

Prevalence and Genotypic Distribution of Bovine Leukemia Virus across Asian Regions: Insights into Economic Significance and Clinical Staging

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Abstract

This study critically reviews the literature on the prevalence and genotypic distribution of Bovine Leukemia Virus (BLV) in Asia, specifically targeting enzootic bovine leukosis, a chronic disease affecting cattle's life and milk production along with reduced fat yield, that causing economic losses due to increased susceptibility to pathogens. European countries are eradicating BLV but Asia has a high prevalence, particularly in Asian cattle. Enzootic bovine leukosis (EBL) has distinct clinical stages: asymptomatic, persistent lymphocytosis, and lymphosarcoma. It typically occurs in adult animals, with 60% being asymptomatic during the aleukemic stage, 30% developing persistent lymphocytosis, and 5% developing lymphoma. Databases like PubMed and Google Scholar were used to search molecular and prevalence studies from 2000-2024, focusing on Asian countries. Prevalence of bovine leukosis (BL) in Asia varies significantly, with high rates in central and eastern regions. BLV is a global disease, has increased in Asia with highest prevalence in central Asia about 40.0–84.0% (Kazakhstan), 35% in Eastern Asia (Japan), and 58.3% in Southeast Asia (Thailand). BLV genotypes G1, G4, G6, G7, G12, G3, G4, G6, G7, and G10 are prevalent in various regions of Asia, with G1 being most prevalent globally. These genotypes might be spread due to animal trading, and animal domestication, affecting various countries. This review provides the current status of enzootic bovine leukosis in Asia, emphasising the need for a closed trading system to control the disease.

Keywords: Bovine Leukemia Virus (BLV), Genotype, Prevalence, Cattle, Asia

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1. Introduction

Bovine leukemia virus is an oncogenic RNA virus belonging to the Deltaretrovirus genus of the Retroviridae family. It causes Enzootic Bovine Leukosis (EBL) and targets B cells, leading to B lymphocytotropic (Mousavi et al., 2014; Olaya-Galán et al., 2022). It is also known by other names like Bovine lymphomastitis, bovine lymphosarcoma or malignant lymphoma of cattle. It is included among the five important diseases of cattle. The virus was isolated for the first time in Germany. BLV can be transmitted vertically and horizontally through blood-contaminated procedures like shared needles, reproductive sleeves, and natural breeding

(Kiugu, 2018). Vertical transmission includes intrauterine infection, particularly in dams with high viral loads (Sajiki et al., 2017) and through colostrum and milk (Quadros et al., 2024). Horizontal transmission involves the transfer of infected cells via direct contact, insect bites, and contaminated iatrogenic procedures (Joris et al., 2021).

Leukosis was first reported by Leisering in 1871 when he found yellowish nodules and tumors in the body (Leisering, 1871). Bovine Leukosis (BL) is characterized by uncontrolled lymphocyte proliferation, immunosuppression, and tumor development in a variety of organs. It induces a lifetime infection by integrating its genetic material into host cells (Juliarena et al., 2017). One of the most crucial

concerns about BLV infection is that it remains asymptomatic in multiple animal species. BLV infects all mammalian species, but cattle are more inherently infected due to the presence of AP3D1 receptors (Olaya-Galán et al., 2022). It is transmitted horizontally by blood (infected B lymphocytes), milk (especially colostrum), and intrauterine infection (Shrestha et al., 2024). Buffaloes were naturally infected and susceptible to infection when challenged with BLV. Countries pursuing the eradication program should screen buffaloes as they could be a permanent threat to free status if remain infected (Feliziani et al., 2017; Molnar et al., 2000; Romero et al., 1981). The majority of sheep that have been artificially infected died from leukemia (Florins et al., 2008).

