The Development of a Bovine Leukemia Virus Control Program

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The Development of a Bovine Leukemia Virus Control Program

by

Alessa Evelyn Traute Kuczewski

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

GRADUATE PROGRAM IN VETERINARY MEDICAL SCIENCES

CALGARY, ALBERTA

DECEMBER, 2019

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Abstract

North American dairy herds are commonly infected with bovine leukemia virus (BLV), with production-limiting effects, reduced animal welfare and consumer concerns. The overall goal of this thesis was the development of an adaptable on-farm BLV control program. To summarize important background knowledge and understand all aspects of BLV control, I performed the following: 1) Available literature concerning BLV transmission and control was reviewed and summarized. 2) Five commercially available ELISA were evaluated and compared, using 160 serum samples from Alberta cattle. 3) Economic impacts of BLV and its control were evaluated by creating an economic model of an average Alberta dairy farm. 4) Motivators and barriers for Alberta dairy farmers to change behavior on farm and implement BLV control measures were investigated by analyzing conversations with farmers as well as veterinarians. 5) In those conversations, dairy farmers’ and veterinarians’ opinions toward various BLV control measures were sought to adjust the BLV control program. 6) Based on findings, a risk assessment tool was designed to identify and weigh on-farm behavior that could cause transmission of BLV between animals. When this risk assessment tool was used on 11 Alberta dairy farms, its results, in combination with serum test results, led to the recommendation of tailored best-management practices aimed at preventing BLV transmission between animals. Implementation, within-herd prevalence, and seroconversions were measured. 7) Finally, all findings were summarized and discussed. In conclusion, recommendations for BLV control have not changed over a long interval, as general principles remain relevant. Additionally, identification of BLV-infected animals is easy and reliable. Although BLV control relies on financial investments, it has an overall economic net benefit. Motivating
farmers to implement BLV control is dependent on knowledge and control measures considered feasible by the farmer. Finally, implementation of on-farm BLV control reduced within-herd BLV prevalence for the majority of participating farms.

*Keywords*: bovine leukemia virus, BLV, bovine leukosis, dairy industry, control program, ELISA, comparison, evaluation, farm economics, economic evaluation, on-farm change, motivation, farmer motivation
Preface

This thesis consists of 6 manuscripts: 2 have been published and 4 will be submitted for publication in November/December 2019. Permission from the publishing journals has been obtained.

The following manuscripts are included in this thesis:

Chapter 2:

Kuczewski, A., Orsel, K., Barkema, H. W., Mason, S., Erskine, R., van der Meer, F. Scoping literature review: on-farm bovine leukemia virus transmission and control

Chapter 3:


Chapter 4:


Chapter 5:

Kuczewski A., Adams C., van der Meer F. Motivation and barriers to control bovine leukemia virus on farm

Chapter 6:

Kuczewski A., Adams C., van der Meer F. Dairy farmers’ and veterinarians’ opinion about on farm bovine leukemia virus control measures

Chapter 7:

Kuczewski A., Mason S., van der Meer F. Trial implementation of a newly developed bovine leukemia virus control program on 10 Alberta dairy farms
Statement of Contribution

Alessa Kuczewski identified, collected, and reviewed the literature for Chapter 2. The study in Chapter 3 was designed in collaboration with Karin Orsel, Herman Barkema, Wendy Hutchins, David Kelton and Frank van der Meer. Additionally, Herman Barkema and David Kelton aided with sample localization and collection in Chapter 3. Henk Hogeveen, Karin Orsel, Jada Thompson, and Eldon Speckman contributed to the model design and manuscript content in Chapter 4. Robert Wolf supported programming and model design in Chapter 4. Cindy Adams and Frank van der Meer helped designing questionnaires and provided feedback during data analysis (Chapter 5, 6). Herman Barkema helped identify veterinarians for interviews (Chapter 5, 6). Steve Mason aided in the design of an on-farm risk assessment tool (Chapter 7). All quantitative data were cleaned and analyzed by Alessa Kuczewski with support from Dr. Frank van der Meer, Steve Mason, and Diego Nobrega, when needed (Chapter 3, 4, 7). All focus groups, interviews, as well as the bovine leukemia virus trial implementation were conducted by Alessa Kuczewski. Audio recordings were transcribed, coded, and analyzed by Alessa Kuczewski. Jesse Schuster helped in conducting farmer focus groups and proof reading of transcripts (Chapter 5,6). Molly Kavanagh aided with transcription of interviews during the trial implementation (Chapter 5). All manuscripts were drafted by Alessa Kuczewski under the guidance and feedback of Frank van der Meer. All co-authors provided critical review and feedback of the manuscripts before journal submission. Permission has been obtained from all co-authors and the publishing journals to reprint the manuscripts in this thesis.
Acknowledgements

First off, I would like to thank my supervisor Dr. Frank van der Meer for his unwavering support, his patience and trust in me and my capabilities. You have challenged me to grow and I wouldn’t be the researcher and veterinarian I am today without your help.

I would also like to thank my supervisory committee: Dr. Herman Barkema, you brought me here and supported me from day 1. Who would have guessed that one short visit at a sheep farm would change my life so much?! Dr. Karin Orsel, thank you so much for your support throughout the past years. Your advice, patience, and care for my well-being have been a gift. Dr. Steve Mason, without your support, advice, time, and energy I wouldn’t be where I am today. Thank you. Dr. Ron Erskine, thank you for having my back not only from a different country, but also a different time zone. Your wealth of knowledge and expertise have had a big influence on this thesis, I wouldn’t have wanted to miss it.

I would also like to thank Dr. John Kastelic for his editing magic. Thank you for ensuring that our manuscripts’ writing quality reflected the quality of the research that was conducted.

This thesis would have not been possible without the support of the dairy farmers and veterinarians in Alberta. From the bottom of my heart: Thank you. Your input, feedback, dedication, and willingness to volunteer your cows for me and your readiness to put up with me time and time again was my favorite part of this project, the results of this thesis are for you!
Molly Kavanagh, my one summer student throughout grad school: Thank you so much for your enthusiasm, dedication, and help. You made my life so much easier and helped me over a mountain of work. Thank you!

My fellow grad students. I can’t thank you enough. Your support, guidance, the tears we cried together, the laughs we shared, and friendships we built, made this a truly magical experience and I am so grateful for each and every single one of you! A special thank you goes to the people that helped me with all the sampling and processing. Thank you for dealing with me on those stressful days. Antonia, you deserve special recognition for spending days with animals you are scared of and hours in the lab, processing samples with me. Thank you!

A sincere “thank you” also goes to the funding agencies that made this project possible: Alberta Livestock and Meat Agency (ALMA), Alberta Forestry and Agriculture, Alberta Milk (all Edmonton, AB, Canada), as well as the Clinical Research Fund of the Faculty of Veterinary Medicine (University of Calgary, AB, Canada) made sure I was able to be here.

Last but not least, I want to thank my family and friends. My parents Ingrid and Herwig. Without your constant moral and financial support, I would have never been able to be here. Thank you for doing everything in your power to allow me to follow my dreams, even though it meant I’d be so far away. My sister, Chantal. I don’t know what I’d do without you. I am sorry I am so far away, but oh so grateful that you are there for me, always. Dustin. I can’t put into words how much your support means to me. You were always there to deal with the best and the worst. Thank you. And finally: Denis and Annabelle. Thank you for being my “Canadian parents” without question or hesitation.
Thank you for welcoming me into your home and lives, for providing me with so much support, for all the adventures and the countless hours I was able to spend on Chubby’s back. If I wouldn’t have met you, my life would have likely taken a very different path. Thank you.
Dedication

To my family.
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<td>AB</td>
<td>Alberta</td>
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<tr>
<td>AI</td>
<td>Artificial Insemination</td>
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<td>AJDI</td>
<td>Alberta Johne’s Disease Initiative</td>
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<td>AGIDT</td>
<td>Agar Gel Immunodiffusion Test</td>
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<td>BLV</td>
<td>Bovine Leukemia Virus</td>
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<td>BMP</td>
<td>Best Management Practice</td>
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<tr>
<td>CFREB</td>
<td>Conjoint Faculties Research Ethics Board</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<td>CQM</td>
<td>Canadian Quality Milk</td>
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<td>DRECA</td>
<td>Dairy Research and Extension Consortium of Alberta</td>
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<tr>
<td>EBL</td>
<td>Enzootic Bovine Leukosis</td>
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<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<td>Polymerase Chain Reaction</td>
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<td>Prairie Diagnostics Saskatoon</td>
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<td>Risk Assessment</td>
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<td>Saskatchewan</td>
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<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
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<td>WBC</td>
<td>White Blood Cell</td>
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<td>Western Canadian Dairy Seminar</td>
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CHAPTER 1: Summarizing Introduction

1.1 Background
Bovine Leukemia Virus (BLV) is a retrovirus that targets bovine B-lymphocytes. The virus’ reverse transcriptase can transcribe BLV’s genomic RNA into DNA and the integrase can then integrate this copy of the virus’ genome (provirus) into the host’s DNA. Consequently, the infection cannot be cleared and animals will be infected lifelong (Goff, 2013). The number of integrated provirus copies varies between infected animals and is called proviral load (Jimba et al., 2010, Jimba et al., 2012). The infection with BLV results in lymphocytosis in approximately 30% of infected animals. Approximately 5% of infected animals develop enzootic bovine leukosis (EBL), fatal tumors that cause the premature death of affected animals and are considered an animal welfare issue (Schwartz and Levy, 1994, Bartlett et al., 2014). Additionally, the infection impairs affected animals’ immune systems (Frie and Coussens, 2015), causing less efficient responses to vaccines and potentially pathogens (Erskine et al., 2011, Frie et al., 2016). Moreover, infected animals’ milk production as well as longevity are reduced (Bartlett et al., 2013, Nekouei et al., 2016, Norby et al., 2016), thereby causing considerable economic losses to the dairy industry (Ott et al., 2003, Rhodes et al., 2003). In addition, the herd prevalence in North America has reached approximately 90% (VanLeeuwen et al., 2005, Scott et al., 2006, LaDronka et al., 2018), while BLV has been eradicated in many European countries, as well as in the dairy herds of New Zealand and Australia (Voges, 2012, Queensland Government Department of Agriculture and Fisheries, 2016, European Commission, 2017), therefore, causing more economic losses due to export restrictions. Additionally, it has recently been claimed that BLV could play a role in
human breast cancer (Buehring et al., 2015, Buehring et al., 2017). Even though no causative relationship between BLV exposure and the development of breast cancer was proven, these findings could have detrimental effects on the dairy industry by causing customer concerns and decreased dairy consumption. Consequently, control and eradication of BLV should be considered a priority within the dairy industry. Nonetheless, due to BLV’s biology, its control and/or eradication is dependent on the replacement of infected animals with uninfected ones. This can be achieved by eliminating BLV test-positive animals, by physically separating test-positive and test-negative animals, or by implementing best management practices (BMP) that are aimed at preventing the transmission of BLV between animals (Rodriguez et al., 2011, Bartlett et al., 2014). However, BLV control in Canada is dependent on the farmers’ initiative as there are no mandatory control programs in place. Despite the availability of some voluntary BLV control programs, BLV control has not been a priority in North America, as demonstrated by the high herd and within-herd prevalence (Brunner et al., 1997, Canadian Food Inspection Agency, 2003, Cornell University College of Veterinary Medicine, 2019). Nonetheless, the increased financial damage as well as emotional burden for dairy farmers has caused Alberta dairy farmers to voice the need for a BLV control program. Therefore, in order to address dairy farmers’ concerns, guarantee the competitiveness of their produce, and to decrease the economic damage BLV causes, the development of a voluntary BLV on-farm control program was initiated by a research team at the University of Calgary and will be described in this thesis.
1.2 Thesis summary

The overall goal of this PhD project was to approach the development of a BLV control program holistically, by investigating all important aspects of BLV control, as well as by including important stakeholders in the development process:

First, a literature review was conducted to summarize available knowledge about BLV transmission as well as options for on-farm BLV control. Additionally, the role of proviral loads for BLV control was investigated (Chapter 2). Second, BLV control is dependent on the identification of infected animals. Therefore, in order to evaluate commercially available tests’ performance, five different ELISA were compared and assessed using Alberta cattle samples (Chapter 3). Third, detailed information on the economic impact of the infection with BLV as well as on the financial investment and benefit of different BLV control strategies was unknown. Therefore, the economic impact of the infection with BLV as well as the economic benefit of BLV control on farm was evaluated by creating an economic model (Chapter 4). The findings of the first three chapters provided researchers, farmers, and other stakeholders with important background knowledge on BLV control. Fourth, a better understanding of motivators and barriers was needed to increase awareness and understand how more farmers could be motivated to implement BLV control measures on their farm. Therefore, motivators and barriers for dairy farmers to change on-farm behavior and implement on-farm BLV control measures was investigated by conducting focus groups and interviews with farmers as well as interviews with veterinarians. Additionally, the farmers’ and veterinarians’ perception of motivators and barriers was compared (Chapter 5). Fifth, the farmers’ as well as veterinarians’ opinions towards on-farm BLV control measures was
investigated (Chapter 6) in order to adjust the developed BLV control program to Alberta farmers’ needs. Sixth, this information was used to establish an on-farm risk assessment tool, which was evaluated by using it on 11 Alberta dairy farms. The introduction, as well as implementation of BLV control measures and their impact on within-herd BLV prevalence were monitored for approximately one year (Chapter 7). Finally, all findings were summarized and generally discussed (Chapter 8).

1.3 Research objectives

The objectives that were meant to be met by the conducted research were the following:

Objective chapter 2: To understand transmission routes of BLV between animals, to evaluate available options for BLV control, to understand the role of proviral load in BLV control, as well as to identify gaps in knowledge.

Objective chapter 3: To evaluate and compare different commercially available ELISA.

Objective chapter 4: To understand the cost of BLV infection and the related economic cost-benefit of BLV control programs.

Objective chapter 5: Assess factors that motivate producers to consider, implement, and continuously use a control program on their farm as well as to define the barriers that producers experience during the implementation process.

Objective chapter 6: To thoroughly understand dairy farmers’, as well as veterinarians’ opinions about different BLV control measures.

Objective chapter 7: To evaluate and improve a newly designed BLV risk assessment and associated on-farm BLV control measures by implementing them on a small number of Alberta dairy farms in order to identify and address potential shortcomings.
1.4 References


http://dx.doi.org/10.1371/journal.pone.0134304.


CHAPTER 2: Scoping Review: Bovine leukemia virus: Transmission, control, and eradication

2.1 Abstract

Bovine leukemia virus (BLV) infection is endemic in North American dairy herds and has production limiting effects. A literature review of available manuscripts since 1995 concerning BLV transmission and its control was conducted to identify the most recent available literature. While available literature confirmed known transmission routes (possibly contact, blood, natural breeding, in utero, colostrum, milk), detailed information on specific risks for transmission of BLV are still missing (e.g. contact, hoof trimming knives). Eradication of BLV was demonstrated to be possible by combined management, segregation, and/or culling approaches. However, the sole implementation of best management practices (BMP) aimed at the prevention of BLV transmission only decreased within-herd BLV prevalence but has not yet resulted in eradication of BLV from a herd. Additionally, the role of different proviral loads in infected animals was investigated. Animals with a high proviral load seem to be more likely to infect other animals, whereas animals with a very low proviral load seem to have low risks to transmit BLV to other animals. This finding could be used when controlling BLV in high prevalence herds. In conclusion, detailed, large scale studies investigating the role of specific transmission routes while taking the proviral load of infected animals into account are necessary. Moreover, the successful control and eradication of BLV by using BMP only, should be further investigated.
2.2 Introduction

Bovine leukemia virus (BLV) is a member of the family *Retroviridae* and is able to infect bovine B-lymphocytes (Goff, 2013). Natural infection does not only occur in cattle (*Bos taurus, Bos indicus*), but also in water buffalo (*Bubalus bubalis*), for example. Other species such as sheep (*Ovis aries*) can be infected experimentally (EFSA Panel on Animal Health and Welfare, 2015). Following infection, BLV integrates its genetic material into the host’s genome, creating a provirus, which is an identical DNA copy of the virus’ RNA genome (Goff, 2013). This way BLV establishes a lifelong persistent infection. The infection with BLV in cattle results in an impaired immune system, reduced cow longevity as well as decreased milk production, and may cause the development of Enzootic Bovine Leukosis in some infected animals (EBL; fatal lymphosarcoma; Schwartz and Levy, 1994, Bartlett et al., 2014, Frie and Coussens, 2015). These tumors cause reduced production, premature death, and welfare concerns in affected animals. Therefore, the eradication of BLV has been a priority in the European Union (EU) since 1964 (EFSA Panel on Animal Health and Welfare, 2015). Since then, many Western European countries were able to eradicate the disease from their cattle population (European Commission, 2017). Similarly, Australia and New Zealand eradicated BLV from their dairy herds (Voges, 2012, Queensland Government Department of Agriculture and Fisheries, 2016). However, BLV is still a common infection in North American dairy herds (~90% herd prevalence, ~40% within-herd prevalence on average; Nekouei et al., 2015, LaDronka et al., 2018, Kuczewski et al., 2019), even though several voluntary BLV control programs were initiated (Brunner et al., 1997, Canadian Food Inspection Agency, 2003).
Eradication programs in the EU and elsewhere applied test and cull approaches, whereby frequent testing of all susceptible cattle and the subsequent slaughtering of test-positive animals led to a reduction in prevalence and eventual removal of the virus from the herds (Burki, 1982, Mammerickx, 1984). Nonetheless, when eradication was initiated in Europe, within-herd and between-herd prevalence of BLV infection in European herds was low (Burki, 1982, Nuotio et al., 2003). As the individual within-herd prevalence in North America varies, different approaches to effective BLV control strategies are necessary. While slaughtering test-positive animals would be possible in low-prevalence herds, it is oftentimes not economically feasible or practical in high-prevalence herds. Then, alternative approaches to control BLV can be implemented, such as the segregation of BLV test-positive animals from negative animals, and/or the implementation of best management practices (BMP) aiming to reduce or prevent the transmission of BLV among animals (Johnson et al., 1985, Shettigara et al., 1989). Recommendations concerning these approaches are generally based on studies and data from 1960 – 1990 (Rodriguez et al., 2011). However, the North American dairy industry has changed considerably since then. Tie-stalls have often been replaced by free-stalls and herd size has been increasing, for example (Barkema et al., 2015). Additionally, new insights in the biology of BLV, especially its epidemiology, could improve BLV control and eradication strategies. It was, for example, hypothesized that animals with different proviral loads pose different BLV transmission risks (Juliarena et al., 2007, Jimba et al., 2010). The strategic culling of high risk (high proviral load) animals could therefore reduce transmission risk, when culling of all positive animals is not a viable option, thereby reducing transmission risk and within-herd prevalence. Therefore, a scoping literature
review was conducted to provide an overview of the available knowledge on BLV transmission and control, to identify gaps in knowledge, and to assess the applicability of historic and recent research to the current situation in the dairy industry.

2.3 Materials and Methods

2.3.1 Eligibility criteria
This literature review focused on published journal papers and relevant findings in theses in German and English, according to the first author’s language abilities. The topics included in this review were: 1) BLV transmission in cattle: Literature concerning the transmission among other species like sheep or goat, as well as in vitro studies were excluded. The last thorough review on BLV transmission was published in 1997 (Hopkins and DiGiacomo, 1997). Therefore, only literature about transmission studies from 1995 onwards was included. 2) BLV control: As this review focuses on on-farm BLV control, it will include literature reporting results of studies using (voluntary) BLV 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 Panel on Animal Health and Welfare, 2015). 3) BLV proviral load: Literature pertaining to the potential role of selecting cattle based on their BLV proviral loads as part of control efforts was also reviewed. All studies were considered for parts 2 and 3 without a restricting time frame.
2.3.2 Information sources

The following sources were used: ProQuest Dissertations & Theses Global (Ann Arbor, MI, USA; www.proquest.com), CAB Abstracts (Wallingford, Oxfordshire, UK; www.ebscohost.com), PubMed (Bethesda, MD, USA; www.ncbi.nlm.nih.gov/pubmed), and Web of Science (www.webofknowledge.com/WOS) (Grindlay et al., 2012).

2.3.3 Search

A search was conducted in all databases and summarized in a literature search worksheet on July 25 and August 7, 2019.

1) 'bovine leukemia virus' OR ‘enzootic bovine leukosis' OR ‘bovine leukosis’ OR ‘bovine lymphosarcoma’ OR 'bovine type c oncovirus'

2) ‘control*’ OR ‘control program*’ OR ‘eradic*’ OR ‘eradication program*’ OR ‘segregat*’ OR ‘remov*’ OR ‘prevent*’ OR ‘manag*’

3) ‘bovine’ OR ‘cattle’ OR ‘cows’ OR ‘dairy cattle’

4) ‘infection’ OR ‘transmission’ OR ‘transmission rate’ OR ‘infection rate’ OR ‘vectors’ OR ‘risk factors’ OR ‘spread’ OR ‘risk’

5) 4 AND 3 AND 1

6) 1 AND 2 AND 3

7) ‘Proviral load’ OR ‘provirus’

8) 1 AND 3 AND 7
2.3.4 Selection of sources of evidence

All sources identified in the process outlined above were downloaded and imported into EndNote (Clarivate Analytics, Philadelphia, USA). For further analysis EndNote, Microsoft Excel, and Microsoft Word (365 ProPlus, Microsoft Corp., Redmond, WA, USA) were used. Subsequently, the following steps were taken: First, duplicates were removed and all titles were screened for relevance. Sources that were not applicable to the researched topics were eliminated. If the information provided in the title was insufficient to make an informed decision, the abstract, when available, was evaluated. If neither abstract, nor full text were available, sources were not considered. Secondly, references were sorted into groups based on the 3 mentioned topics (transmission, control, proviral load) according to their title. Thirdly, abstracts were screened and sources that were not applicable were eliminated. Fourthly, sources were sorted into more detailed groups (i.e. routes of transmission, models and risk analyses, control and eradication attempts) to generate an overview of available literature. Fifthly, a review of each source was conducted by reading the full text. Finally, sources were summarized based on: author, title, year of publication, language of article, country, where research was conducted, sample size, if applicable, diagnostics used, if applicable, intervention and results, allowing for a fair comparison.

2.4 Results

2.4.1 Search results

Results for all searches on all databases are presented in Table 2-1.
2.4.2 Selection of sources of evidence

Detailed numbers of sources and the selection process is presented in Figure 1. In total, 8285 sources were downloaded. After the removal of duplicates, 4768 sources were screened. Finally, 72 sources were reviewed in detail.

2.4.3 Transmission

A short summary of the historic findings of the previous review of Hopkins and DiGiacomo (1997) and a comparison with literature as of 1995 reviewed here is presented in Table 2-2. Additionally, in recent years, risk assessment models were developed in order to identify the causes of BLV transmission on farm (Table 2-3). The factors identified were included in the following paragraphs. Generally, known routes of BLV transmission are animal-to-animal contact, blood contact, during natural breeding, vertical transmission like in utero transmission, peripartum, through milk and/or colostrum. Additionally, some herd characteristics and management strategies were identified to play a potential role in BLV transmission. The sources were categorized accordingly. A thorough summary of what is known about BLV proviral load’s role in the transmission and control of BLV is included in the final paragraph of the results section.

Animal-to-animal contact. In order to understand whether virus transmission through nasal excretions or saliva could be a possibility, BLV’s presence in these fluids was investigated. One Japanese study found BLV provirus in corresponding blood (35/50 PCR positive), nasal excretions (14/48 PCR positive), and saliva (6/47 PCR positive)
samples from the same cows (Holstein-Friesian, Japanese black, crossbred; Yuan et al., 2015). The housing system in this study was not further discussed. Nonetheless, this could be an explanation that, when housed in tie-stalls, dairy cows neighboring BLV-positive cows had a higher risk of becoming test-positive than cows 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 condition. Additionally, the following risk factors for transmission were identified using risk analysis models: Direct contact, especially in loose beef and dairy housing situations (Kobayashi et al., 2014, Kobayashi et al., 2010), intergenerational contact, such as exposure of heifers to older animals in dairy herds (Sargeant et al., 1997). Contact between calves and adult cattle in beef herds as well as comingling bred dairy heifers and adult dry dairy cows led to an increased within-herd BLV prevalence (Kobayashi et al., 2014). In addition, housing and on-farm management could influence BLV infection rates: indoor housing of dry dairy cows during winter (Sargeant et al., 1997), the housing of dairy calves in hutches during the winter (Sargeant et al., 1997), or the feeding of total mixed ration (TMR) to dairy heifers in shared feed bunks (Erskine et al., 2012b) were all associated with a higher risk for BLV infection. These findings might indicate that contact transmission of BLV among animals has been underestimated thus far and could point toward the potential role of stocking density in the transmission of BLV.
**Blood.** Transmission of blood between animals is generally known as an important risk factor for the spread of BLV as low volumes of blood contain enough infected lymphocytes to transmit the virus to another animal.

**Injections and equipment.** Reusing needles (Erskine et al., 2012b, Ramírez Vásquez et al., 2016), dry cow selenium injections (Erskine et al., 2012b), and gouge dehorning have been identified as BLV transmission risk factors through potential blood contamination (Kobayashi et al., 2010, Erskine et al., 2012b) on dairy operations in multiple cross sectional risk analysis studies. However, vaccinations were associated with a reduced prevalence of BLV on dairy farms in another cross sectional risk analysis study (Chi et al., 2002), possibly acting as a surrogate measure for good biosecurity management on farm. Additionally, the implementation of single use of needles for injections did not reduce the risk of new seroconversions on three commercial dairy farms (Ruggiero, 2019). Even though individual animal testing was performed and herds had different prevalences (25.3-74.4%), it is possible that other (iatrogenic) transmission routes caused the seroconversion, as detailed information on management practices was missing in the latter report. Despite these disagreeing findings, transmission of BLV between animals via blood is well-established. However, it is possible that only transitioning to single-use needles in the presence of alternate routes of transmission, cannot decrease infection rates sufficiently to decrease the within-herd prevalence.

**Rectal palpation.** Only one study (Kohara et al., 2006) has investigated rectal palpation as a mode of transmission for BLV. In this study, 3 out of 4 BLV-negative Holstein-Friesian steers that were rectally palpated once a week for 4 weeks for 3 minutes, using a BLV blood contaminated sleeve became BLV AGIDT and PCR-
positive. Generally, the within-herd prevalence of dairy farms rises when the number of rectal examinations increases (Erskine et al., 2012b). While it has been proven that the transmission of BLV via rectal palpation is possible, the quantification of transmission of BLV under realistic on-farm circumstances remains unclear. Similarly, even though contaminated gloves are considered a risk factor for BLV transmission, it is unclear if the implementation of single-use rectal examination sleeves will positively influence the BLV status on dairy farms (Nekouei et al., 2015, Ruggiero, 2019).

**Insects.** The absence of fly control (Erskine et al., 2012b) or the presence of blood-sucking insects on dairy and beef farms (Kobayashi et al., 2010, Kobayashi et al., 2014) were identified as risk factors for BLV infection in cross sectional risk analyses. Whereas this could be an indicator for BLV transmission through insects, it could also be a confounding factor for other on-farm factors influencing BLV transmission, like general hygiene, for example. Recent studies confirmed that genetic material of BLV can be detected through PCR in biting flies; however, natural transmission could not be demonstrated to Holstein or Aberdeen Angus steers and heifers despite the presence of PCR positive insects (Panei et al., 2019). Additionally, studies that used an experimental transmission approach, using injection of homogenized insects or insect parts could not demonstrate transmission by subcutaneous injection of a tick homogenate to sheep (Morris et al., 1996), or inconsistent transmission following injection (depth of injection not reported) with homogenized biting fly mouth parts (Panei et al., 2019). Nonetheless, seroconversion rates decreased when measures to decrease the number of biting flies were implemented on dairy and beef farms (Ooshiro et al., 2013, Kohara et al., 2018). Even though the role of biting flies in the transmission of BLV has been studied, no
definitive answer to their role can be given. Additionally, studies considering the role of other common insects, like lice, for example are missing.

