also be a source of error when mouse monoclonal conjugates are used. Neither AGID nor ELISA discriminates maternal passive antibodies from an active immune response, and do not provide evidence of infection in its early stage (Ballagi-Pordány et al., 1992). Detection of specific antibodies to BLV by AGID or ELISA is not always sensitive enough to detect all BLV-infected cattle (Eaves et al., 1994; Klintevall et al., 1994).

3.2.4. Detection of BLV provirus

Over the last two decades PCR methods to detect proviral DNA of BLV have been developed. The majority of assays are based on a single PCR (Naif et al., 1990; Jacobs et al., 1992; Agresti et al., 1993; Kelly et al., 1993; Eaves et al., 1994); however, semi-nested (Mirsky et

al., 1993) or the nested PCR provide a much higher sensitivity compared to single PCR. Modifications known as PCR-ELISA (Rola and Kuzmak, 2002) and PCR-EIA (Naif et al., 1992) have also been applied to detect proviral DNA. Furthermore, an *in situ* PCR, allowing the detection of minute quantities of BLV DNA directly in intact cells or tissue sections without DNA extraction has been developed (Xie et al., 1997; Duncan et al., 2005; Kubis et al., 2007).

Real time PCR is a suitable method to detect proviral DNA in infected cattle with low, transient, or undetectable antibody levels during the early phase of infection (Kuckleburg et al., 2003; Lew et al., 2004; Heenemann et al., 2012). For diagnostic purposes, sequences of BLV genome located within *pol* and *env* gene are considered the most appropriate target for provirus detection, allowing detection of all BLV genotypes (Kuckleburg et al., 2003; Heenemann et al., 2012; Rola-Luszczak et al., 2013).

Detection of proviral DNA offers several advantages. In young calves it is possible to detect BLV infection in the presence of colostral antibodies. The PCR assay is suitable for differentiating lymphomas induced by BLV from those associated with sporadic bovine leukosis (Klintevall et al., 1994). Finally, PCR methods, especially in real time format, are recommended to elucidate the disease status of animals with inconclusive ELISA results in blood serum (Rola-Luszczak et al., 2013).

3.3. Epidemiology of EBL (TOR 2)

3.3.1. History of epidemiology in Europe

Early descriptions of lymphomas in dairy cattle in Germany date back to the 19th century (Siedamgrotzky, 1878). Studies from the beginning of the 20th century provided important contribution to the identification and understanding of leukosis in cattle as a distinct entity (Knuth and Volkmann, 1916). Several studies over the next decades provided clear evidence that leukosis was an expanding problem (see OIE resolutions in Appendix A), showing both increased incidence and extending its geographical borders from an initial narrow focus around Memel in East Prussia (now Klaipeda in Lithuania). For many years the cause of leukosis remained elusive and attempts to link it with both genetic and environmental factors, as well as a hypothetical infectious agent similar to the avian leukosis agent were made.

In Europe, there was a gradual westward spread of the infection during the first half of the 20th century (Götze et al., 1956), but not all newly infected European countries became infected from mainland Europe. Thus, BLV was introduced into the UK with breeding animals imported from Canada in 1968 and 1973 (Davies et al., 1980). In Denmark, EBL was concentrated in an area that only accounted for 15% of the total cattle population (Bendixen, 1957; Flensburg and Streyffert, 1977), and the annual incidence of diagnosed lymphomas in this area did not exceed 20 per 100,000 head of cattle.

The first attempts to eradicate the disease were made before the causative agent of EBL was discovered. Early epidemiological studies, and observations that EBL was transmissible from affected animals to others by inoculation of blood or cellular material were suggestive of an infectious nature of the disease. It was also well known, that herds with a high incidence of lymphomas had many animals with persistent lymphocytosis. The attempts to eradicate EBL in 1950s and 1960s were based on elimination of herds (Denmark; Germany) or animals (Estonia and former USSR) with persistent lymphocytosis (Bendixen, 1989; Viltrop and Laht, 1996).

In countries, where herd elimination was practiced, the prevalence of EBL was significantly reduced (Stougaard and Flensburg, 1976; Bendixen, 1989). In contrast, in Eastern European countries the selective slaughter of PL animals proved insufficient, as the herd prevalence

continued to increase, further augmented by concentration of animals into larger herds. In Estonia and Lithuania the proportion of infected herds reached 80–90% by the mid-1980s (Viltrop and Laht, 1996; Acaite et al., 2007). In the beginning of 1980s, prevalence studies were conducted in most of the EU and many other European countries on the basis of serological surveys. It appeared that the BLV prevalence in most EU countries with a few regional exceptions was rather low. In Luxemburg EBL has never been registered and in the Netherlands less than 10 infected herds were found. In the Republic of Ireland 13 infected herds were discovered in 1978 following an import of breeding animals from Canada in 1974; the infected animals were rapidly eliminated by test and slaughter. In other EU countries the national herd prevalence remained below 10% (Batho et al., 2008). During the same period the herd prevalence was found to be much higher in EU neighbouring countries, e.g. Slovenia 21,4% and Hungary 58,1% (Burny and Mammerick, 1986).

3.3.2. Prevalence

Prevalence estimates of BLV infection are based on antibody testing in serum and/or milk and may be given as either herd prevalence or within-herd prevalence, reflecting the spread of the virus within and between herds. In the EU, official country or regional freedom from EBL requires that at least 99.8% of herds are free from BLV infection according to set criteria in Council Directive 64/432/EEC.

World-wide

Following the Second World War, lymphoma in cattle was reported in all continents e.g. North America (Theilen et al., 1963), Australia (Clague and Granzien, 1966), Argentina (Sen et al., 1968), Japan and Russia (Ruzina et al., 2013). In most cases the spread has been linked with trade of dairy cattle for breeding.

EBL is regularly reported to OIE by many countries. In 2012 the presence of disease or infection was reported by 51 countries or territories including 3 African, 6 Asian, 18 European and 21 American countries as well as Australia and 1 territory in Oceania.

In countries where compulsory eradication or control schemes are not implemented, the infection tends to become endemic. Murakami and co-workers (2013) have established that the animal-level prevalence of BLV infected cattle in Japan has increased 10-fold since the 1980s reaching 35,2% in 2011. The prevalence of infected dairy and beef herds was 78% and 69%, respectively, with a strong north-south gradient. An increase in herd prevalence has also taken place in Canada. In the 1980s the national dairy herd prevalence was estimated to be about 45% (Reed, 1981). In 2006 the prevalence in the province of Alberta was 87% (Scott et al., 2006) and in the province of Manitoba 60% (VanLeeuwen et al., 2006). In Argentina, the animal-level and herd prevalence reached 33% and 84%, respectively, by 2001 (Trono et al., 2001). In Chile, a herd prevalence of 59% has recently been reported (Felmer et al., 2009). In the United States the prevalence of infected dairy herds in two nationwide studies conducted in 1996 and 2007 were 89,0% and 83,9%, respectively (APHIS, 2008).

In Australia a national voluntary eradication program has been implemented in dairy herds. It has never been attempted to eradicate the infection from Australian beef herds, where the prevalence has been historically very low except for some states in tropical Australia (Kirkland and Rodwell, 2005). In 2010 Australia declared provisional freedom of EBL, and the National Dairy Enzootic Bovine Leucosis Eradication Program aimed for the Australian dairy herd to be EBL free by 2013 (Ryan, 2013). As of December 2013, all but 2 of 5356 dairy herds are declared free from BLV (NAHIS, 2013). New Zealand set up a control program in 1997 and has been free from EBL in dairy herds since 2008 (Anonymous, 2012).

In the Russian Federation BLV is endemic in most of the provinces although national compulsory eradication programs are in place (Rosselkhoznadzor, 1999). Officially, a herd is considered EBL positive only if persistent lymphocytosis or lymphomas have been detected. The animal prevalence of BLV infection according to official statistics has shown a moderate decline over the period 1996-2010 from 12.3% to 7,5% of serologically tested cattle over 6 month of age (Gulyukina, 2011). In the Krasnodar region the animal prevalence has been reduced from 50% to 5% over the last 8 years due to a systematic control program based on serology (Donnik and Tikhonov, 2013). In Ukraine the number of seropositive cattle have been reduced from 359598 to 2316 over the period 2000-2012 (Aranci and Rudyashko, 2013).

EU

A major effort to eliminate BLV-infected herds was made during the period 1993–2010 where EU-co-financed eradication programs were undertaken, leading to official freedom in most MS and a very low level of infected herds in the remaining MS (Figure 1). More than €40 millions were co-funded for this purpose between 1993 and 2009 (see Figure 2).

Official country freedom from EBL requires that 99.8% of herds are certified free from BLV (Council Directive 64/432/EEC). This implies that some infected herds may remain whilst retaining officially free status of the country (see Appendix B). EBL is still present in some of the newest Member States – Romania, Bulgaria (Sandev et al., 2015), Hungary, Croatia and Estonia as well as in Greece. Also certain regions of Poland, Italy and Portugal are not officially free of BLV. Most of the countries are close to achieving the officially free status, or have already reached the desired prevalence level (< 0.2% herds infected) and are in process of applying for free status.

