Epidemiology and genetic diversity of bovine leukemia virus

Meripet Polat¹,², Shin-nosuke Takeshima¹,²,³ and Yoko Aida¹,²,³*

Abstract

Bovine leukemia virus (BLV), an oncogenic member of the Deltaretrovirus genus, is closely related to human T-cell leukemia virus (HTLV-I and II). BLV infects cattle worldwide and causes important economic losses. In this review, we provide a summary of available information about commonly used diagnostic approaches for the detection of BLV infection, including both serological and viral genome-based methods. We also outline genotyping methods used for the phylogenetic analysis of BLV, including PCR restriction length polymorphism and modern DNA sequencing-based methods. In addition, detailed epidemiological information on the prevalence of BLV in cattle worldwide is presented. Finally, we summarize the various BLV genotypes identified by the phylogenetic analyses of the whole genome and env gp51 sequences of BLV strains in different countries and discuss the distribution of BLV genotypes worldwide.

Keywords: Bovine leukemia virus (BLV), BLV diagnostic approaches, BLV genotyping methods, BLV epidemiology

Background

Bovine leukemia virus (BLV) is a retrovirus, an oncogenic member of the Deltaretrovirus genus, and the causative agent of enzootic bovine leukosis (EBL) [1, 2]. The Deltaretrovirus genus also includes human T-cell lymphotropic virus types I and II (HTLV-I and -II) and simian T-cell lymphotropic virus (STLV) [3, 4]. EBL is a contagious lymphoproliferative disease of cattle, characterized by B-cell lymphosarcoma, which occurs throughout the world [2, 5]. Although BLV can infect various immune cell populations, including CD5⁺ IgM⁺ and CD5⁻ IgM⁺ B-cells; CD2¹, CD3¹, CD4⁺, CD8⁺, and γ/δ T-cells; monocytes; and granulocytes in peripheral blood and lymphoid tissues of cattle [6–11], BLV-induced tumors usually arise from the CD5⁺ IgM⁺ B-cell subpopulation [12].

BLV infection can result in a variety of clinical outcomes [2]. The majority of BLV-infected cattle are asymptomatic carriers of the virus, neither showing any clinical signs nor any changes in lymphocyte count; however, a recent study showed that although lymphocyte counts were not elevated in BLV-infected but clinically normal cattle, CD5⁺ IgM⁺ B-cells were increased [11], and there is substantial evidence suggesting that BLV-infected but clinically normal cattle may exhibit a degree of immunological dysregulation leading to economic losses for various reasons including reduced milk production [13], a high incidence of infectious disease [14], and reproductive inefficiency [15]. Approximately one-third of infected cattle develop a benign form of non-malignant proliferation of untransformed B-lymphocytes, termed persistent lymphocytosis (PL). PL is typically characterized by a permanent and stable increase in the number of CD5⁺ IgM⁺ B-cells circulating in the peripheral blood. Less than 5% of infected cattle develop malignant B-cell lymphoma originating from mono- or oligo-clonal accumulation of CD5⁺ IgM⁺ B-cells after a relatively long period of latency. This malignant form of B-cell lymphoma is predominantly detected in cattle over 4–5 years old [16]. Such malignancies induce disruption of the spleen and remarkable enlargement of the lymph nodes, which can be visible under the skin. BLV-induced neoplastic cells can penetrate into the abomasums, right auricle of the heart, intestine, kidney, lung, liver, and uterus. The clinical signs of BLV-induced tumors are varied and primarily involve digestive disturbance, weight loss, weakness, reduced milk production, loss of appetite, and enlarged lymph nodes [17].

* Correspondence: aida@riken.jp
¹Viral Infectious Diseases Unit, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
²Nano Medical Engineering Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
Full list of author information is available at the end of the article

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**BLV genome structure**

The BLV genome consists of 8714 nucleotides (nt) [18] including essential structural protein and enzyme coding genes and a pX region, flanked by two identical long terminal repeats (LTRs) (Fig. 1a). The structural protein and enzyme coding genes, namely, *gag*, *pro*, *pol*, and *env*, have essential and indispensable roles in the viral life-cycle, viral infectivity, and the production of infectious virions [19–24]. The *gag* gene of BLV is translated as the precursor, Pr45 Gag, and processed to generate three mature proteins [19, 23]: the matrix protein, p15, which binds viral genomic RNA and interacts with the lipid bilayer of the viral membrane [25]; the capsid protein, p24, which is the major target of the host immune response, with high antibody titers against this molecule found in the serum of infected animals [26, 27]; and the nucleocapsid protein, p12, which binds to packaged genomic RNA [28] (Fig. 1b). The *env* gene encodes the mature extracellular protein, gp51, and a transmembrane protein, gp30 [19]. The pX region, which is located between *env* and the 3′ LTR [2], encodes the regulatory proteins Tax and Rex, and the accessory proteins R3 and G4 (Fig. 1a). The regulatory proteins are important for regulation of viral transcription, transformation of BLV-induced leukemogenesis, and nuclear export of viral RNA into the cytoplasm [29–36]. The R3 and G4 accessory proteins contribute to the maintenance of high viral loads [37, 38]. In addition to the genes described above, the BLV genome also contains RNA polymerase-III-encoded viral microRNAs (miRNAs) between the *env* and pX regions. Viral miRNAs are strongly expressed in preleukemic and malignant cells, and may have roles in tumor onset and progression [39, 40] through their effects on proviral load and consequently viral replication in the natural host [41]. Besides, Van Driessche et al. revealed the recruitment of positive epigenetic marks on BLV miRNA cluster, inducing strong antisense promoter activity [42]. They also identified *cis*-acting elements of an RNAPII-dependent promoter [42].