**Natural breeding.** 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 was associated with a higher BLV within-herd prevalence of dairy farms in a risk analysis (Erskine et al., 2012b). Nonetheless, the BLV status of the bulls used by the herds examined in the study was not known. BLV 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), therefore representing a gap in knowledge about BLV transmission. Notably, many of these reports do not allow for a thorough evaluation of the quality of the techniques used and therefore it cannot be ruled out that samples were contaminated with lymphocytes. Additionally, BLV was detectable in beef bull smegma (Benitez et al., 2019b), which could play a role in transmission through 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 to 500 proviral copies/50 ng of genomic DNA in blood) were able to infect uninfected animals (Mekata et al., 2018). These findings suggest that transmission of BLV via natural breeding is possible, but might not be caused by semen, but by micro-abrasions and could be dependent on the proviral load in infected bulls.
**Vertical transmission.**

In utero/Peripartum. Embryo transfer is known to not cause cow-to-cow transmission of BLV, if done with the necessary precautions to avoid BLV transmission (e.g. single-use of needles and examination sleeves). However, there is a risk that the transferred embryo will be infected by the recipient dam (dairy cattle; Fukai et al., 1999). Similarly, dairy and beef calves can acquire BLV from their dams through vertical transmission (Meas et al., 2002, Mekata et al., 2015). This was confirmed by Sajiki et al. (2017), who showed that BLV genomic sequences were identical in Holstein dams and their infected calves. Finally, the BLV proviral load in the cow influences the probability of transmission to the calf. Dairy and beef cows in two studies with a high proviral load (defined as >400 copies/10ng DNA or >3,000 copies/50ng DNA) were more likely to transmit BLV to their calves than animals with a lower proviral load (<400 copies/10 ng DNA or ≤10 copies/50ng DNA); 14/29 and 4/4 calves were infected in the high proviral load groups vs. 9/95 and 0/2 calves infected in the low proviral load groups (Mekata et al., 2015, Sajiki et al., 2017). The influence of proviral loads on the likelihood of transmission could be an explanation for former varying findings concerning the vertical transmission of BLV (Hopkins and DiGiacomo, 1997).

Colostrum/Milk. Even though other studies have shown the transmission of BLV to calves via colostrum and/or milk (Hopkins and DiGiacomo, 1997), the quantification of the risk of BLV transmission via colostrum and milk remains a gap of knowledge. Additionally, the protective effect of maternal antibodies is a possibility. Maternal antibodies in dairy calves that received colostrum and milk from BLV-positive dams are
detectable until 3-9 months of age (Meas et al., 2002, Nagy et al., 2007), which may prevent BLV infection (Nagy et al., 2007, Kobayashi et al., 2010). Interestingly, other pathogens could play a role in BLV transmission from dam to their calves, but it is unclear and has not been thoroughly investigated yet. Nonetheless, transmission from BLV-positive dairy cows to their calves could not be demonstrated in 15 BLV mono-infected dams; but, when cows were coinfected with bovine immunodeficiency virus (BIV) and BLV, 5/9 calves became and remained BLV PCR positive (Meas et al., 2002). Finally, a small study reported on the effect of freezing and thawing of cells derived from a Holstein cow’s colostrum; colostrum cells from 3 BLV-infected dams were rendered non-infectious for 1 sheep (Kanno et al., 2014). The freezing and thawing of colostrum to prevent the transmission of BLV to calves is a commonly recommended management practice (Ruiz et al., 2018). However, strong evidence to support this method is not available.

**Herd characteristics and management strategies.** Many risk analysis models also identified herd characteristics that could increase the risk for or protect from BLV infection. Past detection of clinical EBL in a herd (Kobayashi et al., 2014, Nekouei et al., 2015), and increasing within-herd BLV infection rates (Sevik et al., 2015) were associated with increasing BLV prevalence on beef and dairy farms. Not practicing closed herd management is an important risk factor whereby the introduction of untested animals (Casal et al., 1990, Kobayashi et al., 2014, Nekouei et al., 2015, Sevik et al., 2015, Sun et al., 2015), or the use of a heifer rearer (Kobayashi et al., 2014) and larger herd size (Sevik et al., 2015, Sun et al., 2015) pose risks of BLV infection for beef and
dairy herds. Therefore, having a closed herd could decrease the risk of introduction of BLV into a herd and decrease the risk of BLV transmission and new infections (Nekouei et al., 2015). Cattle breed has been identified as a potential risk for BLV infection. In a Turkish study, Holstein dairy cattle had a higher risk of being BLV-infected compared to Brown Swiss dairy cattle (Sevik et al., 2015). However, the examined population consisted of almost twice as many Holstein as Brown Swiss cattle. Although import was not mentioned in this study (Sevik et al., 2015), it could be a possible explanation for increased prevalence within a breed as the import of Holstein cattle from North America is known to cause the occurrence of BLV within herds (Kavanagh, 1981, Suh et al., 2005). In the same study, increasing age within a herd was associated with an increased within-herd prevalence (Sevik et al., 2015), highlighting that older dairy cattle generally have a higher infection rate compared to younger ones (Erskine et al., 2012c). 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 in others (Scott et al., 2006), resulting in no clear understanding of the role of location for BLV prevalence. Finally, one study failed to find risk factors that could explain the within-herd prevalence in beef herds (Bezerra et al., 2019). However, only 16 herds were included in the latter study.

2.4.4 Experiences with voluntary on-farm bovine leukemia virus control and eradication

On-farm BLV control in dairy herds is generally determined by the fate of BLV-positive cattle. Therefore, testing is an integral part of BLV control, as it allows for the
identification of BLV-positive animals, as well as to measure the within-herd prevalence. The 3 control strategies commonly used separately or in combination are: culling of positive animals, physical segregation of positive and negative animals in separate barns or separate pens within the same barn, and the implementation of BMP aimed at the reduction of BLV transmission (see Table 2-4 for a list of reported combinations).

Overall, the majority of the studies reported on the effect of control in a single herd (14 studies), whereas 9 studies reported on more than 1 herd. The maximum number of herds included in a study was 9 herds. The following tests were used in the reported studies to detect BLV infected animals: AGIDT, ELISA, white blood cell counts, PCR, syncytia assay, RIA and/or infectivity tests. Resulting in different test characteristics and therefore possibly in different accuracy of prevalence estimations. The studies describing culling and segregation approaches rarely describe the timing of slaughter and/or segregation (Shettigara et al., 1986, Ruggiero and Bartlett, 2019). In addition, reported management practices aimed at prevention of BLV transmission varied among studies (Table 2-5). Of the reviewed studies, 15 out of 23 reported that the eradication of BLV on farm was achieved successfully, whereby eradication was defined as the lack of detection of new BLV-positive animals within a herd by the used tests (Roberts and Bushnell, 1982, Kaja et al., 1984, Shettigara et al., 1986, 1989, Pannwitz et al., 1987, Brenner et al., 1988, Wang and Onuma, 1992, Yoshikawa et al., 1992, Meszaros et al., 1994, Molloy et al., 1994, Deren et al., 2003, Suh et al., 2005, Lojkic´ et al., 2013, Ruggiero and Bartlett, 2019). Eradication was achieved by testing and culling (Roberts and Bushnell, 1982, Molloy et al., 1994, Ruggiero Brenner et al., 1988, and Bartlett, 2019), by testing and segregation (Brenner et al., 1988, Yoshikawa et al., 1992), by testing, culling, and
segregation (Shettigara et al., 1986, Wang and Onuma, 1992, Deren et al., 2003), by the combination of testing, segregation, and management changes (Shettigara et al., 1989, Suh et al., 2005), as well as through the implementation of culling and management strategies combined (Kaja et al., 1984) or through the combination of culling, segregation, and management strategies (Pannwitz et al., 1987, Meszaros et al., 1994, Lojkic’ et al., 2013). Nonetheless, none of the studies reporting on testing and management strategies only were able to eradicate BLV from a herd. The time from the start of the study to eradication ranged from 10 months to 14 years, whereas initial within-herd prevalence ranged from 2.2 to 70%. This shows that eradication of BLV is possible despite a high within-herd prevalence. Additionally, it becomes apparent that BLV eradication can be a lengthy progress. Even though lower within-herd prevalence generally seemed to result in faster elimination of BLV, a low within-herd prevalence did not always result in fast elimination of the infection (Table 2-5). Of the remaining studies, several reported that a reduction in within-herd prevalence was achieved using the following approaches: test and cull strategies (Flensburg, 1976, Molloy et al., 1994, Ruggiero and Bartlett, 2019, Ruggiero et al., 2019), test and segregation strategies (Brener et al., 1986), testing and management strategies (Ferrer, 1982, Ruppanner et al., 1983a, Sprecher et al., 1991), combined testing, culling, and segregation strategies (Itoh et al., 1990, Ruggiero et al., 2019), or a testing, segregation, and management based approach (Ferrer, 1982, Johnson et al., 1985). Finally, two studies reported no reduction or even an increase in within-herd prevalence despite the implementation of testing and management approaches or a testing and culling approach (Gutierrez et al., 2011, Ruggiero and Bartlett, 2019). Eradication and reduction of BLV prevalence seemed to be
dependent on the commitment to BLV control. The more vigorously control efforts were implemented, the more efficient BLV control seemed to be possible, however uncontrollable or unknown factors might disrupt eradication efforts.

### 2.4.5 Bovine leukemia virus proviral load

Recently, the effects of proviral load in BLV-infected animals on transmission dynamics has received increased interest. Even though proviral load was measured differently, depending on the reporting study [copies/50ng DNA (Sajiki et al., 2017, Mekata et al., 2018), copies/ug of DNA (Juliarena et al., 2007), copies/10⁵ cells (Benitez et al., 2019a, Benitez et al., 2019b, Kobayashi et al., 2019, Ruggiero et al., 2019), percentage of peripheral white blood cells infected (Alvarez et al., 2013, Gutierrez et al., 2014, Merlini et al., 2016), relative proviral load in comparison to 18S reference gene (Gutierrez et al., 2011, Gutierrez et al., 2012, Gutierrez et al., 2015], it was determined that infected animals can harbour different amounts of provirus in their cells, expressed as very low to high proviral loads. This information was considered important, because animals with high proviral loads might have a higher likelihood to infect other animals, whereas the ability of animals with a low proviral load to infect other animals could be negligible (Jimba et al., 2010). It is still unclear whether the proviral load within an infected individual remains constant once established (Gutierrez et al., 2014, Merlini et al., 2016), or can change over time (Ohno et al., 2015, Nishiike et al., 2016). Nonetheless, BLV-infected animals with a high proviral load were more likely to develop lymphocytosis and/or progress to EBL (Jimba et al., 2010, Nishiike et al., 2016, Kobayashi et al., 2019), whereby the proviral load may increase with the progression of infected animals from
aleukemic to lymphocytotic stages of the disease (Ohno et al., 2015). In turn, animals with lymphocytosis likely have a higher proviral load than animals 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). However, animals could have a high proviral load and a normal lymphocyte count (Juliarena et al., 2007). Early on, it became clear that animals with lymphocytosis and/or EBL form a higher risk to transmit BLV to uninfected animals (Kettmann et al., 1980). Lymphocytotic cattle had higher absolute lymphocyte counts than non-lymphocytic cattle, whereby 25-35% of the circulating lymphocytes had integrated proviruses. In cows without lymphocytosis only 5% of the circulating lymphocytes had integrated proviruses. Therefore, the blood volume needed from animals with lymphocytosis to infect another animal was smaller compared to the volume needed from animals without lymphocytosis (Buxton and Schultz, 1984). Moreover, as described before, dams with a high proviral load have a higher probability to infect their calves in utero and/or peripartum than animals with a lower proviral load (Mekata et al., 2015, Sajiki et al., 2017) and have increased levels of provirus as well as anti-BLV antibodies in their colostrum (Gutierrez et al., 2015) compared to animals with a low proviral load. However, a negative correlation between proviral load and BLV antibody titres in milk was identified in another study (Jaworski et al., 2016). In order to better understand the practical applicability of proviral loads for on-farm BLV control, two different kinds of studies were conducted: Either animals with a low proviral load were introduced into a herd of BLV test-negative animals, or animals with a high proviral load were removed from a BLV-infected herd, and the BLV seroconversion rates and within-herd prevalence were monitored. Thus, the 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). Finally, the removal of high proviral load animals in 3 commercial dairy herds, resulted in a decreased BLV seroconversion rate and BLV within-herd prevalence within 2.5 years (Ruggiero et al., 2019). Therefore, the monitoring of the proviral load in BLV infected animals and their use in management decisions could be an effective part of on-farm BLV control.

2.5 Discussion

The objective of this scoping review was to discuss available literature on BLV transmission and control efforts, as well as to understand the possible role of proviral load in future control programs. Additionally, gaps in knowledge were identified. Since the review on BLV conducted by Hopkins and DiGiacomo in 1997, 72 studies were conducted and included in this manuscript. Largely, the results of studies concerning the transmission of BLV before and after 1995 are not in conflict. However, the knowledge gaps identified by Hopkins and DiGiacomo were still relevant: The relative importance of different management factors for the transmission of BLV (e.g. feeding of dam’s colostrum and milk, biting insects, use of breeding bulls) remains unknown, as the results of different studies are inconsistent. Additionally, other, potentially relevant factors for BLV transmission have still not been investigated (for example the transmission risk of blood-contaminated hoof knives), leaving important knowledge gaps. Moreover, it was noted that multiple studies did not report enough information for a thorough evaluation of
applied methods and/or participating herds’ circumstances, preventing a realistic assessment of the studies’ quality. Even though different models have been created to examine transmission risk factors, the resulting information is still limited. Nonetheless, the identified risk factors in modelling studies agreed to a large extent with the risk factors identified in observational studies. Additionally, the results of risk analyses have highlighted a potential role of animal-to-animal contact for the transmission of BLV that would warrant further investigation.

Thus far, BLV eradication was only successful when culling and/or segregation strategies were applied. None of the studies reporting on the sole use of implementing management practices resulted in eradication of the virus from the farm. However, none of the reporting studies monitored herds long enough to provide a definitive answer on whether eradication of BLV on farm was possible by using BLV BMP only. Additionally, other studies reported no change or an increase in BLV within-herd prevalence despite the implementation of BMP (Gutierrez et al., 2011, Ruggiero, 2019). This could indicate that transmission prevention through BMP was incomplete, either because some transmission routes have not been identified yet or because not all necessary control measures were implemented on farm. Similarly, diagnostic methodologies have improved over time and more reliable methods are commonly used (Beier and Siakkou, 1994, Rola-Luszczak et al., 2013). Therefore, studies using tests with low sensitivity, like for example AGIDT, might have missed animals that would have been identified as positive with a nowadays commonly used modern ELISA. Likewise, eradication efforts could be accelerated by the use of modern serological methods or molecular diagnostics like PCR, as BLV-positive animals can be identified earlier and more reliably. Therefore, the time frames
documented in historical eradication studies might not be applicable to the current situation and the true impact of control strategies might have not been measured correctly. Nonetheless, the reported studies can give valuable insight into the potential for success of the chosen control strategies. In addition, the dairy industry has changed since the majority of the described BLV control studies were conducted. It is, therefore, important to consider whether the published literature is still relevant to the current situation. Even though the structure of the dairy industry changed, the most common management practices remained the same. For example, vaccinations, calf care, and milking practices are performed similarly. However, there has been an increase in use of vaccinations and hormone treatments, as well as 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 recently (e.g. needles, animal-to-animal contact), other transmission factors could lose their relevance (e.g. colostrum and milk). Hence, large-scale, longitudinal studies focusing on different, relevant transmission routes (e.g. colostrum, milk, tools, animal-to-animal contact) and on-farm BLV eradication based on the implementation of BMP are necessary. Besides the role of BMP, the importance of biosecurity measures should be highlighted. By implementing biosecurity protocols, like for example the establishment of a closed herd, the introduction of BLV into a herd could be avoided and implemented BLV control measures would not be undermined. Additionally, multiple studies elaborated on the apparent relevance of proviral load for the progression of the disease to lymphocytosis.
and/or EBL, as well as on the transmission of BLV among animals (Mekata et al., 2015, Benitez et al., 2019a, Kobayashi et al., 2019). Even though a unified consistent reporting on the measurement of BLV proviral load would make comparisons more relevant, the identification and elimination of high proviral load animals within a herd appears to accelerate BLV eradication (Juliarena et al., 2016, Ruggiero et al., 2019). However, to date, the reliable identification of animals with a high proviral load is dependent on relatively expensive PCR-based methods, hindering the uptake of this tool (Leach et al., 2010, Sorge et al., 2010). Using an ELISA-based antibody titration has potential as a cheaper alternative as higher p24 (BLV antigen) antibody titres are correlated with a higher proviral load (Gutierrez 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 the use of 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). Additionally, by taking the proviral load of examined animals into account, some of the contradictory results of the described observational studies could possibly be explained (e.g. transmission of BLV through colostrum or rectal palpation only in some cases).

Finally, eradication attempts could possibly be further accelerated by the breeding of BLV-resistant cattle (Juliarena et al., 2008, Esteban, 2009), as cattle with different BoLA class II haplotypes appear to have a different risk to develop high proviral loads and some are therefore at a lower risk to progress to lymphocytosis and EBL. However, the change of the genetic makeup of an entire herd can be time consuming and can be complex as it
is determined by many factors. It should therefore only be considered as part of a long-term strategy.

2.6 Conclusions

The scoping review of available literature concerning the control of BLV resulted in the conclusion that knowledge as well as knowledge gaps have remained consistent over time. While the main known BLV transmission routes (blood, *in utero*, colostrum, milk) were confirmed, the risk connected with specific management practices within dairy operations (e.g. animal-to-animal contact, hoof trimming, feeding raw milk) remained inconclusive. Moreover, other transmission routes might remain unidentified, possibly explaining unsuccessful control attempts. Additionally, the role of proviral load information for on-farm BLV control deserves further attention. This method appears to be promising, especially in high within-herd prevalence herds, where the sole change of management might not be able to eliminate BLV. Nonetheless, more studies are needed to fill knowledge gaps in BLV transmission and to understand the importance of animals’ proviral load for on-farm BLV control.
2.7 References

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http://dx.doi.org/10.1186/1746-6148-6-1.

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Ruggiero, V. J. 2019. Field Studies on the Control of Bovine Leukemia Virus in Dairy Cows. PhD. Michigan State University, Ann Arbor, Michigan, USA.


### Table 2-1. Results of database searches.

<table>
<thead>
<tr>
<th>Search</th>
<th>Date</th>
<th>Database</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) ‘bovine leukemia virus’ OR ‘enzootic bovine leukemia’ OR ‘bovine leukemia’ OR ‘bovine lymphosarcoma’ OR ‘bovine type c oncovirus’</td>
<td>July 25, 2019 and August 7, 2019</td>
<td>2,326</td>
</tr>
<tr>
<td>2) ‘control*’ OR ‘control program*’ OR ‘eradic*’ OR ‘eradication program*’ OR ‘segregat*’ OR ‘remov*’ OR ‘prevent*’ OR ‘manag*’</td>
<td>July 25, 2019</td>
<td>10,147,880</td>
</tr>
<tr>
<td>4) ‘infection’ OR ‘transmission’ OR ‘transmission rate’ OR ‘infection rate’ OR ‘vectors’ OR ‘risk factors’ OR ‘spread’ OR ‘risk’</td>
<td>July 25, 2019</td>
<td>4,927,442</td>
</tr>
<tr>
<td>5) 4 AND 3 AND 1</td>
<td>July 25, 2019</td>
<td>976</td>
</tr>
<tr>
<td></td>
<td>6) 1 AND 2 AND 3</td>
<td>July 25, 2019</td>
</tr>
<tr>
<td>---</td>
<td>------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>7) ‘Proviral load’ OR ‘provirus’</td>
<td>July 25, 2019 and August 7, 2019</td>
<td>4331</td>
</tr>
<tr>
<td>8) 1 AND 3 AND 7</td>
<td>July 25, 2019 and August 7, 2019</td>
<td>188</td>
</tr>
</tbody>
</table>
Table 2-2. Review of bovine leukemia virus (BLV) transmission. Summarized after Hopkins & DiGiacomo (1997)

<table>
<thead>
<tr>
<th>Transmission route</th>
<th>Risk as identified by Hopkins and DiGiacomo, 1997</th>
<th>Risk as identified in current review</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen</td>
<td>None, unless contaminated</td>
<td>BLV detected in 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</td>
</tr>
<tr>
<td>Ova/Embryos</td>
<td>None, if recipient animals are BLV free</td>
<td>No additional studies identified since 1996</td>
</tr>
<tr>
<td>In utero</td>
<td>Transplacental infection after immunocompetency (approx. 3 months 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 EBL have highest risk for transmission</td>
<td><em>In utero</em> transmission of BLV is possible (0-40% calves to positive dams infected); animals with high proviral load have higher risk for vertical transmission of BLV</td>
</tr>
<tr>
<td>Colostrum/Milk</td>
<td>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 colostral antibodies</td>
<td>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</td>
</tr>
<tr>
<td>Dehorning/Surgeries</td>
<td>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</td>
<td>No additional studies identified since 1996</td>
</tr>
<tr>
<td>Activity</td>
<td>Description</td>
<td>Evidence</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Tattooing/Ear Tags</td>
<td>Risk for transmission through tattooing; mention of ear tagging as similar risk, but no evidence; lack of transmission by brucellosis vaccine</td>
<td>No additional studies identified since 1996</td>
</tr>
<tr>
<td>Immunization/Injection/Venipuncture</td>
<td>Highly efficient transmission by inoculation with infected blood: transmission of 1uL of blood caused infection (s.c., i.d., i.m., i.v.); infectivity is dependent on number of lymphocytes in blood; animals with lymphocytosis are infective at lower doses of lymphocytes than animals with normal lymphocyte count; i.v. and venipuncture have likely higher risk than s.c., i.d., i.m. injections</td>
<td>No additional studies identified since 1996</td>
</tr>
</tbody>
</table>
| Breeding/Reproductive management:     | AI, natural service: low risk;  
Semen, Embryos: no risk, if not contaminated with lymphocytes;  
Reuse of equipment and/or examination sleeves for AI: risk for transmission, but no evidence | BLV detected in semen and smegma samples; no transmission through natural breeding by bulls with very low proviral load, some transmission through bulls with low proviral load |
<p>| Natural service/AI/ET                | Rectal mucosa, bleeding from reproductive tract as sources of blood; Controversial results: risk for transmission dependent on circumstances: experimental transmission by blood inoculation or visibly blood contaminated gloves is possible; animals with lymphocytosis could have higher risk for transmission than aleukemic animals; | Experimental transmission with visible blood is possible |</p>
<table>
<thead>
<tr>
<th>Infection Category</th>
<th>Transmission Details</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact transmission</td>
<td>Nasal secretions, saliva: BLV detected inconsistently; transmission dependent on lymphocytes in fluid; prevalence within grouped animals correlated with risk of infection</td>
<td>Nasal secretions, saliva: BLV detected inconsistently; increased risk for BLV infection, if uninfected animals are housed next to infected animals in tie-stalls</td>
</tr>
<tr>
<td>Confinement during calving</td>
<td>Decrease in circulating antibodies around parturition, exposure to blood, tissue, fluids around birth (depending on calving area); controversial results</td>
<td>No additional studies identified since 1996</td>
</tr>
<tr>
<td>Insects/Arthropods</td>
<td>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</td>
<td>PCR detection of BLV in insects; inconsistent results in inoculation transmission experiments; decreased seroconversion rates, when insect-cattle contact could be prevented</td>
</tr>
</tbody>
</table>
Table 2-3. Overview of risk analysis models.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Model</th>
<th>Sample</th>
<th>Data collection</th>
<th>Identified risks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bezerra et al., 2019</td>
<td>Brazil</td>
<td>Multiple logistic regression</td>
<td>16 herds, 160 samples</td>
<td>Questionnaire</td>
<td>No risks identified for BLV</td>
</tr>
<tr>
<td>Casal et al., 1990</td>
<td>Spain</td>
<td>Path analysis</td>
<td>92 herds</td>
<td>Questionnaire</td>
<td>Risk for transmission of BLV between herds; introduction of new animals, number of animals introduced, prevalence in herd of origin</td>
</tr>
<tr>
<td>Chi et al., 2002</td>
<td>Canada</td>
<td>Tobit regression analysis</td>
<td>90 herds, 2604 cattle</td>
<td>Questionnaire</td>
<td>Vaccination: reduced prevalence of exposure for BLV</td>
</tr>
<tr>
<td>Erskine et al., 2012b</td>
<td>USA</td>
<td>One-way ANOVA, simple linear regression, multivariate analysis</td>
<td>113 herds</td>
<td>Interview</td>
<td>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</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Methodology</td>
<td>Data Details</td>
<td>Data Collection</td>
<td>Results and Findings</td>
</tr>
<tr>
<td>------------------------------</td>
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<td>------------------------------------</td>
<td>--------------------------------------------------</td>
<td>-----------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Heald et al., 1992</td>
<td>Canada</td>
<td>Logistic regression</td>
<td>268 herds, 998 cows, AGID</td>
<td>Production records, mail survey</td>
<td>Increased odds for positive animals within a herd: calving in separate calving pens in the winter (alternative mostly tie-stalls), calves in hutches in winter; decreased risk: calving in separate calving pens in the summer (alternative mostly pasture)</td>
</tr>
<tr>
<td>Kobayashi et al., 2014</td>
<td>Japan</td>
<td>Zero-inflated negative binomial model</td>
<td>563 dairy farms, 490 beef farms, 20 animals per herd tested</td>
<td>Interview</td>
<td>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</td>
</tr>
<tr>
<td>Kobayashi et al., 2010</td>
<td>Japan</td>
<td>Mixed logistic regression analysis</td>
<td>139 farms (110 positive), 90 filled out questionnaires</td>
<td>Questionnaire</td>
<td>Risk factors for within-farm transmission of BLV: loose housing, dehorning, large number of horseflies in the summer; protective: feeding colostrum from dam to calf</td>
</tr>
<tr>
<td>Nekouei et al., 2015b</td>
<td>Canada</td>
<td>Zero-inflated negative binomial regression model</td>
<td>315 dairy farms, 9-45 cows/farm sampled (ELISA)</td>
<td>Questionnaire from 272 herds</td>
<td>Risk for increased within-herd prevalence: clinical cases of leukosis in past 12 months, herds with purchased animals with unknown BLV status, changing gloves in Eastern Canada, no association in Western Canada; Western herds more likely to be infected than Eastern herds; closed herds more likely to be negative</td>
</tr>
<tr>
<td>Authors</td>
<td>Location</td>
<td>Methodology</td>
<td>Herd Size</td>
<td>Source Material</td>
<td>Findings</td>
</tr>
<tr>
<td>---------------------</td>
<td>----------</td>
<td>----------------------</td>
<td>-----------</td>
<td>----------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Ramírez Vásquez et al., 2016</td>
<td>Colombia</td>
<td>Logistic regression</td>
<td>29 herds, 1003 cattle</td>
<td>Interview</td>
<td>Increased risk for seropositivity: reuse of needles</td>
</tr>
<tr>
<td>Sargeant et al., 1997</td>
<td>Canada</td>
<td>Multivariable analysis</td>
<td>102 herds, 1330 cows</td>
<td>Records, questionnaire</td>
<td>Negative association between herd-level milk production and BLV status; positive association between weaning age and purchasing animals from outside sources and BLV status; increased risk of BLV: housing calves in hutches/separate buildings, winter: contact of heifers to older animals, dry cow housing</td>
</tr>
<tr>
<td>Scott et al., 2006</td>
<td>Canada</td>
<td>Generalized linear model</td>
<td>77 herds, 2819 samples</td>
<td>Questionnaire</td>
<td>No variance between BLV seroprevalence and agroecological regions in Alberta</td>
</tr>
<tr>
<td>Sevik et al., 2015</td>
<td>Turkey</td>
<td>Generalized mixed linear model</td>
<td>1116 herds, 28982 samples</td>
<td>Records</td>
<td>Increased risk for increased seroprevalence: increasing cattle age, herd size, cattle breed, purchased cattle, increase in prevalence with age</td>
</tr>
<tr>
<td>Sun et al., 2015</td>
<td>China</td>
<td>Multivariate analysis</td>
<td>113 herds, 3674 cattle</td>
<td>Questionnaires</td>
<td>Risk factors for BLV infection: herd size and presence of cattle introduced from other farms</td>
</tr>
</tbody>
</table>
Table 2-4. Summary of reported control strategies and their combinations.