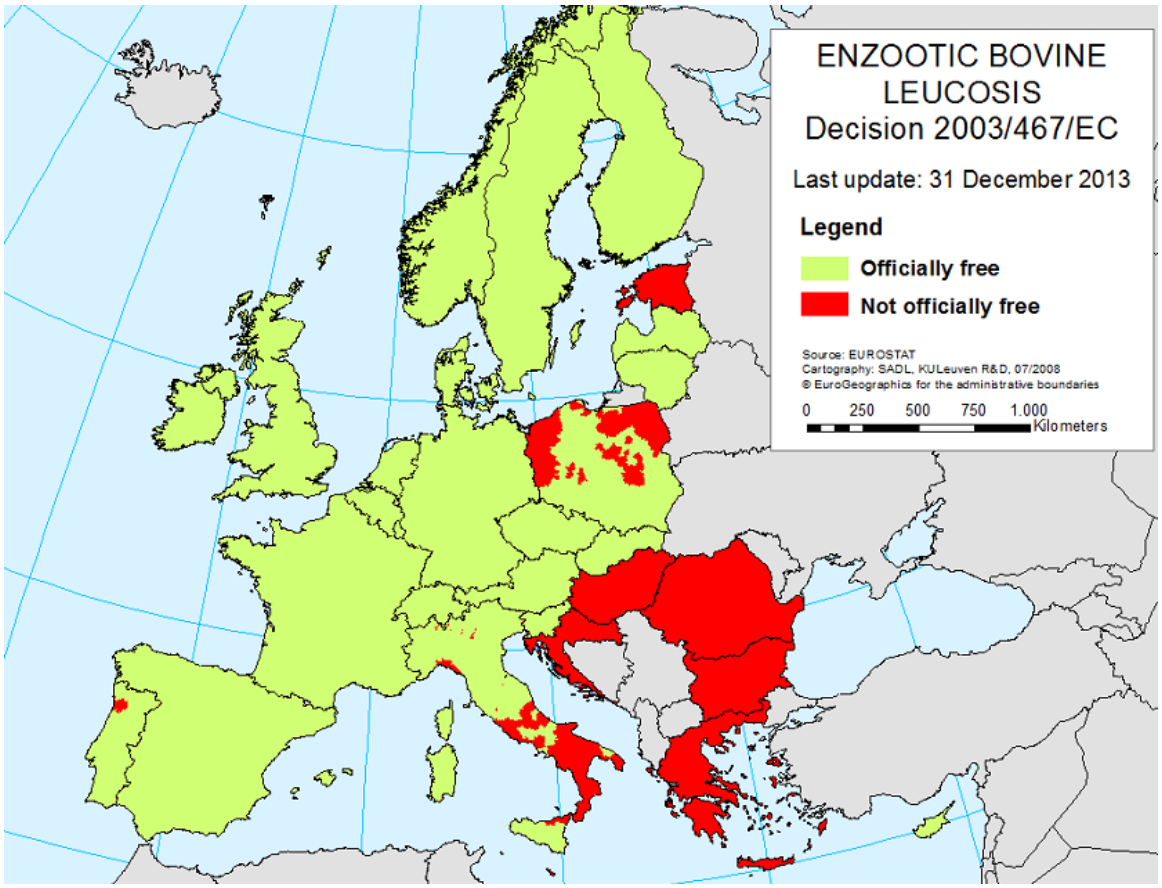
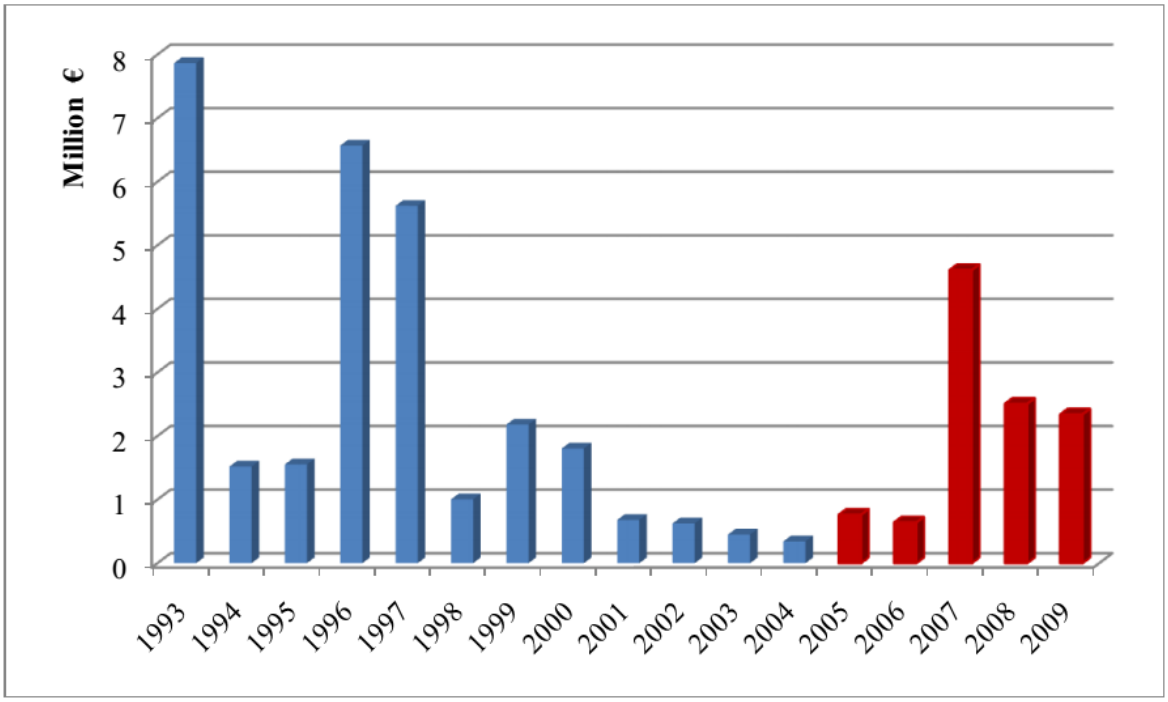


Figure 3: Officially free EU Member States according to Directive 64/432 as of 31 December 2013



Red colour indicates MS enlargement.
Source: Report on the outcome of the EU co-financed animal disease eradication and monitoring programmes in the MS and the EU as a whole. SANCO 2011.

Figure 4: EBL, EU co-funding payments 1993–2009

3.3.3. Routes and speed of transmission

The modes of transmission of BLV have been extensively investigated over the years in experimental and observational studies. Transplacental transmission and/or peripartum infection has been documented and may account for 10–25% of infections (Ferrer and Piper, 1981; Meas et al., 2002; Mekata et al., 2014). However, the main routes of transmission are horizontal, and any mechanism able to transmit blood or infected lymphocytes between animals should be considered. Attempts to infect sheep using semen, urine, nasal secretions, skin scrapings and saliva from infected cattle have been unsuccessful (Takatori et al., 1982; Gatei et al., 1989; Dimmock et al., 1991) with a few exceptions (Roberts et al., 1982; Ressang and Boer, 1984).

Feeding of colostrum from BLV-positive cows may in some cases confer protection through passive antibodies when given to new-born calves (Nagy et al., 2007). On the other hand, it has been convincingly shown that colostrum and milk from infected cows is a risk factor if fed untreated to calves (Romero et al., 1983; Lassauzet et al., 1989). The timing of feeding colostrum containing both antibodies and infected cells appear to be critical, i.e. if fed while uptake of passive antibodies is still functional, calves tend to be protected, whereas, if fed after closure of gut uptake of antibodies, calves appear to have a certain risk of becoming infected. A freeze-thaw cycle has been shown to eliminate infectious BLV from colostrum (Kanno et al., 2013). Milk from infected cows that was treated by a simulated high-temperature short-time pasteurization did not infect sheep and cell-culture derived BLV heated to 60°C or higher for 1 minute did not infect inoculated cells (Baumgartener et al., 1976).

EBL is not a vector borne disease; however, haematophagous insects may contribute to the spread of BLV within a herd by mechanically transferring lymphocytes via biting. The importance of this route of transmission in natural conditions varies in different countries and regions (Hopkins and DiGiacomo, 1997). There is some evidence that horse flies (*Tabanus*

spp.) may have greater potential to transmit BLV within herds in natural conditions (Foil et al., 1989; Manet et al., 1989; Hasselschwert et al., 1993; Kobayashi et al., 2010). Presence of flies in a stable has been reported as a risk factor for higher within-herd prevalence of BLV infection (Kobayashi et al., 2010; Erskine et al., 2012b; Kobayashi et al., 2014).

An important mode of transmission for BLV is iatrogenic. Virus can be transmitted by the use of blood-contaminated needles, instruments for tattooing or dehorning (Lassauzet et al., 1990), via rectal palpation using contaminated gloves, as well as by contaminated vaccines and other immunological products (DiGiacomo et al., 1987). Avoiding iatrogenic spread may reduce substantially the speed of within-herd transmission of the infection (DiGiacomo et al., 1987; Sprecher et al., 1991; Suh et al., 2005). Neglecting biosecurity measures aimed to avoid iatrogenic modes of transmission appear to be a risk factor for high within-herd BLV seroprevalence (Kobayashi et al., 2010; Erskine et al., 2012b).

The use of milking machines has also been shown to be a risk factor compared to manual milking (Mammerickx et al., 1978; Fernandes et al., 2009). The transmission of mastitis pathogens by milking machines, if not properly maintained and managed, is well known (Edmondson, 2001) and it is conceivable that the same mode of transmission may also contribute to the within-herd spread of BLV.

Risk of transmission of BLV via semen or embryos has been considered negligible (Monke, 1986; DiGiacomo et al., 1990). However, both natural mating with infected bulls as well as artificial insemination performed in an infected herd ignoring biosecurity measures may lead to transmission of the virus from infected animals to susceptible ones due to intense direct contact on mating (Erskine et al., 2012b), or through contaminated instruments or sleeves for rectal palpation at artificial insemination (Hopkins and DiGiacomo, 1997).

The rate of direct contact transmission between animals is strongly dependent on within herd prevalence of the herd (Lassauzet et al., 1990; Dimmock et al., 1991). Herd management factors (like housing system, calving management) may impact the spread of the virus within herd (Dimmock et al., 1991; Kobayashi et al., 2010, Kobayashi et al., 2015). It has been estimated that 0.62 (CI95% 0.37-0.89) animals are infected by one infectious animal introduced into a fully susceptible population during a 5-month interval (~1,5/year) in Japan (Tsutsui et al., 2010). Monti and co-workers have estimated the infection transmission parameter (β) based on data obtained in Argentinian cattle herds as 2.9/year (95% CI 1.9–3.7) and, combined with a relatively long infectious period, it resulted in a reproductive ratio (R_0) as high as 8.9. In a simulated transmission study the latter authors found that it would take 30 years to reach a quasi-stationary within-herd prevalence of 80–90% (Monti et al., 2007b).

Longitudinal studies of infected dairy herds have shown that the incidence rate (determined by seroconversion and/or detection of provirus) varies in different age groups. Thus perinatal transmission to new-born calves is only observed in a minority of births (11.5%) from infected dams (Gutierrez et al., 2011) while the incidence increases during first pregnancy and lactation. Although a large number of potential risk factors have been identified, the features of transmission are not known in detail and even strict measures to prevent iatrogenic transmission have not always been able to significantly reduce new infections (Gutierrez et al., 2011).

3.3.4. Persistence

In an animal population

In countries without control schemes the infection tends to persist in the domestic cattle population. In Japan, Canada and Argentina the prevalence has increased during recent decades (see above).

BLV infection has been described occasionally in sheep. In Germany and Brazil single seropositive animals were found (Pannwitz et al., 1988; Del Fava et al., 2010), whereas higher rates of seroconversion were observed in Venezuela in one flock over a one-year period (Marín et al., 1982). In South Africa 20.5% of a flock of 481 Merino sheep kept in a farm with BLV-infected cattle were BLV seropositive (Green et al., 1988). In Stavropol region of the Russian Federation, BLV infection was detected in 5 out of 16 studied sheep farms (Abakin 2004). In a study conducted in Japan, BLV infection was not discovered among sheep (Giangaspero et al., 2013).

BLV infection has been detected and may persist in two other farmed bovine species, Asian water buffalo (*Bubalus bubalis*) and Zebu *(Bos indicus)*. The prevalence among water buffalo was 27.6% in Philippines (Mingala et al., 2009), in Cambodia 16.7% (Meas et al., 2000a), in Pakistan 10.3% (Meas et al., 2000b), and 4.2% in Maranhão, Brazil (Chaves et al., 2012). Marin et al. (1982), Singh et al. (1988) and Jiménez et al. (1995) have reported detection of BLV antibodies in Zebu *(Bos indicus)*.

The presence of BLV in wild animals has been studied only occasionally. In a study conducted among wild European bison (*Bison bonasus*) in Poland one seropositive animal was discovered (Kita and Anusz, 1991). In a similar study among free-ranging American bison (*Bison bison*) at Yellowstone National Park in USA BLV infection was not detected (Taylor et al., 1997). BLV antibodies were not found in free-ranging red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*) in 6 national parks of Germany (Frölich et al., 2006), nor in black-tailed and mule deer (*Odocoileus hemionus*) in California (Chomel et al., 1994). Recently, BLV infection was demonstrated in a 13-month-old farmed alpaca (*Vicugna pacos*) with lymphomas in USA (Lee et al., 2012).

Serological evidence of BLV infections in other species is most likely the result of spill-over from bovine animals or unverified false positive testing results. There is no evidence to suggest that any significant reservoir of BLV exists among species other than cattle.

In the environment

BLV is cell associated and is not excreted in its free form into the environment. Infected cells may survive for a limited time in blood or milk. Infected cells are sensitive to freezing (Kanno et al., 2013) and high temperatures (Baumgartener et al., 1976) and are readily inactivated by UV light (Graves and Jones, 1981), thereby losing the ability to replicate and transmit BLV. However, in vitro at 4°C the BLV in cells survived in blood containing anticoagulant and BLV antibodies for at least two weeks. In these experiments, the virus survived for at least four weeks in blood without BLV antibodies (Roberts et al., 1981).

3.3.5. Likelihood of introduction and spread

Given that 14 officially EBL-free MS reported the presence of BLV-infected herds in 2013 (Appendix B) the question about risk of introduction of BLV into the EU is not the only concern. Nevertheless, it must be stated that any uncontrolled import of live cattle from third countries not free from EBL would represent a high likelihood of repeated introduction of BLV into free areas of the EU.

An important aspect when implementing regional freedom from EBL is to reduce the likelihood of reinfection and reintroduction of BLV into free herds. The EU Member States have devoted considerable effort and resources to EBL control and this investment has been based on the adherence to rules laid down in the OIE International Animal Health Code, which guarantee the absence of reinfection from imported cattle (Toma et al., 1990).