**BLV diagnosis**

A variety of techniques have been developed for diagnosis of BLV and implemented worldwide. These diagnostic methods can be assigned into two main groups, consisting of antibody-based serological tests and detection of the proviral genome by nucleic acid-based polymerase chain reaction (PCR) assays (summarized in Table 1).

*Serological tests*

For indirect BLV diagnostic methods, particularly antibody-based tests, antibodies recognizing the p24 capsid protein encoded by the *gag* gene and the extracellular gp51 protein encoded by *env-gp51* are targeted. This is because antibodies against these proteins are produced shortly after BLV infection, can be detected 2–3 weeks post-infection, and remain detectable for the life of the host animal [43]. Therefore, antibodies against these proteins are targeted for BLV diagnostics using conventional serological techniques such as

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**Fig. 1** Schematic representations of the BLV genome structure (a) and viral particle (b). The structural and enzymatic genes, *gag*, *pro*, *pol*, and *env*; regulatory genes, *tax* and *rex*; accessory genes R3 and G4; and microRNA (miRNA) are indicated in (a). Proteins encoded by structural and enzymatic genes, including the Env glycoproteins (gp51 and gp30) encoded by the *env* gene, the Gag proteins (p12, p24, and p15) encoded by the *gag* gene, reverse transcriptase and integrase (RT-IN) encoded by the *pol* gene, and protease (Pro) encoded by the *pro* gene are indicated in (b)
| Diagnostic assay | Sample                      | Target                      | Advantages                                      | Disadvantages                                                                 | References |
|------------------|-----------------------------|-----------------------------|-------------------------------------------------|-------------------------------------------------------------------------------|------------|
| **Serological test** |                             |                             |                                                 |                                                                                |            |
| AGID             | Serum                       | Antibodies (p24, gp51)      | Specific, simple, and easy to perform            | Less sensitive and inconclusive                                                | Aida et al., 1989 [47] |
|                  |                             |                             | Large scale screening                           | Cannot evaluate disease states of infected cattle                             | Wang et al., 1991 [48] |
|                  |                             |                             | Less expensive                                   |                                                                                | Monti et al., 2005 [49] |
|                  |                             |                             | Rapid                                           |                                                                                | Kurdi et al., 1999 [50] |
|                  |                             |                             |                                                  |                                                                                | Jimba et al., 2012 [43] |
|                  |                             |                             |                                                  |                                                                                | Naif et al., 1990 [55] |
|                  |                             |                             |                                                  |                                                                                | Zaghawa et al., 2002 [52] |
|                  |                             |                             |                                                  |                                                                                |                        |
| **ELISA**        | Serum Milk                  | Antibodies (p24, gp51)      | Specific and sensitive                           | False negatives (cattle in early infection phase) False positive (maternally derived antibodies) Cannot evaluate disease states of infected cattle A number of controls and a plate reader required Results require interpretation | Naif et al., 1990 [55] |
|                  | Bulk milk                   |                             | Large scale screening Time saving                |                                                                                | Burridge et al., 1982 [56] |
|                  |                             |                             | Time saving                                      |                                                                                | Schoepf et al., 1997 [53] |
|                  |                             |                             |                                                  |                                                                                | Kurdi et al., 1999 [50] |
|                  |                             |                             |                                                  |                                                                                | Monti et al., 2005 [49] |
|                  |                             |                             |                                                  |                                                                                | Jimba et al., 2012 [43] |
|                  |                             |                             |                                                  |                                                                                | Zaghawa et al., 2002 [52] |
|                  |                             |                             |                                                  |                                                                                |                        |
| **PHA**          | Virus particle               | BLV glycoprotein            | Sensitive Specific detection of BLV              | Affected by pH and temperature Hemagglutination activity reduced by trypsin, potassium periodate, and neuraminidase | Fukai et al., 1999 [51] |
| **RIA**          | Serum                       | Antibodies (p24)            | Sensitive Able to detect BLV during the early period of infection | Cannot be used for mass screening | Levy et al., 1977 [54] |
|                  |                             |                             |                                                  |                                                                                | Nguyen et al., 1993 [57] |
| **Proviral DNA detection** | Blood PBMC | Provirus                     | Direct, fast, sensitive A variety of samples can be used BLV detection during the early phase of infection or in the presence of colostrum antibodies Can detect new infections, before the development of antibodies to BLV | Unable to detect BLV when the proviral load is too low Cross contamination occurs easily Requires specific primers Requires equipment (PCR machine) False negatives in the presence of PCR inhibitory substances in samples Requires internal control Needs confirmatory testing, such as sequencing | Monti et al., 2005 [49] |
|                  | Tumor sample                |                             |                                                  |                                                                                | Kurdi et al., 1999 [50] |
|                  | Buffy coat                  |                             |                                                  |                                                                                | Zaghawa et al., 2002 [52] |
|                  | Milk                        |                             |                                                  |                                                                                | Tajima et al., 1998 [64] |
|                  | Somatic cells               |                             |                                                  |                                                                                | Tajima et al., 2003 [61] |
|                  | Semen Saliva                |                             |                                                  |                                                                                |                        |
|                  | Nasal secretions            |                             |                                                  |                                                                                | Somura et al., 2014 [68] |
| **Real-time PCR** | Blood PBMC                  | Provirus                     | Direct, fast, sensitive Low risk of contamination A variety of samples can be used Distinguishes EBL from SBL BLV can be detected during the early phase of infection or in the presence of colostrum antibodies Quantitative measurement of proviral load | Requires internal control Requires positive controls of different concentrations Requires specific primers and probes Require equipment (real-time PCR machine) Expensive Complicated sample preparation procedure | Lew et al., 2004 [69] |
|                  | Tumor sample                |                             |                                                  |                                                                                | Jimba et al., 2010 [70] |
|                  | Buffy coat                  |                             |                                                  |                                                                                | Jimba et al., 2012 [43] |
as agar gel immunodiffusion (AGID) [43, 47–50], passive hemagglutination assay (PHA) [43, 51], enzyme-linked immunosorbent assay (ELISA) [43, 49, 50, 52, 53], and radio immunooassay (RIA) [54]. Most of these serological methods aim to detect antibodies in bovine serum and milk, and the supernatants of BLV-infected cell cultures. AGID is relatively inexpensive and can be used to screen many serum samples simultaneously; however, it is not sufficiently sensitive [55] and it is not suitable for analysis of milk samples. ELISA is a highly sensitive and easily implemented procedure, and can be used to analyze both serum and milk samples; however, it requires a number of controls and produces both false-negative result in serum samples from cattle in the early phase of infection [55] and false-positive results in calves that contain maternally-derived antibodies [56]. PHA aims to detect BLV glycoproteins, but, PHA test efficiency is sensitive to pH, temperature, and trypsin. RIA is suitable for diagnosing BLV soon after animals are exposed, but not suitable for the purpose of mass screening [57]. Overall, these antibody-based detection methods cannot be used to test calves les than 6 months old, due to the presence of maternal antibodies, which may trigger false-positive results [58].

