Invited review: Bovine leukemia virus—Transmission, control, and eradication

Alessa Kuczewski,¹ Karin Orsel,² Herman W. Barkema,² Steve Mason,³ Ron Erskine,⁴ and Frank van der Meer^{1*}

¹Department of Ecosystem and Public Health, University of Calgary, Calgary, AB T2N 4Z6, Canada

²Department of Production Animal Health, University of Calgary, Calgary, AB T2N 4Z6, Canada

³Agromedia International Inc., Calgary, AB T2L 0T6, Canada

⁴Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing 48824

ABSTRACT

Bovine leukemia virus (BLV) infection, endemic in North American dairy herds, has production-limiting effects. A literature review of available papers published since 1995 concerning BLV transmission and its control was conducted. Although confirmed transmission routes were reviewed (blood, natural breeding, in utero, colostrum, and milk), there is still a lack of detailed information on other specific risks for transmission (e.g., contact transmission and hoof-trimming knives). Eradication of BLV has been achieved by combined management, segregation, and culling approaches. In contrast, although sole implementation of best management practices aimed at prevention of BLV transmission has decreased within-herd BLV prevalence, it has not eradicated BLV from a herd. Therefore, control and eradication of BLV by best management practices only should be further investigated. Additionally, the role of proviral load in infected cattle was investigated. Cattle with a high proviral load seem to be more likely to infect others, whereas those with a very low proviral load seem to have low risks of transmitting BLV. Information on proviral load could be taken into account when controlling BLV in high-prevalence herds. In conclusion, there is a need for detailed, large-scale studies investigating roles of specific transmission routes, knowing proviral load of infected individuals.

Key words: bovine leukemia virus, transmission, control, eradication

INTRODUCTION

Bovine leukemia virus (BLV), a member of the family *Retroviridae*, is able to infect bovine B-lymphocytes (Goff, 2013). Natural infection is not limited to cattle

(*Bos taurus*, *Bos indicus*) but also occurs in, for example, water buffalo (*Bubalus bubalis*) and capybaras (*Hydrochoerus hydrochaeris*). Some species (e.g., sheep, *Ovis aries*) can be infected experimentally (EFSA AHAW Panel, 2015), but the disease process differs from cattle because they develop tumors earlier and more frequently following challenge. Many other species generate an immune response upon experimental challenge with this virus (deer, rabbits, rats, guinea pigs, cats, dogs, sheep, rhesus monkeys, chimpanzees, antelopes, pigs, goats, and buffalo; OIE, 2018). Following infection, BLV integrates its genetic material into the host's genome, creating a provirus, an identical DNA copy of the virus' RNA genome (Goff, 2013), enabling BLV to establish lifelong persistent infection. Infection with BLV in cattle impairs immune system function, reduces cow longevity, and decreases milk production; in addition, it may culminate in development of enzootic bovine leukosis (EBL; fatal lymphosarcoma; Schwartz and Levy, 1994; Bartlett et al., 2014; Frie and Coussens, 2015). These tumors reduce production and animal welfare and cause premature death. Therefore, eradication of BLV has been a priority in the European Union since 1964 (EFSA AHAW Panel, 2015). Many Western European countries have eradicated BLV from their cattle population (European Commission, 2015). Similarly, New Zealand eradicated BLV from its dairy and beef herds, whereas Australia still has a low prevalence in its beef herds (Voges, 2012; Queensland Government Department of Agriculture and Fisheries, 2016). However, BLV infection is still common in North American dairy herds, averaging ~90% herd prevalence and ~40% within-herd prevalence (Nekouei et al., 2015; LaDronka et al., 2018; Kuczewski et al., 2019) despite the existence of several voluntary BLV control programs (Brunner et al., 1997; Canadian Food Inspection Agency, 2003).

Eradication programs in the European Union and elsewhere applied test and cull approaches, whereby frequent testing of all susceptible cattle and cull-

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*Corresponding author: Frank.vandermeer@ucalgary.ca

ing test-positive individuals reduced prevalence and eventually removed the virus from herds (Burki, 1982; Mammerickx, 1984). In contrast to the North American situation, within-herd and between-herd prevalence of BLV in the European herds was still low when eradication was initiated (e.g., 6.1% of tested herds infected, 2.4% of tested cattle infected; Burki, 1982; Nuotio et al., 2003). As the within-herd prevalence in North American herds varies significantly, various approaches to effective BLV control strategies are likely necessary. Whereas culling test-positive animals would be possible in low-prevalence herds, it is often not economically feasible or practical in high-prevalence herds. In those herds, alternative approaches to control BLV can be implemented, such as segregation of BLV test-positive cattle and implementation of best management practices (BMP) aiming to reduce or prevent within-herd BLV transmission (Johnson et al., 1985; Shettigara et al., 1989). Recommendations concerning these approaches are generally based on studies and data from 1960 to 1990 (Rodríguez et al., 2011). However, the North American dairy industry has changed considerably since then. For example, tiestalls have often been replaced by freestalls and herd size keeps increasing (Barkema et al., 2015). Additionally, new insights in the biology of BLV, especially its epidemiology, could enable the adjustment of BLV control and eradication strategies to the North American situation. It was, for example, hypothesized that proviral load could affect BLV transmission risks (Juliarena et al., 2007; Jimba et al., 2010). When culling all positive cattle is not a viable option, strategic culling of high-risk (high proviral load) cattle could reduce transmission risk and within-herd prevalence.

BLV CONTROL SINCE 1997: AN UPDATE

A critical review of the literature published since 1997 was conducted to provide an overview of available knowledge on BLV transmission and control, identify gaps in knowledge, and assess the applicability of both historic and recent research to the current situation in the dairy industry. The last thorough review on BLV transmission was published in 1997 (Hopkins and DiGiacomo, 1997). Therefore, only literature regarding transmission studies from 1995 onward is included here (Table 1; Figure 1). A short summary of the historic findings of the previous review of Hopkins and DiGiacomo (1997) and a comparison with the literature as of 1995 reviewed herein is presented (Table 2). Topics included in this review are as follows: (1) BLV transmission in cattle (thus, the literature concerning transmission among other species such as sheep or goats as

well as in vitro studies was excluded); (2) BLV control, including studies using voluntary control on individual or multiple farms (for information about mandatory national control programs, we refer to an extensive review by the European Food Safety Authority; EFSA AHAW Panel, 2015); and (3) BLV proviral load, regarding the potential of selecting cattle for culling based on proviral loads.

Additionally, in recent years, risk-assessment models were developed to identify on-farm causes of BLV transmission (Table 3); the risk factors identified are described in the following paragraphs. Generally, likely routes of BLV transmission are animal-to-animal contact, blood contact during natural breeding, vertical (e.g., in utero) transmission, peripartur transmission, and transmission through milk, colostrum, or both (Rodríguez et al., 2011; Lairmore, 2014). Additionally, some herd characteristics and management strategies with potential roles in BLV transmission were identified. Sources were categorized and a comprehensive summary of the role of proviral load in transmission and control of BLV is included in the final paragraph of the Results section.