The spread of the infection seen in Europe in the last century until about 1990 and the continued spread seen in other continents where no BLV control measures are implemented

can serve as a good indicator as to what might happen, if current restrictions to control BLV-infection were not in place. The increased prevalence of BLV seen in other continents is partly due to structural changes towards larger units and herd size. This development has also taken place in Europe during the period where eradication was undertaken, and it must be assumed that the dairy herds in Europe are highly permissive to spread of BLV if introduced and left uncontrolled.

Considering that BLV has a very restricted mode of transmission between herds, i.e. by close contact between live animals, a description of movements of live cattle may also provide a good indication of the potential spread of BLV in a cattle population. For more than a decade cattle in the EU have been uniquely identified and all movements recorded during their lifetime (Reg. 1760/2000). This offers a unique opportunity to perform network analysis based on recorded animal movements (Bigras-Poulin et al., 2006; Natale et al., 2009; Dubé et al., 2011; Mweu et al., 2013). The descriptive analysis of a cattle movement network provides important information on the basic characteristics of the connections between holdings using concepts like in- and outgoing infection chains, degree, density, clustering and fragmentation (Nöremark et al., 2011). These estimates may be used to model outbreak scenarios and estimates of epidemic size.

The extent of movements between MS can be summarized by TRACES data from 2012 (SANCO, 2014), where 135275 bovine animals were moved from non-EBL free MS for breeding and 515379 for fattening.

An estimate of the time needed before BLV infection can be expected to be detected by lymphoma development after introduction of BLV into a free herd was made using a basic age-structured model. The following parameters were included in the modelling: annual cull rate, age at infection, case age distribution and basic reproduction ratio. The details of parameter estimation and calculations are described in Appendix D.

Assuming an R_0 equal to 2 (Monti et al., 2007a; Tsutsui et al., 2010) in a high replacement herd, the probability of detecting the first infected animal is about 20%, which would be about 5 years after introduction. The probability of detecting second generation infection is $1-(0.8)^2$ = 36%, and with an expected generation time of about 4 years, and about 6 years for development of clinical disease, it will take about ten years to detect second generation infection. There is a probability of about 60% that third generation infection will be detected. Thus, the infection is likely to be detected between 10 and 15 years after introduction, but longer delays are also possible (p=17%).

3.4. Impact of the infection and disease (TOR 3)

The development of B-cell derived lymphomas is the main manifestation of infection with BLV. Many attempts have been made to quantify the health and economic impact of BLV-infection and subsequent lymphoma development, but also effects on production parameters (milk yield, culling, reproductive performance) and possible effects on immune-competence have been studied. The impact of EBL is mainly on dairy herds, whereas there is only limited incentive to control EBL in beef herds. Based on the conclusion that BLV does not have a reservoir outside cattle, it was also concluded that any impact on biodiversity and environment would be negligible.

A systematic review of published scientific literature was undertaken to identify, appraise, and synthesise publicly available scientific information on the impact of EBL in dairy herds considering the presence of lymphomas and the loss of production due to the infection. The detailed protocol of the systematic review and its results are available in Annex A.

The search strategy used to identify relevant studies comprised two elements, i) a series of strings aiming at capturing articles where EBL is reported and ii) a string identifying the papers in which the exposure to the infection is measured using one of the tests of interest.

The search identified a total of 5,181 articles. Title and abstract screening led to the exclusion of 4,926 articles. Of the 255 articles eligible for full text screening 221 were found not meeting the eligibility criteria, 3 did not report data suitable for the data extraction phase and 31 were finally considered eligible for inclusion in the systematic review.

The reliability of the studies to be included in the systematic review was appraised by study design and outcome considering the following domains of potential source of bias:

- Comparison/Confounding bias;
- Attrition/Exclusion bias;
- Selective reporting bias;
- Information/Detection bias for the outcome.

A critical appraisal tool was developed to define the elements to be considered for the appraisal.

For each outcome the studies were assigned to three different 'tiers of reliability' along with clear explanation for their allocation. From a methodological point of view, Tier 1 represents studies at lower risk of bias while Tier 3 the ones at higher risk.

A study was classified in Tier 1 if all the domains of potential source of bias were rated as definitively low or probably low. Tier 3 comprises studies where more than two domains of potential sources of bias were rated as probably high or definitively high. A study was classified in Tier 2 when it did not fall in Tier 1 or 3.

The studies were additionally classified into studies not using statistical models, where the final outcome of interest was directly extracted from the data recorded in the field e.g. a mean, and studies using statistical models, where the final outcome of interest was provided by using a model (univariate or multivariate).

Several factors may influence the impact of EBL on production and the magnitude. The direction of the potential biases identified in the systematic review could not be assessed. Hence, in order to reach final conclusion on the EBL impact, it was decided to consider only studies falling under Tier 1.

3.4.1. Lymphomas

Systematic review

The systematic review led to the identification of one single study (Thurmond et al., 1985b). This was a cohort study conducted over 2.5 years in which 7,760 animals were enrolled. Rates of malignant lymphoma (ML) in California dairy cattle and relationships between ML and presence of antibodies to BLV were investigated. Sera from slaughtered California Holstein dairy cows were tested by agar gel immunodiffusion for presence of antibodies to the gp51 and p24 antigens of BLV. Highly elevated prevalence rates of ML in gp51 and p24 antibody-positive cows were 172.6 and 511.5 per 10,000 cows, respectively. Out of 2,521 gp51-seropositive animals, 36 lymphomas were reported, while out of 5,239 seronegative animals, 1 lymphoma was identified (2.3 per 10,000 negative animals). The frequency of lymphoma was thus 75 times higher among BLV-infected cows than among BLV-free cows.

Narrative review

Any attempt to quantify the impact of lymphoma development will depend on lymphomas actually being diagnosed and reported and this presents particular problems for EBL. A fraction of lymphoma cases may lead to sudden death or euthanasia at farm level followed by rendering with or without clinical diagnosis. Historically, it has been estimated that about 15% of lymphoma cases were rendered without a diagnosis being made (Bendixen, 1963). The remaining cases will be sent for slaughter, where meat inspection will identify the majority (2013). Early stages and non-invasive visceral lymphomas may be subject to local condemnation only and therefore may remain unreported.

Compulsory notification of lymphoma cases became an integral part of control schemes both in national and EU legislation on eradication of EBL (Council Directive 78/52/EEC). However, with the advent of serological and virological diagnosis, there has been an apparent shift in focus with less suspect lymphomas being notified and examined. Contemporary farm-level figures of death, euthanasia and rendering caused by EBL are not being recorded in Europe or elsewhere, and are only available from limited studies of selected farms.

In the most recent annual report on bovine and swine diseases 2013 from DG SANCO (see Appendix B) 14 MS reported the presence of BLV-infected herds (total 27,114 infected herds); however, very few suspect cases and no confirmed slaughterhouse cases of lymphoma were reported. Given the number of infected herds, it seems highly unlikely that no lymphoma cases have developed in the EU dairy herds. Furthermore, not all MS apply specific PCR testing for BLV on suspect lymphomas. The current surveillance system probably suffers under-reporting of lymphoma cases, hence contemporary figures and data from meat inspection do not allow for an assessment of the real frequency of lymphomas in EU.

Lymphoma frequency

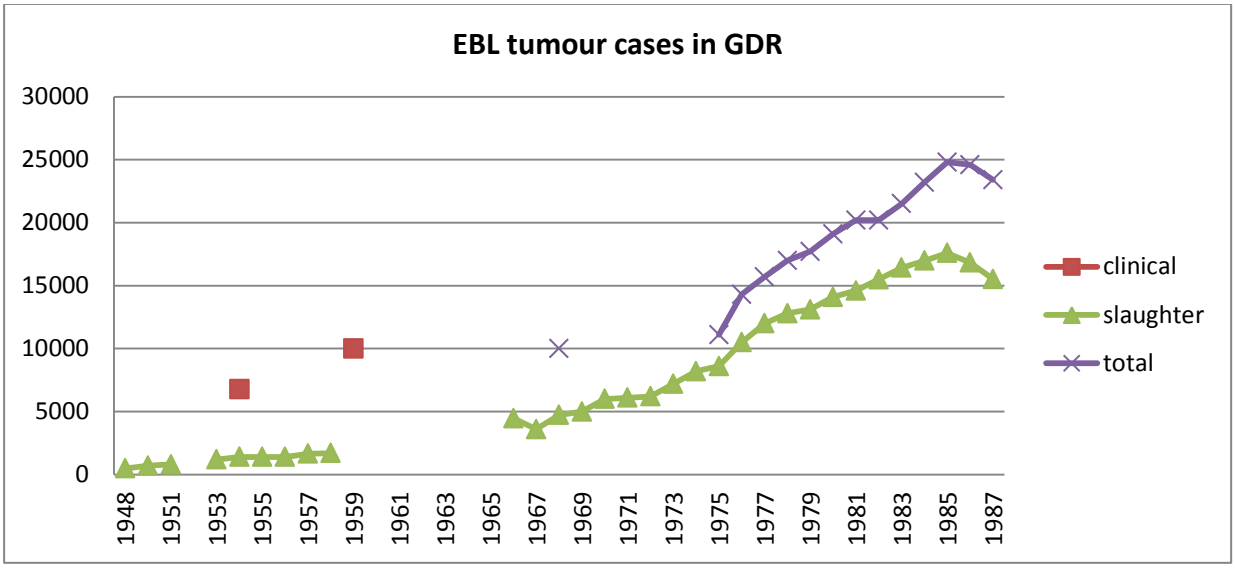
The development of lymphomas is a terminal manifestation of BLV-infection, hence lymphomas are recorded at the end of the productive life of animals, either at slaughter (where lymphomas may have been the reason for slaughter) or in the herd due to sudden death or euthanasia. The number of animals developing lymphomas may be recorded per year in the population at risk (period prevalence), but more often it is recorded at slaughter during meat inspection as the prevalence of animals condemned due to lymphoma.

One of the major difficulties in comparing the lymphoma impact over time is that early studies were based on lymphoma incidence without knowing the prevalence of BLV-infection, whereas later studies mainly reported serological testing without recording lymphoma incidence. In large herds with consolidated, long-standing infection the annual lymphoma incidence among dairy cows may rise to 1–2% (Bendixen, 1963). On a regional level period prevalences of 1% in dairy cows have been recorded in the past in Germany, corresponding to 2–5% of adult cows developing disease. This latter figure is now also seen in Argentina in herds with a high prevalence (Gutierrez et al., 2011), where the first BLV-infected animals were detected in 1978, and where the prevalence has since risen to a high level (Trono, 2011).

Detailed figures on lymphoma incidence are available from the former German Democratic Republic (GDR), which also had the highest prevalence of EBL in Europe before and in the early phases of eradication (Wiesner, 1967; Wittmann, 1993). Some figures are based on clinical diagnosis, whereas others are compiled from meat inspection data and therefore not directly comparable. Thus, in 1956 an estimated 7,000 clinically diagnosed lymphoma cases were recorded (questionnaire data from veterinary practitioners) while in the same period approx. 1,500 cases (0.1% of all slaughtered cattle) were identified during meat inspection

(Mieth et al., 1970). It was also estimated that rendering would add an additional 15% of undiagnosed lymphoma cases to the overall number.

From 1975 to 1987 the total number of registered lymphoma cases in GDR increased from 11,100 annually to 23,400 (Figure 3), the latter number corresponding to a period prevalence of 1% (Kautzsch and Schlüter, 1990). This figure covers a large variation of both between-herd prevalence and within-herd prevalence. After 1987 the number of infected herds in GDR was reduced rapidly due to systematic eradication. A further improvement was seen following a significant reduction of the cattle population after 1989 by slaughter, targeting slaughter of BLV-infected herds (Mieth and Prange, 1997).



Compiled from Meyer, 1961; Mieth et al., 1970; Kautzsch and Schlüter, 1990; Mieth and Prange, 1997.

Figure 5: Lymphoma cases in the German Democratic Republic 1948–1987

In Sweden, lymphomas were diagnosed in approximately 1% of slaughtered cows during the early 1960s from high-prevalence regions (Järplid, 1964). In the former USSR the frequency of lymphomas in slaughtered animals was 58 per 100,000 in 1980 (Abakin 2004).