<table>
<thead>
<tr>
<th>Control strategy</th>
<th>Success of strategy</th>
<th>Number of studies</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test and culling of all BLV test positive animals</td>
<td>Eradication achieved</td>
<td>4</td>
<td>Brenner et al., 1988, Molloy et al., 1994, Roberts and Bushnell, 1982, Ruggiero and Bartlett, 2019</td>
</tr>
<tr>
<td></td>
<td>Reduction of within-herd prevalence</td>
<td>4</td>
<td>Flensburg, 1976, Molloy et al., 1994, Ruggiero et al., 2019, Ruggiero and Bartlett, 2019</td>
</tr>
<tr>
<td></td>
<td>Increase in within-herd prevalence</td>
<td>1</td>
<td>Ruggiero and Bartlett, 2019</td>
</tr>
<tr>
<td>Test and segregation of all BLV test positive animals</td>
<td>Eradication achieved</td>
<td>3</td>
<td>Brenner et al., 1988, Yoshikawa et al., 1992</td>
</tr>
<tr>
<td></td>
<td>Reduction in within-herd prevalence</td>
<td></td>
<td>Brener et al., 1986</td>
</tr>
<tr>
<td>Test and segregation of young stock</td>
<td>No new infections</td>
<td>1</td>
<td>Ferrer, 1982</td>
</tr>
<tr>
<td>Test and implementation of best management practices to avoid BLV transmission</td>
<td>Reduction of within-herd prevalence</td>
<td>3</td>
<td>Ferrer, 1982, Ruppanner et al., 1983, Sprecher et al., 1991</td>
</tr>
<tr>
<td></td>
<td>No change in within-herd prevalence</td>
<td>1</td>
<td>Gutiérrez et al., 2011</td>
</tr>
<tr>
<td>Test, culling, and segregation of BLV test positive animals</td>
<td>Eradication achieved</td>
<td>3</td>
<td>Deren et al., 2003, Shettigara et al., 1986, Wang and Onuma, 1992</td>
</tr>
<tr>
<td>Test, segregation of BLV test positive animals, and implementation of best management practices to avoid BLV transmission</td>
<td>Reduction of within-herd prevalence</td>
<td>2</td>
<td>Itoh et al., 1990, Ruggiero et al., 2019</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Eradication achieved</td>
<td>2</td>
<td>Shettigara et al., 1989, Suh et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Reduction of within-herd prevalence</td>
<td>2</td>
<td>Ferrer, 1982, Johnson et al., 1985</td>
</tr>
<tr>
<td>Test, culling of BLV test positive animals, and implementation of best management practices to avoid BLV transmission</td>
<td>Eradication achieved</td>
<td>1</td>
<td>Kaja et al., 1984</td>
</tr>
<tr>
<td>Test, culling and segregation of BLV test positive animals, as well as implementation of best management practices to avoid BLV transmission</td>
<td>Eradication achieved</td>
<td>3</td>
<td>Lojkic´ et al., 2013, Mészáros et al., 1994, Pannwitz et al., 1987</td>
</tr>
</tbody>
</table>
### Table 2-5. Overview of bovine leukemia virus (BLV) on-farm control reports.

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

<table>
<thead>
<tr>
<th>Kaja et al., 1984</th>
<th>AGID</th>
<th>Summer 1980-March 1983</th>
<th>2.5 years</th>
<th>1</th>
<th>introduction of test negative animals, quarantine of new animals, elimination of test positive animals, frequent testing, ET(^6), AI(^5), needles(^2), syringes(^7), disinfection(^4)</th>
<th>133 cows introduced, 3 removed, because of seroconversion; 104 heifers introduced, 6 removed, because of BLV positive status; no infections by ET or AI, eradication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>Methodology</td>
<td>Duration</td>
<td>Number</td>
<td>Description</td>
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<tr>
<td>Meszaros et al., 1994</td>
<td>AGID</td>
<td>8 years</td>
<td>1,248</td>
<td>1,248 cows, 62% within-herd prevalence; testing before and after colostrum; at 2.5, 6 months, every two months afterward, then every three, then every 6; 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</td>
<td>Gradual replacement of positive herd, eradication</td>
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<tr>
<td>Molloy et al., 1994</td>
<td>AGID, viral antigen</td>
<td>3 years</td>
<td>126-304</td>
<td>126-304 animals, 19-39% within-herd prevalence; selective culling based on antigen level</td>
<td>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</td>
<td></td>
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<tr>
<td>Study</td>
<td>Test Method</td>
<td>Duration</td>
<td>Testing Intervals</td>
<td>Animals Tested</td>
<td>Test Results</td>
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<tr>
<td>Pannwitz et al., 1987</td>
<td>hematology, then AGID</td>
<td>1980-1982</td>
<td>2 years</td>
<td>1 herd, 300 cows, 420 replacements, 180 feedlot animals, beef cattle; 0.3% hematologic positive animals, 8.9% AGID positive; housed in 2 free stall barns; pasture calving, calf on foot with dam; testing all animals 6 months and older in 4-6 month intervals; segregation and culling of positive animals, management: needles, disinfection, disinfection of barn</td>
<td>6 tests until eradication</td>
<td></td>
</tr>
<tr>
<td>Roberts and Bushnell, 1982</td>
<td>AGID</td>
<td>N.R.</td>
<td>42 months</td>
<td>1794 animals tested, 86 positive at first test; test and cull</td>
<td>16 animals positive at second test, third test and after: all animals negative = eradication</td>
<td></td>
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<tr>
<td>Study</td>
<td>Methodology</td>
<td>Time Period</td>
<td>Duration</td>
<td>Herd Size</td>
<td>Prevalence</td>
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<tr>
<td>Ruggiero et al., 2019</td>
<td>PCR, ELISA</td>
<td>Start in fall 2015/spring 2016</td>
<td>2-2.5 years</td>
<td>3 farms; proviral load: very high (≥100,000 copies/105 cells), high (≥50,000 to &lt;100,000 copies/105 cells), moderate (≥16,000 to &lt;50,000 copies/105 cells), or low (&gt;0 to &lt;16,000 copies/105 cells). Lymphocyte counts: very high (≥10.0 × 10⁹/L), high (≥7.5 to &lt;10.0 × 10⁹/L), or normal (&lt;7.5 × 10⁹/L); herd managers were encouraged to prioritize culling of animals with high lymphocyte counts and proviral load, encouragement of segregation of high PVL/WBC cows.</td>
<td>Incidence decrease on all farms (13.8 to 2.2); within-herd prevalence decrease on all farms (62.0 to 20.7%)</td>
<td></td>
</tr>
<tr>
<td>Ruggiero and Bartlett, 2019</td>
<td>ELISA</td>
<td>August 2015-March 2017</td>
<td>2.5 years</td>
<td>3 herds, 150-850 milking cows; &lt;5% within-herd prevalence (2.2-3.2%); test and cull</td>
<td>eradication of BLV from adult herd after 3 years from 1 farm, prevalence increase (&gt;5%) on one farm, prevalence decrease on one farm (1.5%)</td>
<td></td>
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<tr>
<td>Study</td>
<td>Test Method</td>
<td>Years</td>
<td>Duration</td>
<td>BLV Prevalence</td>
<td>Prevention Measures</td>
<td>Results</td>
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<tr>
<td>Ruppaner et al., 1983</td>
<td>Lymphocyte count, AGID</td>
<td>1979-1981</td>
<td>3 years</td>
<td>1</td>
<td>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)</td>
<td>Decrease in prevalence (newborn calves 62.5 to 58.4%)</td>
</tr>
<tr>
<td>Shettigara et al., 1986</td>
<td>AGID</td>
<td>Start in 1981</td>
<td>3 years</td>
<td>9</td>
<td>38-175 animals, 3 herds &lt;10% within-herd prevalence, 4 herds 11-30% within-herd prevalence, 2 herds &gt;30% within-herd prevalence; test and segregation, removal of positive animals within 30 days; retesting, testing every 6 months after more intensive testing regiments</td>
<td>2-3 tests in less than 4 months until eradication on all herds</td>
</tr>
<tr>
<td>Study</td>
<td>Method</td>
<td>Start-End</td>
<td>Duration</td>
<td>Testing Frequency</td>
<td>Description</td>
<td>Outcomes</td>
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<tr>
<td>Shettigara et al., 1989</td>
<td>AGID</td>
<td>1982-1987</td>
<td>5 years</td>
<td>6</td>
<td>28-139 animals, test and segregate (&gt;200m between animals): segregation of BLV positive, negative, replacement cattle; testing every 3 months until no more new positives, then every 6 months; colostrum and milk BLV free or pasteurized</td>
<td>3 herds negative after one AGID (≤8% within-herd prevalence), 2 herds negative after 3 tests (12% within-herd prevalence), 1 herd after 4 tests (24.5% within-herd prevalence)</td>
</tr>
<tr>
<td>Sprecher et al., 1991</td>
<td>AGID</td>
<td>1987-1989</td>
<td>2 years</td>
<td>1</td>
<td>Needles, disinfection, dehorning, milk replacer, colostrum pasteurization; comparison of heifer age cohorts</td>
<td>Decrease in prevalence in all cohorts: 0.76-0.73 to 0.44-0.17</td>
</tr>
<tr>
<td>Suh et al., 2005</td>
<td>ELISA</td>
<td>February 2000-April 2004</td>
<td>4 years</td>
<td>1</td>
<td>491 animals, 163 negative; testing every 3-5 months; test and segregate, only introduction of negative heifers/purchases; sampling of calves before colostrum, colostrum and milk from negative dams, needles, syringes, sleeves, AI, disinfection</td>
<td>Eradication</td>
</tr>
<tr>
<td>Source</td>
<td>Test</td>
<td>Duration</td>
<td>Interval</td>
<td>Test Count</td>
<td>Prevention and Results</td>
<td></td>
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<tr>
<td>Wang and Onuma, 1992</td>
<td>AGID, ELISA</td>
<td>May 1987-October 1990</td>
<td>3 years</td>
<td>2</td>
<td>9 serial tests, farm A: 400 animals, first 4 tests: 3 to 6 month intervals, positive animals segregated or culled; farm B: bull facility, 40 bulls, 2-3 AGID/year</td>
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<td>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 and BLV positive bulls, segregation of positive bulls in 1986, no positive results in 4 tests</td>
<td></td>
</tr>
<tr>
<td>Yoshikawa et al., 1992</td>
<td>AGID</td>
<td>September 1977-November 1991</td>
<td>14 years</td>
<td>1</td>
<td>Approx. 400 cows, 3.52% initial within-herd prevalence; 25 tests within 14 years, test and segregation of positive animals</td>
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<td>eradication after 5 tests (August 1979)</td>
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</tr>
</tbody>
</table>

1 N.R. = not reported
needles = single use of needles

sleeves = single use of examination sleeves

disinfection = disinfection of contaminated equipment and surgical instruments

AI = artificial insemination

ET = embryo transfer

syringes = single use of syringes
Figure 2-1. Selection of sources of evidence.

- Sources identified through searching multiple databases (n=8285)
- Sources after duplicates removed (n=4768)
- Sources screened by title (n=4768)
- Sources excluded (n=3310)
- Sources screened by title and abstract (n=628)
- Sources excluded (n=470)
- Sources assessed for eligibility by abstract and text when available (n=158)
- Sources excluded (unable to retrieve article, wrong language, not original research, not applicable research) (n=86)
- Sources reviewed (n=72)
CHAPTER 3: Short Communication: Evaluation of 5 different ELISA for the
detection of bovine leukemia virus antibodies

3.1. Abstract

Although Canadian dairy herds have been infected with bovine leukemia virus (BLV) for years, recent research has put new emphasis on the potential negative effects of this infection. Consequently, BLV control is becoming more favorable; however, BLV control cannot be successful without identifying infected animals. Bovicheck BLV (Biovet, Saint-Hyacinthe, QC, Canada) is currently the only assay licensed by the Canadian Centre for Veterinary Biologics. The first goal of this study was, therefore, to determine the reproducibility of the Bovicheck BLV assay for serum samples derived from Canadian cattle. The second goal was to evaluate and compare 5 different ELISA and determine their test characteristics using serum samples from Canadian herds. The considered ELISA were Bovicheck BLV, ID Screen BLV Competition (IDvet, Grabels, France), Idexx Leukosis Serum X2 Ab Test (Idexx Europe B.V., Hoofddorp, the Netherlands), Svanovir BLV gp51-Ab (Svanova, Uppsala, Sweden), and the Serelisa BLV Ab Mono Indirect (Synbiotics, Lyon, France). Eighty serum samples from Canadian cattle provided by Prairie Diagnostic Services (PDS; Saskatoon, SK, Canada) and an additional 80 serum samples from Canadian dairy and beef herds were used for the study. The Bovicheck BLV assay yielded the same results for all PDS-derived samples, implying a high level of reproducibility and robustness of this assay. Additionally, the comparison of the assays’ results showed high agreement between assays, with Cohen’s kappa values between $\kappa = 0.91$ and $\kappa = 1$. Furthermore, using original test results of the
field samples as true status, relative diagnostic sensitivity and specificity were calculated.
Relative diagnostic sensitivity of all tests was 100%. False-positive results were probable; therefore, the following relative diagnostic specificities were determined: 100% for Bovicheck BLV, Idexx Leukosis Serum X2, and Svanovir BLV; 95% for ID Screen BLV; and 97% for Serelisa BLV. When considering other test characteristics, ID Screen BLV is exceptional due to considerable practical advantages.

3.2 Short Communication

Bovine leukemia virus (BLV) infection is highly prevalent in North American dairy cattle (VanLeeuwen et al., 2005, Scott et al., 2006, Bartlett et al., 2014). The infection not only causes tumors in some infected cattle (Schwartz and Levy, 1994), but recent research also suggests that it might result in decreased milk production, decreased cow longevity, and an impaired immune system (Erskine et al., 2012a, Bartlett et al., 2013, Frie and Coussens, 2015). Due to these effects on animal health and production, efforts have been made to fully understand efficient BLV control (Bartlett et al., 2014, EFSA Panel on Animal Health and Welfare, 2015, Nekouei et al., 2015). However, as with all retroviruses, BLV integrates its DNA into the host genome, thereby creating a provirus (Goff, 2013), making it impossible for the host to clear the virus despite the production of neutralizing antibodies. Therefore, successful control of BLV is dependent on the accurate identification and removal or segregation of infected animals. Whereas using PCR allows for the detection of the provirus as early as 7 d after infection, detecting antibodies is only possible at least 3 wk postinfection (Klintevall et al., 1994, Nagy et al., 2007). However, the detection of antibodies against BLV has proven to be a reliable,
cost-effective, and quick way to identify infected animals (Burny et al., 1980, Monti et al., 2005, Nekouei et al., 2016). It should be noted, though, that the performance of diagnostic tests also depends on the characteristics of the population in which they are used (Dohoo et al., 2014). It is therefore valuable to evaluate these tests before use in the context of a control program. Bovicheck BLV (Biovet, Saint-Hyacinthe, QC, Canada) is currently the only assay licensed by the Canadian Centre for Veterinary Biologics (http://inspection.gc.ca/active/netapp/veterinarybio-bioveterinaire/vetbioe.aspx#table-heading). Because BLV control and testing go hand in hand, the robustness of an assay is a very important test characteristic. Therefore, the first goal of our study was to determine the reproducibility of the Bovicheck BLV assay for serum samples derived from Canadian dairy cows. Various diagnostic tests are commercially available; thus, the second goal of our study was to evaluate and compare the performance of 5 different commercially available ELISA and their test characteristics using serum samples from Canadian herds. Five different commercially available assays were evaluated: Bovicheck BLV, ID Screen BLV Competition (IDvet, Grabels, France), Idexx Leukosis Serum X2 Ab Test (Idexx Europe B.V., Hoofddorp, the Netherlands), Svanovir BLV gp51-Ab (Svanova, Uppsala, Sweden), and the Serelisa BLV Ab Mono Indirect (Synbiotics, Lyon, France). Serum samples from 220 animals of western Canadian dairy herds were provided by Prairie Diagnostic Services (PDS; Saskatoon, SK, Canada). These samples (referred to as PDS samples) were submitted between November 2015 and March 2016 to PDS for BLV diagnostics and included 108 samples with a previous positive, as well as 112 samples with a previous negative test result. Whereas the specific bovine breed for each sample was unknown, it should be noted that Canadian dairy herds consist to 93% of Holstein
cattle (Canadian Dairy Information Centre, 2016). The PDS samples allowed an
evaluation of the reproducibility for Bovicheck BLV in 2 different laboratories. In
addition, we collected serum samples from BLV antibody positive cows (n = 54), as well
as serum samples from BLV antibody-negative cows (n = 62); collectively they will be
referred to as field samples. The 54 positive field samples were obtained from 3 Alberta
dairy herds. Inclusion criteria for the BLV antibody-positive samples were previous
testing of the milking herd for BLV and the willingness of the farmer to make these
results available. These samples were initially analyzed with a milk ELISA (supplied by
AntelBio Inc., Lansing, MI) by CanWest DHI (Guelph, ON, Canada) or a serum ELISA
(Svanova, no more details available to authors) by Idexx Laboratories (Markham, ON,
Canada). The results of these tests were used to classify animals as positive. Whereas
different antibody tests have successfully been used to eradicate BLV from infected herds
and for export purposes (Shettigara et al., 1986, Suh et al., 2005, World Assembly of the
OIE, 2016), currently no reference test exists for BLV diagnostics. However, the
probability of a true positive test result rises with age (Erskine et al., 2012b). Therefore,
40 samples were selected from the pool of 54 positive field samples from animals over 4
yr of age and were included as true positive. The 62 negative field samples were collected
from a certified BLV-free Alberta beef herd (n = 31) and the dairy herd of the University
of Guelph (n = 31). This herd is considered to be free of BLV, as all adult animals tested
negative in at least 2 consecutive tests (Bovicheck BLV) performed by the Animal Health
Laboratory (Guelph, ON, Canada). Forty randomly selected samples, 20 from each BLV-
free herd, were included as true negative samples. All field samples were collected
between April and July 2016. Each serum sample was divided into 2 to 4 aliquots and
stored at −40°C until used. The study was approved by the Veterinary Sciences Animal Care Committee of the University of Calgary (VSACC AC15–0159). Tests were performed and analyzed per the manufacturers’ instructions (available upon request from first author), including both positive and negative controls as supplied with each kit. The European Union requires the detection of the E05 standard BLV serum in a 1/10 dilution before assays can be licensed for diagnostic purposes (World Assembly of the OIE, 2016). This standard serum was included on each plate in a serial dilution in sterile PBS Buffer (10^0–10^-4) as an additional control (kindly provided by the Enzootic Bovine Leukosis OIE Reference Laboratory, Leipzig, Germany). To control for day-to-day variation, 1 ELISA plate from each kit was evaluated per day. Included on each plate were duplicates of a positive and negative control as provided by the ELISA company, a blank (empty wells), the EU 05 standard serum dilution series, and 40 samples. The same sample aliquot was used for all 5 plates. In these series of tests, a total of 160 different samples were tested (80 PDS samples and 80 field samples): 40 negative and 40 positive samples were randomly selected from the PDS sample collection. Of these selected PDS samples, 20 positive and 20 negative samples were added in random order to each of 2 test plates. The 40 positive field samples were chosen depending on the sampled cow’s age, and the 40 negative field samples were chosen randomly from the 2 negative herds, as described above. The person executing the tests was not blinded to the original results. All assays met the validity criteria, required by the manufacturers’ instructions. The statistical analysis was performed in R 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). To evaluate agreement beyond chance between original tests and tests of the current study, as well as between tests in the current study, Cohen’s kappa values
were calculated. These calculations were done by using the results of all samples. Additionally, relative diagnostic sensitivity and specificity were calculated based on results of the field samples only, as they were considered true positive and true negative. Samples, whose outcome was classified as suspect according to the assay’s instructions, were considered positive for the calculation of results. The fact that the test executing person was not blinded to the original test results might introduce potential bias into the study. However, randomization of the samples and the automated analysis of the test plates should minimize this bias. All PDS-derived samples yielded the same results when retested with Bovicheck BLV in our study, executed in Calgary, indicating a high level of reproducibility and robustness of this assay. Additionally, the original test results agreed almost perfectly ($\kappa = 0.94$) to perfectly ($\kappa = 1$) with the results of all tests evaluated in our study (Table 3-1). Based on previous evaluations, a high agreement between assays was expected (Simard et al., 2000, Monti et al., 2005); this was confirmed in our study. Represented by high Cohen's kappa values, the agreement between tests was classified as almost perfect ($\kappa = 0.91$) to perfect ($\kappa = 1$), suggesting very similar performances (Table 3-2). Nevertheless, whereas Bovicheck BLV and Idexx Leukosis Serum X2 agreed with all original test results, ID Screen BLV, Svanovir BLV, and Serelisa BLV disagreed with some of the original test results (Table 3-4). All samples previously classified as positive (PDS and field samples), yielded a positive result in all assays in our study. Results only varied for 8 samples previously classified as negative (Table 3-4). One sample (sample 5; Table 3-4) was classified as positive or suspect by 2 tests, whereas 7 samples (samples 1–4, 6–8; Table 3-4) were classified as positive or suspect by only 1 assay. These discrepancies between results can be due to incorrect identification as positive or negative
in the original tests or the tests used in our study, seroconversion since the last recorded testing, or a more sensitive cut off that resulted in lower specificity. However, 3 of the samples (samples 6–8, Table 3-4) for which test results did not agree, originated from the Canadian Food Inspection Agency-accredited BLV-negative herd in Alberta (samples evaluated with Bovicheck BLV and Svanovir BLV; Bianca Morel, supervisor, Laboratory Veterinary Services, Canadian Food Inspection Agency, St. Hyacinthe Laboratory-Diagnostic and Methods Validation, Animal Health, Saint-Hyacinthe, Quebec, Canada, personal communication). Although we were unable to obtain additional samples from these animals, this herd is a closed herd and retested yearly by the Canadian Food Inspection Agency, which suggests that these were false-positive results. The additional 5 samples with discordant results (samples 1–5; Table 3-4) were part of the PDS sample group. As no further information on the tested animals was available, no assumption about their true status could be made. However, verifying an individual animal’s status could be done by retesting, because antibody titers rise over the first weeks following infection (Cockerell and Rovnak, 1988, Nagy et al., 2007, EFSA Panel on Animal Health and Welfare, 2015). This means that initially low titers might result in a false-negative or suspect result due to insufficient analytic sensitivity, but should be detectable when animals are retested at a later time point. Alternatively, suspect results could be confirmed by PCR, which can detect the presence of proviral DNA in B lymphocytes (Heenemann et al., 2012).

Considering the field samples as truly positive and truly negative, the values for relative diagnostic sensitivity and specificity for the tests evaluated in our study were as follows. All samples originally classified as positive were identified as positive by all tests in our
study. Therefore, relative diagnostic sensitivity of all tests was 100% (95% CI = 87–100%). Comparing the original test results and test results from our study for field samples, false-positive results can be observed. Consequently, relative diagnostic specificities were determined for Bovicheck BLV, Idexx Leukosis Serum X2, and Svanovir BLV to be 100% (95% CI = 87–100%), for ID Screen BLV to be 95% (95% CI = 83–99%), and for Serelisa BLV to be 97% (95% CI = 87–100%). We were not provided with any additional information on the animals that were tested by PDS. Therefore, we could not make any inferences about the true infection status of these animals and did not include these in the calculations; however, discrepancies between the results for PDS derived samples indicated that the estimated values for specificity were likely overestimates.

A 100% relative diagnostic sensitivity is unlikely to be true; for example, acutely infected animals before seroconversion would not present as positive in our tests. These uncertainties could be reduced, for example, by increasing the sample size or through parallel testing of the samples using PCR. Even though the PDS samples were selected randomly and represent most stages of infection, a valuable addition would be a controlled infection trial.

In addition to test agreement, relative diagnostic sensitivity and specificity, other assay characteristics were investigated (Table 3-4). First, instructions are vital for diagnostic test assays, as different people with different background knowledge and expertise should be able to perform these tests (Crowther, 2009). Whereas Bovicheck BLV, Idexx Leukosis Serum X2, and Svanovir BLV provide instructions in at least 2 languages, ID Screen BLV and Serelisa BLV only provide instructions in English. Second, handling of
tests was examined; Bovicheck BLV suggested to perform sample preparation on a different plate before administering it to the wells of the coated assay plate, resulting in an additional laboratory step. Similarly, Bovicheck BLV and ID Screen BLV required the dilution of the washing buffer and the preparation of the test conjugate. Incubation times were the shortest for ID Screen BLV, at 90 min, and the longest for Serelisa BLV, at 150 min per plate. Overall, ID Screen BLV had the shortest turnaround time. This assay also allowed incubation of samples and test conjugate at room temperature. All other assays required incubation at 37°C, necessitating additional equipment. Finally, except for Svanovir BLV, all assays allow for the classification of results as suspect, which identified them as needing confirmation or retesting. Overall, all assays performed well; however, 2 tests should be highlighted. First, Bovicheck BLV is readily available and the only BLV antibody assay licensed in Canada. Second, ID Screen BLV has considerable practical advantages due to its short turnaround time, its ability to test entirely at room temperature, and ease of handling. Based on the results of the current study, all evaluated tests could be considered for the accreditation by the Canadian Centre for Veterinary Biologics.
3.3 References


paratuberculosis, Neospora caninum, bovine leukemia virus, and bovine viral
diarrhea virus infection among dairy cattle and herds in Alberta and

leukemia virus infection in commercial dairy herds using the agar gel

immunosorbent assay for the diagnosis of bovine leukosis: Comparison with the
agar gel immunodiffusion test approved by the Canadian Food Inspection


antibodies against bovine leukemia virus, bovine viral diarrhea virus,
Mycobacterium avium subspecies paratuberculosis, and Neospora caninum in

World Assembly of the OIE. 2016. Manual of diagnostic tests and vaccines for terrestrial
animals 2016. Section 2.4., Chapter 2.4.10., Enzootic Bovine Leukosis. European
Food Safety Authority, Parma, Italy.
Table 3-1. Cohen’s kappa values (95% confidence intervals) for original test results and test results found in this study.

<table>
<thead>
<tr>
<th>Test Kit</th>
<th>All Samples</th>
<th>PDS Samples⁶</th>
<th>Field Samples⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovicheck BLV¹</td>
<td>1.00 (95% CI: 1.00-1.00)†</td>
<td>1.00 (95% CI: 1.00-1.00)†</td>
<td>1.00 (95% CI: 1.00-1.00)†</td>
</tr>
<tr>
<td>ID Screen BLV²</td>
<td>0.98 (95% CI: 0.94-1.00)†</td>
<td>1.00 (95% CI: 1.00-1.00)†</td>
<td>0.95 (95% CI: 0.88-1.00)†</td>
</tr>
<tr>
<td>IDEXX Leukosis Serum X2³</td>
<td>1.00 (95% CI: 1.00-1.00)†</td>
<td>1.00 (95% CI: 1.00-1.00)†</td>
<td>1.00 (95% CI: 1.00-1.00)†</td>
</tr>
<tr>
<td>SVANOVIR BLV⁴</td>
<td>0.98 (95% CI: 0.94-1.00)†</td>
<td>0.95 (95% CI: 0.88-1.00)†</td>
<td>1.00 (95% CI: 1.00-1.00)†</td>
</tr>
<tr>
<td>Serelisa BLV⁵</td>
<td>0.94 (95% CI: 0.88-0.99)†</td>
<td>0.9 (95% CI: 0.80-1.00)†</td>
<td>0.98 (95% CI: 0.93-1.00)†</td>
</tr>
</tbody>
</table>

¹ Bovicheck BLV (Biovet, Saint-Hyacinthe, QC, Canada)
² ID Screen BLV Competition (IDvet, Grabels, France),
³ IDEXX Leukosis Serum X2 Ab Test (IDEXX Europe B.V., Hoofddorp, the Netherlands)
⁴ SVANOVIR BLV gp51-Ab (SVANOVIR BLV; Svanova, Uppsala, Sweden)
⁵ Serelisa BLV Ab Mono Indirect (Serelisa BLV; Synbiotics, Lyon, France)
⁶ Samples made available by Prairie Diagnostic Services (PDS, Saskatoon, SK, Canada) (n=80)
⁷ Samples from Alberta dairy herds, Alberta Bovine Leukemia Virus (BLV)-free beef herd, and Ontario BLV-free dairy herd. (n=80)
† P < 0.01
Table 3-2. Agreement between the five ELISAs as determined by Cohen’s kappa (95% confidence intervals).