Horizontal Transmission

Herd Characteristics and Management Strategies. Many risk analysis models identified herd characteristics that could alter the risk of BLV infection; these models were based on cross-sectional studies. Having a closed herd could decrease the risk of introduction of BLV into a herd and therefore decrease risk of BLV transmission and new infections (Nekouei et al., 2015). Conversely, previous detection of clinical EBL in a herd (Kobayashi et al., 2014; Nekouei et al., 2015) or increasing within-herd BLV infection rates (Şevik et al., 2015) were associated with increasing BLV prevalence on both dairy and beef farms. Introducing untested replacements (Casal et al., 1990; Kobayashi et al., 2014; Nekouei et al., 2015; Şevik et al., 2015; Sun et al., 2015), use of an independent heifer-rearing operation to manage replacements (Kobayashi et al., 2014), and larger herds (Şevik et al., 2015; Sun et al., 2015) all increased risks of BLV infection for dairy and beef herds.

Cattle breed is a potential risk for BLV infection. In a Turkish study, Holstein cattle had a higher risk of being BLV infected compared with Brown Swiss cattle (Şevik et al., 2015). Furthermore, although cattle origin was not mentioned, it could have contributed, as importation of Holsteins from North America has increased occurrence of BLV in other countries (Kavanagh, 1981; Suh et al., 2005). In the same study, increasing age within a herd was associated with an increased within-

Table 1. Results of database searches

Search	Date	Database ¹			
		Web of Science	CAB Abstracts	PubMed	ProQuest
(1) "bovine leukemia virus" OR "enzootic bovine leukosis" OR "bovine leukosis" OR "bovine lymphosarcoma" OR "bovine type c oncovirus"	Jul. 25, 2019, and Aug. 7, 2019	2,326	5,763	3,864	84
(2) "control*" OR "control program*" OR "eradication" OR "eradication program*" OR "segregat*" OR "remov*" OR "prevent*" OR "manag*" OR "bovine" OR "cattle" OR "cows" OR "dairy cattle"	Jul. 25, 2019	10,147,880	3,949,738	7,701,920	2,725,104
(3) "infection" OR "transmission" OR "transmission rate" OR "infection rate" OR "vectors" OR "risk factors" OR "spread" OR "risk"	Jul. 25, 2019, and Aug. 7, 2019	434,012	582,451	429,372	437,411
(4) "infection" OR "transmission" OR "transmission rate" OR "infection rate" OR "vectors" OR "risk factors" OR "spread" OR "risk"	Jul. 25, 2019	4,927,442	2,022,815	3,894,736	2,045,893
(5) 4 AND 3 AND 1	Jul. 25, 2019	976	4,793	1,458	69
(6) 1 AND 2 AND 3	Jul. 25, 2019	434	1,420	927	59
(7) "proviral load" OR "provirus"	Jul. 25, 2019, and Aug. 7, 2019	4,331	1,533	4,507	5,606
(8) 1 AND 3 AND 7	Jul. 25, 2019, and Aug. 7, 2019	188	204	227	418

¹Web of Science: <https://www.webofknowledge.com>; CAB Abstracts: <https://www.cabdirect.org/cabdirect/>; PubMed: <https://pubmed.ncbi.nlm.nih.gov/>; ProQuest: <https://www.proquest.com/>.

Figure 1. Selection of sources of evidence.

herd prevalence (Şevik et al., 2015), highlighting that older dairy cattle generally have a higher infection rate than younger cattle (Erskine et al., 2012b).

Geographical regions had different within-herd prevalence on dairy and beef operations in some studies (Gnad et al., 2004; Nekouei et al., 2015) but not others (Scott et al., 2006), resulting in no clear understanding regarding the role of location in BLV prevalence. Finally, 1 study failed to identify risk factors that could

Table 2. Review of bovine leukemia virus (BLV) transmission¹

Way of transmission	Risk as identified by Hopkins and DiGiacomo, 1997	Risk as identified in current review	References referred to in current review
Horizontal transmission			
Confinement during calving	Decrease in circulating antibodies around calving, exposure to blood, tissue, fluids around birth (depending on calving area). Controversial results	No additional studies identified since 1996	
Animal-to-animal contact	Nasal secretions, saliva: BLV detected inconsistently. Transmission dependent on lymphocytes in fluid Prevalence within grouped animals correlated with risk of infection	Nasal secretions, saliva: BLV detected inconsistently Increased risk for BLV infection if uninfected animals are housed next to infected animals in tiestalls Single use of needles and rectal examination sleeves did not alter the risk of BLV infection	Kobayashi et al., 2015; Yuan et al., 2015 Ruggiero, 2019
Immunization, injection, venipuncture	Highly efficient transmission by inoculation with infected blood: transmission of 1 µL of blood caused infection (subcutaneous, intradermal, intramuscular, intravenous) Infectivity depends on number of lymphocytes in blood; animals with lymphocytosis are infective at lower doses of lymphocytes than animals with normal lymphocyte count Intravenous injections and venipuncture likely have higher risk than subcutaneous, intradermal, and intramuscular injections High risk for transmission for gouge dehorning without cleaning between calves Mention of tail docking, castration, supernumerary teat removal, growth implants as similar risk, but no evidence Risk for transmission through tattooing Mention of ear tagging as similar risk, but no evidence Lack of transmission by brucellosis vaccine	No additional studies identified since 1996	
Dehorning, surgerites	Rectal mucosa, bleeding from reproductive tract as sources of blood. Controversial results: risk for transmission dependent on circumstances; experimental transmission by blood inoculation or gloves visibly contaminated with blood is possible; animals with lymphocytosis could have higher risk for transmission than aleukemic animals; increased frequency and vigor of palpations have increased risk for transmission	No additional studies identified since 1996	
Tattooing, ear tags	Controversial results: dose-dependent transmission of BLV via inoculation of insect parts; transmission dependent on lymphocytosis status of BLV-positive animals; the more natural the experimental conditions, the less transmission can be proven Controversial association between season (correlated with insect populations) and incidence	Experimental transmission with visible blood is possible Single use of needles and rectal examination sleeves did not alter the risk of BLV infection	Kohara et al., 2006; Ruggiero, 2019
Transrectal palpation	AI, natural service: low risk Semen, embryos: no risk if not contaminated with lymphocytes Reuse of equipment or examination sleeves for AI: risk for transmission, but no evidence	PCR detection of BLV in insects. Inconsistent results in inoculation transmission experiments Decreased seroconversion rates when insect-cattle contact could be prevented BLV detected in semen and smegma samples	Morris et al., 1996; Ooshiro et al., 2013; Kohara et al., 2018; Panet et al., 2019
Insects, arthropods	None, unless contaminated	No transmission through natural breeding by bulls with very low proviral load; some transmission through bulls with low proviral load	Choi et al., 2002; Asadpour and Jafari, 2012; Erskine et al., 2012b; Khamesipour et al., 2013; Mekata et al., 2018; Benitez et al., 2019a,b
Breeding and reproductive management: natural service, AI, embryo transfer	BLV detected in some semen and smegma samples No transmission through natural breeding by bulls with very low proviral load; some transmission through natural breeding by bulls with low proviral load	BLV detected in some semen and smegma samples No transmission through natural breeding by bulls with very low proviral load; some transmission through natural breeding by bulls with low proviral load	Choi et al., 2002; Asadpour and Jafari, 2012; Khamesipour et al., 2013; Mekata et al., 2018; Benitez et al., 2019a,b
Semen			