**Proviral DNA detection**

BLV can integrate into dispersed sites within the host genome [59] and appears to be transcriptionally silent in vivo [60–62] and remain in cellular genomes, even in the absence of detectable BLV antibodies. Indeed, transcription of the BLV genome in fresh tumor or peripheral blood mononuclear cell samples from infected individuals is almost undetectable by conventional techniques [60, 63]. Interestingly, one copy of the full-length proviral genome can be detected in BLV-infected cattle throughout the course of the disease [64]. Another study also demonstrated that BLV-induced tumors and BLV-infected cells contain provirus, with approximately four copies of proviral DNA in each tumor [65]. Hence, in addition to the routine diagnosis of BLV infection using the conventional serological techniques described above, nucleic acid-based PCR methods can greatly accelerate the detection of BLV prevalence.

A variety of PCR methods, including standard PCR [49, 50], nested PCR [33, 52, 64], real-time quantitative PCR (qPCR) [43, 66–71], and direct blood-based PCR [72, 73], have been extensively applied worldwide for BLV detection (Table 1). A variety of genes in the BLV genome are targeted for detection of BLV infection prevalence by direct diagnostic PCR methods, including the LTR region [43, 70, 71, 73–77], and the gag [78], pol [69, 79, 80], env [55, 79], and tax [68, 79] genes.

Importantly, the BLV provirus copy number is generally very low compared with that of host genes therefore, the majority of PCR systems designed to detect BLV used a nested design [64, 74, 76]. These nested assays are extremely sensitive, but also obtain false-positive results due to DNA contamination. However, the method requires expensive real-time PCR machines and reagents and involves difficult sample preparation protocols. Recently, a novel blood-based PCR system that amplifies target DNA regions without a requirement for DNA isolation and purification was developed [72, 73]. The assay can detect BLV provirus with high specificity and at low cost, facilitating timely identification of BLV-infected cattle.

As discussed above, PCR-based genome screening methods for diagnosis of BLV broaden the range of samples that can be used, increase testing sensitivity, specificity, and efficiency, and are less time consuming. PCR also allows the detection of BLV infection in cattle several weeks before it is possible to detect antibodies [81]; however, PCR-based provirus screening involves complicated sample preparation processes, which can

| Diagnostic assay               | Sample Type | Target | Advantages                                      | Disadvantages                                                                                                                                                                                                 | References       |
|--------------------------------|-------------|--------|------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| AGID agar gel immunodiffusion  | Blood       | Provirus | Cost-effective; No need for DNA purification; Low risk of contamination | Unable to detect BLV when the proviral load is too low; Results in failure if there are mismatches between the PCR primers and BLV sequences; Relatively low sensitivity | Tawfeeq et al., 2013 [67] |
| BLV bovine leukemia virus, EBL enzootic bovine leukosis, ELISA enzyme-linked immunosorbent assay, PHA passive hemagglutination assay, RIA radio immunoassay | Blood-based PCR | Blood Provirus | Cost-effective; No need for DNA purification; Low risk of contamination | Unable to detect BLV when the proviral load is too low; Results in failure if there are mismatches between the PCR primers and BLV sequences; Relatively low sensitivity | Brym et al., 2013 [66]; Takeshima et al., 2015 [71]; Nishimori et al., 2016 [72]; Takeshima et al., 2016 [73] |

Table 1 Summary of common techniques used for diagnosis of BLV prevalence (Continued)
lead to false-positive results if cross contamination occurs. In addition, PCR-based BLV detection methods require specific laboratory facilities, including PCR machines, and the design of specific primers and probes is also necessary. The CoCoMo algorithm, a method used to design degenerate primer sets that amplify all available sequences within a target region. Recently, the BLV-CoCoMo-qPCR assay was developed to measure the BLV proviral load with extremely high sensitivity and to amplify both known and novel BLV variants [43, 70, 71]. This assay enabled us to demonstrate that the proviral load correlates not only with BLV infection capacity but also with BLV disease progression [43, 82], and identification of risk factor associated with increased BLV proviral load in infected cattle [82, 83] and detection of BLV provirus in nasal secretion and saliva samples [84].