In the United States, the latest available published figures from the Food Safety and Inspection Service (FSIS, 2002) for slaughter condemnation (disposition) are from fiscal year 2002 where, out of 5,175,861 cows slaughtered, 38 were condemned ante mortem and 25,037 (0.48%) condemned post mortem due to lymphoma (Table 1). Compared with the period 1958–1967 (Migaki, 1969) this represents an overall increase of 500% in the prevalence of malignant lymphoma in adult slaughtered cattle. More recent data have been published (White and Moore, 2009), showing that the proportion of dairy cows condemned post-mortem due to lymphoma had increased to approximately 0.8% for the period 2005–2007. The frequency of condemnation of dairy cows due to malignant lymphoma in Californian slaughterhouses over the period 1979–1982 was 0.8% (Thurmond et al., 1985a). Although a link between lymphoma and BLV-infection has not been established in these slaughterhouse data, it can be assumed that more than 99% of such lymphomas are induced by BLV, based on the data of Thurmond et al. (1985b).

Table 2: Number of slaughtered dairy cows condemned due to lymphoma in the US, 1998–2007

Year	Cows slaughtered	Condemned post mortem due to lymphoma	% condemned

1998	5,886,745	20,907	0.36
1999	5,342,619	19,479	0.36
2000	5,269,576	24,070	0.46
2001	6,214,474	28,315	0.46
2002	5,175,861	25,037	0.48
2005–2007	7,138,997	58,387	0.82

These figures should be considered minimum figures of the actual lymphoma prevalence, because they do not include the cases dealt with at farm-level. Furthermore, a fraction of carcasses may have been approved after partial dressing and the gross diagnosis attained 86% accuracy only (Smith, 1965). A detailed study of tumours identified at slaughter (Reisinger, 1963) found that more than 90% of slaughtered cows with malignant lymphoma were sent to slaughter due to clinical illness or being unthrifty.

In Canada, official figures from meat inspection covering the period from 1999 onwards are published by Agriculture and Agri-Food Canada (http://www.agr.gc.ca/). The figures are based on total numbers of adult cattle and therefore include both dairy and beef cattle (Table 3).

Table 3: Number of slaughtered adult cattle condemned due to lymphoma in Canada, 1999–2014

Year	British Columbia – Saskatchewan – Manitoba	Alberta	Ontario	Quebec – Atlantic	CANADA
1999	2.25[(a)]	0.23	5.52	37.68	4.08
2000	1.71	0.28	4.57	36.05	3.58
2001	1.39	0.31	0.14	38.78	2.97
2002	1.05	0.33	0.02	39.15	2.87
2003	1.33	0.3	0.04	51.21	3.47
2004	0.14	0.37	1.22	37.68	3.1
2005	1.74	0.41	2.88	32.96	3.39
2006	2.36	0.61	5.29	36.31	4.38
2007	1.36	0.58	1.94	37.22	3.33
2008	1.36	0.32	0.15	44.47	3.02
2009	8.42	0.35	0.26	54.57	3.26
2010	20.99	0.6	0.51	48.77	3.53
	West		**Ontario**	**Quebec – Atlantic**	**CANADA**
2011	0.29		1.78	47.85	3.43
2012	0.14		4.69	42.06	2.57
2013	0.08		7.39	13.13	1.9
2014	0.33		5.58	11.32	1.62

(a): condemned per 10,000 slaughtered

Until 2012 approximately 0.5% of slaughtered animals in the high-incidence region (Quebec-Atlantic) were condemned due to lymphoma. The significant drop seen in 2013 and 2014 remains unexplained.

Table 4 summarizes reported prevalence and cumulative incidence figures. The figures are based on denominators including a variable proportion of non-infected animals. Farm-level

lymphoma cases are also not included, therefore these figures should be considered minimum estimates of the overall lymphoma frequency.

Table 4: Summary of lymphoma frequency figures

Country	Period prevalence	Cumulative incidence
East Germany 1985	1%	(2–5%)*
Sweden 1960		1%
USA 2002		0.48%
USA 2005–2007		0.8%
California 1979–1982		0.8%
Canada 1999–2014		0.5%

*calculated, depends on annual replacement rate

EBL has been successfully eradicated from a number of MS. In these countries, there is clearly no disease impact. In countries, where eradication is still ongoing, residual, infected herds tend to be recently infected herds, not detected in previous rounds of screening. Such herds will not have been infected sufficiently long time for lymphomas to develop. Therefore, the disease impact of lymphomas is expected to be low or absent in Member States with low prevalence of infection, but not yet officially free.

The current surveillance system probably suffers under-reporting of lymphoma cases, hence contemporary figures and data from meat inspection do not allow an assessment of the real frequency of lymphomas in EU.

There is some uncertainty in estimating the frequency of lymphomas due to BLV infection. This is mainly due to the fact that lymphomas are not all recorded due to deaths on farms and partial condemnation at the abattoir that are not recorded. Furthermore, a part of the lymphomas are not due to BLV infection, but this part might be very low and can be ignored. Furthermore, figures on lymphoma prevalence from a given country cannot be extrapolated easily to other countries, because they are highly dependant on the prevalence of BLV infection in the area and on the age at culling that differs strongly between countries, depending on farming intensification and dairy versus beef production.

In countries with modern dairy production systems and no control program for EBL, the best estimate of the cumulative lymphoma incidence in BLV-infected cows is 1–2%. In high-prevalence herds the cumulative lymphoma incidence among dairy cows may reach 5%.

3.4.2. Production

The effect of BLV infection on the life span and productivity of dairy cows has been studied using different approaches and designs. However, due to a strong and complex influence of age and breeding value on BLV prevalence, milk production and culling rate, it has proven difficult to draw firm conclusions from most published studies. This section is based on a systematic review of scientific papers that have studies contemporary dairy herd profiles (Annex 1).

Milk production

The impact of BLV infection on milk yield is difficult to assess from observational studies because both parameters are strongly influenced by age, herd size, lactation number and genetic potential and because high milk yield is correlated with increased risk of transmission. A simple comparison of milk yield between seronegative and seropositive cows

within a herd usually finds that seropositive cows have higher yield than seronegative cows (Jacobs et al., 1991; Pollari et al., 1992; Da et al., 1993). This is partly due to increasing BLV-prevalence with increasing lactation number, but it is also observed within each lactation group. The latter finding may be a result of increased susceptibility and/or exposure to BLV due to genetic and management factors (Pollari et al., 1992).

Several studies have shown a link between the bovine leukocyte antigens (BOLA) and susceptibility or resistance to BLV (see Miyasaka et al. (2013) and references therein) and one study has demonstrated the link between breeding value and likelihood of BLV infection (Wu et al., 1989). Thus, seronegative cows may not provide an appropriate comparator when assessing the impact of BLV infection on milk yield in within-herd studies. Furthermore, some studies only included complete lactations and excluded the last lactation in which the cow was culled. Given that the effects of BLV infection would be expected to be late manifestations this may also have diminished any observable difference.

Given the difficulty of assessing the impact of BLV infection on milk production, the choice of study design and use of appropriate statistical methods to correctly account for important confounders is considered important. The results of the systematic review related to milk production outcomes are summarised in Table 5 below.

In the study of Ott et al. (2003) the reduction was estimated to be 9.5 kg per cow (total herd) per year for each percentage-point increase in the within-herd prevalence of BLV-infected cows. In the study of Erskine et al. (2012a) the corresponding figure was estimated to be 10.5 kg per cow (total herd) per year. This would correspond to a reduction of milk production in BLV-infected cows of 950–1050 kg/cow per year.

In conclusion, a minor, but statistically significant reduction in milk yield has been demonstrated in BLV-infected animals.

Table 5: Outcome of systematic review of milk yield for papers with low risk of bias

Author	Measured outcome	Results	Notes
Heald et al., 1992	305 day me, BCA index, estimated difference from herd mean	Negative animals 2.55 (SE: 0.99) Positive animals: 3.33 (SE: 2.19)	No statistically significant difference. Within-herd comparison.
Ott et al., 2003	Yearly milk yield, modelled with many covariates	Keeping fixed all the other parameters a change of 5% in the prevalence at herd level induces a loss of 47.4 kg per cow of milk production.	None
Erskine et al., 2012a	Yearly milk yield, multiple regression model at herd level	Interpretation offered by the authors is: '…every 0.1 increase in the proportion of cattle positive for BLV was associated with a decrease of 140 kg of milk production…'	None

Reproduction

Calving interval was chosen as an indicator of reproductive problems in the systematic review. The results of the systematic review are summarised in Table 6.

Two studies were identified. The paper of Heald et al. did not report any statistically significant association between BLV infection and calving interval. Tiwari et al. found a statistically significant association between BLV infection and calving interval after the first lactation, but not from the second lactation onwards.

Overall, these studies did not indicate an association between BLV infection and a prolonged calving interval.

Table 6: Outcome of systematic review on calving interval for papers with low risk of bias

Author	Measured outcome	Results	Notes
Heald et al., 1992	Estimated mean difference in months from herd mean, standard error (SE)	Seronegative animals: -0.16 (SE: 0.11) Seropositive animals: 0.47 (SE: 0.26)	No statistically significant difference.
Tiwari et al., 2003	Linear mixed model for calving interval	Statistical association with seropositivity after 1st lactation. Increase of 1.97 log days (95%CI 0.29; 3.65) No statistical association with calving interval from second lactation onwards.	None

Mastitis

The influence on mastitis has been examined by comparing somatic cell counts in infected and uninfected cows. The results of the systematic review are summarised in Table 7.

One study was identified, and it did not report any statistically significant association between BLV infection and mastitis.

Table 7: Outcome of systematic review on mastitis for papers with low risk of bias

Author	Measured outcome	Results	Notes
(Heald et al., 1992)	Natural logarithm of Somatic Cell Count. Estimated mean difference, with standard error (SE), from herd mean.	Seronegative animals: 0.073 (SE:0.037) Seropositive animals: 0.19 (SE:0.064)	No statistically significant difference.

Culling

In a given herd that maintains a constant size, the culling rate will determine the distribution of age and lactations of the cows. Culling itself can be either voluntary (number culling) or forced, and the reasons for culling usually differ according to lactation number. Low milk yield is a significant reason for culling in early lactations. On the other hand, forced culling due to a variety of disease conditions (including EBL) tends to increase with age and lactation number. Within-herd studies of the effect of EBL should therefore preferably be stratified according to lactation number. Most studies group EBL status according to

seropositivity, which does not allow for a precise linking in time, because the time of infection remains unknown, and because the effect of BLV infection is delayed by months or years.

Considerable expense is incurred in raising a dairy cow to approximately 2 years of age when they start producing milk. Herds with poor longevity quickly find themselves populated with first and second-lactation animals, which have not yet achieved their full milk production potential (Erskine et al., 2012a).

Cow longevity measured as average number of lactations may carry a significant lowering component of early cullings due to low milk yield and this would obscure effects of EBL in late lactations. Therefore, studies should preferably focus on the effect of EBL on survival rates from 3rd lactation and higher.

The results of the systematic review are summarised in Table 8. Overall, 4 studies were identified. In three of the four papers a statistically significant, negative association between seropositivity and premature culling was reported. No conclusions on the statistical association could be drawn from the other paper since it did not report any dispersion measure.

In conclusion, a statistically significant association between BLV-infection and premature culling has been demonstrated.