Continued

Table 2 (Continued). Review of bovine leukemia virus (BLV) transmission¹

Way of transmission	Risk as identified by Hopkins and DiGiacomo, 1997	Risk as identified in current review	References referred to in current review
Ova, embryos	None, if recipient animals are BLV free	No additional studies identified since 1996	
Vertical transmission			
In utero, peripartum	Transplacental infection after immunocompetency (~3 mo of gestation) 0.5–18% of calves from infected dams born BLV positive Aleukemic animals have small risk for transmission, animals with lymphocytosis have increased risk for transmission, animals with enzootic bovine leukosis have highest risk for transmission	In utero transmission of BLV is possible (0–40% of calves to positive dams infected) Animals with high proviral load have higher risk for vertical transmission of BLV	Fukai et al., 1999; Meas et al., 2002; Mekata et al., 2015; Sajiki et al., 2017
Colostrum, milk	Detection of BLV in colostrum and milk of BLV-positive cows Transmission possible for milk and colostrum Calf's age could have protective effect Protective effect of colostrum antibodies	Transmission of BLV through colostrum and milk is possible (0–100%) Possible protective effect of colostrum from BLV-positive dams Freezing–thawing seems to inhibit infectivity of colostrum	Meas et al., 2002; Nagy et al., 2007; Kanno et al., 2014; Ruiz et al., 2018

¹Summary of experimental studies after Hopkins and DiGiacomo (1997). Results of risk analysis models are not included.

explain within-herd prevalence in beef herds (Bezerra et al., 2019), although only 16 herds were included.

Animal-to-Animal Contact. To determine the potential for virus transmission through nasal excretions or saliva, presence of BLV in these fluids was investigated. In a Japanese study (Yuan et al., 2015), Holstein-Friesian, Japanese Black, and crossbred cows were sampled and BLV provirus was detected in blood (35/50 PCR positive), nasal excretions (14/48 PCR positive), and saliva (6/47 PCR positive).

When housed in tiestalls, dairy cows neighboring BLV-positive cows had a higher risk of becoming test positive than those with BLV-negative neighbors (Kobayashi et al., 2015). However, no detailed information on cow management was provided in this publication. Therefore, it cannot be excluded that iatrogenic transmission or transmission by other factors caused BLV infection in this housing.

Additionally, the following risk factors for transmission were identified using risk analysis models: direct contact, especially in loose-housed dairy and beef cattle (Kobayashi et al., 2010, 2014), and intergenerational contact, such as exposure of heifers to older cattle in dairy herds (Sargeant et al., 1997). Contact between calves and adult cattle in beef herds, as well as commingling pregnant dairy heifers and adult dry dairy cows, increased within-herd BLV prevalence (Kobayashi et al., 2014). In addition, housing and on-farm management were associated with higher within-herd risk for BLV infection, including indoor housing of nonlactating dairy cows during winter (Sargeant et al., 1997), housing of dairy calves in hutches during the winter (Sargeant et al., 1997), or group feeding dairy heifers a TMR in communal feed bunks (Erskine et al., 2012a). Based on these findings, it is possible that the relevance of contact transmission of BLV among cattle has been underestimated.

Blood. Transmission of blood between cattle is generally recognized as an important risk factor for the spread of BLV, as low volumes of blood contain enough infected lymphocytes to transmit the virus.

Injections and Equipment. Reusing needles (Erskine et al., 2012a; Ramírez Vásquez et al., 2016), selenium injections given to nonlactating cows (Erskine et al., 2012a), and gouge dehorning were identified as BLV transmission risk factors via potential blood contamination (Kobayashi et al., 2010; Erskine et al., 2012a) on dairy operations in multiple cross-sectional risk analyses. However, vaccinations were associated with reduced prevalence to BLV on dairy farms in a cross-sectional risk analysis (Chi et al., 2002), perhaps acting as a surrogate measure for biosecurity.

Additionally, implementation of single-use needles for injections did not reduce risk of new seroconversions

Table 3. Overview of risk analysis models for bovine leukemia virus (BLV)

Reference	Country	Model	Sample	Data collection	Identified risks
Bezerra et al., 2019	Brazil	Multiple logistic regression	16 herds, 160 samples	Questionnaire	No risks identified for BLV
Casal et al., 1990	Spain	Path analysis	92 herds	Questionnaire	Risk for transmission of BLV between herds; introduction of new animals, number of animals introduced, prevalence in herd of origin
Chi et al., 2002	Canada	Tobit regression analysis	90 herds, 2,604 cattle	Questionnaire	Vaccination: reduced prevalence of exposure for BLV
Erskine et al., 2012a	United States	1-way ANOVA, simple linear regression, multivariate analysis	113 herds	Interview	Risk for increased BLV within-herd prevalence: natural breeding, dry cow selenium injections, TMR for heifers, gouge dehorning, reuse of needles, no fly control, number of pregnancy checks
Heald et al., 1992	Canada	Logistic regression	268 herds, 998 cows, agar gel immunodiffusion assay	Production records, mail survey	Increased odds for positive animals within a herd: calving in separate calving pens in the winter (alternative mostly tiestalls), calves in hutches in winter
Kobayashi et al., 2010	Japan	Mixed logistic regression analysis	139 farms (110 positive), 90 filled out questionnaires	Questionnaire	Decreased risk: calving in separate calving pens in the summer (alternative mostly pasture)
Kobayashi et al., 2014	Japan	Zero-inflated negative binomial model	563 dairy farms, 490 beef farms, 20 animals/herd tested	Interview	Risk factors for within-farm transmission of BLV: loose housing, dehorning, large number of horseflies in the summer.
Nekouei et al., 2015	Canada	Zero-inflated negative binomial regression model	315 dairy farms, 9–45 cows/farm sampled (ELISA)	Questionnaire from 272 herds	Protective: feeding colostrum from dam to calf Risk factors for increased within-herd prevalence: past detection of clinical leukemia, blood-sucking insects, purchase of animals; dairy: heifer rearer, loose housing; beef: direct contact between calves and adult cattle
Ramírez Vásquez et al., 2016	Colombia	Logistic regression	29 herds, 1,003 cattle	Interview	Risk for increased within-herd prevalence: clinical cases of leukosis in past 12 mo, herds with purchased animals with unknown BLV status, changing gloves in Eastern Canada
Sargeant et al., 1997	Canada	Multivariable analysis	102 herds, 1,330 cows	Records, questionnaire	No association in Western Canada Western herds more likely to be infected than eastern herds; closed herds more likely to be negative Increased risk for seropositivity: reuse of needles
Scott et al., 2006	Canada	Generalized linear model	77 herds, 2,819 samples	Questionnaire	Negative association between herd-level milk production and BLV status; positive association between weaning age and purchasing animals from outside sources and BLV status
Şevik et al., 2015	Turkey	Generalized mixed linear model	1,116 herds, 28,982 samples	Records	Increased risk of BLV: housing calves in hutches or separate buildings; winter: contact of heifers to older animals, dry cow housing
Sun et al., 2015	China	Multivariate analysis	113 herds, 3,674 cattle	Questionnaires	No variance between BLV seroprevalence and agroecological regions in Alberta Increased risk for increased seroprevalence: increasing cattle age, herd size, cattle breed, purchased cattle Increase in prevalence with age Risk factors for BLV infection: herd size and presence of cattle introduced from other farms