**Other methods**

In addition to the techniques described above, other BLV diagnostic approaches, including detection of viral proteins by western blotting [21, 31, 33, 85], a syncytium formation assay [85], and detection of BLV antigens by indirect immunofluorescent assay [47], have also been described.

**BLV genotyping and identification of ten distinct genotypes**

Studies of BLV genotypes for phylogenetic and epidemiological analyses have primarily focused on the *env* gene, the *env* gp51 gene in particular, because of its biological functions. The extracellular gp51 protein has key roles in the viral lifecycle and is indispensable for viral entry into host cells [20, 86]. In addition, because of the surface localization of the gp51 glycoprotein, it is also the target of neutralizing antibodies [87]. The conformational epitopes, F, G, and H, located in the N-terminal half of gp51, are important in syncytium formation and viral infectivity [87, 88]. Therefore, the *env* gp51 sequence region is frequently used for BLV phylogenetic analysis.

Over the years, a number of methods have been applied for BLV genotyping, as summarized in Table 2. In the early days of BLV genotyping, researchers clustered or genotyped BLV strains from different geographical regions based on restriction fragment length polymorphisms (RFLP) of PCR-products, generated using various restriction enzymes [86, 89–96]. BLV clusters and genotypes were named after the geographical region of sample isolation, such as “Argentine type” or “Australian type”, or with reference to phylogenetic clustering (e.g., “cluster one”). A total of seven BLV clusters/genotypes were determined by PCR-RFLP [91]; however, PCR-RFLP genotyping studies were not consistent or comprehensive.

In 2007, Rodriguez et al. reported sequencing of the *env* gene (all of gp51 and part of gp30) of 28 BLV field strains, performed phylogenetic analysis of these sequences in comparison with published sequence data representative of established genetic groups by neighbor-joining, maximum likelihood, and Bayesian inference methods, and assigned BLV sequences into seven genotypes [97]. Subsequently, a new genotype, genotype-8, was identified in BLV samples from Croatia by Balic et al. [98], who concluded that BLV may be more divergent than previously thought, speculating that additional genotypes might be discovered in the future. Indeed, the presence of eight BLV genotypes was later confirmed in different geographical locations [74, 77, 99–101]. Finally, in 2016, the novel BLV genotypes, genotype-9 and -10, were discovered in Bolivia [75], Thailand [102], and Myanmar [76], a totaling ten BLV genotype clusters (Fig. 2). Previously, almost all phylogenetic studies of BLV genotypes focused on the partial or entire *env* gene. However, for the first time in their study [75, 76], Polat et al. successfully concluded the existence of genotypes -1, -2, -4, -6, -9 and -10 among ten BLV genotypes (Fig. 3) by phylogenetic analysis using complete sequences of BLV strains newly determined by next generation sequencing and sequencing cloned, overlapping PCR products in their studies, and using complete BLV genome sequences available in the database (NCBI & DDBJ). These phylogenetic analysis of complete BLV genomes demonstrated that each BLV genotype encodes specific amino acid substitutions in both structural and non-structural gene regions.

**BLV prevalence**

BLV has spread to all continents via the trade in breeding animals, and is prevalent in cattle worldwide. BLV infection levels vary between and within countries, as shown in Table 3 (data obtained on March 17th, 2017; updated and detailed information is available at http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statuslist) [17, 103]. BLV eradication programs and control measures have been established in European Community member countries since the second half of the twentieth century, and eradication programs have been very successful in the majority of western Europe [104–107]; indeed, some countries, including Denmark, Finland, Switzerland, Estonia, The Netherlands and Poland, are completely free of BLV [104, 108–110]. Despite the majority of countries in Western Europe being free from disease, EBL still exists in eastern European nations, including Poland, Ukraine, and Croatia [98, 100, 111–113]. In
**Table 2** Summary of BLV genotyping methods

| Genotyping method | Amplified BLV region | Amplicon size (bp) | Enzymes | Phylogenetic approaches | Classification result | Reference |
|-------------------|----------------------|--------------------|---------|-------------------------|-----------------------|-----------|
| PCR-RFLP          | Partial env-gp51 region | 444                | *Bam*<sub>H</sub>*<br>*Bgl*I, *Hae*III, *Bcl*I, *Pvu*I, *Dra*I, *Hind*III, *Hpa*II, *Stu*I, *Taq*I | 7 groups: A, B, C, D, E, F, G | Fechner et al., 1997 [90]<br>Licursi et al., 2002 [91] <br>Asfaw et al., 2005 [95] |
| RFLP + sequencing | Partial gp51 sequencing | 400–444            | *Bam*<sub>H</sub>, *Bcl*I, *Pvu*I, *Gmb*H | NJ; MP; ML | RFLP-based type: Australian type, Argentine type, Belgium type, Japanese type; Sequence-based type: Argentine cluster, European cluster, Japan and German isolate cluster; groups I–IV; or genotypes 1–8 | Monti et al., 2005 [49] <br>Felmer et al., 2005 [93] <br>Camargos et al., 2007 [122] |
| PCR-sequencing    | Partial gp51 sequencing | 346–444            | NJ; ML; BI | Japanese group, Argentine group, European group; or genotypes 1–8 | Camargos et al., 2002 [121]<br>Licursi et al., 2003 [92] <br>Matsumura et al., 2011 [98] <br>Rola-Luszczak et al., 2013 [99] <br>Polat et al., 2015 [74] <br>Ochirkhuu et al., 2016 [77] <br>Polat et al., 2016 [75, 76] |
| Sequencing of partial or full gp51 gene sequences | 444–903 | NJ; ML; BI | Up to 10 BLV genotypes | Moratorio et al., 2010 [126] <br>Balic et al., 2012 [97] <br>Lee et al., 2015 [100] <br>Lee et al., 2016 [101] |
| Sequencing of env (full gp51 and/or gp30 genes) | up to 1548 | NJ; ML; BI | Consensus cluster, US Californian cluster, European cluster, Costa Rican cluster; or genotypes 1–10 | Zhao et al., 2007 [109] <br>Rodriguez et al., 2009 [96] <br>Yang et al., 2016 [131] |
| Full BLV genome sequencing | BLV complete genome | 8714 | ML | genotypes −1, −2, −4, −6, −9, and −10 | Polat et al., 2016 [75, 76] |