BLV is a persistent infection, with 70% of infected animals remain asymptomatic, 30% developing lymphocytosis, and 10% developing leukemia/lymphoma (Barez et al., 2015). Only about one-third of the infected cattle experience persistent lymphocytosis and in 5% cases, it may evolve to lymphosarcoma (Trono et al., 2001). BL has a significant economic impact due to reduced milk production, diminished fertility, increased susceptibility to other diseases, and premature culling of infected animals (Nakada et al., 2022). EBLV leads to restrictions on international trade, which is a major concern for livestock due to its economic and animal welfare implications (WOAH). In Asia, there is a gap in research on bovine leukemia virus, and the notable lack of comprehensive studies on its prevalence and genetic diversity. Additionally clinical staging and economic loss due to enzootic bovine leukosis. The review summarizes recent research on BLV prevalence and molecular characterization in Asia, highlighting its significant economic impact and being crucial for cattle health and livestock.

2. Economical impact of BLV

Enzootic bovine leukosis is a chronic disease that has detrimental effects on cattle's life and milk production (Nekouei et al., 2016). Economic losses due to enzootic bovine leukosis can decrease milk production, fertility, life span, and condemnation at slaughter (Khudhair et al., 2016a). Higher BLV prevalence has been associated with reduced herd milk production and a shortened cow's lifespan (Erskine et al., 2012; Nekouei et al., 2016). BLV milk positivity was associated with loss of milk production at the individual cow level (305-d mature-equivalent yields), especially among the older cows (Norby et al., 2016). The association of subclinical mastitis with BLV infection was also investigated. Estimated economic loss per animal was \$418.59 and the annual loss was \$6,097,225 due to mastitis caused by BLV. These animals become more susceptible to

pathogens due to immunosuppression. So, BLV infection causes a significant economic loss in the dairy industry (Nakada et al., 2023). On average, there was a loss per animal of about \$59 in a BLV-positive herd and a 218kg milk reduction. About a 2.7% decline in milk production occurs in test-negative cows. These findings were quite comparable to earlier studies as well (Ott et al., 2003). Fat yield also seemed to be highly affected due to BLV, especially with progressing age and the condition of persistent lymphocytosis (PL). Over a period of 6 years, the annual economic loss was calculated to be \$90.72 and \$325.08 for animals having PL for 2 and 3 years, respectively (Da et al., 1993). In Michigan dairy herds, higher BLV prevalence has been associated with reduced herd milk production and a shortened cow lifespan (Erskine et al., 2012).

In Japan, about 21.6% of carcass weight reduction was occurring due to BLV infection. A US\$1,391,649 significant economic loss resulting from carcass weight loss brought on by BLV infection was investigated (Nakada et al., 2022). Malignant lymphoma was causing 26.87% of dairy and 13.35% of beef carcass condemnation in the US about 274,029 of 7,138,997 cull dairy cows were condemned (White & Moore, 2009). The rate of condemned carcasses due to malignant lymphoma was higher during winter in California and US states (Amirpour Haredasht et al., 2018). BLV-positive animal were 23% more likely to be culled than their BLV-negative herd mates in 112 Michigan dairy herds, indicating its economic impact on US dairy (Bartlett et al., 2013). However, a study on a large dairy farm demonstrated successful BLV reduction through selective culling based on diagnostic testing, reducing BLV ELISA-positive cows to 0.85% after three years (Taxis et al., 2023). The association of subclinical mastitis with BLV infection was also investigated. EBLV leads to restrictions on international trade, which is a major concern for livestock due to its economic and animal welfare implications (WOAH).

3. Prevalence of BLV in Asian Countries

BLV has become prevalent all over the world, at various levels of prevalence. Some studies showed that Japan was the first Asian country where BLV was reported, but complete evidence is not present. The first case of BLV in Japan was reported in 1927 in Iwate Prefecture, and then gradually, cases increased (K. Nishikaku et al., 2022). Since then, Asian nations have been measuring the BLV prevalence. In Asian regions, Prevalence of BLV reported over the previous 24 years is shown in Table 1. In Western Asia, about 25.7% prevalence was calculated in dairy cattle of the UAE (Abu Dhabi) using ELISA (Hassan et al., 2020). In Turkey, BLV prevalence was calculated to be about 1.6% in