<table>
<thead>
<tr>
<th></th>
<th>Bovicheck BLV(^1)</th>
<th>ID Screen BLV(^2)</th>
<th>IDEXX Leukosis Serum X2(^3)</th>
<th>SVANOVIR BLV(^4)</th>
<th>Serelisa BLV(^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovicheck BLV(^1)</td>
<td>0.98 (95% CI: 0.94; 1.00)(^\dagger)</td>
<td>1.00 (95% CI: 1.00; 1.00)(^\dagger)</td>
<td>0.98 (95% CI: 0.94; 1.00)(^\dagger)</td>
<td>0.94 (95% CI: 0.88; 0.99)(^\dagger)</td>
<td></td>
</tr>
<tr>
<td>ID Screen BLV(^2)</td>
<td>0.98 (95% CI: 0.94; 1.00)(^\dagger)</td>
<td>0.98 (95% CI: 0.94; 1.00)(^\dagger)</td>
<td>0.95 (95% CI: 0.90; 1.00)(^\dagger)</td>
<td>0.91 (95% CI: 0.85; 0.98)(^\dagger)</td>
<td></td>
</tr>
<tr>
<td>IDEXX Leukosis Serum X2(^3)</td>
<td>1.00 (95% CI: 1.00; 1.00)(^\dagger)</td>
<td>0.98 (95% CI: 0.94; 1.00)(^\dagger)</td>
<td>0.98 (95% CI: 0.94; 1.00)(^\dagger)</td>
<td>0.94 (95% CI: 0.88; 0.99)(^\dagger)</td>
<td></td>
</tr>
<tr>
<td>SVANOVIR BLV(^4)</td>
<td>0.98 (95% CI: 0.94; 1.00)(^\dagger)</td>
<td>0.95 (95% CI: 0.90; 1.00)(^\dagger)</td>
<td>0.98 (95% CI: 0.94; 1.00)(^\dagger)</td>
<td>0.94 (95% CI: 0.88; 0.99)(^\dagger)</td>
<td></td>
</tr>
<tr>
<td>Serelisa BLV(^5)</td>
<td>0.94 (95% CI: 0.88; 0.99)(^\dagger)</td>
<td>0.91 (95% CI: 0.85; 0.98)(^\dagger)</td>
<td>0.94 (95% CI: 0.88; 0.99)(^\dagger)</td>
<td>0.94 (95% CI: 0.88; 0.99)(^\dagger)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Bovicheck BLV (Biovet, Saint-Hyacinthe QC, Canada)

\(^2\) ID Screen BLV Competition (IDvet, Grabels, France),

\(^3\) IDEXX Leukosis Serum X2 Ab Test (IDEXX Europe B.V., Hoofddorp, the Netherlands)

\(^4\) SVANOVIR BLV gp51-Ab (SVANOVIR BLV; Svanova, Uppsala, Sweden)
Serelisa BLV Ab Mono Indirect (Serelisa BLV; Synbiotics, Lyon, France)

† P < 0.01
Table 3-3. Overview of samples with discordant results between original test results and the assays used in the present study.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sample Source</th>
<th>Original Test Result</th>
<th>Assay</th>
<th>Result of present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PDS¹</td>
<td>Negative</td>
<td>SVANOVIR BLV³</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>PDS¹</td>
<td>Negative</td>
<td>Serelisa BLV⁴</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>PDS¹</td>
<td>Negative</td>
<td>Serelisa BLV⁴</td>
<td>Suspect</td>
</tr>
<tr>
<td>4</td>
<td>PDS¹</td>
<td>Negative</td>
<td>Serelisa BLV⁴</td>
<td>Suspect</td>
</tr>
<tr>
<td>5</td>
<td>PDS¹</td>
<td>Negative</td>
<td>Serelisa BLV⁴</td>
<td>Suspect</td>
</tr>
<tr>
<td>5</td>
<td>PDS¹</td>
<td>Negative</td>
<td>SVANOVIR BLV³</td>
<td>Positive</td>
</tr>
<tr>
<td>6</td>
<td>Beef Herd²</td>
<td>Negative</td>
<td>ID Screen BLV⁵</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>Beef Herd²</td>
<td>Negative</td>
<td>ID Screen BLV⁵</td>
<td>Suspect</td>
</tr>
<tr>
<td>8</td>
<td>Beef Herd²</td>
<td>Negative</td>
<td>Serelisa BLV⁴</td>
<td>Positive</td>
</tr>
</tbody>
</table>

¹ Samples initially tested by and made available by Prairie Diagnostic Services (PDS, Saskatoon, SK, Canada)

² Samples from Alberta Bovine Leukemia Virus (BLV) free beef herd, initially tested by CFIA (Canadian Food Inspection Agency).

³ SVANOVIR BLV gp51-Ab (SVANOVIR BLV; Svanova, Uppsala, Sweden)

⁴ Serelisa BLV Ab Mono Indirect (Serelisa BLV; Synbiotics, Lyon, France)

⁵ ID Screen BLV Competition (IDvet, Grabels, France)
<table>
<thead>
<tr>
<th>Assay</th>
<th><strong>Bovicheck BLV</strong>&lt;sup&gt;1&lt;/sup&gt;</th>
<th><strong>ID Screen BLV Competition</strong>&lt;sup&gt;2&lt;/sup&gt;</th>
<th><strong>IDEXX Leukosis Serum X2 Ab Test</strong>&lt;sup&gt;3&lt;/sup&gt;</th>
<th><strong>SVANO VIR BLV gp51-Ab (screening format)</strong>&lt;sup&gt;4&lt;/sup&gt;</th>
<th><strong>Serelisa BLV Ab Mono Indirect</strong>&lt;sup&gt;5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ELISA Principle</strong></td>
<td><strong>Blocking ELISA</strong></td>
<td><strong>Competitive ELISA</strong></td>
<td><strong>Indirect ELISA</strong></td>
<td><strong>Indirect ELISA</strong></td>
<td><strong>Indirect ELISA</strong></td>
</tr>
<tr>
<td><strong>Antigen</strong></td>
<td>gp51</td>
<td>gp51</td>
<td>gp51+others</td>
<td>gp51</td>
<td>gp51</td>
</tr>
<tr>
<td><strong>Sample Material</strong></td>
<td>Milk, Serum; individual or pooled samples</td>
<td>Serum/plasma; 1-10 individuals</td>
<td>Serum/plasma; 1-10 individuals</td>
<td>Serum/plasma: 1-10 individuals; Milk: 1-50 individuals, bulk tank milk</td>
<td>Serum; 1-10 individuals</td>
</tr>
<tr>
<td><strong>Plates per Assay (No.)</strong></td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td><strong>Multilingual Instructions</strong></td>
<td>French, English</td>
<td>English</td>
<td>English, French, Spanish, Portuguese, German</td>
<td>English, French, Spanish, German</td>
<td>English</td>
</tr>
<tr>
<td><strong>Required sample amount (µl)</strong></td>
<td>100</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total incubation time (min)</strong></td>
<td>100</td>
<td>90</td>
<td>135</td>
<td>130</td>
<td>150</td>
</tr>
<tr>
<td><strong>Calculation of result</strong></td>
<td>Inhibition Percentage</td>
<td>Competition Percentage</td>
<td>Sample to Positive Ratio</td>
<td>Percent Positivity Values</td>
<td>Index Calculation or Analysis of OD&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Suspect result possible</strong></td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>

<sup>1</sup> Bovicheck BLV: Bovine leukemia virus antibody assay developed by Bovicheck.  
<sup>2</sup> ID Screen BLV Competition: In-house developed competition ELISA for BLV.  
<sup>3</sup> IDEXX Leukosis Serum X2 Ab Test: A commercial indirect ELISA for BLV.  
<sup>4</sup> SVANO VIR BLV gp51-Ab (screening format): A commercial screening format indirect ELISA for BLV.  
<sup>5</sup> Serelisa BLV Ab Mono Indirect: A commercial indirect monoclonal antibody-based ELISA for BLV.  
<sup>6</sup> gp51: gamma globulin protein 51.  
<sup>7</sup> OD: Optical Density.
<table>
<thead>
<tr>
<th>Specificity by Manufacturer (95% CI)</th>
<th>100% (95.5 -100)</th>
<th>100% (99.7 -100)</th>
<th>99.9%</th>
<th>99.1%</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity by Manufacturer (95% CI)</td>
<td>100% (94.9 -100)</td>
<td>100% (96.3 -100)</td>
<td>N/A</td>
<td>99%</td>
<td>N/A</td>
</tr>
<tr>
<td>Specificity by present evaluation (95% CI)</td>
<td>100% (87 - 100)</td>
<td>95% (83 - 99)</td>
<td>100% (87 - 100)</td>
<td>100% (87 - 100)</td>
<td>97% (87 - 100)</td>
</tr>
<tr>
<td>Sensitivity by present evaluation (95% CI)</td>
<td>100% (87 - 100)</td>
<td>100% (87 - 100)</td>
<td>100% (87 - 100)</td>
<td>100% (87 - 100)</td>
<td>100% (87 - 100)</td>
</tr>
</tbody>
</table>

1 Bovicheck BLV (Biovet, Saint-Hyacinthe QC, Canada)
2 ID Screen BLV Competition (IDvet, Grabels, France),
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4 SVANOVIR BLV gp51-Ab (SVANOVIR BLV; Svanova, Uppsala, Sweden)
5 Serelisa BLV Ab Mono Indirect (Serelisa BLV; Synbiotics, Lyon, France)
6 Relative diagnostic sensitivity and specificity with matching 95% confidence intervals. Calculations are based on results for all field samples (n=80), considering original test results assigned by the laboratory responsible for the initial testing as “true”.
7 Optical Density
CHAPTER 4 Economic evaluation of 4 bovine leukemia virus control strategies for Alberta dairy farms

4.1 Abstract

Bovine leukemia virus (BLV) is a production-limiting disease common in North American dairy herds. To make evidence-based recommendations to Canadian dairy producers and their consultants regarding cost and financial benefits of BLV on-farm control, an economic model that takes the supply-managed milk quota system into account is necessary. Alberta-specific input variables were used for the presented analysis. A decision tree model program was used to evaluate economic aspects of decreasing a 40% BLV within-herd prevalence on dairy farms by implementing various control strategies over 10 yr. Investigated strategies were (1) all management strategies, including 3 options for colostrum management; (2) some management strategies; (3) test and cull; and (4) test and segregate. Each of these strategies was compared with a no control on-farm approach. The prevalence for this no-control approach was assumed to stay constant over time. Each control strategy incurred specific yearly cost and yielded yearly decreases in prevalence, thereby affecting yearly partial net revenue. Infection with BLV was assumed to decrease milk production, decrease cow longevity, and increase condemnation of carcasses at slaughter from cattle with enzootic bovine leukosis, thereby decreasing net revenue. Cows infected with BLV generated a yearly mean partial net revenue of Can$7,641, whereas noninfected cows generated Can$8,276. Mean cost for the control strategies ranged from Can$193 to 847 per animal over 10 yr in a 146-animal herd. Net benefits of controlling BLV on farm, as compared with not
controlling BLV, per cow in a 146-animal herd over a 10-yr period for each strategy was: Can$1,315 for all management strategies (freezer); Can$1,243 for all management strategies (pasteurizer); Can$785 for all management strategies (powdered colostrum); Can$1,028 for some management strategies; Can$1,592 for test and cull; and Can$1,594 for test and segregate. Consequently, on-farm BLV control was financially beneficial. Even though negative net benefits were possible and expected for some iterations, our sensitivity analysis highlighted the overall robustness of our model. In summary, this model provided evidence that Canadian dairy farmers should be encouraged to control BLV on their farm. Key words: bovine leukemia virus, leukosis, economic evaluation, dairy economics

4.2 Introduction

Bovine leukemia virus (BLV) is a retrovirus that infects lymphocytes in cattle (Radostits et al., 2007) and inserts its genomic material into the host’s genome, thereby causing lifelong infections. This virus is the causative agent of enzootic bovine leukosis (Radostits et al., 2007), characterized by development of tumors in a small subset of BLV-infected cattle. In the absence of a vaccine or treatment, prevention of infection is currently the only way to control spread of the virus (Rodriguez et al., 2011). Bovine leukemia virus is common in North American dairy cattle (Bartlett et al., 2014; Nekouei et al., 2015), with the herd prevalence in Alberta dairy herds reported to be ~90% (Scott et al., 2006). With only limited efforts to control BLV, the current herd prevalence is assumed to be at least this high. By causing tumors and negatively affecting infected cattle’s immune system, BLV has adverse effects on animal welfare (Bartlett et al., 2014;
Frie and Coussens, 2015) and economics of dairy farms due to premature death of dairy cows (Rhodes et al., 2003) and condemnation of carcasses after slaughter (Agriculture and Agri-Food Canada, 2017c). In addition, BLV infection can decrease milk production (Sargeant et al., 1997, Ott et al., 2003, Erskine et al., 2012a) and cow longevity (Brenner et al., 1989, Bartlett et al., 2013). These deleterious effects of BLV infection on the main economic determinants of a dairy farm highlight the importance of this virus infection, not only for dairy farms but also for the dairy industry as a whole (Pelzer, 1997, Trainin and Brenner, 2005). A high BLV prevalence, combined with its negative effects, make implementation of on-farm control programs desirable (Bartlett et al., 2014). Bovine leukemia virus control programs generally use interventions to reduce virus transmission, thereby preventing new infections. Ultimately, test-positive cattle can be replaced with test-negative ones, thereby decreasing the within-herd prevalence. This can be achieved by implementing best management practices that prevent new infections, through segregating or culling infected cattle or a combination of these methods (Rodriguez et al., 2011, Bartlett et al., 2014). Regardless, on-farm disease control investments will be weighed against (economic) advantages of eradication in a farmer’s decision process (Ritter et al., 2017). Economic evaluations highlighting economic consequences of a BLV infection have been published, but vary in the economic losses taken into account (Chi et al., 2002, Ott et al., 2003, Rhodes et al., 2003). Unlike the free-market system for dairy production in the United States and many other countries, the Canadian dairy industry is a supply chain-managed system with production quotas. Dairy farmers in a free market system often keep the number of cows on their farms constant due to space constraints, resulting in a relatively constant milk production. However, under these...
conditions milk production could potentially be increased by breeding efforts, disease control, and feed adjustments, for example; this would result in a direct increase in revenue as well as in a decrease in cost. This is not applicable to supply chain-managed systems, as the amount of milk that is produced has to stay constant, whereas the number of cows to reach a production goal can be decreased, thereby decreasing cost. The net revenue is thus only indirectly affected. For this reason, an economic model relevant for the Canadian dairy industry has to take the supply chain-managed system into account. Additionally, only 1 evaluation considered cost for a test and management-based control program (Rhodes et al., 2003). A thorough comparison of different control program variations and their economics is not yet available. The objective of our study was to estimate economic impacts of BLV infection and its control. We modeled partial cost and net revenue for 4 variations of a BLV control program, using a decision tree model program to simulate effects of various on-farm BLV control strategies on the within-herd BLV prevalence and, thereby, on the farm’s partial net revenue. Furthermore, all control programs were compared with no on-farm control to estimate the net benefit of the BLV control strategies.

4.3 Materials and Methods

Our economic evaluation examined partial cost and net revenues related to an on-farm BLV control program. The evaluation was based on Canadian guidelines for economic evaluation of health technologies (Canadian Agency for Drugs and Technologies in Health, 2017). A model was created with TreeAge Pro 2017 (TreeAge Software, Inc., Williamstown, MA). Calculation outputs were further analyzed in Excel (365 ProPlus,
Microsoft Corp., Redmond, WA). The perspective adopted was that of an average Alberta dairy farmer. Input data were obtained from publications and reports produced by Alberta Milk (Edmonton, AB, Canada), CanWest DHI (Guelph, ON, Canada), and the Government of Canada. Whenever possible, Alberta-specific input data were used. During the analyzed period, the within-herd prevalence of BLV was modeled to change yearly due to the chosen control strategy and to stay constant for no implemented control. Based on this prevalence, partial cost and net revenues were calculated at the end of each year.

4.3.1. Modeling

The model was constructed to allow for comparison of each strategy’s outcome after strategies were implemented. A detailed list of model variables and inputs is provided in Table 4-1. The initial decision node branched off to 4 control programs, as well as to a no-control program option. Within each strategy, a Markov model with 10 stages (each stage representing 1 yr) was applied to mimic a 10-yr course of on-farm BLV control. The model was analyzed with Monte Carlo simulation (microsimulations), running 5,000 trials. Results were summarized as mean per individual animal in a 146-adult cow herd (average size of Alberta dairy herds). The range of variation for these results can be found in Table 4-6. Average partial cost and net revenues for each strategy were examined. The initial within-herd prevalence was the same for all strategies. Every cycle, the within-herd prevalence was influenced by 2 events: (1) depending on the control strategy, the within-herd prevalence was reduced accordingly; and (2) an infected animal could be purchased and introduced into the herd as a replacement animal. The probability
for the latter event to happen was calculated as the probability of introducing an infected animal = fraction of open herds × number of cows introduced per year × herd prevalence in Alberta × within-herd prevalence.

The probability not to introduce an infected animal was 1 – (probability of introducing an infected animal). If an infected animal was introduced, the change in prevalence equaled (prevalence decrease according to strategy) + (1/herd size). However, if no infected animal was introduced, the prevalence was reduced by the strategy’s assigned yearly reduction in prevalence (Table 4-2). This change in prevalence was tracked over time and kept within limits of 0 to 100% to enable a continuous reduction in prevalence. The resulting within-herd prevalence at the end of each cycle was the basis for calculating each strategy’s partial cost and net revenue for that cycle. In addition, the model included the assumption that 1 management strategy was adhered to throughout the 10-yr period, thereby enabling comparison of each strategy’s outcome.

**4.3.2. Considered strategies for BLV control and the respective partial cost**

Various options are available to control BLV transmission. We considered the most commonly recommended strategies (Rodriguez et al., 2011, Bartlett et al., 2014), namely (1) testing and implementing best management practices, split up according to 3 different options for colostrum management (freezing or pasteurizing colostrum, or using powdered colostrum replacer); (2) testing and implementing a subset of best management strategies; (3) testing and culling; and (4) testing and segregating. For all strategies, sensitivity and specificity of all diagnostic tests were assumed 100% (Monti et al., 2005, Walsh, 2013, Nekouei et al., 2016). As a testing protocol for all strategies, we tested all
animals in the adult herd in the first year of the model, whereas only negative animals and replacement animals were retested in the following years. Test-positive animals were considered positive for the rest of their lives, as they cannot clear infection (Radostits et al., 2007). Cost for each strategy, including expenses for testing, as well as investments made for implemented measures, are detailed below. The strategy, all management strategies considered all commonly recommended best management practices that aim at preventing transmission of BLV between animals. All management strategies included fly control, disinfection of equipment, the purchase and use of an electric dehorner, single-use of needles, and single use of examination sleeves for AI as well as transrectal pregnancy diagnosis (palpation or ultrasonography). Using a new examination sleeve before a rectal examination on each cow resulted in the veterinarian taking longer to conduct regular reproductive examinations. As veterinarians are commonly paid on an hourly basis for these visits, an additional 30 s (examination sleeve removal and replacement) per cow were added. Moreover, colostrum management was an important part of this strategy. Three common variations are used for prevention of BLV transmission: (1) freezing colostrum [all management strategies (freezer); Kanno et al., 2014] whereby the purchase of a freezer is required; (2) pasteurizing colostrum [all management strategies (pasteurizer)], requiring the purchase of a pasteurizer; or (3) feeding powdered colostrum replacer instead of fresh colostrum [all management strategies (powdered colostrum); Godden, 2008]. The cost for the purchased freezer or pasteurizer were assumed invested in the first year of the analyzed period. Because implementing control measures might cause prolongation of daily routines, a daily increase of labor for 30 min was assumed. The strategy some management strategies
represented a subset of all management strategies and was therefore assumed less effective, although less labor-intensive and expensive than all other options. The considered best management practices were the same as for all management strategies, excluding increased fly control and colostrum management. The next possibility was the test-and-cull strategy. In this strategy, emphasis was placed on slaughtering test-positive cattle as part of the regular turnover of the herd. To prevent virus transmission and new infections, best management practices [see all management strategies (freezer)] were assumed implemented. In addition, culling a BLV-positive cow over a BLV-negative cow could potentially result in culling the better producing animal. Therefore, an opportunity cost was added. The test-and-segregate strategy implied that test-positive animals were housed separately from test negative animals, combined with measures of the all management strategies (freezer) option, thereby limiting the probability of spreading virus within the herd. This strategy considered cost for structural changes in the barn to facilitate segregating positive from negative animals. This cost was also assumed invested in the first year and added to the all management strategies (freezer) expenses. All partial cost were discounted by a rate of 1.5% (Canadian Agency for Drugs and Technologies in Health, 2017) to account for the time value of money. The last possibility was no implementation of any of the above-mentioned best management practices aimed at preventing transmission of the virus (no control). This was not assigned any cost.
4.3.3. Yearly reduction in within-herd prevalence for each strategy

(Effectiveness)

We found no recent numbers regarding average within-herd prevalence of BLV in Alberta. Therefore, initial within-herd prevalence for all strategies was assumed to be 40%. We were able to confirm this estimate by analyzing a convenience sample made available by CanWest DHI (Guelph, ON, Canada). The median within-herd prevalence of BLV in this data set was approximately 39% (Table 4-3). The reduction in within-herd prevalence depended on the control strategy considered. Values were based on literature when possible and on expert opinion if no literature was available. The within-herd prevalence was assumed to decrease by 25% annually for all management strategies. We assumed that some management strategies would be less effective than all management strategies. The annual decrease in within-herd prevalence was therefore assumed to be 15%. Generally, disease control efforts are more effective in the early stages, whereas the effectiveness tends to decrease with the within-herd prevalence; therefore, we deemed a nonlinear decrease in prevalence appropriate. For the test-and-cull strategy, a yearly decrease in prevalence as part of the regular turnover of the herd was assumed (CanWest DHI, 2017, Government of Canada, 2017). On average, 39.6% of a dairy herd is replaced every year (CanWest DHI, 2017). This turnover-rate consists of voluntary and involuntary culling of animals (Ansari-Lari et al., 2012); however, only the voluntary culling rate could be influenced by the producer’s personal decisions. The voluntary culling rate accounted for 24.5% of the total turnover-rate (Government of Canada, 2017). Therefore, if a producer decided to make BLV the only reason for voluntary culling, a maximum of 9.7% of the herd could be replaced based on BLV infection each
year (VolCullrate). Assuming that no new infections occur and that herd size and culling rates stay constant, this allowed for 14.6 animals of the herd to be culled because of BLV, every year, thereby reducing the within-herd prevalence (FinPrev). Therefore, the number of infected animals was assumed to decrease consistently every year:

Decrease in prevalence for test and cull = [(FinPrev × herd size) − (VolCullrate × herd size)]/herd size.

The annual reduction in prevalence for the test-and-segregate strategy was calculated as follows: Housing BLV-positive and BLV-negative animals in separate pens seemingly prevents new infections efficiently (Johnson et al., 1985). However, the within-herd prevalence would not decrease immediately, as the infected animals would not leave the herd as soon as they were identified. The replacement of BLV-positive animals with BLV-negative animals would result in a decrease in within-herd prevalence over time. The yearly turnover rate of the herd was assumed to stay constant (39.6%, CanWest DHI, 2017); however, BLV-positive animals were found to be 23% more likely than their BLV-negative herd mates to leave the herd (Bartlett et al., 2013). Therefore, increasing the yearly risk of being culled to 48.7%. These assumptions resulted in the following annual decrease in within-herd prevalence for the test-and-segregate strategy:

Decrease in prevalence for test and segregate = [((within-herd prevalence × herd size) − (risk for culling × within-herd prevalence × herd size))/herd size.

For the option of not implementing any control strategy, the within-herd prevalence was assumed to remain constant at 40% over time. We assumed that new infections in the adult herd were prevented by assuming perfect compliance with prevention protocols, as well as no fly or contact transmission. Additionally, animals that were leaving the herd
were assumed to be replaced by test negative animals, except for cow purchases (see above), thereby reducing within-herd prevalence.

4.3.4. Partial net revenues

Fixed expenditures (e.g., building maintenance) were not included in the calculations; therefore, partial net revenues were calculated for all strategies. These were calculated as mean per animal in a 146-animal herd for a 10-yr period. Calculated partial net revenues were the sum of partial net revenues of uninfected cattle (revenue UI) and partial net revenues of infected cattle (revenue I). The probability of being infected or uninfected was represented by the prevalence and incorporated into the estimate as 

\[(1 − \text{prevalence}) \times \text{revenue UI} + \text{prevalence} \times \text{revenue I}\].

Revenues for uninfected cattle were assumed to be generated by milk and slaughter revenue, but reduced by raising replacement animals:

\[
\text{Revenue UI} = (\text{milk production} \times \text{milk price}) + [\text{replacement rate} \times \text{carcass weight} \times \text{slaughter value} \times (\text{HR} − 1)] − [\text{replacement rate} \times \text{heifer cost} \times (\text{HR} − 1)],
\]

where milk production represented the average per cow per year, milk price the value per liter, replacement rate the percentage of a herd replaced annually, carcass weight the average carcass weight of a cow, slaughter value the price per kilogram, and heifer cost the cost of raising replacement heifers. The hazard ratio (HR) is the higher probability to be culled of infected animals relative to uninfected animals, and (HR − 1) is the probability of uninfected animals to be culled, which is less likely than infected animals. Partial net revenue for infected animals was also assumed to be represented by milk and slaughter revenues and reduced by cost related to raising replacement animals.
Additionally, partial net revenue was assumed to be decreased by reduced milk production (Brenner et al., 1989, Emanuelson et al., 1992, Ott et al., 2003) and reduced longevity (Bartlett et al., 2013) of infected animals, as well as by development of enzootic bovine leukosis (Schwartz and Levy, 1994, CanWest DHI, 2017) and subsequent condemnation of affected carcasses. However, because Canada’s dairy industry is based on a milk quota system, the revenue of selling milk was assumed to stay constant over time and not directly affected by BLV within-herd prevalence. Nevertheless, a farm’s net revenue was decreased, due to indirect cost, because of BLV-related reductions in milk production. This was due to the cost of additional feed, housing, and maintaining more cattle to generate the same amount of milk as in an uninfected herd. Additionally, because infected cattle were assumed to be replaced by uninfected cattle, the replacement animal only had to produce 97% (3% less milk production of infected animals, see below) of the amount of milk a healthy, uninfected cow would generate in order to produce the same amount of milk as an infected cow.

Therefore, partial net revenue generated by infected animals was calculated as

Revenue I = (milk production × milk price) + (replacement rate × carcass weight × slaughter value × HR) – (replacement rate × carcass weight × slaughter value × lymphosarcoma) – (1 – milk loss) × (replacement rate × heifer cost × HR) – [milk loss × (dry feed + breeding + veterinary cost + labor + manure removal + calf value)],

where dry feed represents the feed necessary for maintenance of a dry cow, breeding is the average cost for breeding a cow per year, veterinary cost is the average annual cost related to veterinary expenses per animal, labor is the average annual labor necessary per animal, manure removal is the annual average cost for disposing of one animal’s manure,
and calf value is the value of a calf that could be born and sold if the number of animals would not decrease. As the prevalence of BLV changes in each stage, revenue changes accordingly. All revenues were discounted by a rate of 1.5% (Canadian Agency for Drugs and Technologies in Health, 2017). The difference between the different control strategies and the no control strategy was calculated as

\[
\text{Net benefit} = \text{partial net revenue (control strategy)} - \text{cost (control strategy)} - \text{partial net revenue (no control)}.
\]

This represents the net benefit producers implementing BLV control would accumulate as opposed to producers who would not control BLV on their farm. Moreover, for the various control strategies, net benefit per year per cow was calculated.