Table 8: Outcome of systematic review on culling for papers with low risk of bias

Author	Measured outcome	Results	Notes
Heald et al., 1992	Estimated mean percentage of culling rate.	Seronegative: 19.4 Seropositive: 24.1	No dispersion measure provided, not possible to say if there is any statistical difference.
Thurmond et al., 1985c	Culling rate (%) range.	The Mantel-Byar test was used to test if cull rates were independent from serological status. Cull rates were higher in seropositive animals above 3 years of age as compared to seronegative animals (P=0.008).	None
Pollari et al., 1993	Cox regression model for culling	Hazard ratio > 1 for cows over 36 months of age.	Statistically significant difference for seropositive animals above 54 months of age.
Bartlett et al., 2013	Cox proportional hazards model. Outcome: days to culling (including both death and culling).	Hazard ratio for seropositive positive animals compared to seronegative animals was 1.23 (p = 0.000)	None

		Hazard ratios for seropositive positive animals (OD>=0.1) compared to seronegative animals (OD<0.1) were 0.1<=OD<0.25: 1.08 (p=0.314) 0.25<=OD<0.5: 1.25 (p=0.001) OD>0.50: 1.40 (p=0.000)	

3.4.3. Welfare

The development of lymphomas is accompanied by chronic illness, progressive loss of body condition, weakness, anaemia and anorexia, attributable to infiltration of lymphomas into various internal organs (Marshak et al., 1962). Lymphomas are not likely to be detected until they cause conspicuous pathophysiological manifestations. The animal welfare consequences in terms of duration and severity may vary according to the location and magnitude of the spread of lymphomas in organs, e.g. heart, kidneys, lungs, central nervous system or gastrointestinal system. Overall, animals are likely to suffer when lymphomas have progressed beyond early stages for the following reasons (Merck, 2014):

- Lesions in the heart, which is most common in EBL, seem to resemble chronic heart disease with symptoms like asthenia, tachycardia, dyspnoea and increased jugular venous pressure. Lesions in the right atrium cause arrhythmias, murmurs or heart failure.
- Infiltration into bronchial, mediastinal and cervical lymph nodes contributes to hyperpnoea or dyspnoea and tracheal constriction.
- Lesions in the abomasum lead to abdominal pain cause anorexia, diarrhoea and constipation.
- Extradural spinal lesions lead to compression of the cord or spinal nerves resulting in pelvic limb paresis.
- Lesions in the spleen lead to rupture and exsanguination into the peritoneal cavity.
- Uterine lesions cause reproductive failure and abortion.
- Lesions in the liver cause jaundice and liver failure.
- Lesions in kidney and ureter cause severe abdominal pain and renal failure.
- Retrobulbar lesions cause protrusion of the eyeballs resulting in keratitis and eventually proptosis.

Due to the above listed factors, it is likely that BLV-infected cattle suffer considerably during the last months of their lives, in particular at later stages of tumour progression.

3.5. Control and prevention (TOR 5)

Following the recommendations from OIE in 1964 and 1967, many countries initiated programs to control EBL with the aim of reducing economic losses and to reduce the spread of the disease by trade of breeding animals.

The feasibility, availability, proportionality, effectiveness and impact of the disease prevention and control measures are directly linked to the herd management, the applied eradication strategies and the measures for maintaining disease freedom.

3.5.1. Control measures

Herd management

Numerous attempts have been made world-wide over the last decades to reduce the impact of EBL in dairy herds by lowering the within-herd prevalence of BLV-infected animals. Some positive effects have been observed in dairy herds when implementing rigorous management procedures. Nevertheless, the prevalence of BLV has been steadily rising, possibly augmented by structural changes towards larger herd sizes.

As long as herd freedom has not been achieved, there are a number of management practices that should be implemented to support control schemes in place. These practices aim at reducing the spread of BLV within the herd as much as possible; hence, a solid knowledge about modes of transmission is important to optimize such practices. The most important practices that should be followed are (Evermann, 2014):

- Use only milk from BLV-negative cows or milk replacer to feed calves. Milk from BLV-infected cows can be used after freezing or heat treatment
- Use chemical dehorning or cautery
- Use disposable needles or needles sterilised by boiling between animals
- Clean and disinfect ear tattoo implements between animals
- Wash stomach tubes and drenching guns between animals
- Use separate gloves for rectal exploration
- Wash and disinfect all equipment used to assist calving
- Use separate calving paddocks for BLV-infected and uninfected cattle
- Remove calves from cows within 24 hours of birth but after intake of colostrum
- Implement a fly control program

Eradication

In general, eradication of a disease having a long incubation period can be accomplished only by programs that include all infected animals, without restricting attention to the 'tip of the iceberg', represented by actual outbreaks of the disease. This applies particularly to EBL, where most of the infected animals will complete their economic life without showing signs of the disease, or even being suspected of infection (Toma et al., 1990).

Applying stringent management tools in dairy herds may lead to a certain reduction of within-herd prevalence, but will not be able to eliminate the infection (Gutierrez et al., 2011). Hence, the only sustainable method to obtain freedom from BLV is by eliminating infected animals. This has now been accomplished in most European countries after 40–50 years of control efforts. Russia initiated control programs in 1965 and has reached a fairly low prevalence by now. Most recently, Australia and New Zealand have also managed to eliminate BLV from their dairy herds. One of the main incentives for New Zealand to embark on an eradication program has been to secure trade in breeding animals, being the world's largest exporter of dairy products (Anonymous, 2012).

Beef herds can remain a significant reservoir for BLV and no distinction is made between dairy and beef herds regarding official freedom from EBL in EU legislation. The same is true

for the OIE code on EBL; furthermore, a partial eradication scheme in the dairy sector would not qualify for freedom using the principles of compartmentalisation.

The experience of European countries has shown that the application of conventional disease control measures can overcome the disease and lead to a very low impact, mainly deriving from maintaining surveillance for freedom from disease.

Test and slaughter

Early control measures were based on identification of lymphomas and haematological testing. In Denmark, where a control program was initiated in 1959, herds having one animal with leukotic tumours or persistent lymphocytosis were classified as suspect and kept under observation for at least 2 years (Flensburg and Streyffert, 1977). If no further cases of lymphocytosis or lymphomas developed during this period the herd was released from observation. Otherwise, i.e. when multiple cases were identified, the owner was offered compensation for slaughtering the whole herd. This led to a gradual but substantial reduction in the herd prevalence of EBL, so that a low rate of serological reactors remained for the final phase of the eradication of the disease (Gottschau et al., 1990).

Following the introduction of serological tests, an animal-based test and slaughter policy was adopted in Europe, replacing previous programs based on haematological testing. Considering the slow spread of BLV, an interval of at least 4 months after removal of BLV-positive animals should be allowed between consecutive testing. Test and slaughter has proven to be very efficient in obtaining freedom from BLV, and this approach was further corroborated by the advent of suitable ELISAs for bulk milk testing, allowing for rapid and cost-effective identification of BLV-infected dairy herds.

The 'test and slaughter' strategy is based on detection and prompt culling of infected animals. As an additional measure, culling of the offspring of infected animals should be considered due to the possibility of perinatal transmission of the virus. Selective segregation of animals according to their proviral load (Gutierrez et al., 2011; Mekata et al., 2015) or level of viral antigen expression in lymphocyte cultures (Molloy et al., 1994) can be used to prioritize the culling of BLV infected cattle with the highest risk of transmission in heavily infected herds.

Freedom can only be achieved if the rate of removal of positive animals exceeds the annual incidence of infection and this becomes increasingly difficult with increasing within-herd prevalence. A test and slaughter scheme would not be feasible if the within-herd prevalence is close to 100%, reflecting that the annual incidence approaches the annual turn-over of animals in the herd. In such situations a test and separate scheme may be applied in an attempt to gradually reduce the prevalence to a level that would allow switching to test and slaughter. The feasibility and proportionality of this control strategy will depend on the level of within-herd prevalence and the culling rate in the herd.

Test and separate

The strategy is based on physically separating (often in a separate barn) the BLV-infected cattle from uninfected ones, and gradual elimination of infected animals by increased culling rate in the group of infected animals. The seronegative group of animals is regularly tested serologically and antibody positive animals separated or eliminated. In the final stage of the eradication program the 'test and slaughter' strategy is applied. Safe management practices should be applied to avoid spread of BLV between animals.

This strategy is suitable and feasible for herds with high within-herd prevalence, where immediate culling of infected animals is not economically sustainable. Various modifications of this strategy have been used in different countries, and EBL has been successfully

eradicated from herds with high prevalence by using this strategy (Shettigara et al., 1986; Shettigara et al., 1989; Wang and Onuma, 1992; Viltrop and Laht, 1996; Suh et al., 2005; Acaite et al., 2007).

It can be concluded that the proportionality of the the disease control measures can be regarded as appropriate. The impact of control measures does not hamper on-farm production processes. The possible negative impact on animal welfare is limited to blood sampling (if milk samples cannot be obtained) of animals for testing.

Post-mortem inspection rules of slaughtered animals require that animals with tumours be identified and disposed of as unfit for human consumption, irrespective of EBL control programs. Thus the impact of EBL surveillance at slaughterhouse inspection is limited to costs of laboratory examination.

It can be stated that the effectiveness of the the disease control measures is directly linked to herd management measures. A systematic elimination of infected animals is the only sustainable way to obtain freedom from BLV. A stringent herd management in dairy herds (e.g. only milk from BLV- cows to feed calves, strict hygiene when performing minor surgery and rectal exploration, fly control, etc.) in dairy herds may lead to a reduction of within-herd prevalence but will not be able to eliminate the infection entirely. The impact of lymphoma development on animal welfare can only be reduced by implementing a control program that reduces the prevalence of BLV infection.

Depending on the within-herd prevalence 'test and slaughter' and 'test and separate' are both feasible strategies for disease eradication.

3.5.2. Vaccination

During the last few decades, a series of attempts have been performed to develop a vaccine against BLV. All vaccines based on inactivated virus, infected cell extracts, or purified proteins failed to efficiently protect against BLV challenge, despite inducing a strong specific neutralizing humoral immune response (Miller and Van Der Maaten, 1978). Other versions based on recombinant vaccinia viruses produced neutralizing humoral and cytotoxic responses, but were also inefficient in cows (Portetelle et al., 1991). DNA vaccines containing the env gene under the control of the cytomegalovirus promoter only yielded partial protection to BLV challenge.

Failure of these vaccines was likely due to inadequate or short-lived stimulation of all components of the immune system. Ideally, the optimal vaccine should therefore contain a large number of viral factors permanently stimulating the immune response. Attenuated derivatives of BLV proviruses meet these requirements. A first generation of these genetically simpler viruses contained gag, pol and env genes in a single genome under the control of spleen necrosis virus regulatory sequences. The vaccine was partially effective in rat and rabbit models, but was not evaluated in cows due to regulatory limitations.

Recently, a novel approach based on the use of a recombinant live-attenuated BLV provirus has been proposed (Rodríguez et al., 2014). The rationale behind this strategy relies on the deletion of genes required to induce lymphoma pathogenesis but retaining those involved in replication. The key parameter was to obtain a strain that replicates at very low levels while efficiently stimulating the host immune response.

By a combination of mutations and deletions, a promising vaccine is now available for experimental trials. The vaccine is infectious in cattle, replicates at reduced levels and elicits strong humoral and cytotoxic immune responses. This vaccine is currently being tested in a large-scale trial in Argentina.

3.5.3. Prevention measures

Maintaining disease freedom

Freedom from EBL is defined at the herd level in both the OIE animal health code and Council directive 64/432, requiring that all animals in each herd over 24 months of age have tested negative for BLV antibody. The criteria for obtaining and maintaining country freedom differ between OIE and Council directive 64/432 in several points (highlighted in Appendix C).

Proving freedom

According to Dir. 64/432 freedom can be maintained by surveillance for lymphomas at post-mortem inspection of slaughter animals, once all adult animals have been tested serologically negative within the first five years after official freedom has been granted. If a complete serological test has not been carried out it may be replaced by annual testing of all animals more than 12 months of age in 1% of randomly selected herds.

According to OIE criteria surveillance for lymphomas is an integral part of obtaining freedom from EBL, i.e. moving from an infected status where lymphomas are expected to occur, whereas maintenance of freedom from EBL is based solely on a serological survey carried out annually on a random sample sufficient to provide a 99% level of confidence of detecting EBL if it is present at a prevalence exceeding 0.2% of the herds.

It is important to note that the wording in OIE requires the surveillance to be able to **detect** a prevalence of 0.2% or higher, and that any detection of the agent would lead to loss of free status, at least clearly specified for compartments. On the other hand, Dir. 64/432 **allows** freedom to be obtained and maintained as long as less than 0.2% of herds are infected. From a disease transmission point of view this is only justifiable, if appropriate restrictions apply to infected herds. If this is the case, the limit of 0.2% is arbitrary.