on 3 commercial dairies (Ruggiero, 2019). Even though individual cattle were tested and herds had varying within-herd prevalence (25–74%), perhaps other (iatrogenic) transmission routes caused seroconversion, as details regarding management practices were missing. Transmission of BLV via blood is well-established route (Hopkins and DiGiacomo, 1997). However, it appears that only transitioning to single-use needles, thereby eliminating needles as a vehicle to transfer blood between cows, cannot decrease infection rates sufficiently to lower the within-herd prevalence.

Transrectal Palpation. Only 1 study (Kohara et al., 2006) investigated transrectal palpation as a mode of transmission for BLV. In that study, 3 of 4 BLV-negative Holstein-Friesian steers that were transrectally palpated 3 min/wk for 4 wk, with the examiner wearing a palpation sleeve contaminated with BLV-positive blood, became BLV agar gel immunodiffusion test and PCR positive. No negative control animals were included. Generally, within-herd prevalence of dairy farms increases with increasing number of transrectal examinations (Erskine et al., 2012a). Although transmission of BLV via transrectal palpation is possible and contaminated palpation sleeves are considered a risk factor for BLV transmission, the probability of transmission of BLV under realistic on-farm circumstances remains unclear. Similarly, it is unclear whether implementation of single-use sleeves would reduce BLV status on dairy farms (Nekouei et al., 2015; Ruggiero, 2019).

Insects and Arthropods. The absence of fly control (Erskine et al., 2012a) or presence of blood-sucking insects on dairy and beef farms (Kobayashi et al., 2010, 2014) were identified as risk factors for BLV infection in cross-sectional risk analyses. Whereas this could be an indicator for BLV transmission via insects, it could also be a confounding factor for other on-farm factors influencing BLV transmission (e.g., general hygiene). Genetic material of BLV in biting flies can be detected by PCR (Panei et al., 2019); however, despite the presence of PCR-positive insects, natural transmission to Holstein or Aberdeen Angus steers and heifers was not confirmed (Panei et al., 2019). Additionally, there was no evidence of transmission following subcutaneous injection of a tick homogenate to sheep (Morris et al., 1996) and inconsistent transmission following injection (depth of injection not reported) of homogenized biting fly mouth parts (Panei et al., 2019). Although the role of biting flies in BLV transmission has been studied without yielding definitive answers, BLV seroconversion rates on dairy and beef farms were decreased when measures to decrease number of biting flies were implemented (Ooshiro et al., 2013; Kohara et al., 2018). Studies considering the role of other common biting and sucking insects (e.g., lice) are missing.

Natural Service. Hopkins and DiGiacomo (1997) concluded in their review that semen is not a source of BLV infection as long as it is not contaminated with BLV-infected lymphocytes. However, in a recent cross-sectional study, natural breeding of dairy cattle was associated with higher within-herd BLV prevalence (Erskine et al., 2012a), although BLV status of herd bulls was unknown. Bovine leukemia virus has been detected in bull semen in some studies (Sharifzadeh et al., 2011; Asadpour and Jafari, 2012; Khamesipour et al., 2013) but not in others (Choi et al., 2002; Benitez et al., 2019b), representing a gap in knowledge regarding BLV transmission. In many of these reports, detailed assessment of techniques used was not possible; therefore, whether samples were contaminated with lymphocytes cannot be excluded. Additionally, BLV was detected in beef-bull smegma (Benitez et al., 2019b), implying a potential role in transmission during natural breeding. Finally, breeding beef and dairy bulls with low proviral loads (175.90 proviral copies/10⁵ cells in smegma and <100 proviral copies/50 ng of genomic DNA in blood, respectively) were unable to transmit BLV (Mekata et al., 2018; Benitez et al., 2019a), whereas breeding dairy bulls with a higher proviral load (100–500 proviral copies/50 ng of genomic DNA in blood) were able to infect uninfected cattle (Mekata et al., 2018). Based on these findings, we inferred that transmission of BLV during natural breeding is possible; although it may not be caused by semen, it may be due to microabrasions and could depend on proviral loads in bulls.

Vertical Transmission

In Utero and Peripartum. Embryo transfer does not cause cow-to-cow transmission of BLV if done with appropriate precautions (e.g., single-use needles and transrectal examination sleeves; Fukai et al., 1999). Nonetheless, calves can generally acquire BLV from their dams through vertical transmission (Meas et al., 2002; Mekata et al., 2015). This was confirmed in a report (Sajiki et al., 2017) that BLV genomic sequences were identical in Holstein dams and their infected calves. Additionally, a cow's BLV proviral load influences rate of transmission to her calf. In 2 studies, dairy and beef cows with a high proviral load (>400 copies/10 ng of DNA or >3,000 copies/50 ng of DNA) were more likely to transmit BLV to their calves than those with a lower proviral load (<400 copies/10 ng of DNA or ≤10 copies/50 ng of DNA). Specifically, 14/29 and 4/4 calves were infected in the high proviral load groups versus 9/95 and 0/2 calves infected in the low proviral load groups (Mekata et al., 2015; Sajiki et al., 2017). Effects of proviral loads on likelihood of transmission

could explain contradictory findings regarding vertical transmission of BLV (Hopkins and DiGiacomo, 1997).