**BI** Bayesian inference, **BLV** bovine leukemia virus, **NJ** neighbor-joining, **ML** maximum-likelihood, **MP** maximum-parsimony, **RFLP** restriction fragment length polymorphism
addition, in Italy, Portugal, Belarus, Latvia, Greece, Romania, and Bulgaria, BLV is present, although disease is either absent or limited to specific areas [103].

Nationwide BLV eradication and control programs were introduced in Australia and New Zealand in 1983 and 1996, respectively, and 99.7% of Australian dairy herds were declared free from EBL in December 2013, while those in New Zealand have been free from BLV-induced EBL since 2008 [113, 114].

In North America, an epidemiological study of BLV prevalence in US dairy cattle conducted by the Department of Agriculture’s National Animal Health Monitoring System demonstrated that 83.9% of dairy cattle were BLV-positive at herd level and 39% of beef
herds had at least one BLV-infected animal [115]. In Canada, studies of BLV prevalence revealed that up to 37.2% of cows and 89% of herds were BLV-positive [116–118]. BLV is also present in both beef and dairy cattle in Mexico [119]; however, disease is either absent or limited to specific areas [17] (accessed on 22 Dec 2016).

In South America, relatively high levels of BLV prevalence have been observed, and BLV-induced leukemia is present in the majority of countries. In Brazil, BLV prevalence varies among states, with infection rates ranging from 17.1% to 60.8% [120–123]. Individual and herd level BLV prevalence in Argentina are as high as 77.4% and 90.9%, respectively [75, 95, 124]. Moreover, individual infection rates between 19.8% and 54.7% have been reported in Chile, Bolivia, Peru, Venezuela, Uruguay, Paraguay, and Columbia [75, 94, 125–131].