A low level of sporadic lymphoma-suspect tumours are to be expected in a BLV-free population. In one study it was estimated that about 1.5 suspect lymphomas per 10,000 slaughtered animals were not associated with BLV-infection (Thurmond et al., 1985b). This baseline level might therefore serve as an indicator of the effectiveness of monitoring for lymphomas by post mortem inspection in free areas.

Bio-exclusion

According to Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine, the transport of bovine animals to another Member State is authorised if the animals are accompanied by a health certificate attesting compliance with certain requirements for EBL. For herds with an official EBL free status, it is required that newly introduced animals come from herds with the same health status and are subjected to an individual test for BLV prior to introduction if they are over 12 months of age. A test for BLV is not required if the animal originates in a Member State or part of a Member State recognized as officially EBL free. Given that 10–25% of BLV infections occur perinatally or during milk feeding (Ferrer and Piper, 1981; Gutierrez et al., 2011; Lassauzet et al., 1991; Meas et al., 2002; Mekata et al., 2014), animals less than 12 months of age do represent a potential mode of uncontrolled spread of BLV.

Early detection

If BLV is introduced into a hitherto free country it may take 5 years or more before lymphomas appear. The surveillance for lymphomas at post-mortem inspection of all slaughtered adult cattle will therefore primarily be of value in an eradication phase, where it may help to identify herds with long-standing infections and lymphomas.

A systematic surveillance based on bulk milk testing with a system sensitivity approximating 1 (Hutchison and Martin, 2005) will definitely detect newly infected herds several years before development and detection of lymphomas. The degree of confidence required by the OIE for maintenance of freedom from EBL can be achieved by testing a representative sample of dairy herds annually. In practice, however, it may be more cost-effective to continue testing every herd at least once each year (Hutchison and Martin, 2005) or to coordinate sampling with surveillance for bovine brucellosis. Risk-based sampling for serological surveillance has been suggested as a means to increase the sensitivity of detecting infected herds (Blickenstorfer et al., 2011; Reist et al., 2012).

Current experience from France has shown that newly infected herds are identified on the basis of serological testing at an early stage without development of lymphomas (Bendali and Perrin, 2014). Overall, the data suggest that active surveillance and clinical surveillance complement one another well. The total cost incurred by the French State in 2013 for the control (prophylaxis and slaughtering) of EBL amounted to €12,246, most of which were allocated to laboratory analysis.

Due to the food safety requirement for identification of tumours at post-mortem slaughter inspection, testing for EBL lymphomas may still represent a feasible and inexpensive surveillance system component complementing milk/blood surveillance in dairy cattle. It must be stressed that all suspect tumours should be confirmed by testing for the presence of BLV by PCR or, when a serum sample can be obtained, simply by testing for BLV antibody. Histological examination can ascertain whether the tumour is invasive or not (to determine if total condemnation is required) but it cannot distinguish between sporadic tumours and those induced by BLV.

It can be concluded that the proportionality of the the disease prevention measures including the measures for maintaining disease freedom can be regarded as appropriate and favourable. The proportionality of these measures can be mirrored by the surveillance activities conducted in the MS. In regions free of EBL continued surveillance is based on a combination of serological testing of adult animals and identification of tumours at slaughter.

Criteria for maintaining country freedom from EBL differ substantially between OIE and EU. OIE prescribes only serological testing whereas EU allows for surveillance for lymphomas only (detailed comparison in Appendix C). For dairy herds an active surveillance based on bulk milk testing is the method of choice.

4. Conclusions

ToR 1: the disease profile and significance comprising the morbidity and mortality rates (both quantitative and qualitative) of the disease in animal populations at country or regional level

Particular features of the EBL disease profile are the slow spread between animals and long time period from infection with BLV to appearance of lymphomas.

The significance of the disease, taking into account morbidity and mortality rates, is directly linked to the prevalence of infection at regional, country and herd level, and it is also influenced by the age profile of the herd. The morbidity and mortality due to EBL in the EU is currently negligible as a consequence of strict control measures applied since the 90s. During the last 6 years only 35 lymphoma cases have been reported from all MS. However, underreporting cannot be excluded.

The full manifestations of BLV infection cannot be expected to occur until all cows in the herd have been at risk of becoming infected at a young age.

In early stages of the infection flu-like symptoms may occur, while at later stages of infection immune dysregulation develops, leading to increased susceptibility to other infections.

About 30–50% of infected animals develop a persistent lymphocytosis. During this phase, morbidity is characterized by weakness and opportunistic infections, leading to a loss in milk production.

In high-prevalence herds lymphomas may develop in up to 5% of infected animals, predominantly in adult cattle older than 3 years. A range of clinical manifestations may develop, depending on organ involvement and degree of dysfunction, e.g. lymphadenopathy, asthenia, weight loss, constipation, tachycardia, posterior paresis, exophthalmos, and fever.

Lymphomas are not likely to be detected until they cause conspicuous pathophysiological manifestations. Once developed, BLV-associated lymphomas invariably lead to death.

There is no unequivocal evidence for an etiological role of BLV in human diseases.

ToR 2: THE ASSESSMENT OF THE PERSISTENCE OF THE DISEASE IN AN ANIMAL POPULATION OR IN THE ENVIRONMENT AND THE ROUTES AND SPEED OF TRANSMISSION OF THE DISEASES BETWEEN ANIMALS, THE DISTRIBUTION OF THE DISEASE IN THE EU, AND, THE RISK OF ITS INTRODUCTION

Although within-herd transmission of BLV is slow and progressive, it is highly likely that it will persist in a herd if control measures are not applied.

Serological evidence of BLV infection in species other than cattle is most likely the result of spill-over from bovines or unverified false positive testing results. There is no evidence to suggest that any significant reservoir of BLV exists among other species, including wildlife.

Peripartum transmission of BLV from infected cows to offspring occurs in 10–15% of calvings. Milk from infected cows consistently contains BLV, and feeding of non-treated bulk milk to young stock substantially contributes to within-herd transmission.

A high density of haematophagous insects is also correlated with increased within-herd transmission of BLV, but the importance of this route of transmission in natural conditions varies in different countries and regions. However, it is considered unlikely that haematophagous insects play a significant role in transmitting BLV between herds.

Iatrogenic transmission of BLV has contributed to spread via use of blood-contaminated needles, instruments for tattooing or dehorning as well as rectal palpation using contaminated gloves. The use of milking machines has also been shown to be a risk factor.

Considering that BLV is almost exclusively transmitted between herds by movement of live cattle, the trade patterns currently seen in Europe will provide efficient channels for spread of BLV between herds throughout Europe if no testing or certification is implemented.

Due to efficient eradication and control measures over the last decades, 21 MS are currently officially free from EBL. However, targeted surveillance still identified residual cases of EBL in 14 MS in 2013.

Uncontrolled movement of live cattle and genetic material from countries or regions not free from EBL would represent a high likelihood of re-introducing BLV into free regions of the EU.

ToR 3: THE IMPACT OF THE DISEASE ON AGRICULTURAL PRODUCTION CONSIDERING THE LEVEL OF PRESENCE OF THE DISEASE IN THE UNION, THE LOSS OF PRODUCTION DUE TO THE DISEASE AND ITS IMPACT ON ANIMAL WELFARE, THE BIODIVERSITY AND ENVIRONMENT

EBL has been successfully eradicated from a number of MS. In MS not yet officially free, the disease impact is considered negligible.

In third countries with endemic BLV infection, the impact of the EBL is considerable, being proportional to prevalence, due to production losses and carcass condemnation at slaughter.

The current EU surveillance system probably suffers under-reporting of lymphoma cases, hence contemporary figures and data from meat inspection do not allow an assessment of the true incidence of lymphomas in EU.

In countries with modern dairy production systems and no control program for EBL, the best estimate of the cumulative lymphoma incidence in BLV-infected cows is 1–2%.

In high-prevalence herds the cumulative lymphoma incidence among dairy cows may reach 5%.

A statistically significant reduction in milk yield is observed in BLV-infected animals. Longevity is also reduced, evidenced by premature culling, whereas a significant effect on calving interval and mastitis has not been established.

Overall, animals will suffer when lymphomas have progressed beyond an early stage. The welfare consequences in terms of duration and severity may vary according to the location and magnitude of organ involvement.

Available evidence indicates that BLV does not pose a threat to any wild species and that wild species do not constitute a reservoir for BLV.

ToR 4: THE EXISTENCE OF SUITABLE DIAGNOSTIC METHODS AND DISEASE CONTROL TOOLS

Suitable and sensitive methods have been developed and made commercially available for serological diagnosis of BLV infection. The indirect ELISA has proven a suitable control tool for screening of bulk milk samples.

In young calves it is possible to determine an underlying BLV infection in the presence of colostral antibodies by PCR.

Histological examination can support the diagnosis of malignant lymphomas, but is not able to distinguish between sporadic tumours and those induced by BLV.

The PCR can complement serology for confirmatory testing, especially in cases of weak positive or uncertain results in AGID and/or ELISA. Additionally, the PCR is suitable for detection of BLV infection in young calves with colostral antibodies.

To confirm EBL in an animal with tumours (detected clinically or post-mortem), a BLV specific PCR or BLV-antibody test must be performed.

ToR 5: THE FEASIBILITY, AVAILABILITY, PROPORTIONALITY, EFFECTIVENESS AND IMPACT OF THE DISEASE PREVENTION AND CONTROL MEASURES

The feasibility and availability of the disease prevention and control measures towards a successful eradication of EBL in the entire EU is given.

A test and slaughter scheme is feasible when the within-herd prevalence is moderate to low. As an additional measure culling of the offspring of infected animals should be considered due to the possibility of perinatal transmission of the virus.

Control measures should include animal movement restrictions for infected herds.

A test and slaughter strategy has been used successfully to eradicate EBL, even from herds with high animal prevalence.

A test and separate scheme is suitable for herds with high within-herd prevalence, where immediate culling of infected animals is not economically feasible. This strategy is based on physically separating (often in a separate barn) BLV infected cattle from uninfected ones and

gradual elimination of infected animals from the farm by increased culling frequency in the infected group. Safe management practices should be implemented to avoid transmission of the virus.

Vaccination is currently not an option for controlong and preventing EBL. A promising experimental vaccine is currently being evaluated for efficacy and might become an alternative option for high prevalence herds in the future.

The proportionality of the the disease prevention and control measures can be regarded as appropriate and is reflected by the surveillance activities in the MS, which is done according to the EBL-status.

In regions free of EBL continued surveillance is based on a combination of serological testing of adult animals and identification of lymphomas at slaughter. For dairy herds an active surveillance based on bulk milk testing is the method of choice.

Due to the food safety requirement for identification of tumours at post-mortem inspection, testing for EBL lymphomas represents a feasible and inexpensive additional surveillance system component.

Criteria for maintaining country freedom from EBL differ substantially between OIE and EU. OIE prescribes only serological testing whereas EU allows for surveillance for lymphomas only.

The effectiveness of the disease prevention and control measures is directly linked to the surveillance strategy applied. In addition, a systematic elimination of infected animals is the only sustainable way to obtain freedom from BLV.

A stringent herd management in dairy herds (e.g.: only milk from BLV- cows to feed calves, strict hygiene when performing minor surgery and rectal exploration, fly control, etc.) may lead to a reduction of within-herd prevalence but will not eliminate the infection entirely.

The impact of lymphoma development on animal welfare can only be reduced by implementing a control program that reduces the prevalence of BLV infection.

The impact of prevention and control measures do not hamper on-farm production processes. The possible negative impact on animal welfare is limited to blood sampling (if milk samples cannot be obtained) of animals for testing.

5. Recommendations

It is recommended that the standardisation method of the agar gel immunodiffusion test described in Annex D, chapter II section B of Directive 64/432 should be brought in line with the methodology proposed in the OIE Manual.