### 4.3.5. Within-herd prevalence and partial net revenue

Herd-based yearly partial net revenues were examined according to different levels of the within-herd prevalence. Instead of examining revenue per head within a herd, the partial net revenue for the entire herd for 1 yr was calculated as herd-based partial net revenue = number of infected animals \times revenue I + number of uninfected animals \times revenue UI.

### 4.3.6. Variability and uncertainty

A one-way sensitivity analysis was performed for all input variables as provided in Table 4-1. Combined with the comparison of the net benefit for each control strategy, this facilitated identification of variables with the main sources of uncertainty and the largest effect on the economically preferred choice.
4.3.7 Scenario analysis

To show the effect of having an open herd compared with a closed herd, 2 scenario
analysis were conducted. First, a closed herd scenario, where no animals are purchased
and therefore no infected animals are introduced into the herd. Second, an open herd
scenario, where animals are purchased and introduced into the herd every year.

4.4. Results

4.4.1. Cost

Mean cost in the first year for each control strategy per animal in a 146-animal herd,
excluding cost of testing but including investments for hardware (e.g., dehorner,
pasteurizer, barn alterations), were Can$29 for all management strategies (freezer),
Can$101 for all management strategies (pasteurizer), Can$75 for all management
strategies (colostrum), Can$11 for some management strategies, Can$39 for test and cull,
and Can$55 for test and segregate. Mean cost for the following years for each control
strategy per animal in a 146-animal herd, excluding cost of testing, were Can$20 for all
management strategies (freezer), Can$20 for all management strategies (pasteurizer),
Can$73 for all management strategies (colostrum), Can$9 for some management
strategies, Can$30 for test and cull, and Can$33 for test and segregate. The cost for
testing was added annually. Mean costs per animal over a 10-yr period including testing
in a 146-animal herd are shown in Table 4-4. The range of variation is shown in Table 4-6.
4.4.2. Partial net revenue

Based on described formulas, annual mean partial net revenue of an infected cow was Can$7,641, whereas mean partial net revenue of an uninfected cow was Can$8,276. Partial net revenue for each strategy over a 10-yr period per animal in a 146-animal herd is provided in Table 4-4. The range of variation is shown in Table 4-6.

4.4.3. Herd-based partial net revenue

The herd-based partial net revenue per year was found to be between Can$1,115,647 for a 100% within-herd prevalence and Can$1,208,234 for a 0% within-herd prevalence for a herd with 146 animals. A list of levels of the within-herd prevalence from 0 to 100% in 10% increments with matching herd based partial net revenue is provided in Table 4-5.

4.4.4. Net benefit

Mean net benefit per cow in a 146-animal herd over a 10-yr period compared with no control for each strategy was Can$1,315 for all management strategies (freezer), Can$1,243 for all management strategies (pasteurizer), Can$785 for all management strategies (powdered colostrum), Can$1,028 for some management strategies, Can$1,592 for test and cull, and Can$1,594 for test and segregate (Table 4-4).

4.4.5. Variability and uncertainty

The test-and-segregate strategy was the preferred strategy for most variables in the one-way sensitivity analysis. Test and cull was the most common alternative. Increasing the yearly change in prevalence for one strategy at a time allowed us to change the preferred
strategy to the respective control strategy. Similarly, an initial within-herd prevalence of 0% made all strategies less efficient than no implementation of control strategies on farm. An initial within-herd prevalence of 10% resulted in a positive net revenue for all strategies, except for all management strategies (pasteurizer) and all management strategies (colostrum). All management strategies (pasteurizer) was shown to be economically beneficial for a within-herd prevalence of 20%, whereas all management strategies (colostrum) resulted in a positive net benefit for a within-herd prevalence of 30%. Through sensitivity analysis, the following variables had the biggest effect on mean net revenue (in order of importance): years of implementation of on-farm control (Can$16,191 to Can$117,421 for low and high variable inputs for the test-and-segregate strategy, respectively); milk price (Can$71,783 to Can$87,097); average milk production per cow per year (Can$79,749 to Can$86,529); HR (Can$89,188 to Can$82,861); cost to raise replacement heifers (Can$84,495 to Can$83,031); slaughter value (Can$83,557 to Can$84,232); initial within-herd prevalence (Can$84,108 to Can$83,441); replacement rate (Can$84,267 to Can$83,649); herd size (Can$83,659 to Can$83,982), and cost for testing (Can$83,951 to Can$83,747; Figure 4-1). Variables representing years of implementation of on-farm control, initial within-herd prevalence, as well as variables representing the main drivers for the annual reduction in within-herd prevalence (e.g., HR for test and segregate) for each strategy were additionally able to generate a negative net benefit, when entering assigned low values (Table 4-1).
4.4.6 Scenario analysis

According to results of the scenario analysis, partial net revenue after 10 yr per animal in a 146-animal herd averaged Can$41 (range = 22–68) higher when no infected animals were introduced as opposed to introducing animals (1.39 animals per year; Van Biert, 2016) with a 54% chance of being infected (90% herd prevalence, with average 40% within-herd prevalence) into the herd every year (Table 4-4).

4.5. Discussion

The present model resulted in similar findings as other economic analysis in that BLV-infected cattle yielded lower partial net revenue than uninfected cattle (Chi et al., 2002, Ott et al., 2003, Rhodes et al., 2003). All of these reports and others (Pelzer, 1997, Trainin and Brenner, 2005) concluded that BLV caused economic losses. However, we found wide variation in factors that were taken into account and, consequently, in monetary loss attributed to BLV. Chi et al. (2002) only considered treatment and mortality losses due to BLV. In addition, Ott et al. (2003) considered mainly milk losses due to BLV infection. Rhodes et al. (2003) considered subclinical and clinical losses of BLV and examined, in detail, the cost of BLV infection. However, those authors only considered a free market milk price and did not take a quota system into account. Our analysis did not examine subclinical and clinical losses of BLV in as much detail (cost of treatment and possible fetal wastage were not considered). However, to our knowledge, the model presented herein is the only model specifically incorporating a quota system. This is important, as not only Alberta but also Canada as a whole relies on a supply managed system. In our economic model, implementation of any one of several on-farm
BLV control strategies resulted in a positive net benefit, making BLV control economically beneficial. Although one other study (Rhodes et al., 2003) reached the same conclusion for a test and manage control program and a within-herd prevalence of at least 12.5%, similar analyses for other control strategies are missing. Based on comparisons of various strategies considered in the present model, test and segregate was the most beneficial. This was expected and can be explained by the highest rate of yearly reduction in prevalence, resulting in the highest net benefit, compared with other strategies. According to available literature (Johnson et al., 1985, Suh et al., 2005), test-and-segregate control programs are highly efficient in preventing new infections, therefore resulting in a rapid increase in income. Although this strategy requires structural and organizational changes for dairy operations that might be difficult to implement, it offers an effective way of preventing new infections when uninfected replacement animals are difficult to realize. Even though these changes could be implemented in a reversible manner, segregation strategies for other diseases (e.g., mastitis) were found it to be rather unpopular due to the necessity of additional labor and altered routines (Huijps et al., 2009). In contrast to economically beneficial on-farm BLV control, negative net benefits were possible for some input variations in the sensitivity analysis. The same variables were also identified among the 10 variables with the biggest effect on the model. However, none of the iterations causing negative net benefits were unexpected. Low input values (1–3 yr, depending on the considered strategy) for the number of years of implementation of on-farm control resulted in a negative net benefit. Years was also identified as the variable with the biggest effect on the model. The more stages of the model (years of implementation) were run, the more revenue accumulated,
with an increasing effect on the result. Additionally, to control BLV efficiently, a long-term commitment is necessary (Brunner et al., 1997); consequently, a timespan of 10 yr was considered in this analysis. Interestingly, according to this model, BLV control became economically beneficial after a relatively short period. The reduction in prevalence of 15% per year for the some management strategies strategy resulted in a positive net benefit when continued over at least 2 yr (data not shown). Similarly, for a control program to be beneficial, the controlled disease has to be present on farm. Therefore, a very low or no within-herd prevalence of BLV resulted in a negative net benefit, whereas a higher prevalence and therefore more negative effects of the disease increased benefits of controlling the disease, confirming results of Rhodes et al. (2003). Consequently, within-herd prevalence ranked number 7 in the sensitivity analysis for the test-and-segregate strategy. Nevertheless, this might underestimate the true situation. Best management practices commonly recommended for BLV control will decrease the within-herd prevalence of BLV; however, they can be beneficial in additional ways. Pasteurizing colostrum, for example, can also improve calf health by reducing risks for calf scours and other diseases, thereby reducing calf morbidity including the necessity of treatment and mortality, resulting in additional economic benefits not considered in this model (Godden et al., 2012, Wolf et al., 2014). Finally, low input values for the effectiveness of each strategy also generated negative net benefit. This was unsurprising, as BLV control has to be efficient in reducing within-herd disease prevalence to increase net revenue. Other variables identified to have an effect on overall outcomes of this analysis were also readily explained. Milk price as well as the average yearly milk production per cow (ranked 2 and 3, respectively, in sensitivity analysis for the test and
segregate strategy) are fundamental determinants of income of a dairy farm and therefore expected to affect results. These variables undergo fluctuation in the dairy industry and the inputs represent variability among years and herds. This is also true for slaughter value (rank 6 in the sensitivity analysis for test and segregate). Similarly, high cow longevity means more revenue, because fewer replacement animals are necessary and surplus cattle can be sold. The uncertainty around HR for premature culling and the variability around the replacement rate therefore affect the net revenue of this analysis (ranks 4 and 8 in sensitivity analysis for the test-and-segregate strategy), because they influence the number of replacements needed and, therefore, the cost of raising them. The identification of the variable for cost to raise replacement heifers mirrors variability in cost to raise these animals (rank 5 in the sensitivity analysis for test and segregate).

However, independent of input values for these variables, BLV control remained economically beneficial. Test and cull most commonly resulted in a higher net revenue than test and segregate in the sensitivity analysis. Test and segregate was the most efficient strategy in our model; however, it was more costly than test and cull. This combination allowed the test-and-cull strategy to result in the highest net revenue for some variables in the sensitivity analysis. Nevertheless, one limitation of our analysis was the small amount of available data for input variables, which means that some model inputs were only estimates and therefore had higher uncertainty. However, we used average values for Alberta, wherever possible, and the sensitivity analysis incorporated the range of realistic alternative values, making the analysis very applicable. Well-documented studies concerning implementation of best management practices and their influence on the within-herd prevalence of BLV were not readily available; therefore,
only estimates could be used. It is impossible to reflect reality with a model such as ours. The actual decrease in prevalence on a certain farm is likely to differ from what is presented here, as it is highly dependent on the individual farm’s circumstances. However, values solely based on within-herd prevalence, without the assumption of a constant decrease in prevalence over time (Table 4-5), suggested that any decrease in BLV within-herd prevalence would result in an increased annual net revenue for the farmer. Similarly, the sensitivity analysis for the annual decrease in prevalence for the all control strategies (freezer) and all control strategies (pasteurizer) strategies showed that a decrease in prevalence of at least 5% every year would result in a positive net benefit in the considered time span.

The within-herd prevalence was assumed to stay constant over time when no control measures were implemented. This might underestimate the true situation, as the within-herd prevalence would probably rise over time in a newly infected herd. This assumption likely led to a conservative estimate, and the benefit of controlling BLV over not controlling might be higher than portrayed here. Nonetheless, based on sensitivity analysis, the model was highly robust and a valuable source of information for economic considerations of a BLV control program. The assumption of perfect diagnostic tests introduces some additional uncertainty.

Although sensitivity and specificity of BLV ELISA are high, they are not perfect (Walsh, 2013, Nekouei et al., 2016, Kuczewski et al., 2018). Consequently, some cattle might be identified as test negative even though they are infected; this could delay reductions in within-herd BLV prevalence. In contrast, false-positive results are also possible, which could result, depending on the strategy, in culling of an uninfected cow and the un
necessary premature slaughter of an animal and ensuing losses. However, chances of culling a BLV-negative animal are very low (~1%, based on 40% within-herd prevalence in a 146-animal herd and an ELISA test with 95% specificity and sensitivity). Similarly, if compliance is lacking and not all best management strategies are followed consistently, the decrease in BLV within-herd prevalence might be delayed. The results of different studies were not clear on the importance of biting flies in the transmission of BLV between animals (Weber et al., 1988, Erskine et al., 2012c). However, the issue of potential transmission of BLV through biting flies was addressed by incorporating increased fly control in our model.

The cost for testing infected animals could be increased or decreased by using various testing strategies. Using serum samples enables testing calves and heifers before lactation. Alternatively, testing of a subset of animals allows for estimation of within-herd prevalence while reducing cost for antibody testing (Erskine et al., 2012b).

Even though BLV infection likely has a negative effect on the infected animals’ immune system, and thereby health (Frie and Coussens, 2015), this effect was only considered partially in this analysis by taking production effects and higher risk for culling into account. In reality, these costs would be increased by treatment and other disease-specific costs. Consequently, the present results probably underestimated the true benefit of controlling BLV on farm. Assuming that BLV-infected animals are more likely to have lower milk production and less likely to successfully eliminate pathogens (Ott et al., 2003, VanLeeuwen et al., 2010, Erskine et al., 2012a), eradicating BLV will make voluntary culling space available. This culling space could be used to increase overall performance of the herd by reducing the BLV within-herd prevalence more quickly as
well as by replacing animals with a lower milk production with animals with a higher genetic potential for milk production, resulting in fewer animals needed to meet quota requirements. Consequently, herd size could be decreased, resulting in fewer expenses for animal maintenance. Alternatively, overall milk production or quota could be increased. The decrease in herd size would result in an additional yearly benefit of Can$2,172.55 per animal.

Even though the present analysis uses Alberta-specific data as an example, outcomes could be used as a guideline for Canada and other supply-managed systems in general. Whereas some characteristics vary between provinces (e.g., herd size, housing system), the entire Canadian dairy industry relies on a quota system. Additionally, taking the results of the sensitivity analysis into account, a smaller herd size resulted in smaller, but overall positive net benefits. The housing for dairy cattle differs in that tie stalls are more common in eastern Canada than in Alberta. This might have an influence on interanimal contact, and therefore on the risk of BLV transmission between animals, depending on how different farms are managed (e.g., pasture time and housing of dry cows). However, most risk factors for BLV transmission are independent of the housing system, and quantifying the difference in risk of transmission is therefore difficult to realize and likely does not have a big effect on transmission risk. To get a better understanding of the disease and to populate economic models with more detailed information, future research should examine development of BLV prevalence when no control is pursued, as well as when specific management changes (e.g., one-time use of needles) are implemented. Additionally, implementation of on-farm control programs could be closely monitored, to learn about the true economic effects of BLV and its control.
4.6. Conclusions

Based on the model we developed and presented here, implementation of any of the 4 proposed on-farm control strategies (all management strategies, some management strategies, test and cull, and test and segregate) resulted in a positive net benefit for the average dairy farm with 146 cows and a 40% within-herd prevalence of BLV, independent of the strategy chosen. In addition, the model also highlighted considerable economic losses due to BLV infection. These results should encourage implementation of BLV control programs and eradication of BLV on dairy farms.
4.7. References


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<td>Additional equipment for ‘test and segregate’</td>
<td>Farm supply store, May 2017</td>
<td>4 additional gates; shovel, broom: 3 each over 10 years; 1 additional</td>
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<td>Government of Alberta, 2015</td>
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<td>Average value for one calf</td>
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<td>99% isopropyl alcohol, 2 bottles per year</td>
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<td>Herd Health</td>
<td>Canadian Veterinary Medical Association and Alberta Veterinary Medical Association, 2017</td>
<td>Cost for pregnancy diagnosis by veterinarian; 1 examination per cow per year, additional 30 seconds per cow to change examination glove</td>
<td>2.23</td>
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<td>Herd prevalence in Alberta</td>
<td>Scott et al., 2006</td>
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<td>0.87</td>
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<td>HR (Hazard Ratio)</td>
<td>Bartlett et al., 2013</td>
<td>BLV infected animals are more likely to leave the herd than uninfected animals</td>
<td>1.23</td>
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<td>Labor cost per cow per year</td>
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<td>302.37</td>
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<td>Milk price (CAD/l)</td>
<td>Alberta Milk, 2016, 2017</td>
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<td>0.81</td>
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<td>131.4</td>
<td>87.6</td>
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<td>Opportunity Cost</td>
<td>Expert Opinion</td>
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<td>100</td>
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<td>Pasteurizer</td>
<td>Golden Calf Company (Bloomer, WI, USA) (personal communication with customer support) Milk Taxi (Holm &amp; Laue GmbH &amp; Co. KG, Westerrönfeld, Germany) (personal communication with Chinook Dairy Service Ltd., Chilliwack, BC)</td>
<td>1 pasteurizer over 10 years</td>
<td>1,15</td>
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<td><strong>Powdered colostrum</strong></td>
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<td>20.99</td>
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<td><strong>Purchase rate</strong></td>
<td>Dube et al., 2011, Van Biert, 2016</td>
<td>Open herds × no. cows purchased per year</td>
<td>0.79</td>
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<td><strong>Reduced milk production due to BLV infection</strong></td>
<td>Brenner et al., 1989, D'Angelino et al., 1998, Emanuelson et al., 1992, Ott et al., 2003, Tiwari et al., 2007</td>
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<td>0.03</td>
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<td><strong>Replacement rate</strong></td>
<td>CanWest DHI, 2017</td>
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<td>38.9</td>
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<td><strong>Risk of a slaughtered animal to suffer from lymphosarcoma</strong></td>
<td>CanWest DHI, 2017, Schwartz and Levy, 1994</td>
<td>Risk of developing LS when infected with BLV × percentage of cows older than 3 days lactation × within-herd prevalence</td>
<td>Values dependent on the respective within-herd prevalence</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Slaughter value (CAD/kg)</strong></td>
<td>Statistics Canada, 2017</td>
<td></td>
<td>2.08</td>
<td>1.3</td>
<td>3.14</td>
</tr>
<tr>
<td><strong>Testing</strong></td>
<td>CanWest DHI (personal communication with customer support)</td>
<td></td>
<td>8</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Veterinary cost per cow per year</td>
<td>Heikkila and Van Biert, 2010, Manitoba Agriculture, 2015, Van Biert, 2016</td>
<td>166.97</td>
<td>137.11</td>
<td>175.29</td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>Within-herd prevalence</td>
<td>Table 4-2</td>
<td>0.4</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Table 4-2. Effectiveness or reduction in bovine leukemia virus (BLV) within-herd prevalence per year for each strategy

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Reference</th>
<th>Change in Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>All management strategies</td>
<td>Expert Opinion</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low: 0, High: 50%</td>
</tr>
<tr>
<td>Some management strategies</td>
<td>Expert Opinion</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low: 0, High: 50%</td>
</tr>
<tr>
<td>Test and cull</td>
<td>CanWest DHI, 2017, Government of Canada, 2017</td>
<td>((Within-herd prevalence * Herdsize) - Voluntary culling rate * Herdsize) / Herdsize</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NA¹</td>
</tr>
<tr>
<td>Test and segregate</td>
<td>Bartlett et al., 2013, CanWest DHI, 2017</td>
<td>((Within-herd prevalence * Herdsize) - Replacement rate * Hazard Ratio * Within-herd prevalence * Herdsize) / Herdsize</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>No control</td>
<td>Assumption: no change over time</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NA</td>
</tr>
</tbody>
</table>

¹Value depends on result of the displayed formula.
Table 4-3. Prevalence estimation based on bovine leukemia virus (BLV) herd tests conducted by CanWest DHI (Guelph, ON, Canada; results for 90% of herd tested)

<table>
<thead>
<tr>
<th>Province</th>
<th>All</th>
<th>Alberta</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>263</td>
<td>37</td>
<td>165</td>
<td>28</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>No. positive</td>
<td>236</td>
<td>31</td>
<td>148</td>
<td>26</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Herd prevalence</td>
<td>0.90</td>
<td>0.84</td>
<td>0.90</td>
<td>0.93</td>
<td>0.91</td>
<td>0.95</td>
</tr>
<tr>
<td>Median within-herd prevalence</td>
<td>49.0</td>
<td>39.5</td>
<td>50.0</td>
<td>52.4</td>
<td>68.4</td>
<td>31.5</td>
</tr>
<tr>
<td>Range within-herd prevalence</td>
<td>1.69-100</td>
<td>6.67-91.11</td>
<td>2.0-88.1</td>
<td>1.69-90.63</td>
<td>44.64-88.04</td>
<td>1.82-100</td>
</tr>
</tbody>
</table>
Table 4-4. Results of simulation, comparing four on-farm bovine leukemia virus (BLV) control strategies. All results are presented in Canadian Dollars.

<table>
<thead>
<tr>
<th>STRATEGY</th>
<th>No control</th>
<th>All management strategies (freezer)</th>
<th>All management strategies (pasteurizer)</th>
<th>All management strategies (powdered colostrum)</th>
<th>Some management strategies</th>
<th>Test &amp; cull</th>
<th>Test &amp; segregate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cost per animal&lt;sup&gt;1&lt;/sup&gt; over 10 years</td>
<td>0</td>
<td>317</td>
<td>389</td>
<td>847</td>
<td>193</td>
<td>353</td>
<td>464</td>
</tr>
<tr>
<td>Mean partial net revenue&lt;sup&gt;2&lt;/sup&gt; per animal&lt;sup&gt;1&lt;/sup&gt; over 10 years</td>
<td>82,248</td>
<td>83,563</td>
<td>83,491</td>
<td>83,033</td>
<td>83,276</td>
<td>83,840</td>
<td>83,842</td>
</tr>
<tr>
<td>Average net benefit&lt;sup&gt;3&lt;/sup&gt; per year per animal&lt;sup&gt;1&lt;/sup&gt;</td>
<td>-</td>
<td>131.5</td>
<td>124.3</td>
<td>78.5</td>
<td>102.8</td>
<td>159.2</td>
<td>159.4</td>
</tr>
<tr>
<td>Partial net revenue&lt;sup&gt;2&lt;/sup&gt; per animal&lt;sup&gt;1&lt;/sup&gt; over 10 years: scenario (infected cattle can be introduced every year)</td>
<td>82,248</td>
<td>83,539</td>
<td>83,467</td>
<td>83,009</td>
<td>83,246</td>
<td>83,830</td>
<td>83,827</td>
</tr>
<tr>
<td>Partial net revenue&lt;sup&gt;2&lt;/sup&gt; per animal&lt;sup&gt;1&lt;/sup&gt; over 10 years: scenario (no infected cattle are introduced)</td>
<td>82,248</td>
<td>83,593</td>
<td>83,521</td>
<td>83,063</td>
<td>83,314</td>
<td>83,853</td>
<td>83,862</td>
</tr>
</tbody>
</table>
1Per animal in a 146-animal herd, 40% initial within-herd prevalence

2Partial net revenue = partial net revenue (strategy) – cost (strategy)

3Net benefit = partial net revenue (strategy) – cost (strategy) – partial net revenue (no control)
Table 4-5. Annual partial net revenue based on within-within herd prevalence of bovine leukemia virus (BLV) infection in Canadian Dollars per 146-animal herd.

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>1-Prevalence</th>
<th>Number of animals infected</th>
<th>Number of animals uninfected</th>
<th>Partial net revenue(^1) infected animals</th>
<th>Partial net revenue(^1) uninfected animals</th>
<th>Total partial net revenue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>146</td>
<td>0</td>
<td>1,115,647</td>
<td>0</td>
<td>1,115,647</td>
</tr>
<tr>
<td>0.9</td>
<td>0.1</td>
<td>131.4</td>
<td>14.6</td>
<td>1,004,082</td>
<td>120,823</td>
<td>1,124,905</td>
</tr>
<tr>
<td>0.8</td>
<td>0.2</td>
<td>116.8</td>
<td>29.2</td>
<td>892,517</td>
<td>241,647</td>
<td>1,134,164</td>
</tr>
<tr>
<td>0.7</td>
<td>0.3</td>
<td>102.2</td>
<td>43.8</td>
<td>780,953</td>
<td>362,470</td>
<td>1,143,423</td>
</tr>
<tr>
<td>0.6</td>
<td>0.4</td>
<td>87.6</td>
<td>58.4</td>
<td>669,388</td>
<td>483,294</td>
<td>1,152,682</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>73</td>
<td>73</td>
<td>557,823</td>
<td>604,117</td>
<td>1,161,940</td>
</tr>
<tr>
<td>0.4</td>
<td>0.6</td>
<td>58.4</td>
<td>87.6</td>
<td>446,259</td>
<td>724,941</td>
<td>1,171,199</td>
</tr>
<tr>
<td>0.3</td>
<td>0.7</td>
<td>43.8</td>
<td>102.2</td>
<td>334,694</td>
<td>845,764</td>
<td>1,180,458</td>
</tr>
<tr>
<td>0.2</td>
<td>0.8</td>
<td>29.2</td>
<td>116.8</td>
<td>223,129</td>
<td>966,588</td>
<td>1,189,717</td>
</tr>
<tr>
<td>0.1</td>
<td>0.9</td>
<td>14.6</td>
<td>131.4</td>
<td>111,565</td>
<td>1,087,411</td>
<td>1,198,975</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
<td>146</td>
<td>0</td>
<td>1,208,234</td>
<td>1,208,234</td>
</tr>
</tbody>
</table>

\(^1\)Partial net revenue = revenue generated by infected/uninfected animals, respectively, per year.
Table 4-6. The range of variation displayed in 95% confidence intervals (CI) for partial net revenue and cost for all control strategies, as well as for no control. All values are in Canadian Dollars.

<table>
<thead>
<tr>
<th>STRATEGY</th>
<th>Mean cost per animal(^1) over 10 years</th>
<th>95% CI</th>
<th>Mean partial net revenue per animal(^1) over 10 years</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No control</td>
<td>0</td>
<td>N/A</td>
<td>82,248</td>
<td>82,248.04; 82,248.04</td>
</tr>
<tr>
<td>All management strategies (freezer)</td>
<td>317</td>
<td>316.88; 317.67</td>
<td>83,880</td>
<td>83,844.71; 83,910.44</td>
</tr>
<tr>
<td>All management strategies (pasteurizer)</td>
<td>389</td>
<td>388.80; 389.59</td>
<td>83,880</td>
<td>83,844.71; 83,910.44</td>
</tr>
<tr>
<td>All management strategies (powdered colostrum)</td>
<td>847</td>
<td>846.44; 847.23</td>
<td>83,880</td>
<td>83,844.71; 83,910.44</td>
</tr>
<tr>
<td>Some management strategies</td>
<td>193</td>
<td>192.64; 193.66</td>
<td>83,469</td>
<td>83,423.35; 83,507.56</td>
</tr>
<tr>
<td>Test &amp; cull</td>
<td>353</td>
<td>351.93; 354.86</td>
<td>84,193</td>
<td>84,171.03; 84,204.39</td>
</tr>
<tr>
<td>Test &amp; segregate</td>
<td>464</td>
<td>464.12; 464.63</td>
<td>84,306</td>
<td>84,284.80; 84,326.45</td>
</tr>
</tbody>
</table>

\(^1\)Per animal in a 146-animal herd, 40% initial within-herd prevalence
Figure 4-1. Tornado diagram of sensitivity analysis for variables with the highest impact on net revenues (net revenue-cost) of the ‘test and segregate’ strategy.¹

¹‘Years’ was excluded due to high range of results (CAD 16,191 to CAD 117,421 for low and high variable inputs for the ‘test and segregate’ strategy): the effect of this variable would have decreased visibility of other variables’ range.
CHAPTER 5: Motivation and barriers to control bovine leukemia virus on farm

5.1. Abstract

The bovine leukemia virus (BLV) herd and within-herd prevalence as well as BLV’s negative impact are high in North America. However, BLV control is dependent on a change in farmer behavior. Therefore, the goal of this study was to understand farmers’ motivators and barriers to consider, implement, and continuously adhere to best management practices (BMP) aimed at the control of BLV. This was achieved by conducting 4 focus groups with Alberta dairy farmers, 11 interviews with Alberta veterinarians focused on dairy herds, as well as multiple interviews with 11 Alberta dairy farmers participating in a BLV control program trial implementation. Through thematic analysis the following process in the change of on-farm behavior could be identified: First, the initiation of behavior change on farm caused by the farmer’s personal attitude, the community around the farmer, lived experiences with BLV and the disease it is causing, or the value of animals to the farmer. Second, the introduction of change, which was found to be dependent on knowledge and the way change was introduced. Third, the implementation of change seemed only possible if old habits and other barriers could be overcome. Additionally, it was found that farmers and veterinarians did not identify the same motivators and barriers. For example, farmers valued a team approach to the solutions of problems and identified the lack of trust into BMP as an important barrier, whereas veterinarians did not mention these factors. Consequently, communication efforts between veterinarian and farmer as well as within farm should be increased in order to generate better mutual understanding. By increasing teamwork, barriers can be
identified, solutions can be found, and the implementation of BLV control measures as well as behavior change on farm become more likely.