2003 based on the Immunodiffusion test and 21% in 2005 based on AGID (Burgu *et al.*, 2005; Meas *et al.*, 2003). In Kazakhstan (Central Asia), BLV seems to be at a high level of prevalence of about 40-84% overall based on PCR test results (Mamanova *et al.*, 2020). In Eastern Asia, Japan has an overall 35% prevalence in both dairy and beef cattle measured in 2013 (Murakami *et al.*, 2013). In Korea and China, BLV is reported at about 10.2% and 10% overall based on ELISA (Kim *et al.*, 2017; Ma *et al.*, 2021). In Taiwan again, a high prevalence of about 99% in dairy cattle at the herd level and 81.8% at the individual level was calculated in 2019 (Hsieh *et al.*, 2019). In Mongolia, a 3.9% overall prevalence was reported based on PCR results of blood from dairy cattle in Tuv, Arkhangai, and Ulaanbaatar (N. Ochirkhoo *et al.*, 2016). In southern Asia in 2017, India had about 9.09% overall prevalence in dairy and breeding cattle (Katoch *et al.*, 2017). In Iran, the prevalence calculated in Isfahan and Bukhtiary provinces was about 22.1%–25.0% overall in cattle, sheep, and camel (with 0% in camel) (Nekoei *et al.*, 2015). In Pakistan, 20% BLV prevalence is

calculated in cattle from the Northwest region based on Indirect ELISA (Khan *et al.*, 2020). The next region is Southeast Asia, in which 9 provinces of Thailand (2016) and 5 provinces of Cambodia (2000) measured a prevalence of 58.3% and 5.3%, respectively (Lee *et al.*, 2016; Meas *et al.*, 2000). The Philippines reported that 23.1% of cattle were affected by BLV in 2015, while Myanmar reported 37.4% of cattle having BLV infection in 2020 (Mamanova *et al.*, 2020; Polat *et al.*, 2015). Limited veterinary resources and infrastructure in many regions make regular testing and disease monitoring difficult. Basic biosecurity practices, such as changing gloves and avoiding needle reuse, are not always followed. The introduction of new animals without knowing their BLV status is common, increasing the risk of spread within herds (Kuczewski *et al.*, 2022). Cultural and management differences, such as communal grazing or uncontrolled breeding, also contribute to the spread of BLV (Kiugu, 2018). High-risk cattle are more likely to spread the virus, emphasizing the importance of monitoring and segregating them.

Table 1. Prevalence of Bovine leukemia virus (BLV) in different regions of Asia

Regions of Asia	Country	Area	Specie	Sam ple Size	Sam ple	Test	Prevalence	Reference
Western Asia	United Arab Emirate	Abu Dhabi	Dairy cattle	957	Bloo d	ELISA	25.7% overall	Hassan <i>et al.</i> (2020)
	Turkey	-	Cattle	-	Seru m	AGID	21% overall	Burgu <i>et al.</i> (2005)
		-	Cattle	300	Bloo d	Immuno diffusion test	1.6% overall	Meas <i>et al.</i> (2003)
	Iraq	-	Cattle	400	Bloo d	PCR ELISA	7% overall	Khudhair <i>et al.</i> (2016b)
Central Asia	Kazakhstan	East Region	Cattle	-	Bloo d	PCR	40.0–84.0% overall	Mamanova <i>et al.</i> (2020)
Eastern Asia	Japan	-	Dairy and Beef cattle	20835	Seru m	ELISA	35% overall 40.9% of Dairy cattle 28.7% of Beef cattle	Murakami <i>et al.</i> (2013)
	Korea	-	Cattle	4498	Seru m	ELISA	10.20% overall	Kim <i>et al.</i> (2017)
	China	-	Cattle Yak Buffalo	-	Bloo d	ELISA PCR AGID	10.00% overall	Ma <i>et al.</i> (2021)
	Taiwan	-	Dairy Cattle	-	Seru m	ELISA PCR	99.1% herd 81.8% indivi dual	Hsieh <i>et al.</i> (2019)
	Mongolia	Tuv, Arkhan gai and	Dairy cattle, native cattle, and yaks	517	Bloo d	PCR	3.9% overall	N. Ochirkhoo <i>et al.</i> (2016)