It is recommended that the polymerase chain reaction (PCR) should be included in Annex D of Directive 64/432. The PCR can complement serology for confirmatory testing, especially in cases of weak positive or uncertain results in AGID and/or ELISA. Additionally, the PCR is suitable for detection of BLV infection in young calves with colostral antibodies. In lymphoma cases where no serum samples are available PCR should be used to differentiate between sporadic and BLV-induced lymphomas.

It is recommended that surveillance for freedom of BLV in EU should be based on the OIE criteria.

Measures to prevent and detect reintroduction should remain in place after the disease-free status has been obtained in order not to lose the resources invested in eradication.

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Glossary

Iatrogenic: Induced unintentionally in a patient by a veterinarian or other professional performing surgical, medical or diagnostic interventions in animals

Lymphoma: Malignant, invasive blood cell tumour that develops from lymphocytes

Provirus: A virus genome that is integrated into the DNA of a host cell, replicating itself through replication of its host cell.

Abbreviations

AGID	agar gel immunodiffusion
AIDS	acquired immunodeficiency syndrome
BFoV	bovine foamy virus
BIV	bovine immunodeficiency virus
BLV	bovine leukemia virus
BOLA	bovine leukocyte antigens
CD-	cluster of differentiation (leukocyte antigens)
EBL	enzootic bovine leukosis
ELISA	enzyme linked immunosorbent assay
HTLV	human T-lymphotropic virus
IFNγ	Interferon gamma
IL-	Interleukin-
OIE	World Organisation for Animal Health
PCR	polymerase chain reaction
PL	persistent lymphocytosis
PTLV	primate T-lymphotropic virus
ToR	Terms of Reference
TNFα	tumour necrosis factor alpha

Appendix A – OIE resolutions

OIE RESOLUTION 1967:

EPIZOOTIOLOGY, DIAGNOSIS AND CONTROL OF BOVINE LEUCOSIS

Leucosis is the most common type of tumour in cattle and causes considerable economical losses in some areas of Europe and North America.

The O.I.E. has in 1964 pointed out the importance of performing research work on the aetiology and epidemiology of this disease and wishes once more to stress the importance of preventing further spread of the disease which seems to occur chiefly by the movement of affected breeding animals.

In order to prevent further spread of the disease it is recommended that:

— All countries and especially those which export cattle for breeding provide diagnostic laboratory facilities to perform haematological control of cattle. This method is at the moment the only satisfactory diagnostic procedure.

— The basis of the evaluation is the determination of the lymphocyte concentration in the circulating blood. It is necessary to determine the upper limits of the normal lymphocyte concentration in healthy animals for specific breeds, ages and regions.

— Any evidence of the presence of leukotic tumours should be carefully assessed and established by blood examination of all animals over 2 years of age in the herd. The examination of a single animal may be misleading.

OIE RESOLUTION 1964:

LEUCOSIS IN CATTLE

The Office International des Epizooties recommends :

1. That all countries initiate epizootiological investigations concerning the presence and incidence of bovine leucosis in their cattle populations. This should be done by recognised diagnostic methods.

2. That all herds affected with the enzootic form should be supervised so that it is no more possible to sell animals for breeding, and to have contacts with other herds. The affected herds should be slaughtered.

3. That countries which want to sell animals for breeding purposes to other countries set up a leucosis control programme, so that the risk for spreading the disease in this way can be restricted to a minimum.

4. That the Veterinary Authorities of the different countries agree upon a system of diagnostic examinations, which are necessary and sufficient for preventing export and import of leucosis-affected breeding animals.

5. That the research work on the etiology and pathogenesis of bovine leucosis should be developed, and the existing diagnostic methods be improved to facilitate the prophylactic measures.

6. That it is desirable to unify the classification of the different forms of leucosis of cattle and of other domesticated animals, including, fowls.

7. That special care should be taken in regard to donor cattle in countries where premunition with blood is used.

OIE RESOLUTION 1953:

V. — LEUCÉMIE DES BOVIDÉS (L).

La leucémie des bovidés est anatomiquement bien caractérisée comme maladie à tumeur; son agent causal est probablement un ultravirus; en premier lieu, la prédisposition de l'animal, est importante.

La démonstration expérimentale du caractère héréditaire, n'a pas pu encore être mise en évidence, alors que la leucose des souris peut être considérée comme héréditaire. Etant donné que la leucose des bovidés et celle des souris sont identiques au point de vue clinique, anatomique et histologique, la conclusion par analogie conduit à la notion que la leucose bovine pourrait être également une maladie héréditaire. De plus, les observations multiples dans la pratique concernant l'apparition familiale constituent un autre argument. On doit tenir compte du caractère, héréditaire de la leucose bovine dans la lutte contre cette maladie; les animaux atteints de leucose seront exclus de l'élevage, sacrifiés, et leurs descendants seront surveillés de près.

La leucose bovine restera à l'étude. En plus des conditions héréditaires et virologiques, il sera nécessaire d'étudier l'influence du milieu dans les contrées où l'on trouve des animaux atteints. Il sera également important de poursuivre des expériences par la méthode de culture des tissus.

Appendix B – Surveillance of EBL in the EU in 2011–2013

2011:

MS or region (1)	Total number of existing bovine		Surveillance (2)						Cases (1)				Percentage of officially free herds
	Herds	Animals	Serological tests			Examination by bulk milk samples			Suspect		Confirmed		
			Number of bovine herds tested	Number of animals tested	Number of infected herds	Number of bovine herds tested	Number of animals or pools tested	Number of infected herds	Tumours	Other causes	Tumours	Other causes	
AT	69,586	1,976,527	3,762	30,295	0	33,358	33,596	0	2	44	0	0	100
BE*	34,540	2,682,370	5,358	19,894	0	0	0	0	10	0	0	0	100
BG	103,383	586,434	15,894	108,672	7,059	0	0	0	0	0	0	0	55.59
CH	41,095	1,591,233	1,248	29,751	0	62	0	0	0	0	0	0	100
CY	313	29,071	55	375	0	214	214 (pools)	0	0	0	0	0	100
CZ	19,658	1,324,350	3,992	69,154	0	0	0	0	0	0	0	0	100
DE	175,330	12,776,773	21,224	719,164	2	51,711	133,063	0	47	111	14	9	99.996
DK	20,384	1,623,400	…	5,904	0	38	…	0	3	0	0	0	100
EE*	4,716	238,684	3,118(*)	19,792	1	3,118(*)	94,325	0	0	0	0	0	99.98
ES	118,431	5,079,456	10,788	291,776	0	1,008	13,117	0	0	0	0	0	100
FI	14,935	914,053	0	0	0	1,449	1449 (pools)	0	22	126	0	0	100
FR	232,592	19,005,674	122,923	223,114	2	18,156	14,455	1	3	70	0	3	99.86
UK(GB)	77,785	8,286,934	0	137	0	1979	2134	0	58	0	0	0	100
UK(NI)	25,677	1,590,452	444	11,116	0	0	0	0	0	0	0	0	100
UK(IoM)	280	32,803	28	945	0	38	4,000	0	0	0	0	0	100
GR*	31,381	708,397	2,683*	6,337*	48*	1,026*	2,272	5	4	0	4	0	98.21
HU*	16,608	756,721	4,007	190,134	62**	21	4,730	0	0	0	0	0	99.55
IE	116061	5828014	2203	51307	0	0	0	0	3	0	0	0	100
IT*	74,167	2,794,720	29,627	714,936	0	11,178	228,096	0	14	0	0	0	99.99
LT	86,207	669,190	45,688	165,184	64	27,208	194,102	73	0	0	0	0	99.83
LU	1,525	187,066	1,525	578	65	798	798	0	0	0	0	0	100
LV	33,998	380,612	10,804	44,147	8	236	30,718	0	0	0	0	0	99.97
MT*	125	13,912	125	12,828	17	0	0	0	0	0	0	0	62.4
NL	51,119	3,912,112	9,029	19,435	0	8,011	8,028	0	29	7	0	0	100
NO	16,400	856,000	1,236	4,758	0	1,226	1,226	0	0	0	0	0	100
PL*	646,016	6,068,806	179,829	1,024,920	131	0	0	0	0	0	0	0	99.97
PT(AZ)*	10,489	261,844	3,367	62,693	1	2,744	5,114	0	0	0	0	0	99.97
PT(others)	48,014	1,232,554	28,658	563,163	25	0	0	0	0	0	0	0	99.91
RO	751,595	2,220,939	725,804	1,361,575	1,791	0	0	0	4	0	0	0	99.76
SE	20,503	1,511,846	3,713	7,603	0	1,783	0	0	14	6	0	0	100
SI	32,225	457,634	0	0	0	0	0	0	1	0	0	0	100
SK	8,635	463,574	1,735	70,240	0	0	0	0	0	0	0	0	100

Source: http://ec.europa.eu/food/animal/liveanimals/bovine/docs/final_report_2011_en.pdf)

2012:

MS or region [1]	Total number of existing bovine		Surveillance [2]						Cases [1]				Percentage of officially free herds
	Herds	Animals	Serological tests			Examination by bulk milk samples			Suspect		Confirmed		
			Number of bovine herds tested	Number of animals tested	Number of infected herds	Number of bovine herds tested	Number of animals or pools tested	Number of infected herds	Tumours	Other causes	Tumours	Other causes	
AT	69,430	1,976,698	3,480	28,101	0	31,241	31,244	0	5	33	0	0	100
BE*	32,475	2,603,148	2,312	29,524	0	0	0	0	0	0	0	0	100
BG	98,177	593,131	98,177	111,192	9,545	0	0	0	0	0	0	0	90,28
CH	40,309	1,577,407	1,040	18,436	0	1,799	3,590	0	0	0	0	0	100
CY*	356	57,438	25	84	0	210	210 pools	0	0	0	0	0	100
CZ	19,439	1,390,111	4,027	74,418	0	0	0	0	0	0	0	0	100
DE	170,000	12,703,512	31,127	824,813	2	41,366	90,766	1	36	645	0	0	100
DK	20,368	1,607,138	na	1,601	0	45	na	0	4	6	0	0	100
EE*	4,496	248,124	2,827	22,041	5	2,827	99,376	0	0	0	0	0	99,89
ES	123,448	5,537,550	7,148	209,240	0	227	9,849	0	0	0	0	0	100
FI*	14,166	912,768	0	0	0	1,312	1,312 pools	0	17	114	0	0	100
FR	224,514	24,095,402	32,703	290,925	0	13,935	11,626	0	3	155	2	0	99,99
UK(GB)	79,174	8,129,760	633	3,223	0	3,704	4,216	0	60	0	0	0	100
UK(NI)	25,776	1,625,446	258	6,774	0	0	0	0	0	0	0	0	100
UK(IoM)	269	31,780	31	720	0	38	4,000	0	0	0	0	0	100
GR*	31,858	704,110	1,978	48,416	25	923	1,077	1	0	0	0	0	98,74
HU*	16,645	815,141	9,529	247,916	104	103	4,401	0	1	0	1	1	99,44
IE	115,787	6,145,469	2,217	43,704	0	0	0	0	3	0	0	0	100
IT*	76,293	2,886,861	30,205	715,582	7	9,861	57,173	0	2	0	0	0	100
LT	79,242	683,972	45,062	122,897	43	24,718	302,420	6	0	0	0	0	99,9
LU	1,512	195,432	1,512	383	0	752	752	0	0	0	0	0	100
LV	31,765	393,097	6,434	36,111	3	810	32,503	0	0	0	0	0	99,99
MT*	121	14,004	121	13,244	7	0	0	0	0	0	0	0	85,95
NL	51,119	3,912,112	4,293	13,754	0	8,140	8,161	0	15	12	0	0	100
NO	15,264	850,257	1,178	4,306	0	1,189	1,189	0	0	0	0	0	100
PL*	610,078	6,057,153	163,953	787,048	61	0	0	0	0	0	0	0	99,99
PT(AZ)*	10,207	263,060	2,254	41,738	0	1,887	37,718	0	0	0	0	0	100
PT(others)	40,395	1,081,495	12,918	201,492	4	0	0	0	0	0	0	0	100
RO	682,802	2,206,005	678,069	1,369,307	1,680	0	0	0	0	0	0	0	99,75
SE	19,561	1,500,293	3,244	7,305	0	1,794	1,794	0	0	4	0	0	100
SI	34,339	456,742	0	0	0	0	0	0	2	0	0	0	100
SK	8,407	492,209	1,947	59,143	0	0	0	0	0	0	0	0	100