BLV infection is widespread in Chinese dairy farms. Infection rates are up to 49.1% among individual dairy cattle, while 1.6% of beef cattle are BLV-positive [132]. Moreover, serological tests revealed that 20.1% of yaks in China were BLV-positive [133]. Epidemiological studies in Japan revealed varying levels of BLV prevalence throughout the country, based on different detection methods [83, 134–136], and BLV infection rates of 40.9% of dairy and 28.7% of beef cattle, with infection rates in animals over 2-years-old reaching 78% in dairy herds and 69% in beef cattle herds [136]. Less than 6% of cattle were infected with BLV in Mongolia (3.9%) [77], Cambodia (5.3%) [137], and Taiwan (5.8%) [48], while a serological survey in Iran revealed that the prevalence of BLV was between 22.1% and 25.4% in that country [138, 139]. Lee et al. [102] demonstrated an average prevalence of BLV of 58.7% in Thailand, reaching maxima of 87.8% and 100% of cattle when assayed using PCR and ELISA, respectively. In Korea, 54.2% of dairy cattle and 86.8% of dairy herds were BLV-positive, whereas only 0.14% of beef cattle were infected with BLV [101]. BLV infection levels in the Philippines ranged from 4.8% to 9.7% [74] while it was 9.1% in Myanmar [76]. BLV infections in Middle Eastern countries are relatively low. The prevalence of BLV infection is approximately 5% in Israel [140], while in Saudi Arabia, 20.2% of...
| Geographical division | Country        | Within country | BLV prevalencea | References                                      |
|-----------------------|----------------|----------------|-----------------|------------------------------------------------|
| Europe                | Andorra        | Nationwide     | BLV-free, 1994  | OIE, 2009 [103]                                 |
|                       | Cyprus         | Nationwide     | BLV-free, 1995  | OIE, 2009 [103]                                 |
|                       | Czech Republic | Nationwide     | BLV-free, 2010  | OIE, 2009 [103]                                 |
|                       | Denmark        | Nationwide     | BLV-free, 1990  | OIE, 2009 [103]                                 |
|                       | Estonia        | Nationwide     | BLV-free, 2013  | OIE, 2009 [103]                                 |
|                       | Finland        | Nationwide     | BLV-free, 2008  | OIE, 2009 [103]                                 |
|                       | Ireland        | Nationwide     | BLV-free, 1999  | OIE, 2009 [103]                                 |
|                       | Norway         | Nationwide     | BLV-free, 2002  | OIE, 2009 [103]                                 |
|                       | Spain          | Nationwide     | BLV-free, 1994  | OIE, 2009 [103]                                 |
|                       | Switzerland    | Nationwide     | BLV-free, 2005  | OIE, 2009 [103]                                 |
|                       | Sweden         | Nationwide     | BLV-free, 2007  | OIE, 2009 [103]                                 |
|                       | Slovenia        | Nationwide     | BLV-free, 2006  | OIE, 2009 [103]                                 |
|                       | UK             | Nationwide     | BLV-free, 1996  | OIE, 2009 [103]                                 |
|                       | The Netherlands | Nationwide     | BLV-free, 2009  | OIE, 2012 [17]                                  |
|                       | Poland         | BLV-free, 2017 |                 | EFSA Panel on Animal Health and Welfare, 2017 [110] |
|                       | Ukraine        | Present        |                 | OIE, 2012 [17]; Rola-Luszczak et al., 2013 [100] |
|                       | Croatia        | Present        |                 | OIE, 2012 [17]; Balik et al., 2012              |
|                       | Italy          | Present        |                 | OIE, 2009 [103]; Molteni et al., 1996 [144]    |
|                       | Portugal       | Present        |                 | OIE, 2009 [103]                                 |
|                       | Belarus        | Present        |                 | OIE, 2012 [17]; Rola-Luszczak et al., 2013 [100] |
|                       | Latvia         | Present        |                 | OIE, 2009 [103]                                 |
|                       | Romania        | Restricted to certain area | OIE, 2009 [103] |
|                       | Bulgaria       | Present        |                 | OIE, 2009 [103]                                 |
|                       | Greece         | Present        |                 | OIE, 2009 [103]                                 |
|                      | Oceania        | Australia      | BLV-free in dairy cattle, 2013 | EPAHW, 2015 [113] |
|                      |                | New Zealand    | BLV-free, 2008  | Chethanond, 1999 [114]                         |
|                      | North America  | USA            | 83.9% dairy cattle; 39% beef cattle, 2007 | APHIS, 2008 [115] |
|                      |                | Canada         | 89% at herd level | APHIS, 2008 [115] |
|                      |                | Nationwide     | 78% at herd level, 1998–2003 | Nekouei, 2015 [13] |
|                      |                | Saskatchewan  | 37.2% at individual level, 2001 | VanLeeuwen et al., 2001 [116] |
|                      |                | Maritime       | 20.8% at individual and 70.0% at herd level, 1998–1999 | VanLeeuwen et al., 2005 [117] |
|                      |                | Maritime       | 30.4% at individual and 90.8% at herd level, 2013 | Nekouei, 2015 [118] |
|                      |                | Mexico         | 36.1% of dairy and 4.0% of beef cattle, 1983 | Suzan et al., 1983 [119] |
|                      | South America  | Brazil         | 17.1% to 60.8%, 1980–1989 and 1992–1995 | Sammara et al., 1997 [120]; D’Angelino et al., 1998 [121] |
|                      |                | Argentina      | 77.4% at individual and 90.9% at herd level, 2007 | Polat et al., 2016 [75] |
|                      |                | Buenos Aires  | 32.85% at individual and 84% at herd level, 1998–1999 | Trono et al., 2001 [124] |
|                      |                | Multiple regions |                           |                                        |
|                      |                | Chile          | 27.9% at individual level, 2009 | Polat et al., 2016 [75] |
dairy cattle tested as BLV-positive [141]. Compared to these countries, BLV infection rates in Turkey are higher, with 48.3% of dairy herds including sero-positive animals [142].

**Table 3** Detailed information on BLV infection levels worldwide (Continued)

| Geographical division | Country | Within country | BLV prevalence | References |
|-----------------------|---------|---------------|----------------|------------|
| Africa                | South Africa | BLV-free, 2012 |                | OIE, 2012 [17] |
|                       | Tunisia | BLV-free, 2005 |                | OIE, 2009 [103] |
|                       | Egypt | BLV-free, 1997 |                | OIE, 2009 [103] |
|                       | Japan | BLV-free, 2007 |                | OIE, 2009 [103] |
|                       | Kazakhstan | BLV-free, 2008 |                | OIE, 2009 [103] |
|                       | Kyrgyzstan | BLV-free, 2008 |                | OIE, 2009 [103] |
|                       | China | Nationwide 73.3% at individual cattle, 2012–2014 | Ohno et al., 2015 [83] |
|                       | Mongolia | 3.9% of dairy cattle, 2014 | Ochirkhuu et al., 2016 [77] |
|                       | Cambodia | 5.3% of draught cattle, 2000 | Meas et al., 2000 [137] |
|                       | Taiwan | 5.8% of dairy cattle, 1986 | Wang et al., 1991 [48] |
|                       | Iran | Nationwide Between 22.1% to 25.4%, 2012–2014 | Nekoei et al., 2015 [138]; Mousavi et al., 2014 [139]. |
|                       | Khorasan Razavi | 29.8% of dairy cattle, 2009 | Mousavi et al, 2014 [139]. |
|                       | Khorasan Shomali | 1.5% of dairy cattle, 2009 | Mousavi et al, 2014 [139]. |
|                       | Thailand | 58.7% of cattle, 2013–2014 | Lee et al., 2016 [102] |
|                       | Philippines | 4.8% to 9.7% of cattle, 2010–2012 | Polat et al., 2015 [74] |
|                       | Myanmar | 9.1% at individual level 2016 | Polat et al., 2016 [76] |
|                       | Korea | 54.2% of dairy cattle and 86.8% of dairy herds; 0.14% of beef cattle, 2014 | Lee et al., 2015 [101] |
| Middle East | Israeli | 5% at individual level | Trainin & Brenner, 2005 [140] |
|                       | Saudi Arabia | 20.2% of dairy cattle, 1990 | Hafez et al, 1990 [141] |
|                       | Turkey | 48.3% of dairy herd | Burgu et al, 2005 [142] |