		Ulaanbaatar						
Southern Asia	India	Himachal Pradesh	Dairy and Breeding cattle	1,511	Blood	AGID	9.09%Overall	Katoch et al. (2017)
	Iran	Isfahan and Bukhtia ry provinces	Cattle Sheep Camel	874	Blood	Nested PCR	22.1%-25.4% overall 5.3% Cattle 22% Sheep 0% Camel	Nekoei et al. (2015)
	Pakistan	Northwest	Cattle	600	Blood	Indirect ELISA	20 % Cattle	Khan et al. (2020)
Southeast Asia	Thailand	9 provinces of Thailand	Cattle	744	Serum	ELISA Nested PCR	58.30% Cattle	Lee et al. (2016)
	Philippines	-	Cattle	-	Blood	qPCR Nested PCR	23.1 % Cattle	Polat et al. (2015)
	Myanmar	-	Cattle	-	Blood	qPCR	37.04% Cattle	Mamanova et al. (2020)
	Cambodia	5 provinces of Cambodia	Draught Cattle	544	Blood	ELISA	5.3% Draught cattle	Meas et al. (2000)

Efforts to reduce BLV prevalence have been made, primarily through the eradication of infected cattle, segregation of BLV-free animals, and vaccination. However, these strategies have been unsuccessful elsewhere due to economic costs, management restrictions, and the lack of an efficient vaccine. Despite successful eradication in some European countries, BLV prevalence is increasing in several nations (Lv et al., 2024). Control strategies include eradication of infected cattle, segregation of BLV-free animals, and vaccination attempts, though these have had limited success due to economic and management constraints. Recent research has explored novel approaches, such as competitive infection with virus deletants to reduce proviral loads (Rodríguez et al., 2011).

4. Pathogenesis and Clinical staging of BLV Infection

Bovine Leukemia Virus (BLV) specifically targets B-lymphocytes and enters these cells and integrates its proviral DNA into the host genome, enabling persistent infection. The viral envelope glycoproteins interact with specific surface receptors on B-lymphocytes to mediate viral entry. Toll-like receptors (TLRs), particularly TLR3, TLR7, and TLR9, are upregulated in infected animals, suggesting their involvement in viral recognition and entry mechanisms.

Once inside the cell, the viral RNA genome is reverse-transcribed into DNA and integrated into the host genome. In veterinary medicine, clinical staging of EBL is a crucial procedure to evaluate the severity and progression of the disease in infected animals. Bovine leukemia is a fatal disorder characterized by neoplastic lymphocytosis and systemic lymphoma, with two types: EBL caused by BLV and SBL, not transmissible, and unknown etiology (Nishimori et al., 2017). The incubation period is key to understanding disease spread and progression. After a long incubation period of 7.0 years, 1.4% of EBL-infected dairy cattle developed lymphosarcoma (Tsutsui et al., 2016). Enzootic bovine leukosis manifests itself in distinct clinical stages based on serologic, hematologic, and clinical observations: asymptomatic, persistent lymphocytosis, and lymphosarcoma as described in Figure 1 (Radostits et al., 2006; Trono et al., 2001). Stage I was characterized by seropositivity without any apparent clinical signs. Stage II referred to as subclinical; there is an elevation in antibody levels but no observable clinical symptoms. Stage III referred to as clinical, the disease becomes evident with visible clinical signs. Clinical signs include Lymphocytosis, swollen lymph nodes, progressive weight loss, decreased milk production, unilateral exophthalmos, and impaired