Source: http://ec.europa.eu/food/animal/liveanimals/bovine/docs/final_report_2012_en.pdf

2013:

MS or region (1)	Total number of existing bovine		Surveillance (2)						Cases (3)				Percentage of officially free herds
	Herds	Animals	Serological tests			Examination by bulk milk samples			Suspect		Confirmed		
			Number of bovine herds tested	Number of animals tested	Number of infected herds	Number of bovine herds tested	Number of animals or pools tested	Number of infected herds	Tumours	Other causes	Tumours	Other causes	
AT	67,496	1,961,479	1,327	10,304	0	1,264	1,266	0	2	0	0	0	100
BE*	31,363	2,585,003	1,890	74,829	0	0	0	0	0	0	0	0	100
BG	93,412	600,043	2,361	14,728	25,220	0	0	0	0	0	0	0	0
CH	39,161	1,560,293	1,287	16,960	0	1,606	3,182	0	0	0	0	0	100
CY*	335	55,959	21	63	0	207	207	0	0	0	0	0	100
CZ	18,789	1,368,602	4,243	71,006	0	0	0	0	0	0	0	0	100
DE	164,666	12,829,056	21,129	662,362	2	33,056	82,502	0	48	74	0	0	100
DK	19,371	1,584,769	n.a	1,526	0	24	n.a	0	5	1	0	0	100
EE*	4,206	261,574	3,226	28,385	2	3,226	115,185	0	0	0	0	0	99,95
ES	121,787	5,756,550	5,020	184,023	0	0	0	0	0	0	0	0	100
FI*	13,464	911,943	0	0	0	1,292	1,292	0	14	30	0	0	100
FR	219,846	19,020,183	28,100	316,792	3	14,284	14,164	1	2	8	0	4	99,96
UK(GB)	77,132	8,348,198	-	2,644	0	3,692	4,021	0	44	0	0	0	100
UK(NI)	24,098	1,587,766	503	13,905	0	0	0	0	0	0	0	0	100
UK(IoM)	273	30,533	14	356	0	38	4,446	0	0	0	0	0	100
GR	38,951	736,229	3,298	78,864	26	994	957	1	0	0	0	0	99,2
HR*	35,707	462,180	37,258	212,367	263	0	0	0	0	0	0	0	2,775
HU*	17,573	848,338	5,046	151,314	34	17	5,754	2	1	0	0	1	99,74
IE	115,765	6,212,293	2,209	53,772	0	0	0	0	21	0	0	0	100
IT*	76,659	3,169,608	30,896	684,855	6	9,448	54,467	0	2	1	0	0	100
LT	79,242	683,972	24,400	90,315	39	4,927	84,372	0	0	0	0	0	99,90
LU	1,387	198,557	2	417	0	728	728	0	0	0	0	0	100
LV	30,897	415,277	10,084	42,317	3	1,374	38,087	0	0	0	0	0	99,99
MT*	189	15,204	189	13,398	7	0	0	0	0	0	0	0	96,3
NL	50,990	3,997,013	5,177	18,761	0	7,917	7,935	0	30	0	0	0	100
NO	14,715	831,994	1,167	4,079	0	1,042	1,042	0	0	0	0	0	100
PL*	576,281	6,140,846	87,043	664,370	47	0	0	0	0	5	0	2	99,99
PT*	44,727	1,428,360	7,577	154,541	2	1,736	39,981	0	0	0	0	0	99,94
RO	656,236	2,223,937	656,236	1,363,831	1,460	0	0	0	0	0	0	0	99,77
SE	18,962	1,496,526	3,132	6,746	0	1,798	1,800	0	18	0	0	0	100
SI	33,917	461,461	0	0	0	0	0	0	1	0	0	0	100
SK	8,608	480,097	1,546	52,806	0	0	0	0	0	0	0	0	100

Source: http://ec.europa.eu/food/animal/liveanimals/bovine/docs/final_report_2013_en.pdf

Appendix C – Criteria for obtaining freedom from EBL according to OIE and 64/432/EEC.

Significant differences are highlighted in red italics.

	OIE	64/432/EEC
Herd obtaining freedom	1a) no evidence of EBL either clinical, post-mortem, or as a result of a diagnostic test for EBL within the previous two years;	no evidence, either clinical or as a result of a laboratory test, of any case of enzootic bovine leukosis in the herd and no such case has been confirmed in the previous two years
	1b) all cattle over 24 months of age have been subjected to a diagnostic test for EBL on two occasions with negative results, at an interval of not less than 4 months during the preceding 12 months	all animals over 24 months of age have reacted negatively during the preceding 12 months to two tests carried out in accordance with this Annex, at an interval of at least four months
Herd maintaining freedom	All cattle in the herd over 24 months of age on the day of sampling should be subjected to a diagnostic test for EBL with negative results at intervals of no more than 36 months and the conditions referred to in point 1a) above continue to be fulfilled	all animals over 24 months of age continue to react negatively to a test carried out in accordance with Chapter II at intervals of three years
Country obtaining freedom	satisfy the following requirements for at least three years: a) all tumours, suspected to be lymphosarcoma, are reported to the Veterinary Authority, and are examined at a laboratory by appropriate diagnostic techniques; b) all cattle with tumours in which EBL has been confirmed or cannot be ruled out are traced back to the herds in which they have been kept since birth; all cattle over 24 months of age in these herds are subjected to an individual diagnostic test for EBL; c) at least 99.8 % of the herds are qualified as EBL free.	(a) at least 99,8 % of the bovine herds are officially enzootic-bovine-leukosis-free Or (b) no case of enzootic bovine leukosis has been confirmed in the Member State for the past three years, and the presence of tumours suspected of being due to EBL is compulsorily notifiable with investigations of cause being carried out, And all animals aged over 24 months in at least 10 % of the herds, selected randomly, have been tested with negative results in accordance with Chapter II in the previous 24 months, Or (c) any other method which demonstrates to a confidence rating of 99 % that less than 0,2 % of herds were infected

	OIE	64/432/EEC
Country maintaining freedom	a) a serological survey should be carried out annually on a random sample of the cattle population of the country or zone sufficient to provide a 99 % level of confidence of detecting EBL if it is present at a prevalence rate exceeding 0.2 % of the herds; For a compartment to maintain its EBL free status, all herds in the compartment should remain free according to Article 11.8.4. and specific surveillance implemented according to Article 4.4.5. has not detected the agent.	(a) all animals slaughtered within the territory of that Member State or region are submitted to official post-mortem examinations at which all tumours which could be due to the EBL virus are sent for laboratory examination, and (b) the Member State reports to the Commission all cases of enzootic bovine leukosis that occur in the region, and (c) all animals which react positively to any of the tests provided for in Chapter II are slaughtered and their herds remain subject to restrictions until re-establishment of their status in accordance with Section D and (d) all animals more than two years old have been tested, either once in the first five years after the status is granted under Chapter II or during the first five years after the grant of the status under any other procedure demonstrating with a certainty level of 99 % that less than 0,2 % of herds have been infected. However, where no case of enzootic bovine leukosis has been recorded in a Member State or in a region of a Member State in a proportion of one herd out of 10 000 for at least three years, …routine serological tests may be reduced provided that all bovine animals more than 12 months old in at least 1 % of herds, selected at random each year, have been subjected to a test carried out in accordance with Chapter II.

Appendix D – Spread model

A basic age-structured model was used to estimate the time it takes before the infection will be detected if it is introduced in a free herd, unsuspecting of the infection.

The cattle population is described by a (constant) annual removal (culling) rate of all animals in the milking herd (ages 2 years and older). For the most common and most problematic example, we assume a replacement rate of 30%. The probability that such animals are still alive at age a is given by

$$f(a) = \mathrm{Exp}[-\mu\,(a-2)]$$

Infection with BLV leads to high infectiousness in about 80% of the animals, i.e. the fraction of effective transmitters is given by λ=0.20. These animals can also develop disease.

In a herd where there is no suspicion regarding BLV, detection of the infection in the herd, will depend on the meat inspection, which may detect tumours, and thus detect the disease in the animal. We assume that when an animal develops clinical disease (tumours), the infection will be detected.

Little good data are available to estimate the age at infection of the duration of the latent and infectious period of this disease, only summarized data on age of cases could be found. We quantify parameters leading to a case age distribution in the field, and verify these parameter values using the results of use Mieth et al, 1970. In this verification we use an annual replacement of the cattle of 20%, assuming this to be relevant for the farming conditions of the period in which these data were collected. Since we have no other good data sources with different age distributions or good experimental data, the age at infection cannot be estimated.

Assuming that most infectious periods start at 2 years old, and a case age distribution as given in Figure xx (graph from Mieth) can be applied, we quantify the probability to survive for an infectious animals which is not submitted to normal culling, by g(a) calculate the probability to develop clinical disease for in infected 2 year old cow as

$$g(a) = \mathrm{Exp}[-0.005\,(a-2)^3]$$

And the age dependent cull due to (clinical) disease is given by

$$\mu_d = g'(a)/g(a)$$

The exact shape of this distribution is not very important for the estimation, as long as it fits the width and peak of the case age distribution sufficiently well. For comparison with the reported data, we need to apply annual culling of 20%, while for modern farming we could apply a culling rate of 33%.

We now proceed with an assessment of the duration to detection (due to tumours detected in clinically diseased animals). We use the basic reproduction ratio (R_0) as the main tool for this assessment. After introduction of the infection in a herd (first infection generation) in the form of an infected heifer of two years old, a second generation of infections is expected to consist of R_0 infectious individuals, a third generation of $(R_0)^2$, etc. The expected number of infections in the n^{th} generation is given by $(R_0)^{(n-1)}$.

The probability of detecting the infection in an average infected animal is not very big, because the probability of such an animal reaching the age of clinical infection is rather small. This probability is strongly influenced by the management of the herd, e.g. the average number of lactations per dairy cow, or replacement rate. The probability of detection can be calculated from

$$P(a) = \int_{2}^{15} \mu_d \, \mathrm{g(a)f(a)da}$$

We only address dairy cattle, from 2 to 15 years of age. Before two years of age, cull hardly applies and above 15, we assume no animals survive. In a high production herd with μ=33% and the above quantified disease parameters, we find that the probability for one infected cow to develop into a clinical case is 21%. If the annual replacement is much lower, the probability of detection is much higher. In a herd with 20% annual replacement, the probability of developing clinical disease is 38%.

N.B. f the age at infection would be above 2 years, for example 3 years, than this probability hardly changes (20%). If only a fraction of 20% of the infected animals would actually develop high infectiousness and clinical disease, then only 4% of the infected animals develop into cases.

Furthermore, in a herd with higher replacement, the reproduction ratio of the infection is lower, because the average infectious period is shorter due to more cull. This leads to an R_0 which can by 30% lower in a herd with 33% replacement as compared to a herd with 20% replacement (see below). In combination with a lower probability of detections, this leads to the conclusion that in herds with high replacement, the detection of introduction of the infection may take much longer than in lower replacement herds.

NB. With normal culling management, the R_0 cannot be brought below 1, since that would require an annual replacement which does not allow the population to survive.

Assuming an R_0 equal to 2 (references…) in a high replacement herd, the probability of detecting the infection in the first infected animal is about 20%, which would be about 5 years after introduction. The probability of detection in the second infection generation is 1-$(0.8)^2$ = 36%, and with an expected generation time of about 4 years, and about 6 years for development of clinical disease, ti will take about ten years, for detection in the second infection generation. The third infection generation has 60% probability of leading to detection. Thus, the infection is likely to be detected between 10 en 15 years after introduction, but longer delay is also possible (p=17%).

For this rather simple model, the reproduction ratio can be calculated using

$$R_0 = \beta \int_{2}^{15} f(a)\mathrm{g(a)da}$$

Where β is the transmission rate of the infectious animals.