Colostrum and Milk. Although transmission of BLV to calves via colostrum, milk, or both has been reported (Hopkins and DiGiacomo, 1997), quantification of the risk of BLV transmission via colostrum and milk remains a knowledge gap. Additionally, maternal antibodies may have a protective effect. Maternal antibodies in dairy calves that received colostrum and milk from BLV-positive dams were detectable until 3 to 9 mo of age (Meas et al., 2002; Nagy et al., 2007), which may prevent BLV infection (Nagy et al., 2007; Kobayashi et al., 2010). Although transmission from BLV-positive dairy cows to their calves was not detected in 15 BLV monoinfected dams, when cows were coinfectd with both BLV and bovine immunodeficiency virus, 5/9 calves became and remained BLV positive based on PCR (Meas et al., 2002). Finally, in a small study, frozen-thawed cells from colostrum of 2 BLV-infected Holstein cows were rendered noninfectious for 1 sheep (Kanno et al., 2014). Although freezing and thawing of colostrum to prevent transmission of BLV to calves is commonly recommended (Ruiz et al., 2018), there is a lack of large-scale studies to support it.

Experiences with Voluntary On-Farm BLV Control and Eradication

As the effect of on-farm BLV control in dairy herds is generally determined by the fate of BLV-positive cattle, testing is critical. It enables identification of BLV-positive individuals and provides a measure of within-herd prevalence. Once BLV-positive cattle are identified, the 3 control strategies commonly used separately or in combination are (1) culling test-positive cattle, (2) physical segregation of test-positive and test-negative cattle in separate barns, pens within a barn, or pastures, and (3) implementation of BMP to reduce BLV transmission (Table 4).

Fourteen studies reported on effect of control in a single herd, whereas 9 studies reported outcomes in up to 9 herds. In the reported studies, BLV-infected cattle were detected with agar gel immunodiffusion test, ELISA, white blood cell counts, PCR, syncytia assay, RIA, and infectivity tests. It is noteworthy that test characteristics for diagnostic tests used will affect the accuracy of these prevalence estimations.

Unfortunately, studies describing culling and segregation approaches rarely provided details regarding timing of culling or segregation (Shettigara et al., 1986; Ruggiero, 2019). In addition, reported focus of management practices to prevent BLV transmission varied among studies (Table 5).

Of the 23 reviewed studies, 15 reported that eradication of BLV on farm was achieved successfully, whereby eradication was defined as failure to detect new BLV-positive cattle based on the test(s) used (details on definition for successful BLV control for each study can be found in Tables 4 and 5; Roberts and Bushnell, 1982; Kaja et al., 1984; Shettigara et al., 1986; Pannwitz et al., 1987; Brenner et al., 1988; Shettigara et al., 1989; Wang and Onuma, 1992; Yoshikawa et al., 1992; Mészáros et al., 1994; Molloy et al., 1994; Dereń et al., 2003; Suh et al., 2005; Lojkić et al., 2013; Ruggiero and Bartlett, 2019). Eradication was achieved by testing and culling (Roberts and Bushnell, 1982; Brenner et al., 1988; Molloy et al., 1994; Ruggiero and Bartlett, 2019); testing and segregation (Brenner et al., 1988; Yoshikawa et al., 1992); testing, culling, and segregation (Shettigara et al., 1986; Wang and Onuma, 1992; Dereń et al., 2003); a combination of testing, segregation, and management changes (Shettigara et al., 1989; Suh et al., 2005); implementation of culling and management strategies combined (Kaja et al., 1984); or combinations of culling, segregation, and management strategies (Pannwitz et al., 1987; Mészáros et al., 1994; Lojkić et al., 2013). The interval from the start of these studies to eradication ranged from 10 mo to 14 yr, whereas initial within-herd prevalence ranged from 2.2 to 70%. Although lower within-herd prevalence generally seemed to hasten elimination of BLV, a low within-herd prevalence did not ensure rapid elimination of infection. It became apparent that BLV eradication can be a protracted progress. (Table 5). However, eradication of BLV may be possible despite high within-herd prevalence.

Of the remaining studies, several reported that a reduction in within-herd prevalence was achieved using the following approaches: testing and culling (Flensburg, 1976; Molloy et al., 1994; Ruggiero and Bartlett, 2019; Ruggiero et al., 2019); testing and segregating (Brenner et al., 1986); testing and management (Ferrer, 1982; Ruppanner et al., 1983; Sprecher et al., 1991); combinations of testing, culling, and segregating (Itoh et al., 1990; Ruggiero et al., 2019); or testing, segregating, and managing (Ferrer, 1982; Johnson et al., 1985). Finally, 2 studies reported no reduction or even an increase in within-herd prevalence despite implementation of testing and management or testing and culling approaches (Gutiérrez et al., 2011; Ruggiero and Bartlett, 2019).

Eradication and reduction of BLV prevalence seemed to be dependent on the commitment of the farmer toward BLV control (i.e., culling or segregation efforts, number of BMP implemented). The more control efforts were implemented, the more efficient BLV control

Table 4. Summary of reported bovine leukemia virus (BLV) control strategies and their combinations

Control strategy	Success of strategy	No. of studies	References
Test and culling of all BLV test-positive animals	Eradication achieved	4	Roberts and Bushnell, 1982; Brenner et al., 1988; Molloy et al., 1994; Ruggiero and Bartlett, 2019
	Reduction of within-herd prevalence	4	Flensburg, 1976; Molloy et al., 1994; Ruggiero and Bartlett, 2019; Ruggiero et al., 2019
	Increase in within-herd prevalence	1	Ruggiero and Bartlett, 2019
Test and segregation of all BLV test-positive animals	Eradication achieved	3	Brenner et al., 1988; Yoshikawa et al., 1992
	Reduction in within-herd prevalence	3	Brenner et al., 1986
Test and segregation of young stock	No new infections	1	Ferrer, 1982
Test and implementation of best management practices to avoid BLV transmission	Reduction of within-herd prevalence	3	Ferrer, 1982; Ruppanner et al., 1983; Sprecher et al., 1991
	No change in within-herd prevalence	1	Gutiérrez et al., 2011
Test, culling, and segregation of BLV test-positive animals	Eradication achieved	3	Shettigara et al., 1986; Wang and Onuma, 1992; Dereń et al., 2003
	Reduction of within-herd prevalence	2	Itoh et al., 1990; Ruggiero et al., 2019
Test, segregation of BLV test-positive animals, and implementation of best management practices to avoid BLV transmission	Eradication achieved	2	Shettigara et al., 1989; Suh et al., 2005
	Reduction of within-herd prevalence	2	Ferrer, 1982; Johnson et al., 1985
Test, culling of BLV test-positive animals, and implementation of best management practices to avoid BLV transmission	Eradication achieved	1	Kaja et al., 1984
Test, culling and segregation of BLV test-positive animals, and implementation of best management practices to avoid BLV transmission	Eradication achieved	3	Pannwitz et al., 1987; Mészáros et al., 1994; Lojkić et al., 2013