5.2 Introduction

Bovine Leukemia Virus (BLV) causes economic damage to the dairy industry by impairing infected animals’ immune systems as well as by reducing their milk production and longevity (Rhodes et al., 2003, Frie and Coussens, 2015). Additionally, a small subset of infected animals will develop fatal tumors and die prematurely (Schwartz and Levy, 1994), which negatively impacts animal welfare and could cause consumer concerns (Bartlett et al., 2014). The Alberta dairy herd prevalence is ~90% (Scott et al., 2006) and there is no vaccine or treatment available. Therefore, control is dependent on the replacement of infected with uninfected animals. This can be achieved by the implementation of best management practices (BMP) aimed at preventing BLV transmission between animals (Rodriguez et al., 2011). BLV control is economically beneficial, but is dependent on long-term BMP implementation (Rhodes et al., 2003, Kuczewski et al., 2019). Historically, compliance with on-farm BLV control has been low in North America (EFSA Panel on Animal Health and Welfare, 2015). This is also true for other voluntary disease control or biosecurity programs (Brennan and Christley, 2013, Ritter et al., 2016). Consequently, research efforts aimed at understanding farmers’ motivators, and barriers to control diseases recently increased (Jansen et al., 2009, Ellis-Iversen et al., 2010, Garforth, 2015). This research showed that farmers are influenced by external factors (e.g. economics), as well as internal factors (e.g. knowledge, pride, a desire for good animal welfare). However, these internal factors have not yet been
extensively studied (Ritter et al., 2017). Moreover, the majority of research was initiated either during the roll-out or after implementation of disease control programs, mainly because their uptake was limited (Jansen et al., 2010a, Sorge et al., 2010). To date, there is limited research available that investigates the farmers’ motivation before and/or during the introduction of a new disease control program (Bell et al., 2009). By investigating these factors before the introduction of a control program, bottlenecks and barriers can be identified and addressed. Simultaneously, by understanding important motivators, participation rates could increase (Roche, 2014, Ritter et al., 2015).

Therefore, to inform a newly developed BLV control program, investigating possible barriers and motivators before its introduction to the dairy industry was considered beneficial (Roche, 2014, Ritter et al., 2015). To gain a more accurate understanding of factors influencing a farmer’s complex decision-making process, as well as factors specific for BLV or the Alberta context, a qualitative approach based on open questions was used. To reach a variety of dairy farmers, focus group sessions were organized. In addition, it is well known that veterinarians play a substantial role in motivating farmers to implement disease control measures (Hop et al., 2011, Ritter et al., 2015). Therefore, their expertise, experience, and opinions were collected during interviews with dairy veterinarians. Furthermore, it was possible to include a longitudinal component in this study through interviews of farmers, who participated in the trial implementation of the newly developed BLV control program in Alberta.

In summary, the goal of these in-depth conversations was to investigate motivators and barriers to consider, introduce, and continuously implement BLV control measures on farm.
5.3 Materials and Methods

5.3.1 Study design

The Alberta dairy industry and a research team at the University of Calgary collaborated on the development of a risk-assessment based on-farm BLV control program. The control program was developed based on the following steps: Firstly, a list of transmission routes and resulting BMP to prevent BLV transmission was created based on literature (Hopkins and DiGiacomo, 1997, review of current literature, manuscript in preparation) and common on-farm practices. Secondly, based on this list, conversations with farmers and veterinarians were sought to ensure the completeness of identified on-farm practices as well as to elucidate the feasibility of the suggested BMP, and to create a better understanding of barriers and motivators for implementing new BMP on farm. This was achieved by conducting 4 focus groups with farmers, as well as 11 interviews with veterinarians. Thirdly, the control program’s risk assessment tool and BMPs were adapted according to the farmers’ and veterinarians’ feedback. Fourthly, the control program was implemented on 11 farms for one year. In order to uncover further barriers and to better understand motivators to control BLV on farm, as well as personal attitudes and opinions, the participating farmers were interviewed five times during the trial implementation.

Here, we will discuss the results focused on motivators and barriers when introducing new BMP on farm, based on the focus groups with farmers, interviews with veterinarians and interviews with farmers throughout the trial implementation. All results directly pertaining the BLV risk assessment and control program will be discussed in two other
manuscripts (Chapter 6, and Chapter 7). Figure 5-1 represents the outline of the study presented here.

All focus group and interview guides were developed through a collaborative approach between the authors and can be found in Appendix I.

The project was approved by the Conjoint Faculties Research Ethics Board (CFREB) of the University of Calgary (REB16-0224). All participants provided written consent before the beginning of the different sessions.

5.3.2 Participants and structure

Establishment of relationships. In order to meet farmers and establish relationships, the first author attended multiple events important to the dairy industry throughout the years from 2015 to 2019 (e.g. Western Canadian Dairy Seminar, WCDS, Red Deer, AB, Canada, Dairy Research and Extension Consortium of Alberta (DRECA) meetings, Edmonton, AB, Canada). Moreover, some farmers participated in multiple parts of the study, grounding the relationships further. The established relationships allowed for the following conversations to be honest and open, which facilitated a deeper understanding of personal drivers, attitudes, and opinions.

Farmer focus groups. We used focus groups as a first step to involve farmers in the development of the control program. Because implementing BLV control on farm is dependent on farmers’ behavior, the goal of the focus groups was to understand barriers and motivators involved in the process of behavior change on farm in general and specifically for BLV. Focus-groups were considered the most suitable approach to
interact with the dairy farmer community, as a group setting was expected to create a more relaxed situation, resulting in open conversations between the first author and the farmers.

Attending Alberta dairy farmers were invited to provide their contact information for possible recruitment for the participation in events regarding BLV and its control at various occasions (e.g. WCDS, Red Deer, AB, Canada; yearly Alberta Milk Delegate Meetings, Edmonton, AB, Canada, etc.), at the same time they were encouraged to share our contact information with fellow farmers. Farmers that provided their contact information were subsequently invited via e-mail to participate in the focus groups. Another invitation was published in the Alberta Milk “Milking Times” magazine. All invitations provided a short summary of the agenda of the day, a time estimate, as well as the request to register for the focus group to facilitate planning. Additionally, one group of farmers contacted the first author directly and initiated the focus group in Taber.

Recruitment took place from February to April 2017 and the focus groups were conducted between April and May 2017. In total, 4 focus groups were conducted in Lethbridge, Red Deer, Leduc, and Taber; regions of Alberta with a high density of dairy farms. The duration of the focus groups varied from 4-6 hours and included a lunch free of charge for all participants.

The first author facilitated the focus groups, while a second researcher took detailed notes. On the days of the focus groups, all participants were provided with a brief overview of the schedule and content of the day. Additionally, a questionnaire was used to gather demographic information (e.g. age, herd size, milk production). The focus groups were conducted in a semi-structured format whereby a focus group guide was
used for consistency between the sessions: A short introductory round was followed by
two pile sorting exercises. Here, every farmer received 10 small pieces of paper and was
asked to first, list the 5 most important matters of concern for him/her on his/her farm and
write each one on a piece of paper. Next, the pieces of paper were organized to reflect the
least important to most important item, as perceived by the farmer, then reorganized to
indicate the easiest to hardest to change. Finally, the same exercise was repeated for the 5
most important issues for the dairy industry according to the farmer’s opinion. All
participants were invited to discuss their notes. This exercise was meant to build trust and
encourage open conversation between the participants. Besides this exercise, the focus
group guide consisted almost exclusively of open questions, aiming at the goals described
above.

*Interviews with veterinarians.* After completion of the farmer focus groups, interviews
with veterinarians were conducted. The goal of these interviews was to learn about the
veterinarians’ perception of motivators and barriers for farmers to implement disease
control measures in general and specifically for BLV, as veterinarians are important
advisors on farm. Moreover, some BMP are dependent on the veterinarian’s initiative
(e.g. changing palpation sleeves during rectal pregnancy examinations), making it
important to understand the veterinarians’ drivers as well.

However, there is only a limited number of veterinary clinics throughout the province
that focuses their services on dairy clients. In addition, the distance between veterinary
clinics servicing dairy farms in Alberta is oftentimes relatively far (e.g. 100 km).
Consequently, scheduling veterinarians in groups was challenging and one-on-one interviews were considered more appropriate.

Veterinary clinics were selected based on their focus on dairy clients and based on their distance to Calgary (<4 hours travel by car). In total, 11 clinics were contacted directly by the first author: contact was initiated via e-mail, alternatively through a phone call. Calls were followed up with e-mails, if an e-mail address was provided. The e-mails provided a short overview and purpose of the interview, as well as all contact information of the researchers. If there was no response, a follow-up phone call was placed after 5-7 days. In total, 11 veterinarians from 9 clinics agreed to participate. All interviews with veterinarians were planned and conducted between September and October 2017. Except one off-site interview, all interviews were conducted directly at the veterinary clinic. Meetings were between 1 and 3 hours long.

All participants were provided with a short explanation of the topics that would be discussed during the interview. Additionally, all participants filled out a short questionnaire on demographics (e.g. age, percentage of clientele that is dairy farms). The interviews were semi-structured, using open-ended questions, thereby providing the veterinarians latitude in their responses.

**Trial implementation.** The final step was a trial implementation of the control program. The trial implementation included sampling and testing of the herd, a risk assessment, the recommendation of specific BMP based on the results of testing and risk assessment, as well as 5 interviews. The goals of the interviews were to: 1) Gain a better understanding of which recommended control measures were considered feasible or unpractical and
why; 2) Further investigate motivators and barriers that promote or hinder the implementation of BLV control on farm. Here, we will mostly discuss the results for the second goal derived from the interviews.

Participants were recruited by the first author based on a convenience sample. Farmers that provided their contact information previously (see focus groups with farmers) and whose farms were within 4-hour driving distance from Calgary, AB, were contacted directly via e-mail (42 contacts) and invited to participate in the trial implementation of the control program. Farmers were recruited in the order of their response until the maximum of 11 farms was reached (total of 19 responses). All 11 farmers were contacted by phone and were provided with an explanation of the trial. All farmers had the opportunity to ask clarifying questions and subsequently agreed to be a part of the trial. The trial implementations started in January 2018 and ended for the last farm in June 2019. One farm stopped participation after the 6-month follow-up interview, due to personal reasons. Sampling and testing were free of charge for all participants.

A schematic overview of the trial can be found in Figure 5-2. During the first meeting, all farmers filled out a short questionnaire about some important demographics (e.g. age, herd size, milk production). Afterward, the risk assessment as well as the first interview were conducted by the first author (approximately 2 hour duration). At the second meeting, all animals on farm that were 12 months and older were sampled for serum testing. During the sampling events no interviews were conducted, but the first author and different helpers spent the day with the farmers. Once the test results were available, they were shared with the participants. Afterward, a phone interview was held between the first author and the farmers. These conversations were used to discuss the test results,
to recommend specific BMP, as well as to conduct a short interview (6 questions, approximately 1 hour duration). After approximately three and six months since the recommendations of best management practices, farmers were visited and interviewed by the first author (approximately 1 hour duration each). These interviews were aimed at discussing the experience of the farmers with the control program so far as well as to understand motivational factors and barriers that were encountered during the process of implementing new BMP. After approximately 13 months since the first sampling, a second sampling took place and a second risk assessment was conducted. The results for the sampling were communicated and a final interview was completed by the first author (approximately 1 hour duration). All interviews followed a semi-structured interview guide that mainly consisted of open questions.

5.3.3 Data analysis

Focus groups, interviews with veterinarians, as well as the interviews with farmers were audio recorded and transcribed verbatim by the first author. All transcripts were compared with the original recording at least once, to ensure accuracy and descriptive validity (Braun and Clarke, 2013).

A thematic analysis was conducted on all transcripts from farmer focus groups, interviews with veterinarians, as well as interviews with farmers that were part of the trial implementation (Braun and Clarke, 2006). The perspective adopted for this study was a critical realist one (Braun and Clarke, 2013). The first author familiarized herself with the data by transcribing, listening, and reading all transcripts multiple times. During the familiarization process, notes were taken, and emerging codes were identified. All further
analysis was done with ATLAS.ti (Scientific Software Development GmbH, Berlin, Germany) as well as with Microsoft Word (365 ProPlus, Microsoft Corp., Redmond, WA). The analysis continued with the establishment of a code book, based on the transcripts of the focus groups with farmers, according to a holistic coding process (Saldana, 2016). Afterward, the interviews with veterinarians were coded based on the created code book, while new codes were added, when necessary. Subsequently, the interviews with farmers participating in the trial implementation were coded in the same manner. Next, all excerpts of holistic codes referring to motivation or barriers in behavior change on farm, either in general, or specifically for BLV, were coded in a more detailed manner by the first author, resulting in a more detailed, separate code book. Throughout the coding process it was noted which codes occurred in which transcript set (focus groups with farmers, interviews with veterinarians, or interviews during trial implementation) to allow for future comparison. Finally, themes and subthemes were identified. Throughout the process, the original transcripts were revisited, and newly emerging codes were incorporated, as well as themes adjusted. Additionally, themes, subthemes, and codes were discussed on regular basis between the authors. Three transcripts of the first two interviews conducted during the trial implementation were coded by the first author as well as by a second researcher. This researcher was trained and mentored by the first author. Both researchers independently read the transcripts and identified applicable codes that were then compared and discussed to guarantee credibility.
5.4 Results

5.4.1 Participants

Farmer focus groups. In total, 24 farmers participated in the four focus groups. Group sizes were 3 (Lethbridge), 2 (Red Deer), 9 (Leduc), and 10 (Taber). Overall, 22 males, and 2 females participated. Participants were born between 1950 and 1990. Twenty-two participants were dairy farmers, 1 was a feed specialist, and 1 was the herd manager of a research herd. The average herd size was 139 milking cows (60-390), average milk production per cow per day was 36 kg (20-40kg/day). 14 farms had not implemented any on-farm BLV control, 9 farms had implemented some BLV control.

Interviews with veterinarians. Nine different clinics were involved in the interviews. In total, 11 veterinarians participated. From 8 clinics, one veterinarian participated. In 1 clinic, all 3 practicing veterinarians participated in a group interview. One veterinarian was female, 10 veterinarians were males. All veterinarians were born between 1940 and 1990. Their clientele consisted from 25 to 100% of dairy farmers.

Trial. In total 11 farms participated in the trial. On 8 farms, the first author communicated with the farmer. On 2 farms, the wife and husband were involved. On 1 farm, the first author communicated with a father and son team. Participating farms had on average 285 animals that were 12 months and older (110-524) and an approximate average number of 153 (range 85-330) milking cows. The average daily milk production was 38 kg (range 34-44). On average, 39.2% of these animals were infected (13-66%).
5.4.2 Data analysis

Based on the described process the following themes and subthemes were identified:
Initiating change, introducing change, and implementing change. Figure 5-3 depicts
identified themes, subthemes, as well as their relationships.

In the text, all participating farmers (focus groups and/or trial implementation) will be
referred to as “farmers”. It was considered appropriate to refer to the two different groups
as one, as some farmers participated in focus groups as well as in the trial implementation
and the identified codes and themes for both events were almost identical. Farmers and
veterinarians did not agree on all topics, therefore it will be indicated which codes and
themes were identified for farmers, veterinarians, or both throughout the results. Based
on the questionnaires and therefore the density of codes within the themes, the first and
third theme were expected to yield a longer analysis. This does not reflect the importance
of the three different themes in the context of behavior change on farm.

5.4.3 On-farm behavior change

Based on the findings presented here, the process of behavior change on farm, can be
separated into 3 main themes that represent 3 consecutive steps: 1) The initiation of
change by creating or using existing motivators for change. BLV and its effects, on-farm
economics, the quota system and its effects on the dairy industry, the animals in a
farmer’s care, the farmers’ personal attitude, as well as the community farmers work and
live within were considered important subthemes here. 2) The introduction of change was
considered dependent on knowledge as well as on the way change was introduced. 3) The
implementation of change was considered dependent on whether old habits can be overcome and whether new habits can be established.

5.4.3.1 Theme 1: Initiating change

Bovine Leukemia Virus/The disease

When asked whether participating farmers and veterinarians considered BLV to be a problem for the dairy industry, the majority agreed. However, when asked whether BLV was a problem on their farm, only some farmers agreed. Upon further investigation it was discovered that BLV was oftentimes considered a problem, when farmers had personal experience with cows that showed clinical signs and/or died due to EBL and were diagnosed following a post-mortem examination by the veterinarian. One farmer, for example, said:

“And it’s a real shame when you have these good cows, [...] that have a couple of lactations in them and all of a sudden, they’re dead.”

The veterinarians echoed this finding and oftentimes referred to these examinations as ‘teachable moments’. Additionally, it was noticed that the severity of the problem was measured by farmers as well as veterinarians by the number of EBL cases. Farmers and veterinarians pointed out that BLV oftentimes was not considered a problem because only a few infected animals developed EBL and the associated clinical signs, as summarized by this veterinarian:

“I think it’s dependent on the prevalence within the herd. Cause for some of our guys, they’re pretty dialed in. Cause they’ve seen 6 dead cows getting dragged out of the barn in a short period of time. Whereas the next person, you know, you might diagnose one and you’ll never see another one for three or four years, so then they just are not as dialed in.”
Farmers might not have considered BLV a problem, even though their herd was infected, because of unspecific and inapparent clinical signs caused by the infection. Therefore, infected animals might have been culled due to a lack of performance without investigating the cause. This might have aggravated the impression that the infection with BLV does not have a considerable impact on the herd. A veterinarian mentioned:

“It’s hard to get someone excited about a disease that they don’t think they have, or just doesn’t seem to be a big impact, if their prevalence or their incidence of clinicals is too low.”

However, some farmers and veterinarians considered BLV a problem, even though they had not encountered EBL cases, but noticed the production limiting effect of the virus on infected animals, like this farmer:

“Well, they have what you get when an animal is down. So, low milk production, low perception rate [sic], more susceptible to mastitis. That’s what I’m assuming anyways. I find it hard to believe that a cow just drops dead.”

In general, farmers that implemented any kind of BLV control tied their motivation to control BLV to the negative effects of the infection, oftentimes combined with a within-herd prevalence they considered “too high”. The veterinarians identified the same pattern. However, the level of infection that was considered “too high” varied considerably amongst farmers. Some farmers expected a within-herd prevalence over 60%, whereas another farmer, for example, said:

“Well, when I did my milk samples that came back 25%, I’m going, ‘Woah! That’s a little high’. So, I wanted to try and do something about it.”

However, other farmers and veterinarians pointed out that a very high within-herd prevalence might result in the feeling that the problem is too big to be solved and that implementing control measures will not make a difference. Additionally, BLV control
requires a long-term commitment, which might have been perceived as difficult by some farmers and was also pointed out by the veterinarians. One farmer said:

“Well, the results, you know. But the results aren’t coming over night. That’s the problem with all these programs. It takes a while before you see it.”

Even though some farmers and veterinarians thought that BLV wasn’t considered an issue by the industry at large, others were motivated by hypothetical future incentives to eliminate BLV, as pointed out by this farmer:

“Second reason is, I think in the future, Canada will have to be like Europe, and probably be Leukosis-free or a certain percentage.”

**Economics/In the end, it’s the bottom line.**

One of the main reasons identified by farmers and veterinarians why farmers did not implement BMP or do not test their animals for BLV, were related to cost. On the other hand, farmers that were motivated to control BLV oftentimes highlighted the preventable financial loss. One farmer summarized it as follows:

“That was our biggest reason. Just the costs of the loss of these good cows.”

Farmers and some veterinarians also mentioned that other farmers could be persuaded to consider BLV control by pointing out the financial damage BLV infection can cause, and that control measures would result in a positive net benefit, as mentioned by this farmer:

“We just have to be shown that the disease is costing us money, before we start spending money.”

A veterinarian said:

“I think, if you convince farmers, that it’s saving them money, compliance would be really good.”
This was highlighted multiple times and economics were generally considered a strong motivating factor to initiate change. Although one farmer pointed out that the economics might have been a reason to start, but not one to continue long-term.

Additionally, financial support was considered a reason to increase the motivation to control BLV by farmers and veterinarians. Both groups pointed out that by supporting testing of herds or supporting the purchase of equipment necessary to implement on-farm control would increase the motivation to control BLV on farm. Test results were oftentimes referred to as ‘eye openers’ or teachable moments by both, farmers and veterinarians. One veterinarian mentioned:

“I think, if we could get funding and get all the herds tested, so they know what their prevalence is. Cause right now, you know they may see a clinical once in a while and that’s about it. But if we show them, [...] you’ve got 40, 50, 60, 80% positive in your herd, and we had some support saying, this is an issue, we need to start going after this.”

Generally, it was pointed out by veterinarians as well as by farmers that the investment had to be worth the outcome. One farmer justified not changing on farm behavior as follows:

“No. Cause they [=specific group of cows] were all positive anyway, I didn’t see any benefit.”

However, the outcome was not limited to financial benefits. Benefits of any kind were considered a reason to invest into BMPs. As pointed out by this farmer:

“Extra time... It’s more labor, that’s all there’s to it. It’s basically just a... It’s [...] more management. [...] hopefully that pays off with less management with less sick cows.”

The veterinarians mentioned that as well.

*The Quota System*
Canada’s dairy industry is managed by a supply managed system (quota system).

Interestingly, the quota system was considered by the veterinarians as well as the farmers to be influencing farmer behavior. Generally, farmer’s quota followed the same trend as the national quota. This meant that the farmer had to adjust milk production within his/her existing facilities. Consequently, quota decreases enabled easier culling decisions, as the pressure to deliver milk lowered. However, the veterinarians pointed out that at the same time the value of individual animals decreased, potentially causing investments for animal care to decrease as well. When quota increases occurred, farmers perceived the need to produce more milk. Consequently, animals might not be culled as readily. One veterinarian reflected on his experience:

“And it all depends on the value of the individual animal, too. Because, when BSE came out, that propelled us very quickly into control, because the individual animal was worth 200$, you didn’t do a lot of individual animal stuff, they got, if it was too serious, you just put them down. But, if they’re short on quota and the milk is worth something, then all of a sudden, the individual animal is worth spending time on.”

**Animals**

The cattle were also considered to play an important role in behavior change. Despite the influence of the quota system on the monetary value, the sentimental value of the individual animals was mentioned as being influential on behaviour by farmers as well as veterinarians. The genetic value of some animals was considered an additional motivating factor to invest more time, money, and effort into their well-being. This veterinarian, for example, noted:

“That herd had experienced a lot of clinical disease and you know, was concerned, because of their genetics as well and wanted to improve...”
Nonetheless, bull calves were generally considered less valuable than heifer calves and therefore received different care.

However, some farmers pointed out that the sentimental value of their cows hindered them from culling animals that imposed a risk on the health status of their herd. Like this farmer for example pointed out:

“I just can’t say goodbye to cows... I have a problem, myself. I hate it to cull cows. I see something good in every cow and I always have a hard time selling them.” – “That way the herd is not cleaning up, maybe. I don’t know. I think I’ll keep more problems that way.”

However, farmers pointed out their animals’ own minds as hindering factor. For example, bulls’ behavior was considered unpredictable, making breeding management challenging. Similarly, it was normal for cattle to spread manure and, when injured, blood throughout the facility, making it difficult to keep facilities clean. One farmer pointed out that:

“...Cause cows will be cows. They like making a mess.”

**Personal attitude/Different characters are motivated by different things**

Farmers and veterinarians were quick to point out that the personal attitude toward a topic is a key criterion when initiating change on farm. A negative attitude was oftentimes connected with older age, or a reluctance to change behavior. On the other hand, the personal attitude was considered very powerful by farmers and veterinarians, when introducing new behavior. One farmer summarized the influence of personal attitude:

“It’s gonna depend mostly, if people really wanted to do it. ‘Cause if you really wanna do it, you’re not gonna care what it costs. But if you don’t care, if you don’t really wanna do it, it’s going to be pretty hard to convince everybody to do anything.”

However, there were many motivators for farmers participating in the trial implementation and/or the focus groups. A good feeling about doing the right thing and
perceived control of the situation were pointed out as motivating factors by farmers.

Farmers and veterinarians pointed out the pride in a well-run farm as a reason for progressive management. One veterinarian said:

“[…], farmers will take pride in their farm, when things are going really well. And this is one way of helping them, get it going well, […].”

A farmer echoed:

“Like, it all depends, if it’s a farm that wants to have a little…[…]… has a little self-esteem, I would say they’d just do it automatically.”

Some farmers liked being ‘the first’ or ‘ahead of a trend’ and felt it was important to be an early adopter. Similarly, a veterinarian mentioned the competitive nature of some farmers.

Some farmers were also motivated by an improvement of their own and their employees’ personal health and quality of life, as well as by trying to assuring prosperity for themselves and future generations. Finally, their own gut feeling was considered to play a role in decision-making for the farmers. One farmer said:

“You know what, I go with my gut feeling. I try to think about it with common sense.”

In the following, different subthemes that summarize factors that influence and are influenced by the personal attitude will be discussed.

**Improvement/Move the operation forward.** Improving their operation and learning from mistakes was considered a strong motivator for many farmers and was also mentioned by the veterinarians. One farmer commented:

“…no matter how slight the improvement is, how can you not want improvement?”
Animal health, animal welfare, cow longevity, production, and milk quality were especially highlighted. Improvement was also measured as improvement over time, compared their current farm performance.

**Teamwork.** Some farmers pointed out the importance of solving problems using a team approach. They pointed out that it was important for them to work together with their veterinarian, feed specialist, hoof trimmer, technician, etc. in order to improve their operation and the industry. One farmer pointed out:

> “These are all specialties, right?! So when we were having milk quality issues, too, we had the vet and the guy from [XXX] there, the milking parlour guy, right, so that they can talk to each other, too. So instead of having to go from him to me and then to the vet and then this way. So the vet is coming this day, I want you there, too, right?! So that all the three of us are there and can talk at the same time.”

**On-farm culture.** On-farm culture was considered to influence farmer’s behavior and was influenced by the farmer’s background and personality. For example, ‘we have always done it this way’ was a common statement amongst farmers: This attitude could have a positive impact on behavior change for some farmers, because they used their old habits as steps toward behavior change. Like this farmer, for example:

> “But as long as I can remember we’ve always had frozen colostrum on the farm, so... So this was just our next step. Start pasteurizing it.”

On the other hand, it could be hindering behavior change, if it was used as an excuse, like in this example:

> “I’ve been doing this forever and the calves are still healthy!”

Additionally, farmers explained their on-farm culture by their European background and the wish to farm with the same standards as they did before they moved their operation to Canada. Besides, Religion and culture were also identified as important influences. For
example, being a part of the ethnoreligious group of Hutterites, made it impossible to introduce some BMP, like replacing bull breeding with artificial insemination. Another farmer based some of his management strategies on his faith:

“God made cows with milk for their babies, why would I give them something else! That’s my theory!”