fertility (Mekata et al., 2019; Santos et al., 2023). Finally, Stage IV represented the end-stage of the disease, where terminal lymphosarcomas or the extensive infiltrations of lymphoid tissue were observed (Burng, 1980; Radostits et al., 2006). About 60–70% of BLV-infected cattle were asymptomatic during the aleukemic stage, but after a latency that extends from a few months to years, 30% developed persistent lymphocytosis (PL), and 5% developed lymphoma, an incurable form of the disease. It mostly occurs in adult animals (Gillet et al., 2007). The vast majority of infected animals exhibited no clinical signs and remained carriers throughout life. All BLV-positive animals did not have lymphocytosis, which affected their health and spread infection through infected blood lymphocytes (Khudhair et al., 2016a; Trono et al., 2001). In cattle, a lymphocyte count exceeding 8000 lymphocytes/ μ L indicated persistent lymphocytosis (PL), a form of leukemic leukosis. A majority (70%) of BLV-positive cattle exhibited atypical lymphocytes (Khudhair et al., 2016b). BLV disrupts immune functioning and increases susceptibility to other infectious diseases (Emanuelson et al., 1992; Erskine et al., 2011). BLV prolongs immunosuppression during the periparturient period by increasing expression of immunological checkpoints in T cells, leading to higher rates of intramammary infections (do Nascimento et al., 2023). Studies have found a correlation between BLV infection and increased susceptibility to other common cattle diseases, particularly reproductive infectious diseases such as Bovine viral diarrhea, Bovine herpesvirus, and Leptospirosis (De Brun et al., 2023). BLV infection is associated with decreased milk production, shortened lifespan, and reduced immune response to immunization (De Brun et al., 2023). Persistent lymphocytosis in enzootic bovine leukosis involved elevated B-lymphocytes and B/T ratio inversion. PL was associated with immune disruptions and heightened vulnerability to infections, indicating its pre-tumoral nature in cattle (Okagawa et al., 2012; Suzuki et al., 2013). Animals remain persistently infected despite the absence of visible signs because it consistently triggers the immune response (Florins et al., 2007). Following infection, humoral and cytotoxic mediators impair the viral replication cycle, allowing only provirus-carrying cells to proliferate mitotically. Infected cells vigorously replicate, indicating a comparatively higher replication rate than normal to persist and multiply within the host (Elemans et al., 2014; Florins et al., 2007). Proviral DNA was found to be integrated a large number of genomic sites in both persistent lymphocytosis and lymph node tumor forms of bovine leukemia (Kettmann et al., 1980). Leisering found yellowish nodules with splenomegaly and tumors of B cell infiltrate in all body tissues like the liver, eyes, skin, lungs, and lymph nodes

(Leisering, 1871). Prevalence varies, with ongoing research focusing on epidemiology, transmission, and control strategies to mitigate losses and potential public health concerns (Lv et al., 2024; Polat et al., 2015). BLV staging can differ between countries based on available resources and diagnostic tools. For example, countries with strong veterinary infrastructure may use advanced methods like proviral load testing, while others rely on basic clinical signs or ELISA due to limited resources. Treatment approaches also vary, with some countries focusing on culling and others emphasizing biosecurity and education.