Table 5. Overview of bovine leukemia virus (BLV) on-farm control reports

Reference	Tests used ¹	Reported time span	Time to eradication, if applicable	No. of herds	Intervention ²	Result
Brenner et al., 1986	NR ²	March 1984 to January 1985	10 mo	1	298 animals; segregation of negative animals; segregation of heifers; introduction of heifers to negative group	No new positives at end of study Prevalence decrease from 60/298 to 36/298
Brenner et al., 1988	AGID	NR	NR	2	Herd A: 8.4% within-herd prevalence, test and cull Herd B: 30.8% within-herd prevalence, test and segregate	Herd A: no more positives after 3 tests (no information on time between tests) Herd B: segregation of positive animals, successive removal from herd Eradication of BLV
Dereń et al., 2003	AGID, ELISA	1992 to 2002	11 yr	3	468 cows; 70% positive, testing every 4 mo until 1997, then twice a year Systematic segregation of positive animals into separate herd and culling of positive cows	
Ferrer, 1982	RIA, AGID, syncytia infectivity test	Until calves were 32–35 mo old	35 mo	1	~400 cows; ≥90% within-herd prevalence at ≥3 yr Identification of negative calves (6–8 mo old): 2 tests within 2–3 mo; calf nursing dam, rearing BLV-negative calves (25) in (partial) isolation from adult herd	2 seroconversions out of 25 calves
Flensburg, 1976	Lymphocyte count, AGID	1965 to 1975	10 yr	1	Test (138–339 animals) and cull (0–18/test) animals and their progeny with high lymphocyte counts (Bendixen key) Testing every 6 mo; 1975: AGID and other serological tests 800 milking cows; ≥85% within-herd prevalence	Reduction after 3 yr, steady for 7 yr (0–3 animals slaughtered/test)
Gutierrez et al., 2011	ELISA, PCR	Start in June 2006	3 yr	1	Test and manage: needles, sleeves, disinfection; colostrum from dam or pooled bank, bulk milk, contact between pregnant heifers and adult cows	No reduction in overall within-herd prevalence
Itoh et al., 1990	AGID	1985 to 1987	2 yr	1	575 cows; 50.2% within-herd prevalence Testing twice a year; test and segregation, gradual culling of positive animals	Reduction in seroconversion and within-herd prevalence (28.6 to 2.5% and 50.2% to 49.2%, respectively) Seroconversion on pasture, no seroconversion in barn
Johnson et al., 1985	AGID	November 1978 to October 1983	5 yr	1	114 lactating cows; 95% within-herd prevalence Test and segregate for 3 yr within same barn, then mixing of positive and negative cows Monthly serologic testing of BLV test-negative animals (6–7 mo and older); segregation of positive and negative animals when 2 consecutive positive AGID tests Management: milking of positive cows last, separate calving pens for positive and negative animals, dam's colostrum for calves, needles, sleeves, AI, electric dehorner Personnel were aware of BLV transmission and control	Reduction of within-herd prevalence: 34% after 3 yr of separation

Continued

Table 5 (Continued). Overview of bovine leukemia virus (BLV) on-farm control reports

Reference	Tests used ¹	Reported time span	Time to eradication, if applicable	No. of herds	Intervention ²	Result
Kaja et al., 1984	AGID	Summer 1980 to March 1983	2.5 yr	1	Introduction of test-negative animals, quarantine of new animals, elimination of test-positive animals, frequent testing, embryo transfer, AI, needles, syringes, disinfection	133 cows introduced, 3 removed because of seroconversion 104 heifers introduced, 6 removed because of BLV-positive status No infections by embryo transfer or AI, eradication Eradication in 2010
Lojkić et al., 2013	AGID (1998, 2002, 2003, 2004), ELISA, PCR (2004, 2008, 2009)	1998 to 2010	12 yr	1	488 cows; 12.5% within-herd prevalence Starting in 2002: test and segregation, slaughter of test-positive animals after pregnancy; needles, sleeves, milk replacer	
Mészáros et al., 1994	AGID	NR	8 yr	1	1,248 cows; 62% within-herd prevalence Testing before and after colostrum, at 2.5 and 6 mo, every 2 mo afterward, then every 3 mo, then every 6 mo Test, management, segregation, and culling. Management: separation of calves, frozen negative colostrum, calf starter, hygienic measures, culling of positive animals, segregation of positive and negative animals within barn by foil wall, separate calving areas for positive and negative animals 126-304 animals; 19-39% within-herd prevalence Selective culling based on antigen level	Gradual replacement of positive herd, eradication
Molloy et al., 1994	AGID, viral antigen expression in lymphocyte cultures	1989 to 1992	3 yr	5		4 herds 19-21% within-herd prevalence; culling of antigen-positive animals 1 herd 39% within-herd prevalence; animals with highest antigen production were culled first; reduction in prevalence 6 tests until eradication
Pannwitz et al., 1987	Hematology, then AGID	1980 to 1982	2 yr	1	1 herd, 300 cows, 420 replacements, 180 feedlot animals, beef cattle; 0.3% hematologic-positive animals, 8.9% AGID-positive animals Housed in 2 freestall barns; pasture calving, calf on foot with dam Testing all animals 6 mo and older in 4- to 6-mo intervals Segregation and culling of positive animals; management: needles, disinfection, disinfection of barn	
Roberts and Bushnell, 1982	AGID	NR	42 mo	4	1,794 animals tested, 86 positives at first test Test and cull	16 animals positive at second test, third test, and after All animals negative = eradication

Continued

Table 5 (Continued). Overview of bovine leukemia virus (BLV) on-farm control reports