Bigger farms were generally considered to adopt new management practices easier by the veterinarians and farmers. This was also true for younger generations, like this veterinarian mentioned:

“It’s generation change. Lots of new generations coming in. I think that’s part of the huge change of what we’re seeing.”

However, some younger generations were hindered by other family members, when trying to change on-farm management.

Community

The community the farmer found himself within was also considered an important influence on farmer behavior and could have an encouraging or a discouraging effect. The industry, consumers, the veterinarian, and their peers were pointed out consistently by farmers. Some were also identified by the veterinarians.

The dairy industry was considered important by both veterinarians and farmers. Priorities within the industry would generally become a priority on farm as well. Additionally, implementing mandatory disease control programs by the industry was considered a possibility by farmers and veterinarians to increase the participation in BLV control. Some pointed out that it would be a way to motivate all farmers. One veterinarian said:

“[...] what you need to do. You need to make it mandatory.”
However, others were doubtful of the effectiveness of this strategy and some farmers as well as veterinarians raised concerns. Another veterinarian noted:

“I think, if you convince farmers, that it’s saving them money, compliance would be really good. If they feel like they’re wasting their time, but they are forced to, because it’s part of their industry, then compliance is real low.”

Consumers were perceived as influential by the farmers, as their concerns were considered to have an impact on on-farm management. Farmers were concerned about the influence consumer opinion could have on the sale of dairy products and pointed out the importance of addressing consumer concerns in a timely fashion.

“You know any time you talk about a disease, in a food animal, consumer perception is strange [...] we need to work with that. Every time we can eradicate or control a disease, I mean we are better off and the whole industry is better off...”

However, in some instances, consumer perception was also identified as a barrier for the implementation of some BMP, for example the timely removal of the calf from its dam after birth. Besides the industry and consumers, the veterinarian was identified as one of the most important influences on farm by the farmers, but also by the veterinarians themselves. Animal health as well as biosecurity were considered to be well understood by veterinarians, and farmers relied on their opinion and advice. Veterinarians were also found to have an influence on the farmers’ problem perception:

“I think if the veterinarian is really interested in something, I think it’s pretty natural that the producer gets really interested in it too.”

However, veterinarians that were not in favor of changing their examination gloves for pregnancy exams were barriers in the implementation of BMPs. One farmer said about this topic:

“But I mean, we’ve had that discussion with our vet multiple times and I don’t think in their minds they really believe it’s gonna make a whole lot of difference.”
Additionally, some veterinarians were perceived as discouraging BLV control by some farmers, because they reported stories about unsuccessful BLV control and other farmers’ doubts of the importance of BLV control. Nonetheless, the lack of good on-farm data, as pointed out by the veterinarians, or the refusal of farmers to share test results with the veterinarian could further complicate the effective implementation of change. One veterinarian mentioned:

“The one thing that happens with the milk testing, is that, you know, the decision to do that and which cows to do, quite often rests with the producer rather than with the veterinarian and so, we lose track a little bit, […] of monitoring the herd results with that.”

Interestingly, when farmers were asked directly whether their peers influence their behavior, the majority answered with no. However, during other discussions, peers were mentioned in an influential role. For example, one farmer pointed out that:

“If you hear good news, sure, you might implement it. If you hear bad news, well you’re gonna avoid it...”

5.4.3.2 Theme 2: Introducing change

Knowledge

When introducing change, knowledge was generally considered important. Veterinarians and farmers were quick to point out that education and discussion about a topic can be used to provide reason, raise awareness, and create motivation to change. Additionally, it was considered important to create realistic expectations, based on knowledge. These veterinarians noted:

“And again, going through with more education, if the program did come out and talking to them I think that would be enough to persuade a very large part of them. So, cause a lot of times they need a reason as to why they are doing something. So, I think education to a certain degree will help compliance.”
“And another thing I really stressed, too, is, you have to make them understand, why it takes a long time to get rid of it. I think that was really helpful.”

Additionally, it was made clear that a lack of knowledge or information can be hindering the process, by veterinarians and farmers.

**How to introduce change**

When change is introduced, it was pointed out by some veterinarians that sudden and extreme changes rarely result in success. On the other hand, gradual change, as well as the implementation of easy, cheap, and incremental changes were considered to help implement change successfully by veterinarians and farmers.

Two veterinarians summarized:

“I like to make it simple. To do one thing at a time, right?!"
“Cause no one likes a black and white change over night”

This was echoed by a farmer and his wife:

“Well, it’s change. People don’t like change.” – “Exactly. So, if you make that step not too high, they can slowly [adapt]… “

However, different farmers perceived different things as easy. For example, one farmer considered changing needles in the milking parlor as very easy, whereas other farmers considered it to be one of the most difficult BMP to implement. Additionally, some farmers also identified the lack of and need for specific, individual standard operating procedures (SOPs). These were considered helpful when introducing change and the lack thereof was considered an important barrier. This farmer explained:

“I think that’s what some of the downfall was, of the Johne’s problem. They were talking about, you should feed, this kind of colostrum to your calf right away, but they forgot all those steps in between. They never bothered coming up with best management practices for the colostrum. So, everybody did something, they thought that was the best […].”
5.4.3.3 Theme 3: Implementing change

Habits and barriers vs. BMPs

It was oftentimes pointed out by the farmers that overcoming old habits was a significant barrier in the implementation of change. One farmer was able to highlight this:

“If humans were only a little more adaptable to change. It would make things easier.”

The additional effort that was necessary for the implementation of some BMP was commonly mentioned as a hindering factor. Some farmers mentioned that even the thought of extra work was a barrier. One farmer’s response to the question why they had not controlled BLV before the trial summarized that:

“Because it’s such a hassle.”

Some farmers pointed out that their own laziness and forgetfulness acted as barriers as well. Additionally, while some BMPs only took a little more effort than old habits, as pointed out by veterinarians and farmers, other BMPs were considered impractical by some farmers. In addition, some farmers were not convinced of the effectiveness of the BMP, had negative experiences with suggested BMP, or feared the side effects. One farmer pointed out for example:

“Well, we’re trying to find an issue to fight the pneumonia in calves. And we were kind of thinking, maybe the antibodies in the colostrum, the maternal, would be better than the replacer.”

The lack of technical service, extra time and/or manpower was considered a complication. Farmers also pointed out that their facilities couldn't be adapted to implement some BMPs. One farmer explained that:

“[…] I really haven’t had too much of time to really implement anything.”
Moreover, the involvement of third parties on farm was identified as a significant barrier to change by veterinarians and farmers. For example, convincing the hoof trimmer to disinfect knives between cows was met with concern. However, some farmers also considered the veterinarians to be a problem. These farmers pointed out:

“So, if you don’t have to ask the vet or the hoof trimmer, that would be better.”
“[… you can’t really tell them what to do.”

Similarly, employees’ compliance with BMP was considered a challenge to some farmers. However, being prepared to implement BMP (e.g. have all necessary equipment at hand) was identified by the farmers as an important way of making change easier. Additionally, it was pointed out by farmers and veterinarians that repetition of the same information by the same, as well as by different sources, and regular reminders were an effective measure to introduce change and establish new habits. A farmer summarized this:

“The more people encourage you, the more you get onto it.”

Regular risk assessments were considered good reminders and were considered a useful tool to implementing change by some farmers as well as some veterinarians, although different information from different sources can be inhibiting change. Similarly, some farmers pointed out that some control programs were designed to be too focused on a complete buy-in of every participant and that control programs should be designed with a more individual approach. One farmer noted:

“And I think you keep a lot more producers interested in those kind of programs, if you’re more of that happy go lucky kind of thing, ‘yeah, you’re making progress. Not as fast as you could, but you’re not spending money, you’re not spending a lot of time, but things are going a lot better in 10 years down the road.’”
Similarly, the feeling of having almost achieved holistic BLV control, helped implement more changes, as mentioned by this farmer:

“Well, the thing is that - that’s the way I see it anyways - a lot of the things we’re already doing. We’re basically, pretty much doing, so just go a little bit further and do it right. It doesn’t take that much more effort, because I think [...] we’re already 75% there.”

Additionally, realising that by implementing one thing, multiple infectious agents were fought was also considered a motivating factor by the farmers:

“This is one disease we are targeting, but it’s not the one disease we are fighting.”

Overall, veterinarians and farmers agreed that changing habits is difficult, but once they are changed, it requires little effort to continue. These two farmers noted:

“It doesn’t make a difference. You still do it, just do it different.”

“Needles are kind of a pain, but, much of the same thing - once you get in the habit of doing it it’s not that big of a deal”

Additionally, if farmers could track their achievements, motivation to long-term behavior change seemed to be possible. A veterinarian talked about his personal experience:

“Yeah, but they can see it on the results, too. It’s you know, all the positive results first and if you tell them about the disease, over time they see less and less and it’s just, it’s I guess it’s quite satisfying for them.”

Finally, one veterinarian summarized the process:

I think you have to set out with a program, right. So, step-by-step program. It has to be as management-friendly, as you possibly can. So that it’s just a simple thing to do and then it has to become habit to them. [...] As a veterinarian you need to reinforce it. Ask the same question time and time again. And they’re gonna say, you asked me that before, [...] but I think that’s what you need to do. Some of these things are veterinarian driven and so, I think that’s probably... And you know, it’s... the milk industry itself can play a very positive role in this.
5.5 Discussion

By investigating farmers’ motivators and barriers before the design of a BLV control program, the control program could be adjusted accordingly. Subsequently, by accompanying the farmers during the introduction as well as implementation of an on-farm BLV control program, a deep understanding of relevant individual motivators and barriers could be achieved. The combination of these components with the veterinarians’ point of view, created a unique dataset.

The goal of this study was to investigate motivators and barriers to consider, implement, and continuously apply BLV control measures on farm. Based on previous research it was expected that the investigated topics were highly amenable to inductive inquiry and likely difficult to understand using more traditional quantitative methods (reviewed by Ritter et al., 2017). Therefore, qualitative methods were chosen. They allow for open conversations and the interviewee to reflect on personal experience, opinions, and attitudes, while facilitating the identification and exploration of internal drivers, therefore resulting in a deeper understanding of the participants’ perspective (Braun and Clarke, 2006). Even though generalizability of the findings is difficult using qualitative methods, a more detailed understanding of internal and external drivers was considered important for the successful implementation of the newly developed BLV control program (Braun and Clarke, 2013). Additionally, because of the sampling strategy, a selection bias was introduced. The voluntary participation of all participants could indicate a higher personal interest in BLV and its control than the average dairy farmer, and therefore findings may not always be applicable to other farmers. However, less than half of the participating farmers in the focus groups had implemented any BLV control on their farms. Moreover,
all participants in the trial implementation were asked to implement additional BMP. Therefore, different barriers and motivators were encountered and discussed. In addition, farmers that had implemented BLV control measures could elaborate on their motivators, barriers, and possible solutions. Similarly, the veterinarians interviewed for this study interact with a variety of producers and were able to report on many different attitudes, opinions, and beliefs that they encounter in their everyday work. Therefore, the results of the study presented here were considered to be applicable to many producers and the results could be useful for BLV and other disease control programs.

During the analysis, we identified a progression of three steps, when trying to change behavior on farm. At first, change was initiated: Awareness was raised through different sources, like the veterinarian, peers, or industry outlets. The personal attitude then seemed to decide whether the farmer was interested or not. This personal attitude in turn was dependent on many factors. For example, the importance of constant improvement of the operation, the joy in teamwork, or the on-farm culture. Additionally, experience with the disease, as well as the economic and quota situation could play a role in the prioritization of the problem. Secondly, once the farmer identified a certain topic as important, the introduction of change began. Farmers frequently sought advice or advisors took the initiative and introduced options for change. The introduction of change seemed to be highly dependent on knowledge about the issue as well as the way the change was introduced. Providing knowledge and communicating realistic expectations seemed to be crucial. Additionally, by introducing incremental (small, easy, inexpensive) changes, the compliance of farmers was considered to be a lot higher, than by introducing changes that were perceived as big and daunting. Thirdly, the actual implementation of
change seemed dependent on whether the farmer considered the BMP feasible and whether he/she was able to overcome old habits as well as other barriers, like bringing up the necessary extra effort or overcoming doubts in the effectiveness of BMP. Similar patterns have been described in other behavior models, like the Health Belief Model, or the Theory of Planned Behavior (Janz and Becker, 1984, Ajzen, 1991). In summary, even though there are some differences in the details, both mentioned models claim that, before a behavior is performed, the farmer would have to acknowledge a problem, perceive it as important enough to be solved, and consider him/herself able to solve the problem (self-efficacy/perceived behavioral control). The Theory of Planned Behavior also includes the intention of changing a behavior before the actual behavior change. The Theory of Planned Behaviour was used to successfully explain English and Welsh dairy farmer behavior, including their motivators and barriers in relation to zoonotic control programs (Ellis-Iversen et al., 2010). One of the most important identified barriers was the absence of belief in self-efficacy, whereas the veterinarian was mentioned as an important motivator. However, they found that even though the general attitude toward the implementation of zoonotic control programs was positive, some farmers were lacking the intent to change their behavior. Therefore, when introducing change, it should be kept in mind that the fulfilling of the three mentioned prerequisites is crucial to the successful implementation of change.

When further analyzing barriers and motivators in our study, some of the identified themes seemed to have a more general applicability than others. For example, that economical information may influence farmers’ decision making was considered an obvious conclusion. Similarly, the economic impact of Johne’s Disease or mastitis to a
farm was an important driver for Canadian dairy farmer behavior (Valeeva et al., 2007). At the same time, financial investments were indicated as an important barrier to overcome. Similarly, financial motivation was more important for farmers that intended to implement zoonotic control measures on farm than for farmers that didn’t have that intent (Ellis-Iversen et al., 2010). In the study presented here, there seemed to be an individual threshold for the financial burden that was acceptable. Even though the economic benefit of controlling BLV was demonstrated in multiple studies (Rhodes et al., 2003, Kuczewski et al., 2019), some farmers seemed reluctant to make investments. When the investment was comparatively small, implementation was perceived easier. It was pointed out that the perceived benefit of a control measure had to outweigh the cost before an investment was considered. However, it was found that a benefit was not always measured in financial terms, but improvements in other areas, like disease prevalence or calf health, for examples, were considered benefits as well. Similarly, financial benefits only made up a small portion of the motivation to implement change on farm (Gramig et al., 2010): The developed model could only explain parts of the farmers’ behavior change with financial motivation, meaning that other motivators were important as well, but they were not further identified. Similarly, dairy farmers that participated in a voluntary Johne’s Disease control program in Ontario who implemented BMP did not only save money and time but also improved general calf health (Sorge et al., 2010). Therefore, when introducing change to a farm in order to control a certain disease, it would be recommendable to introduce the easiest and/or most efficient changes first, track progress to demonstrate the effectiveness of control measures, and introduce changes that require larger investments and/or more effort later on.
Additionally, the industry as a whole was pointed out by participating farmers and veterinarians as an important motivator in this study. This could be explained by a constant stream of information on a certain topic, thereby creating a sense of importance and priority (Jansen, 2010a). Additionally, the establishment of mandatory programs, by the proAction initiative, for example (Dairy Farmers of Canada, 2019), could force farmers to implement new BMP. Canadian dairy farmers participating in focus groups about Johne’s Disease identified proAction as an option for mandatory Johne’s Disease control as well (Roche et al., 2019). Nonetheless, the establishment of mandatory programs generally causes behavior change without an attitude change. Consequently, once the mandatory program expires, farmers tend to stop the encouraged behavior. A similar result could be seen with Johne’s Disease control programs: when financial support was no longer available, participation rates abruptly declined (Barkema et al., 2018, Roche et al., 2019). Therefore, efforts of the industry to increase BLV control should aim at raising awareness and creating internal motivation and an attitude change.

In the study presented here, veterinarians and producers pointed out that knowledge was considered to play an important role in behavior change. It could equip the producer with the necessary information to understand a problem, gage its importance, and make informed decisions. Additionally, a clear understanding of how to introduce certain changes, for example based on well written SOPs, seemed to be of great importance to participating farmers. Ellis-Iversen et al. (2010) identified the lack of knowledge as one of the most important barriers for English and Welsh farmers that were meant to introduce zoonosis control measures on farm. Similarly, dairy farmers participating in a voluntary Johne’s Disease control program had higher self-assessed knowledge than non-
participants (Ritter et al., 2015). Knowledge about the problem, but also about the solution, could provide the producer with a sense of control and could create the perception of being able to solve the problem. This could increase the motivation for change and increase the chances of the change actually happening (Ajzen, 1991).

Another finding of this study was that even though there were some factors that seemed applicable to many farmers, it was noticed that no two participating farmers were motivated or hindered by the same combination of factors. Additionally, the importance of factors varied substantially from farmer to farmer. For example, religious faith was identified as a deciding factor in some farmers’ behavior, but has not been identified in other studies yet. In the study presented here, it seemed that categorization of farmers would not capture the introduction of change on farm appropriately. Even though other researchers have indicated that understanding individual’s motivators and barriers was important, when trying to change behavior, farmers were grouped based on similar traits (Jansen et al., 2010b, Ritter et al., 2016). However, some farmers did not fit in any group. Therefore, in order to create on-farm change it was considered instrumental to understand the individual farmer’s motivation. By understanding behavioral drivers, it would become possible to cater to his/her needs.

Once change was introduced, follow-up, reminders, and receiving the same information from different sources was perceived to be very valuable to encourage long-term behavior change. Ultimately, change in daily routines and habit could be considered very daunting by the farmer, possibly resulting in not implementing anything at all. Dairy farmers in the Netherlands considered it easier to make investments compared to changing habits in order to control mastitis (Huijps et al., 2008). Similarly, farmers
participating in a voluntary Johne’s Disease control program in Ontario found breaking old habits the second biggest barrier when implementing new BMP (Sorge et al., 2010). Therefore, follow-up, understanding, and addressing barriers was considered crucial for implementation and continuation of changed behavior for the farmers in this study. Additionally, it was important to notice that the involvement of external professionals (e.g. hoof trimmer, herd veterinarian) on farm was oftentimes considered a significant barrier by participating farmers, as requests to these groups seem difficult. Additionally, implementing and monitoring the compliance to new BMP by personnel was perceived difficult as seen in other studies (Sorge et al., 2010). This could be improved by changing communication on farm by using relational coordination (Gittell et al., 2010). Improving communication between the herd advisors, the farmer, as well as the employees, could increase every individual’s knowledge, reinforce mutual respect, as well as create common goals that all involved parties want to strive for.

Veterinarians and farmers did not always identify the same motivators and barriers, and farmers highlighted some factors not mentioned by the veterinarians. Teamwork seemed to be valued by producers and could result in highly productive and feasible solutions, but was not emphasised by the veterinarians. Similarly, teamwork was considered an important motivator for Danish dairy farmers for the implementation of health management programs, whereas veterinarians considered economic reasons as more important for the farmers (Kristensen and Enevoldsen, 2008). Additionally, the doubt in the effectiveness of BMP or inconsistent information from different sources was identified to be demotivating to the farmers (Garforth et al., 2013). This could cause insecurity for the farmer, leading to decreased motivation to implement new BMP,
thereby increasing the likelihood that BMP are not implemented at all or are adhered to less strictly. The insecurity toward BMP could be aggravated, if veterinarians did not inquire about the implementation of recommended BMP, or if the farmers chose to not communicate the implementation of new BMP or difficulties with BMP that were recommended to them. Both possibilities were mentioned in some way during the conversations described above. Veterinarians have been trained to address the farmers’ problems and respond to farmers’ needs, but the implementation of new practices, i.e. the solution to a problem, is oftentimes dependent on the farmer. If the farmer doesn’t consider changing his/her behavior important, or if the veterinarian is not aware of important barriers and barriers, the likelihood for change is very minimal. Therefore, communication between veterinarians and farmers has to be optimal in order to address barriers and motivators alike (Kristensen and Enevoldsen, 2008). One way of improving the understanding between farmers and veterinarians, could be by adjusting the way of history taking. Instead of only focusing on the problem presented, the farmer’s context could be taken into account by asking open-ended questions, listening, showing empathy, and by giving the farmer a chance to voice his opinions and concerns (Coe et al., 2008, Ritter et al., 2019). Additionally, another study showed the consequences of suboptimal communication within a small animal medicine setting (Nogueira Borden et al., 2019): By not taking the patient-owner’s context into account, treatment plans were considered unrealistic by the patient-owner, resulting in less than optimal care for the patients. Therefore, it was considered important to identify possible barriers in the patient-owners’ lives, in order to find solutions and guarantee optimal patient care. Similarly, the farmers in the study presented here pointed out that on-farm control programs were oftentimes
designed without the participating producers in mind. Producers are frequently unable or unwilling to participate fully, generally due to external barriers (Sorge et al., 2010, Bruijnis et al., 2013). Through a constructive team approach and applying creative problem solving (Huijps et al., 2009), focused on the goals of the producer, a realistic set of BMP could be identified, resulting in successful disease control. This is an important finding and could result in significantly less frustration for the veterinarians, when trying to convince “hard-to-reach-farmers” (Jansen et al., 2010b) and more successful implementation of control programs.

5.6 Conclusion

In conclusion, this study was able to add valuable information specifically about on farm BLV control and the involved motivators and barriers, but also about the process of behavior change on farm. By using an inductive approach, important motivators as well as barriers that weren’t found before, and their importance in behavior change could be identified (e.g. faith, milk quota). Farmers and veterinarians were able to identify almost the same motivators and barriers. However, some important topics were missed by the veterinarians. By increasing communication efforts, the understanding between farmers and veterinarians could be improved, thereby achieving the successful implementation of disease control programs on farm more efficiently. Therefore, veterinarians could increase their efforts to understand farmers’ individual situations and adjust their advice to specific farmers’ needs. Similarly, farmers could put more emphasis on communication with their herd veterinarian, but also with their employees and other external parties that work on farm (e.g. hoof trimmer). By better understanding each
others’ barriers and motivators, teamwork can be successful, and problems can be identified and solved before they can cause issues on farm.
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Figure 5-1. Outline of all events whose results are described in this chapter.
Rectangles represent events, while circles represent parts of the bovine leukemia virus control program development. The pentagon represents farmer motivation and barriers.

- **Focus groups with dairy farmers**
  - N=4 focus groups
  - April & May 2017

- **Interviews with veterinarians**
  - N=11 veterinarians
  - September & October 2017

- **Trial implementation of BLV control program**
  - N=11 farms
  - January 2018 – June 2019

- **Understanding of practical applicability of BLV control**

- **Adjustment of BLV control program**

- **Understanding of farmers’ motivators and barriers for on-farm change and BLV control**

- **Final adjustment of BLV control program**
Figure 5-2. Outline of bovine leukemia virus (BLV) control program trial implementation.

I. Interview & risk assessment

II. Sampling

III. Phone interview & recommendations

IV. Interview ~3 months after III

V. Interview ~6 months after III

VI. Sampling ~13 months after II

VII. Final interview
Figure 5-3. Representation of themes and subthemes identified in this study and concerning on-farm behavior change

- Initiating Change
  - BLV
  - Economics
  - Quota
  - Animals
  - Improvement
  - Personal Attitude
  - Teamwork
  - On-Farm Culture

- Introducing Change
  - Knowledge
  - Community
  - How-to

- Implementing Change
  - Habits and Hurdles vs. BMPs

- Attitude
  - Teamwork
  - On-Farm Culture
CHAPTER 6: Alberta dairy farmers’ and veterinarians’ opinion about bovine leukemia virus control measures

6.1. Abstract

A high herd and within-herd prevalence of Bovine Leukemia Virus (BLV) infections in the dairy herds of North America and the negative effects thereof caused the Alberta dairy industry to initiate the development of an on-farm BLV control program. Because BLV control is dependent on the commitment of the farmer, potential barriers were meant to be identified and farmers’ and veterinarians’ point of views toward different control options were meant to be understood, thereby allowing for the adjustment of the control program. Conversations with these stakeholders were sought and four focus groups with farmers as well as interviews with eleven veterinarians were conducted. Testing for BLV, the most common BLV control strategies (culling/segregation/management), as well as on farm best management practices (BMP) to prevent the transmission of BLV were discussed. The thematic analysis of these conversations resulted in the following findings: Testing of animals was considered important for BLV control, but the financial investment was prohibitive for farmers. Test and cull as well as test and segregation approaches of test positive animals were considered efficient BLV control measures, but impractical and not feasible in the current Alberta situation. The management of test positive animals by implementing BMP was considered the most realistic BLV control strategy. The most important barriers for suggested BMP were the cost for some BMP, the inconvenience of performing other BMP, as well as difficulties in performing some BMP consistently and well.
Additionally, a lack of knowledge about BLV and its control were identified as an important barrier. On the contrary, it was found that farmers were inclined to implement BMP they considered feasible or that were considered a standard within the industry. Additionally, if BMP increased convenience on farm, they were considered easy to implement. Farmers and veterinarians agreed in many, but not all cases. For example, the single use of examination sleeves was met with differing opinions. In conclusion, it was found that stakeholders’ knowledge about BLV and its control has to be improved in order to increase awareness. In addition, by communicating and understanding barriers and motivators for specific BMP, important barriers could be identified and solutions found, thereby improving BLV control efforts on farm.

6.2. Introduction

Infection of dairy cattle with Bovine Leukemia Virus (BLV) leads to reduction of milk production and cow longevity. Additionally, in some infected animals, this retrovirus can induce fatal cancers in the form of Enzootic Bovine Leukosis or EBL (Schwartz and Levy, 1994, Bartlett et al., 2013, Norby et al., 2016.). Due to the high herd (~90%) as well as within-herd (~40%) prevalence in North America, EBL is a common diagnosis (LaDronka et al., 2018, Kuczewski et al., 2019). Farmers indicate that this frequent occurrence of EBL is a strong motivator to implement BLV control. Moreover, research conducted during the past decade made clear that there is a significant negative impact of subclinical BLV infections (Erskine et al., 2012a, Bartlett et al., 2014, Nekouei et al., 2016). Therefore, the Alberta dairy industry initiated the development of a BLV control program, as no successful formal control program has been established within Canada to
date. Generally, when new on-farm disease control programs are developed, researchers and veterinarians combine their efforts. Then, farmers are informed and expected to implement the suggested practices. However, disease control programs oftentimes fail, mainly when recommended best management practices (BMP) are not implemented rigorously or consistent enough for success (Bell et al., 2009, Jansen et al., 2009, Pieper et al., 2015). Recently, studies were conducted to understand why farmers failed to implement recommended BMP (Sorge et al., 2010, Ritter et al., 2016, Roche et al., 2019). Many internal (e.g. teamwork, practicality) and external (e.g. knowledge, public concerns) motivators and barriers were identified in this research (Kristensen and Enevoldsen, 2008, Ellis-Iversen et al., 2010, Roche et al., 2019). Additionally, it was suggested that the implementation of disease control measures was less likely when voluntary, generic disease control programs were offered, compared to farm-specific recommendations (Jansen, 2010, Ritter et al., 2017). The latter approach was used for Johne’s Disease control programs in Canada: Risk assessments on farm were followed by specific recommendations, which resulted in on-farm management changes leading to a decrease in within-herd prevalence (Sorge et al., 2011, Wolf et al., 2015). However, even though these voluntary disease control programs had a positive outcome for participating farmers, communication with participating and non-participating farmers after the large-scale implementation of these voluntary control programs identified important drawbacks (Sorge et al., 2010, Ritter et al., 2015, Roche et al., 2019). Addressing these drawbacks beforehand might have increased the number of participating farmers and caused a higher success rate. Consequently, throughout the development process of the new BLV control program Alberta dairy farmers were included through focus groups and interviews in
order to mitigate concerns and ensure their support. In addition, on farm management is strongly influenced by the farms’ veterinarians (Garforth et al., 2013, Ritter et al., 2015). Therefore, the veterinarians’ opinion, experiences, and expertise with BLV control as well as in guiding behavior change on farm were considered highly valuable and included in the study.

In summary, to ensure the future success of the implementation of a voluntary BLV control program, the goal of this study was to understand dairy farmers’ as well as veterinarians’ opinions about different BLV control measures.