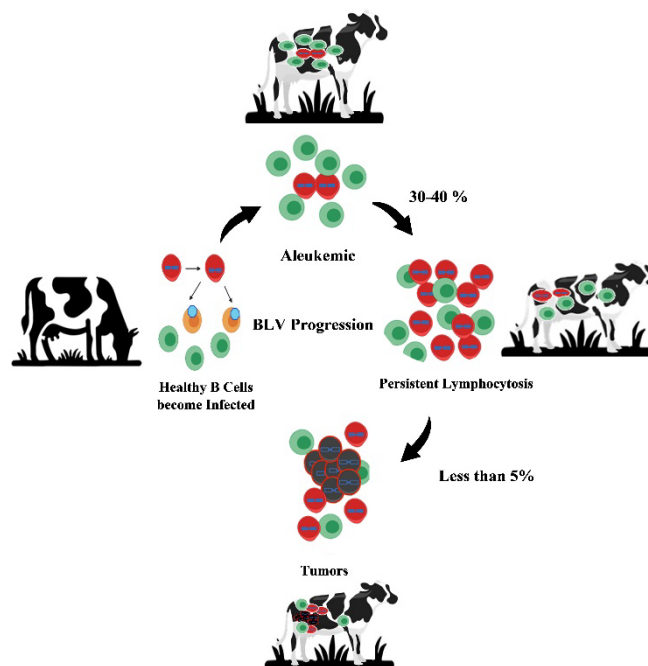


Figure 1. BLV Progression and Clinical Staging

5. BLV Genotypes Distribution in Asian Countries

Bovine Leukemia Virus (BLV) genotypes exhibit mutations in the envelope (env) gene, which influence virulence and immune evasion. Notably, genotypes with changes in immunogenic epitopes are more adept at escaping host immune responses, potentially increasing transmission rates (Zhao et al., 2024). Specific mutations in the BLV genome, particularly in the long terminal repeat (LTR) and Tax regions, are linked to higher proviral loads, which correlate with increased virulence and transmissibility (Pluta et al., 2018). Strains with these mutations are more efficient in viral replication and immune evasion. BLV Genotype G1 is most predominant all over the world, including Asia, Europe, America, and Australia. In Asian countries, G1 is most dominant and continues to spread in all Asian countries having higher prevalence. G6 is the second most distributed genotype in Asian countries

like Thailand, Colombia, Philippines, Iran, Japan, Taiwan, Turkey, Pakistan, Myanmar, Mongolia, Vietnam, China, South Korea, Jordan, and India. G4 is primarily distributed in Europe and American and Europe countries but now it is distributed in Iran, Mongolia, Russia, Turkey, and Kazakhstan. G2 is only found in Japan. G7 is commonly distributed in Russia, Kazakhstan and Mongolia. Another genotype, G10 mostly found in China, Vietnam, Myanmar, and Thailand. G12 is prevalent in Kazakhstan. These genotypes spread in Asia due to animal trading and exchange across national boundaries. The extensive dispersion of BLV genotypes across different regions, even those far apart, may be influenced by the viral transfer facilitated by the movement of live animal populations due to human migration and animal domestication (Reed, 1981).

The apparent BLV genotype distribution across Asia is displayed in Table 2 and Figure 2. In detail, Genotypes G1, G2, G3, G4, G6, G7, G8, G10, and G12 have been detected in all regions of Asia. Genotype G1, G2, and G3 in Japan (Matsumura et al., 2011); G4, G7, and G8 in Russia (Rola-Łuszczak et al., 2013); G1, G6, and G10 in Thailand (Lee et al., 2016); G1, G4, G7 in Mongolia (Nyamsuren Ochirkhuu et al., 2016); G4, G7, and G12 in Kazakhstan (Sultanov et al., 2022); G1 and G6 in Philippines, Jordan and Pakistan (Ababneh et al., 2012; Polat et al., 2015; Rola-Łuszczak et al., 2021); G1 and G4 in Iran and Turkey (Alkan et al., 2021; Degirmenci, 2011; Kazemimanesh et al., 2019); G1, G6 and G10 in Myanmar (Moe et al., 2020); G1, G6, and G10 in Vietnam (Le et al., 2020); G1 and G3 in Taiwan and South Korea (Hsieh et al., 2019; Lee et al., 2015); G6 in India have been investigated (Gautam et al., 2018). Analysis of Bovine leukemia virus's phylogenetic properties and ancestral traits indicates its progenitors originated in Asian regions, but further research is needed to determine its geographical origin. Keeping that in mind, Asia is the most susceptible and potential geographical region for BLV spread. These are commonly cattle species in Asia and are most susceptible to possessing, maintain, and transmitting the virus (Kohei Nishikaku et al., 2022).