BLV prevalence in this table shows BLV infection in certain specific period. Therefore, there might be a change in BLV prevalence in different times APHIS Animal and Plant Health Inspection Service, BLV bovine leukemia virus, EFSA European Food Safety Authority, EAHW European Panel on Animal Health and Welfare, OIE The World Organisation for Animal Health

**Note:** BLV prevalence in each sample collection year; however, no information about sample collection year was provided in some cases

**Distribution of BLV genotypes worldwide**

As mentioned above, phylogenetic analyses of whole genome (Fig. 3) and env gp51 sequences (Fig. 2) of BLV strain showed that BLV can be classified into ten...
genotypes. Three genotypes of BLV, namely genotype-1, genotype-4 and genotype-6, were mainly detected from across the world, as shown in Table 4. Genotype-1 is the most dominant genotype of BLV and is distributed across almost all continents, including Europe, America, Asia, and Australia. In particularly, genotype-1 spread to South and North America, and these continents still have a high prevalence of BLV infection. In addition, genotype-1 continues to spread worldwide, including Asian countries. The second most widely distributed genotype is genotype-4, which is primarily detected in Europe and some American countries. However, it is only found in Mongolia among Asian nations. Interestingly, although genotype-4 used to exist in Europe, it decreased because of BLV eradication in European countries. Genotype 6 may have come from South America and spread to South Asia by animal trading. Of the other genotypes, genotype-2 is restricted to South American countries and is only found in Japan among Asian nations, while genotype-8 is restricted to Europe. Genotypes-5 (in Brazil and Costa Rica) and −10 (in Thailand and Myanmar) are only observed in geographically proximal areas, where there may be an exchange of animals across national boundaries [76, 102]. By contrast, genotypes-7 is distributed across geographically dispersed regions [74, 77].

In detail, in Europe, a total of five different BLV genotypes have been detected (genotypes −1, −3, −4, −7, and −8): genotype-4 in Belarus [100] and Belgium [86, 143]; genotypes-4, −7, and −8 in Russia and Ukraine [100]; genotype-8 in Croatia [98]; genotypes −4 and −7 in Poland [100]; genotypes −3 and −4 in France [86]; genotypes −1 and −4 in Germany [91]; and genotype-7 in Italy [144]. In Australia, only genotype-1 was detected [90]. In North America, genotypes −1, −3, and −4 have been detected in the USA [86, 143, 145], and genotype-1 was reported in the Caribbean [146]. In Central America, genotypes −1 and −5 were detected in Costa Rica [143]. A variety of BLV genotypes (−1, −2, −4, −5, −7, and −9) were detected in South America: genotypes −1, −2, −4, and −6 in Argentina [93, 95, 97, 147, 148]; genotypes −1, −2, −5, −6, and −7 in Brazil [122, 123, 127]; genotypes −4 and −7 in Chile [94]; genotypes −1, −2, −6, and −9 in Bolivia [75]; genotypes −1, −2, and −6 in Peru and Paraguay [75]; and genotype-1 in Uruguay [126]. In Asia, a total of seven BLV genotypes have been confirmed (−1, −2, −3, −4, −6, −7, and −10): genotypes −1 and −3 in Korea [101, 149]; genotypes −1, −2, and −3 in Japan [93, 99, 143, 150]; genotypes −1 and −6 in The Philippines [74]; genotypes −1, −6, and −10 in Thailand [102]; genotypes −1, −4, and −7 in Mongolia [77]; genotype-10 in Myanmar [76]; and genotypes −1 and −6 in Jordan [151].

Based on the European Food Safety Authority panel on animal health and welfare, BLV-induced EBL may have originated and spread widely from an area of Memel in East Prussia (now Klaipeda in Lithuania) [113, 152]. The worldwide distribution of the disease occurred due to the introduction of cattle from European countries into herds in other countries free of the disease, and also through the international trade of bred animals [113]. Interestingly, genotype-4 existed primarily in East Prussia as shown in Table 4. Then, infected cattle were reintroduced into some European countries; for example, BLV was introduced into the UK via bred animals from Canada in 1968 and 1973 [113]. As detailed in some previous publications, the widespread distribution of BLV genotypes within and between distant geographical locations may be driven by the spread of virus through the movement of live animal populations, associated with human migration and animal domestication, and also with viral transmission during close contact between individual animals [97].

Future prospects
It appears that at least ten different BLV genotypes of BLV strains are circulating in various geographical locations worldwide. The completion of whole genome sequencing of these BLV strains has revealed that BLV genomes contain a number of unique genotype specific substitutions not only in the env region, but also in the LTR, Gag, Pro, Pol, Tax, Rex, R3, G4, and miRNA encoding regions, distinguishing each genotype [75]. However, the BLV genome sequences of strains from different geographic origins, especially the important sites on the regulation of viral replication of BLV, are relatively stable and highly conserved among BLV strains, assigned to different genotypes. By contrast, several groups recently reported that the expression or pathogenesis of BLV does not depend on strains, but rather, is related with the specific site of mutation in their BLV genome [153, 154]. These results clearly demonstrate that BLV strain should be determined by full genome sequencing. However, although BLV is present worldwide, BLV genotyping studies are limited to certain areas, as shown in Table 4. Therefore, the accumulation of the full genome sequencing of BLV strains, assigned to different genotypes worldwide may define the genotype-dependent pathogenesis and association between genetic variability in each genotype and its infectivity, and differences in its functions in the future.