Reference	Tests used ¹	Reported time span	Time to eradication, if applicable	No. of herds	Intervention ²	Result
Ruggiero et al., 2019	PCR, ELISA	Start in fall 2015/spring 2016	2–2.5 yr	3	3 farms Proviral load: very high ($\geq 100,000$ copies/ 10^5 cells), high ($\geq 50,000$ to $<100,000$ copies/ 10^5 cells), moderate ($\geq 16,000$ to $<50,000$ copies/ 10^5 cells), or low (>0 to $<16,000$ copies/ 10^5 cells) Lymphocyte counts: very high ($\geq 10.0 \times 10^9/L$), high (≥ 7.5 to $<10.0 \times 10^9/L$), or normal ($<7.5 \times 10^9/L$) Herd managers were encouraged to prioritize culling of animals with high lymphocyte counts and proviral load, encouragement of segregation of high proviral load/white blood cell cows	Incidence decrease on all farms (13.8 to 2.2); within-herd prevalence decrease on all farms (62.0 to 20.7%)
Ruggiero and Bartlett, 2019	ELISA	August 2015 to March 2017	2.5 yr	3	3 herds, 150–850 milking cows; $<5\%$ within-herd prevalence (2.2–3.2%) Test and cull	Eradication of BLV from adult herd after 3 yr from 1 farm, prevalence increase ($>5\%$) on 1 farm, prevalence decrease on 1 farm (1.5%) Decrease in prevalence (newborn calves: 62.5 to 58.4%)
Ruppner et al., 1983	Lymphocyte count, AGID	1979 to 1981	3 yr	1	130 milking cows Test and manage: BLV-free bulls, AI, raising uninfected calves (SIA/RIA test-negative calves: milk/colostrum from negative dams), insect control, disinfection, needles, closed herd (replacement with own animals, only test-negative animals introduced)	2–3 tests in less than 4 mo until eradication in all herds
Shettigara et al., 1986	AGID	Start in 1981	3 yr	9	38–175 animals; 3 herds $<10\%$ within-herd prevalence, 4 herds 11–30% within-herd prevalence, 2 herds $>30\%$ within-herd prevalence Test and segregation, removal of positive animals within 30 d; retesting, testing every 6 mo after more intensive testing regimens	3 herds negative after 1 AGID ($\leq 8\%$ within-herd prevalence), 2 herds negative after 3 tests (12% within-herd prevalence), 1 herd negative after 4 tests (24.5% within-herd prevalence)
Shettigara et al., 1989	AGID	1982 to 1987	5 yr	6	28–139 animals Test and segregate (>200 m between animals); segregation of BLV-positive, BLV-negative, replacement cattle; testing every 3 mo until no more new positives, then every 6 mo Colostrum and milk BLV free or pasteurized	Decrease in prevalence in all cohorts: 0.76–0.73 to 0.44–0.17
Sprecher et al., 1991	AGID	1987 to 1989	2 yr	1	Needles, sleeves, disinfection, dehorming, milk replacer, colostrum pasteurization; comparison of heifer age cohorts	Eradication
Suh et al., 2005	ELISA	February 2000 to April 2004	4 yr	1	491 animals, 163 negatives Testing every 3–5 mo; test and segregate, only introduce negative heifers/purchases; sampling of calves before colostrum, colostrum and milk from negative dams, needles, syringes, sleeves, AI, disinfection	

Continued

Table 5 (Continued). Overview of bovine leukemia virus (BLV) on-farm control reports

Reference	Tests used ¹	Reported time span	Time to eradication, if applicable	No. of herds	Intervention ²	Result
Wang and Onuma, 1992	AGID, ELISA	May 1987 to October 1990	3 yr	2	9 serial tests Farm A: 400 animals; first 4 tests: 3- to 6-mo intervals, positive animals segregated or culled Farm B: bull facility, 40 bulls, 2-3 AGID/yr	Farm A: 30.2% within-herd prevalence, segregation until 5.2% prevalence, then culling, eradication after 8 tests Farm B: 13.8% within-herd prevalence, increase to 30%; elimination of TAA ⁴ - and BLV-positive bulls, segregation of positive bulls in 1986, no positive results in 4 tests Eradication after 5 tests (August 1979)
Yoshikawa et al., 1992	AGID	September 1977 to November 1991	14 yr	1	~400 cows, 3.52% initial within-herd prevalence 25 tests within 14 yr; test and segregation of positive animals	

¹AGID = agar gel immunodiffusion assay.

²Needles = single use of needles; sleeves = single use of examination sleeves; disinfection = disinfection of contaminated equipment and surgical instruments; syringes = single use of syringes; SIA = syncytium induction assay.

³Not reported.

⁴TAA = tumor-associated antigen.

seemed to be, although uncontrollable or unknown factors might disrupt eradication efforts.

BLV Proviral Load

Effects of proviral load in BLV-infected cattle on transmission dynamics have recently received increased interest. The findings were considered relevant to on-farm BLV control, especially in situations where the prevalence at the start of a control program is relatively high. It could provide an alternative or addition to the above-mentioned strategies. Therefore, a summary of these findings was included in the present review and is presented here.

Early on, it became clear that cattle with lymphocytosis, EBL, or both have higher risk of transmitting BLV to uninfected cattle (Kettmann et al., 1980). Lymphocytic cattle had higher absolute lymphocyte counts than nonlymphocytic cattle, whereby 25 to 35% of circulating lymphocytes had integrated proviruses. In contrast, in cows without lymphocytosis, only 5% of circulating lymphocytes had integrated proviruses. Therefore, the blood volume needed from cattle with lymphocytosis to infect other cattle was smaller compared with the volume needed from cattle without lymphocytosis (Buxton and Schultz, 1984).

These findings were followed by multiple studies that evaluated the BLV proviral load in infected animals. Numerous ways to measure and express proviral load were used: copies per 50 ng of DNA (Sajiki et al., 2017; Mekata et al., 2018), copies per microgram of DNA (Juliarena et al., 2007), copies per 10⁵ cells (Benitez et al., 2019a,b; Kobayashi et al., 2019; Ruggiero et al., 2019), percentage of peripheral white blood cells infected (Alvarez et al., 2013; Gutiérrez et al., 2014; Merlini et al., 2016), and relative proviral load compared with 18S reference gene (Gutiérrez et al., 2011, 2012, 2015).

To date, reliable identification of cattle with a high proviral load was dependent on relatively expensive PCR-based methods, hindering utilization (Leach et al., 2010; Sorge et al., 2010). Fortunately, an ELISA-based antibody titration has potential as a cheaper alternative, as p24 (BLV antigen) antibody titers are directly correlated with proviral load (Gutiérrez et al., 2012). Additionally, ELISA optical density values were directly correlated with the negative production effects of BLV infection (Norby et al., 2016). Another cost-effective approach may be blood cell differentiation, whereby increased white blood cell counts could be used as indicators for high proviral loads (Alvarez et al., 2013; Nishiike et al., 2016).

Despite these differences in approaches, it became clear that infected cattle harbor variable amounts of provirus in their cells, generally expressed as low to

high proviral loads. This information was considered important because cattle with a high proviral load may be more likely to infect others, whereas cattle with a low proviral load are very unlikely to infect others (Jimba et al., 2010).

It is still unclear whether proviral load within an infected animal remains constant once established (Gutiérrez et al., 2014; Merlini et al., 2016) or whether it can change over time (Ohno et al., 2015; Nishiike et al., 2016), as the proviral load may increase as individuals progress from aleukemic to lymphocytic stages (Ohno et al., 2015). Nonetheless, BLV-infected cattle with a high proviral load were more likely to develop lymphocytosis, progress to EBL, or both (Jimba et al., 2010; Nishiike et al., 2016; Kobayashi et al., 2019). Similarly, although cattle can have a high proviral load and a normal lymphocyte count (Juliarena et al., 2007), cattle with lymphocytosis likely have a higher proviral load than those with a normal white blood cell count (Juliarena et al., 2007; Alvarez et al., 2013; Ohno et al., 2015; Nakada et al., 2018; Benitez et al., 2019b).