Vaccine development against retroviruses is challenging due to their ability to integrate into the host genome and undergo latency (Abdala et al., 2019). Efforts to create BLV vaccines have included inactivated and live-attenuated approaches, with safety and efficacy being primary concerns (Abdala et al., 2019). Recent research has focused on vaccine development, with (Suarez Archilla et al., 2022) reporting a safe and effective attenuated BLV provirus vaccine that provided sterilizing immunity in 28 out of 29 vaccinated heifers over 48 months. Additionally, (Samad et al., 2022) designed a multi-epitope vaccine using computational analyses, showing potential for generating

an effective immune response. Current control strategies involve early detection and diagnosis, as no widely available effective vaccine exists (Marawan et al., 2021). The discovery of BLV nucleic acid fragments in human breast cancer tissues has raised public health concerns (Lv et al., 2024).

Table 2. BLV Genotype Distribution in different regions of Asia

Regions of Asia	Country	Genotypes			Reference
Northern Asia	Russia	G4	G7	G8	Rola-Łuszczak et al. (2013)
	Turkey	G1	G4	-	Alkan et al. (2021); Degirmenci (2011)
Western Asia	Colombia	G1	G6	-	Corredor-Figueroa et al. (2020)
	Iran	G1	G4	-	Kazemimanesh et al. (2019)
	Jordan	G1	G6	-	Ababneh et al. (2012)
	Kazakhstan	G4	G7	G12	Sultanov et al. (2022)
Central Asia	Japan	G1	G2	G3	Matsumura et al. (2011)
	China	G1	G6	G10	Ababneh et al. (2012); Wang et al. (2018); Yu et al. (2019)
	Taiwan	G1	G3	-	Hsieh et al. (2019)
	Mongolia	G1	G4	G7	Nyamsuren Ochirkhuu et al. (2016)
	South Korea	G1	G3	-	Lee et al. (2015)
	India	G6	-	-	Gautam et al. (2018)
Southern Asia	Pakistan	G1	G6	-	Rola-Łuszczak et al. (2021)
	Philippines	G1	G6	-	Polat et al. (2015)
Southeast Asia	Thailand	G1	G6	G10	Lee et al. (2016)
	Myanmar	G1	G6	G10	Moe et al. (2020)
	Vietnam	G1	G6	G10	Le et al. (2020)

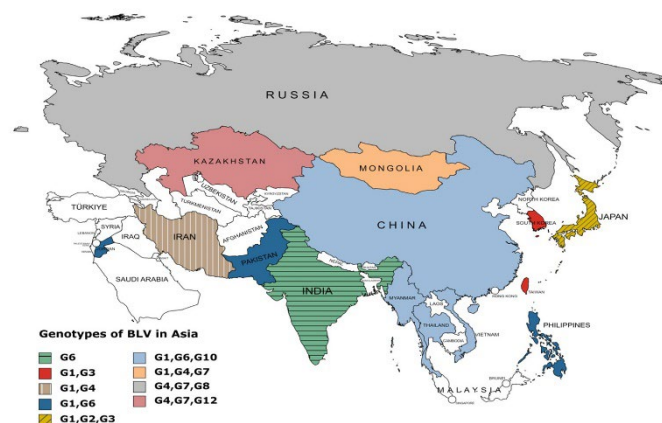


Figure 2. Mapping of BLV Genotype Distribution Across Asia

6. Conclusion

There is a dearth of well-conducted studies on the prevalence and molecular characterization of enzootic bovine leukosis in Asia. This review provides the current status of bovine leukosis which is widely prevalent in Asian countries, and the majority of genotypes circulating in Asia are G1, G2, G3, G4, G6, G7, G8, G10, and G12. The prevalence level of BLV in different regions of Asia is quite variable in every region, relatively high prevalence shows the importance of highlighting this issue. Bovine leukosis is an important disease because it causes significant economic losses, and animals remain carriers throughout their lives. Therefore, the present review helps in the control of EBL by implementing a closed trading system in Asia to test and prevent the introduction of infected animals. Collaboration across countries may also play an important role in standardizing control measures and sharing resources, resulting in more effective and sustainable disease management.

Author contributions

All authors contributed significantly to the writing of this review paper. All authors have reviewed and agreed on the final version of the manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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