Conclusion
BLV is the etiologic agent of EBL, which is the most common neoplastic disease in cattle. It infects cattle worldwide, thereby imposing a severe economic burden on the dairy cattle industry. In this review,
we summarized currently available detailed information on BLV infection worldwide, and indicated that BLV has spread to most countries except for some countries which are completely free of BLV by successful BLV eradication. We also outlined at least ten different BLV genotypes circulating in various geographical locations worldwide and the distribution of these BLV genotypes worldwide. This should be useful information to those investigating BLV for the potential development of diagnostic methods and vaccines, and for reducing the incidence of BLV in herds.

Abbreviations
AGID: Agar gel immunodiffusion; BI: Bayesian inference method; BLV: Bovine leukemia virus; EBL: Enzootic bovine leukosis; ELISA: Enzyme-linked immunosorbent assay; EPAHW: European Food Safety Authority panel on animal health and welfare; HTLV-I &-II: Human T-cell lymphotropic virus types I and II; LTR: Long terminal repeats; miRNA: microRNA; ML: Maximum likelihood method; NJ: Neighbor-joining method; PCR: Polymerase chain reaction; PHA: Passive hemagglutination assay; PL: Persistent lymphocytosis; qPCR: Quantitative PCR; RFLP: Restriction fragment length polymorphism; RIA: Radio immunoassay; STLV: Simian T-cell lymphotropic virus

Acknowledgments
We thank our collaborators for kindly assisting with the large-scale sampling from many farms in the Philippine, Myanmar, South America (Argentina, Peru, Paraguay, Chile and Bolivia) and Japan.

Funding
The studies on BLV were supported by Grants-in-Aid for Scientific Research [A (08021470), A (16H02590), B (10004294), and C (25450405)] from the Japan Society for the Promotion of Science (JSPS), by a grant from Integration Research for Agriculture and Interdisciplinary Fields in Japan (14538311), and by a grant from the Project of the NARO Bio-oriented Technology Research Advancement Institution (the special scheme project on regional developing strategy) (Grant No. 16817983).

Table 4 Worldwide geographical distribution of the ten known BLV genotypes based on env-gp51 sequences

| Geographical division | Country | Genotype | Reference |
|----------------------|---------|----------|-----------|
| Europe               | Belarus | 4        | Rola-Luszczak et al., 2013 [99] |
|                      | Russia  | 4 7 8    | Rola-Luszczak et al., 2013 [99] |
|                      | Ukraine | 4 7 8    | Rola-Luszczak et al., 2013 [99] |
|                      | Croatia | 8        | Balic et al., 2012 [97] |
|                      | Poland  | 4 7      | Rola-Luszczak et al., 2013 [99] |
|                      | Belgium | 4        | Mamoun et al., 1990 [85]; Zhao & Buehring, 2007 [142] |
|                      | France  | 3 4      | Mamoun et al., 1990 [85] |
|                      | Germany | 1 4      | Fechner et al., 1997 [90] |
|                      | Italy   | 7        | Molteni et al., 1996 [143] |
|                      | Austria | 1        | Coulston et al., 1990 [89] |
|                      | America | USA 1 3 4 | Derse et al., 1985 [144]; Mamoun et al., 1990 [85]; Zhao & Buehring, 2007 [142] |
|                      |         | Belgium  | 1        | Yang et al., 2016 [145] |
|                      |         | France   | 3 4      | Mamoun et al., 1990 [85] |
|                      |         | Germany  | 1 4      | Fechner et al., 1997 [90] |
|                      |         | Italy    | 7        | Molteni et al., 1996 [143] |
|                      | Australia | 1        | Coulston et al., 1990 [89] |
|                      | America | USA 1 3 4 | Derse et al., 1985 [144]; Mamoun et al., 1990 [85]; Zhao & Buehring, 2007 [142] |
|                      |         | Belgium  | 1        | Yang et al., 2016 [145] |
|                      |         | France   | 3 4      | Mamoun et al., 1990 [85] |
|                      |         | Germany  | 1 4      | Fechner et al., 1997 [90] |
|                      |         | Italy    | 7        | Molteni et al., 1996 [143] |
|                      | Australia | 1        | Coulston et al., 1990 [89] |
|                      | America | USA 1 3 4 | Derse et al., 1985 [144]; Mamoun et al., 1990 [85]; Zhao & Buehring, 2007 [142] |
|                      |         | Belgium  | 1        | Yang et al., 2016 [145] |
|                      |         | France   | 3 4      | Mamoun et al., 1990 [85] |
|                      |         | Germany  | 1 4      | Fechner et al., 1997 [90] |
|                      |         | Italy    | 7        | Molteni et al., 1996 [143] |
Availability of data and materials
Not applicable

Authors’ contributions
Yoko Aida designed the concept of the review article, edited and revised the manuscript. Meri pivot Polat wrote the manuscript, constructed Tables and Figures, and edited and revised the manuscript. Shin-nosuke Takeshima wrote some part and helped with the revision of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interest.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
1Viral Infectious Diseases Unit, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan. 2Nano Medical Engineering Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan. 3Bovine Leukemia Virus Vaccine Laboratory RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan.

Received: 31 July 2017 Accepted: 24 October 2017
Published online: 02 November 2017

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