Some studies were conducted to better understand the role of proviral load for the risk of transmission of BLV between animals. As described previously, dams with a high proviral load have a higher probability of infecting their calves in utero or peripartum than those with a lower proviral load (Mekata et al., 2015; Sajiki et al., 2017), and they have increased levels of provirus as well as anti-BLV antibodies in their colostrum (Gutiérrez et al., 2015) compared with those with a low proviral load. However, a negative correlation between proviral load and BLV antibody titers in milk was reported (Jaworski et al., 2016). To elucidate practical ramifications of proviral loads for on-farm BLV control, 2 kinds of studies were conducted: (1) cattle with a low proviral load were introduced into a herd of BLV test-negative cattle, or (2) cattle with a high proviral load were removed from a BLV-infected herd and BLV seroconversion rates and within-herd prevalence were monitored. Regarding outcomes, introduction of low proviral load bulls (Mekata et al., 2018; Benitez et al., 2019a) or cows caused no or only a few new infections in BLV test-negative cows and heifers (Juliarena et al., 2016). In addition, removal of individuals with a high proviral load in 3 commercial dairy herds decreased BLV seroconversion rate and BLV within-herd prevalence within 2.5 yr (Ruggiero et al., 2019). Although more research is needed to recommend the use of proviral load monitoring to inform management decisions, it could be a valuable addition to future on-farm BLV control.

DISCUSSION

The objective of this review was to discuss the available literature on BLV transmission and control efforts published since 1995 as well as to elucidate potential utility of proviral load in future control BLV control programs. Additionally, gaps in knowledge were identified. Seventy-two studies published after the review on BLV by Hopkins and DiGiacomo (1997) were included in this paper. Despite the resulting limited temporal scope of the present review, it was considered beneficial to summarize the most recent research on BLV transmission and control.

In general, results of studies concerning transmission of BLV before and after 1995 are not in conflict, whereby knowledge gaps identified by Hopkins and DiGiacomo (1997) still remain relevant. Moreover, it was noted that many studies did not report enough information for a thorough evaluation of applied methods or participating herds' circumstances, precluding realistic assessment of study quality. Even though various models have been created to identify transmission risk factors, the resulting information is still limited. Nonetheless, identified risk factors in models agreed to a large extent with identified and examined routes of transmission in other observational studies. Additionally, results of risk analyses have highlighted a potential role of animal-to-animal contact for transmission of BLV that warrant further investigation.

Thus far, successful BLV eradication was only reported when culling or segregation strategies were applied. However, diagnostic methodologies have improved over time (Beier and Siakkou, 1994; Rola-Luszczak et al., 2013). Therefore, studies using tests with low sensitivity (e.g., agar gel immunodiffusion test) may have missed individuals that would have been identified as positive with the current more sensitive methods (e.g., ELISA) and therefore incorrectly reported successful BLV eradication. Likewise, eradication efforts could be accelerated with modern serological methods or molecular diagnostics (e.g., PCR), as BLV-positive cattle can be identified more reliably and sooner after infection. Therefore, time frames documented in historical eradication studies may no longer be relevant and the true effect of control strategies may have not been accurately measured. Additionally, none of the studies demonstrated that solely implementing management practices resulted in eradication of BLV from the farm. However, unfortunately, none of the studies monitored herds long enough to provide a definitive answer on whether eradication of BLV on farm was possible with only BMP. Other studies reported no change or an

increase in within-herd prevalence despite implementation of BMP (Gutiérrez et al., 2011; Ruggiero, 2019). Perhaps transmission prevention through BMP was incomplete, either because some transmission routes have not yet been identified or because not all necessary control measures were implemented.

In addition, the dairy industry has profoundly changed since the majority of the described BLV control studies were conducted. Consequently, it is important to consider whether the published literature remains relevant. Despite changes in the dairy industry, the most common management practices have remained the same. For example, milking practices are generally similar, although there are more vaccinations and hormone treatments as well as ongoing increases in herd size. Additionally, as technology becomes more sophisticated and affordable, calf management, milking, and other practices change. For example, milk and colostrum are frequently pasteurized before feeding to calves, and milking procedures follow more hygienic protocols (Barkema et al., 2015). Although some risk factors could have increased in importance (e.g., needles, animal-to-animal contact), other transmission factors could lose their relevance (e.g., colostrum and milk). Hence, to make definitive recommendations, large-scale, longitudinal studies focusing on relevant transmission routes (e.g., colostrum, milk, tools, animal-to-animal contact) and on-farm BLV eradication based on implementation of BMP are necessary. In addition, the importance of biosecurity measures should be highlighted. By implementing biosecurity protocols (e.g., establishment of a closed herd), introduction of BLV into a herd could be avoided and implemented BLV control measures would not be undermined.

Additionally, multiple studies expounded on the apparent relevance of proviral load for progression of the disease to lymphocytosis, EBL, or both as well as on transmission of BLV among cattle (Mekata et al., 2015; Benitez et al., 2019a; Kobayashi et al., 2019). Consistent reporting on measurement of BLV proviral load would make comparisons more relevant (Juliarena et al., 2016; Ruggiero et al., 2019). Additionally, by taking proviral load into account, some of the contradictory results of the described observational studies (e.g., transmission of BLV through colostrum or transrectal palpation in only some cases) may be explained.

Finally, eradication attempts could be further accelerated by genetic selection for BLV-resistant cattle (Juliarena et al., 2008; Esteban, 2009), as BoLA class II haplotypes appear to modulate risk of developing high proviral loads and some cattle are therefore at a lower risk of progressing to lymphocytosis and EBL. Regardless, as altering genetic composition of an entire herd is

generally time consuming and complex, it should only be considered as part of a long-term strategy.

CONCLUSIONS

The review of the available literature concerning control of BLV resulted in the conclusion that knowledge as well as knowledge gaps have remained consistent over time. Although the main known BLV transmission routes (blood, in utero, colostrum, milk) were supported, risks connected with specific management practices within dairy operations (e.g., hoof trimming, feeding raw milk) remained inconclusive. Moreover, other transmission routes might remain unidentified, perhaps accounting for unsuccessful control attempts, highlighting the limited body of literature available for BLV control. Additionally, the role of proviral load information for on-farm BLV control deserves further attention. This method appears promising, especially in herds with high prevalence, where the sole change of management may not eliminate BLV. Nonetheless, more studies are needed to fill knowledge gaps in BLV transmission and understand the importance of proviral load for on-farm BLV control.

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