6.3. Materials and Methods

6.3.1 Strategy

The Alberta dairy industry initiated the development of a BLV control program in collaboration with a team of researchers from the University of Calgary. The goal of the collaboration was to develop a BLV control program that is adjustable to farm specific circumstances. To achieve this goal, a risk assessment tool was developed aimed at identifying farm practices which have a high probability to transmit BLV between animals. This formed the basis of the action plan that could be tailored to individual farms and their farmer’s needs.

To start, BLV transmission routes were identified based on existing literature (contact, blood, colostrum, milk), followed by identifying common on-farm practices that could cause transmission through each of these routes. Based on these findings, possible control measures in the form of BMP were compiled. In the next phase, communication between the first author and farmers as well as veterinarians was sought. Farmers and
veterinarians were invited to provide feedback on an array of BLV control approaches. Communication with farmers and veterinarians was facilitated using focus group meetings with small groups of dairy farmers and interviews with veterinarians. All focus groups and interviews were conducted by the first author. These sessions were designed to clarify which BLV control measures were considered (not) practical and feasible on farm, as well as to identify important barriers for implementation for farmers and veterinarians. Based on these findings, the pre-existing BLV control program for Alberta was adjusted to reflect the needs and preferences of farmers and veterinarians.

The project was approved by the Conjoint Faculties Research Ethics Board (CFREB) of the University of Calgary (REB16-0224).

6.3.2 Focus groups with farmers

Four focus-group sessions were organized and conducted in Alberta (Leduc, Red Deer, Lethbridge, Taber) from March to May 2017. All Alberta dairy farmers were invited to attend: Focus groups were advertised during dairy farmer focused events such as the Western Canadian Dairy Seminar (Red Deer, AB, Canada), for example. Additionally, an invitation was printed in the Milking Times magazine, published by Alberta Milk (Edmonton, AB, Canada). Farmers were asked to provide their contact information as well as to register for the focus groups in advance to facilitate further planning. During all focus groups, the practicality, feasibility, and challenges of different BLV control measures were discussed. The goal was to understand which control strategies as well as which BMP were considered feasible or not. Additionally, farmers were asked to explain their reasoning. All focus groups followed a semi-structured focus-group guide that
consisted almost exclusively of open questions (Morgan and Krueger, 1998). Additionally, all participants were asked to perform two paper-based ranking exercises (Morgan and Krueger, 1998). The first ranking exercise focused on the perceived ability of farmers to implement different BLV control measures. The second ranking focused on the perceived importance of the same control measures for the transmission of BLV between animals. Focus group guide and ranking exercise can be found in Appendix I.

### 6.3.3 Interviews with veterinarians

Nine interviews with dairy veterinarians from Alberta, Canada were conducted between September and October 2017. Veterinarians within Alberta, with a focus on dairy farming and within a 4-hour driving distance from Calgary were eligible. These veterinarians were contacted directly and invited to participate. Invitations were sent via e-mail when e-mail addresses were publicly available, alternatively or if there was no response after 1 week, the clinic was contacted by phone. During the phone calls, a short summary of the interview topics was provided, and veterinarians could accept or decline the invitation. When the invitation was accepted, a meeting was arranged. In semi-structured interviews (Braun and Clarke, 2013), the veterinarians’ experience with BLV control on farm was discussed. The goal was to understand which, from the veterinarians’ perspectives, control measures were consistently implemented by farmers as well as which of the control measures were considered challenging. All interviews followed an interview guide that consisted almost exclusively of open questions. Additionally, all veterinarians performed the same ranking exercises as the farmers. Interview guide and ranking exercises can be found in Appendix I.
6.3.4 Data analysis

All focus groups and interviews were transcribed verbatim and compared back to the original recording to ensure accuracy and descriptive validity (Braun and Clarke, 2013). All further analyses were done with ATLAS.ti (Scientific Software Development GmbH, Berlin, Germany) and Microsoft Word (365 ProPlus, Microsoft Corp., Redmond, WA). A thematic analysis was performed on all transcripts. First, a conductive analysis was performed: BLV control is dependent on specific control measures. These measures were discussed during the focus groups as well as the interviews, and were used as initial, holistic codes (Saldana, 2016). Subsequently, an inductive approach was chosen within the initial codes to identify more specific codes. Finally, findings from focus groups with farmers and interviews with veterinarians were summarized and compared.

6.4 Results

6.4.1 Focus groups with farmers

Focus groups were between 4 and 6 hours long and were held in Lethbridge (3 participants), Red Deer (2 participants), Leduc (9 participants), and Taber (10 participants). Participants consisted of 22 males and 2 females, who were born between 1950 and 1990. Aside from one manager of a research herd and one feed specialist, all were dairy farmers. The average herd size of the participating dairy farmers’ herds was 139 milking cows (60-390) with an average milk production of 36 kg per day (20-40 kg/day). Nine farmers had already implemented some BLV control measures, whereas 14 farmers thus far had not.
6.4.2 Interviews with veterinarians

In total, 11 clinics were contacted. Of those, 11 veterinarians from 9 clinics participated. Interviews were conducted with individual veterinarians, except for one clinic, where all three veterinarians participated. The interviews were between 1 and 3 hours long. Ten of the interviewees were male, one was female, and all were born between 1940 and 1990. Their clientele consisted of 25% to 100% dairy farms. All veterinarians had worked with farmers who implemented some BLV control on their farm.

6.4.3 Data analysis

BLV control is often based on testing of all or some animals of the herd. Depending on the test results, different strategies and BMP might be pursued. The control strategies determine the fate of the positive animals. Within a strategy several BMP can be implemented. These BMP aim to prevent the transmission of BLV. However, many BMP can be implemented without testing.

Therefore, in the following sections farmers’ and veterinarians’ opinions about different components of a BLV control program will be presented: 1. Laboratory testing for BLV, 2. the three most common control strategies (test and cull, test and segregate, as well as test and manage), and 3. Individual BLV-specific BMP. An overview of the results can be found in Table 6-2 and 6-3.

6.4.4 Testing

BLV testing is commonly done using ELISAs with a high sensitivity and specificity (Kuczewski et al., 2018). During the focus groups and interviews, different approaches for BLV testing were suggested: 1) ELISA testing all female animals on farm that are older than 12 months using blood and/or milk samples. This approach allows for the most
accurate estimate of the within-herd prevalence as well as individual animals’ test results.

2) ELISA testing a subset of animals. This latter approach provides some flexibility and allows for an estimation of the within-herd prevalence. For example, Erskine et al. suggested to test a group of 40 animals (2012b), whereas the participating farmers and veterinarians suggested to only test heifers. Participating farmers felt that by only testing heifers the effect of implemented control measures could be monitored without having to invest in testing all adult animals.

Generally, testing was considered important by farmers and veterinarians, because it allowed the adjustment of control measures, could help with culling decisions, and monitor progress. One farmer for example mentioned:

“To see how much you want to invest into it [...]. Do I have to get a pasteurizer now or if it’s not that prevalent, maybe I don’t need it. I’d still like to get an idea.”

However, cost for sampling was identified as an important barrier. Consequently, testing the entire herd was often considered important, but not feasible. Testing a subgroup of animals was considered more realistic. Alternatively, farmers and veterinarians argued that free testing should be offered by the industry to increase control efforts by farmers.

On the other hand, one farmer argued that testing for BLV is unnecessary, as BLV control can be done efficiently without testing:

“Because we’re pretty sure that all farms have Leukosis and it really doesn’t change anything in your preventive program. So you don’t have to spend the money if you don’t want to. [...], but you can have a beautiful and effective program without any testing, if you want.”

Additionally, using milk ELISA testing was considered an important option. Farmers and veterinarians were interested in the comparative performance of milk and serum ELISA, but agreed that milk samples would be easy to use for milking animals. However, some
veterinarians voiced concerns, because farmers could initiate the testing and obtain the results without the involvement of a veterinarian. This could cause the veterinarians to be unaware of efforts taken by the farmers to control BLV, as mentioned by this veterinarian:

“The one thing that happens with the milk testing, is that, [...], the decision to do that and which cows to do, quite often rests with the producer rather than with the veterinarian and so, we lose track a little bit, [...], of monitoring the herd results with that.”

6.4.5 Test and cull

When pursuing the test and cull strategy, the goal would be to test the entire adult herd in regular intervals and cull animals with a positive BLV test result. Overall, this was not considered a feasible option by farmers and veterinarians. Most farmers felt that their BLV within-herd prevalence was too high to cull test positive animals and remain economically viable at the same time. However, some farmers as well as veterinarians argued that culling might become an option once the within-herd prevalence decreased to a low level or if culling BLV positive cows would be made mandatory. This farmer mentioned, for example:

“But that’s not going to happen. People aren’t going to cull animals for that. Unless you have one or two. That’s different. But not if you have 60 or 70%.”

Interviewed veterinarians also considered culling to be an efficient way to reduce the within-herd BLV prevalence. Additionally, because of the supply managed system in Canada, culling rates were considered highly dependent on quota fluctuations. When quota decreased, culling was considered to become easier, because less milk would have to be produced and vice versa. Two participating farmers noted that BLV positive cows
were added to their list of cows that could be culled, but weren’t culled immediately. This farmer, for example explained:

“You might not breed them back. Like our cull list is cows we don’t want to breed back, [...]. We still milk them till they quit milking [...]. Those are our culls. It all depends on where you’re sitting on your quota and how many of them replacements you’ve got sitting around and that all influences your cull rate”

6.4.6 Test and segregate

The test and segregate strategy would be based on testing adult animals regularly and segregating test positive from test negative animals, either in a separate pen in the same barn or in a separate barn. Most farmers and veterinarians considered this as an efficient measure to prevent transmission of BLV. Some farmers considered it a possibility, if BLV control had a high priority on farm. Like this farmer for example:

“If you really focus on it, it’s always possible [...]. You could put a few gates in and make a separate pen, [...].”

However, the concept was generally met with concerns as cows were oftentimes grouped based on other characteristics (e.g. age, milking speed). Subdividing these established groups or reorganizing the groups was considered impractical, and not feasible because of the associated increase in workload. This farmer mentioned:

“If it makes sense, it makes sense, but for you it’s totally unpractical.”

Other farmers could not create separate groups for their adult herd because of building restrictions. The veterinarians agreed with the farmers, but suggested to visually mark positive animals with, for example, ear tags instead.

6.4.7 Test and manage

The test and manage strategy would be based on testing the herd on a regular basis and adjusting the management of the herd accordingly. This adjustment could either be done
based on test results or based on the assumption that all animals are test positive and able to infect other animals. Therefore, testing is not necessary for this strategy. Farmers and veterinarians supported this approach, as it would allow the farmer to keep all animals within the existing structure while reducing virus transmission.

*Best management practices*

This section will report on the different BMPs that were considered during the conversations.

**Bull breeding.** Bulls are considered a possible source of infection with BLV for cows, if they are used for natural breeding (Zalucha et al., 2014, Benitez et al., 2019b). Therefore, a suggested BMP for the control of BLV is to not use natural breeding, but to use artificial insemination (AI) exclusively. Most participating farmers used AI, sometimes in combination with a bull in order to breed animals that did not conceive or miscarried following AI (‘clean-up bull’). Notably, within the ethnoreligious group of Hutterites, the use of AI is not an option due to cultural reasons. Farmers and veterinarians agreed that discontinuation of natural breeding within Hutterite managed herds is almost impossible. Similarly, replacing the clean-up bull was considered difficult. The convenience of having a bull was considered more important than the risk of BLV transmission. However, farmers and veterinarians agreed that testing bulls for BLV before purchase should be part of the prevention strategy to avoid the introduction of BLV to the farm. This veterinarian mentioned:

“I test the bulls [...] And then the bulls which are positive we send it back to the seller.”

**Calf management.** The ingestion of colostrum from BLV positive dams is a possible route of infection for calves (Ruiz et al., 2018). In order to prevent newborn
calves from taking up any colostrum, they have to be removed from the dam shortly after birth. This BMP is also important for the prevention of transmission of other diseases (Wolf et al., 2014). Farmers and veterinarians were aware of this BMP, understood its importance, and it was oftentimes part of the farms’ SOPs. The Alberta Johne’s disease initiative (AJDI; Ritter et al., 2015) was mentioned as an important reason for the common implementation of this BMP. This farmer, for example said:

“Through the Johne’s program, a lot of farmers have started to take the calves off within an hour or two. That wouldn’t mean a big change […].”

However, farmers and veterinarians agreed that removing calves timely and consistently was difficult. Even though it was desired for numerous reasons, it was not always possible to do. The two most mentioned restricting factors were calving during the night, as well as a shortage of manpower. Especially on family operated farms, where only limited manpower is available, calf removal could lose priority, for example during harvest season. This BMP was considered easier to implement on larger farms, as there is generally more staff present to monitor calvings. This farmer summarized:

“I think as your farm gets bigger it’s a little easier, more staff around throughout the day, but … I think that’s probably one of the easier things to do is to take the calves away earlier. The nights might be the harder ones and the days might be a little easier […].”

Colostrum. As mentioned above, colostrum is considered a route of transmission for BLV. There are different BMP available to control the risk of transmission: 1) Pasteurize colostrum (Ruiz et al., 2018), 2) freeze colostrum (Kanno et al., 2014), and 3) use colostrum replacement products (Ruiz et al., 2018). Generally, farmers and veterinarians agreed that colostrum was an important factor in BLV control. Additionally, colostrum management was the second most recommended BMP by the veterinarians,
when encouraging BLV control. However, it was pointed out by the farmers that good
SOPs for colostrum management were missing. Additionally, it was noticed that
knowledge on colostrum quality, use, and the effect of freezing and pasteurizing
colostrum was lacking. Many farmers doubted that freezing colostrum from positive
dams would result in rendering the colostrum non-infective. They therefore suggested the
freezing of colostrum from BLV negative dams only. Farmers as well as veterinarians
asked questions about the quality of different colostrum treatments and products.
Although there was some uncertainty, farmers and veterinarians agreed that pooling of
colostrum from multiple dams was less common than feeding colostrum from individual
dams. This was important because pooling of colostrum from multiple dams could
increase the risk of transmission of BLV and other diseases to multiple calves (Jaworski
et al., 2016). The AJDI was mentioned again as a positive influence. This veterinarian
said:

“I think the Johne’s thing helped with that, too, though, that we stressed that [...] no more pooling of colostrum. I think a lot of guys have adopted, [...] single cow to calf and if that’s not working for some reason, the cow has got bad colostrum, blood in the quarter, whatever, then go to powdered for that individual and not mixing that.”

Generally, it was noted by the veterinarians that the higher the priority of disease control,
the higher the priority of colostrum management on farm. Only few of the participating
farmers pasteurized colostrum. However, their experience was positive, and
pasteurization was considered to be convenient and beneficial for calf health, even
though it required additional effort. On the other hand, some farmers considered
pasteurization complicated. Farmers and veterinarians agreed that the most restrictive
factor to the implementation of pasteurization was the cost. Some farmers consequently
chose to freeze colostrum instead. Freezing colostrum was generally considered easier to implement than pasteurizing colostrum by both farmers and veterinarians. Freezing was considered easy and cost effective, although thawing frozen colostrum was considered time consuming and complex by most farmers, as it requires delicate management of thawing temperatures and colostrum storage. These two farmers mentioned:

“Convincing them to just throw it in the freezer would be easier than to go buy a pasteurizer […]”

“I think with the freezing you need a good way of thawing.”

Some veterinarians mentioned that many farmers amongst their clientele were successfully freezing and thawing colostrum. However, one farmer noted that freezing colostrum based on BLV infection status could ignore other important diseases, like Johne’s Disease, for example. Lastly, some farmers decided to feed colostrum replacement products exclusively in order to prevent the transmission of colostrum-borne diseases. Other farmers used replacement products less frequent and only mixed it with colostrum from their own cows to increase the antibody concentration following assessment with a refractometer. Lastly some farmers used it only in emergencies, when the dam’s colostrum was not available. Using colostrum replacement products was generally considered easy, but costly. Additionally, the suitability of these products for the calves was questioned by farmers and veterinarians as the colostrum replacement products’ antibody makeup might not be farm specific. Therefore, veterinarians pointed out that when using these products, careful management was necessary.

**Milk.** Ingestion of milk from BLV positive dams is also considered a risk factor for the infection of calves with BLV (Ruiz et al., 2018). In order to control this route of transmission, farmers should feed pasteurized milk or milk replacement products.
Farmers present at the focus groups, used pooled, untreated milk, pasteurized milk, or milk replacement products to feed calves. The veterinarians mentioned that depending on the quota, milk was either sold or used to feed calves. Additionally, it was pointed out by the veterinarians that feeding milk replacement products is easy, but expensive and that milk pasteurizers are more common than colostrum pasteurizers. This veterinarian said:

“A lot of farmers are getting away from feeding waste milk and they pasteurize, so, [...] a lot of them are on milk replacer.”

**Dehorning.** Disbudding of calves is a common practice in the dairy industry. Depending on the method used (disbudding by heat, surgical removal of the horn bud, or by use of caustic paste), blood contamination of the dehorning instrument can cause the transmission of BLV between calves (DiGiacomo et al., 1985). Farmers and veterinarians agreed that most farmers used heat-based disbudding methods. However, some farmers admitted that they removed horn buds with a gouge dehorner before using heat-based dehorners. This farmer for example mentioned:

“We burn ‘em all, except when we get behind or whatever. It’s not supposed to happen [...]”

Cleaning and disinfecting of instruments between calves was considered easy to do. Similarly, it was noted that fully transitioning to heat-based disbudding would also be easily achievable.

**Needles.** Another risk factor for the transmission of BLV between animals is the reuse of needles for injections (Wilesmith, 1979). Farmers and veterinarians most commonly considered reusing needles as having the highest risk for transmission of BLV between cows. Consequently, the single use of needles was the most commonly recommended BMP by the veterinarians to control BLV on farm. However, not all
participating farmers changed needles after every use. Farmers indicated the increased
time requirement and the convenience of using repeater syringes as barriers. Like this
farmer mentioned, for example:

“We just use repeater syringes. And same with all our hormone shots. We’re
usually doing 1-10 cows on a needle [...]”

Although veterinarians and farmers pointed out that changing needles was more costly, it
was not considered prohibitive to change needles. Generally, farmers and veterinarians
agreed that not reusing needles was easy to do, like this farmer for example:

“One of the easiest changing that you can do.”

This veterinarian echoed:

“I think needles are the most important. To change the needles. And that’s easy to do.
[...] it’s more costly, but people seem like they don’t mind that.”

The veterinarians also mentioned that more and more farmers were adopting this practice
and it seemed to be easy to implement. Farmers and veterinarians supported the change
of needles for other reasons such as animal welfare, prevention of abscesses, and drug
quality (e.g. hormone deterioration due to contamination). However, it was noted by
farmers and veterinarians that some farmers changed needles for all injections, however,
not for oxytocin treatments in the milking parlour. If that was the case, employees
oftentimes provided these injections. This farmer for example explained:

“We change needles for everything except for oxytocin in the milking parlour. It’s just
easier for us, because we have so much hired help, we try to get them done in a certain
time, it’s just extra work for them to do.”

Similarly, one veterinarian mentioned that changing needles seemed harder in larger
herds. Another concern raised by the farmers was the disposal of used needles. Some
farmers were concerned because no disposal service was set up in their area.
Syringes. Even though syringes were considered less important than needles, they were identified as a possible source for transmission of BLV. Farmers and veterinarians were speculating about the risk connected to the syringes, but could both remember incidents, when blood of injected animals could be found in the conus of the syringe. This farmer for example remembered:

“But I have seen that you can get blood in on the tip where you put the needle on. If there’s blood in there, you put a new needle on, you inject the vaccine and the blood.”

Therefore, veterinarians emphasized the single use of syringes, if BLV control has a high priority on farm. However, participating farmers and veterinarians agreed that it would be more difficult to convince farmers in general to change syringes compared to needles. This farmer for example explained:

“I think you should pick up the battle with needles first and maybe leave the syringes in the background […]”

This veterinarian echoed:

“I have a hard time convincing guys to throw a needle out after a single use. Throw out the syringe after a single use... But I agree that it’s definitely a problem. It could easily transmit that way.”

Syringes are generally more expensive than needles and many farmers are using repeater syringes. Cost and convenience were mentioned to have a higher priority than BLV transmission. However, some farmers and veterinarians mentioned that they replaced repeater syringes for injections with small volumes (e.g. vaccines) and used small syringes instead. Before injecting, needles and syringes were prepared, afterward, needles were disposed of, and syringes were either disposed of or disinfected and reused. Syringes for injections with larger volumes (e.g. antibiotics) were often either cleaned
and disinfected between uses or marked with the cow’s identification and cleaned and reused until the treatment was completed.

**Order of treatment.** As an alternative to changing needles and syringes after every use, it was suggested to change the order of treatment and treat test negative animals first and test positive animals afterward. Alternatively, two different syringes could be used: one for positive, one for negative animals. While some veterinarians suggested this to some of their clients and some farmers considered it a feasible alternative, most participating farmers as well as veterinarians considered it more time consuming and pointed out that it would need good organization. These two farmers for example discussed:

“You can go down to the one end and do the positives and then come back and do the negatives, [...]”

“[...] I guess that’s true. If you organize it well enough.”

Additionally, farmers and veterinarians raised concerns, because not all test negative animals remain negative and it would be difficult to assess when animals seroconvert. Additionally, it would make testing all animals within a herd necessary. Overall, it was agreed that changing needles would be easier than changing the treatment order.

**Hoof trimmer.** Because the transmission of BLV between animals only requires small amounts of blood, hoof knives used during hoof trimming, were identified as a possible risk for transmission (Evermann et al., 1986). Therefore, it was suggested to ask the hoof trimmer to disinfect his/her knives between uses. Farmers and veterinarians agreed that the importance would depend on the number of cows that had to be treated with invasive methods. Additionally, it was considered important to distinguish between electric grinders and hoof knives, and whether blood contamination occurs. Generally,
farmers and veterinarians agreed that farmers would have to demand cleaning and
disinfection of knives and equipment from the hoof trimmer. Additionally, the hoof
trimmer would have to change his/her habits, which could be difficult to achieve as well.

This farmer mentioned:

“I think an issue there, too, is the changing habits and that hoof trimmers, I’ve
seen them work, they just go. If you then ask them I need you to do this, they’re ... you
would have to train it.”

This veterinarian noted:

“[…] the farmer has to demand it […] from the hoof trimmer […] if you want to hoof
trim my cows, you have to disinfect at least when you do the ulcers or when you start to
dig in […]”

The farmer’s initiative to require hoof trimmers to disinfect their tools was considered
dependent on the priority of BLV on his/her operation. Even though disinfecting knives
was considered easy to do, disinfecting electric tools was considered difficult.

**Disinfection of equipment.** Because of a possible contamination of equipment
like drenchers, pill-guns, etc. and thus its ability to transmit BLV between animals,
cleaning and disinfection between uses was suggested (Lucas et al., 1993, Yuan et al.,
2015). Generally, farmers agreed that cleaning and disinfection should be easy and quick
to do. The veterinarians agreed and mentioned that they encourage hygiene and
cleanliness for other reasons like transmissible warts as well. This veterinarian for
example mentioned:

“I think for the most part […] other than taggers and stuff like that […]. a lot of them
are conscious about warts. You know, and they know, that if they don’t dip and
disinfect… the ear tag equipment that they’re going to end up getting warts.”

**Fly control.** The transmission of BLV through biting flies is an argued topic
(Buxton et al., 1985, Hasselschwert et al., 1993). Even though transmission isn’t proven,
farmers’ and veterinarians’ opinion about fly control was investigated. Most farmers agreed that they pursued regular fly control during the summer months. However, motivation did not stem from BLV control, but was caused by animal welfare concerns as well as personal convenience. Milking during the summer months could be negatively impacted by the presence of flies, leading to intensified fly control efforts, like mentioned by this farmer:

“We don’t think fly control till August/September, when they start biting the cows so that you can’t milk ‘em.”

The veterinarians agreed and said that fly control was commonly and well implemented on most farms. Most farmers used different chemical fly control, like pour-on products and different kinds of fly poison. However, one farmer was concerned about negatively impacting ‘beneficial insects’ by using chemical products, leading to natural fly control, like parasitic wasps.

**Examination Sleeves.** The reuse of examination sleeves for AI and pregnancy examinations is known to be a risk for the transmission of BLV between animals (Kohara et al., 2006). Farmers commonly perform AI themselves. Farmers and veterinarians agreed that most commonly, either one sleeve was used for one cow or one sleeve was used for two cows. This farmer mentioned:

“Yes. Like I say, we have never used one sleeve for two cows, we would have no reason to.”

Therefore, it would be easy to use one glove for one cow only, as it would not add any extensive time or labour. However, the farmers raised concerns about external AI services requiring specific instruction. Nonetheless, participating farmers agreed that this would be easy to achieve. Most commonly, when veterinarians performed pregnancy
examinations on farm, multiple animals were examined during one visit and the same examination sleeve was used for all animals. In order to prevent the transmission of BLV between animals, ideally, every cow should be palpated using a new sleeve. Alternatively, test negative animals should be examined first, test positive animals could be done next, provided recent test results were available. Farmers and veterinarians agreed that, if BLV control was of high priority on farm, one of the two mentioned options should be used. Additionally, the careful management of examination sleeves was the third most commonly mentioned BMP by the veterinarians. However, the biggest barrier for the implementation, was the additional cost to the farmer. Veterinarians are commonly paid per time unit spend on farm and changing sleeves or changing the order of exams would increase the time necessary to do the task and therefore the cost. This farmer for example explained:

“This is mostly what you want to pay for it I guess. The vet is so much a minute while they are there and it’s gonna take you an extra 20 minutes to do herd health, because of this, well you’re just paying for it that way.”

And this veterinarian noted:

“I know that we have a risk with rectal palpation, but other than in a few herds that […] have knowledge of who the negative animals are and have us palpate the negatives first, we’re not really doing anything in the way of preventive medicine programs […]. The cost would be […] prohibitive for producers.”

Farmers and veterinarians agreed that the farmer would have to initiate the implementation of this BMP. This veterinarian for example mentioned:

“A lot of the herds here are not concerned enough to have us do that. I don’t think anybody in our practice is adament that we change after every cow.”

Some farmers added that the implementation would depend on the veterinarian’s willingness to change his/her routines. Some veterinarians perceived changing
examination sleeves as impractical and frustrating, if multiple animals had to be 
examined. However, other veterinarians either changed all examination sleeves or 
changed the order of examination for some of their clients and mentioned that doing 
either was easier on smaller operations. Additionally, if an ultrasound probe was used, 
additional measures had to be taken to prevent transmission via the probe. This could be 
done by using a separate examination sleeve for the probe or by washing the probe 
between animals. Like mentioned by this veterinarian:

“I think it’s reasonable to have a soap, disinfectant pale [sic] there, if you’re 
gonna change sleeves and just run it over the probe.”

6.4.8 Ranking

Detailed results for the mentioned ranking exercises can be found in Table 6-1. Overall, 
veterinarians and farmers gave similar scores to most items that seemed to represent what 
was mentioned during the conversations. However, there were some disagreements. The 
scores suggested that veterinarians considered testing the entire herd almost as easy as 
testing a subset of animals, contrary to the farmers’ scores. Additionally, farmers scored 
testing all animals on average, as less important than the veterinarians did. Moreover, 
culling of test positive animals was scored as less important by farmers than by 
veterinarians. Changing examination sleeves during pregnancy examinations was scored 
as less important as well as harder to implement by the veterinarians than by the farmers. 
Veterinarians’ scores suggested that they considered it harder to implement the removal 
of calves within 1 hour after birth than farmers did.
6.5 Discussion

The goal of this study was to document dairy farmers’ as well as veterinarians’ opinion about different BLV control measures. This creates the opportunity to adjust a newly developed BLV control program to the needs of the Alberta dairy industry as indicated by the stakeholders. In this way barriers could be identified before they hindered the successful introduction of a BLV control program. In addition, the industry’s attention could be drawn toward BLV, thereby increasing awareness of BLV and its consequences as well as the need for its control.

Because participation in the focus groups was voluntary, participants might have been biased towards a willingness and interest to control BLV. Nine participants had already implemented some form of BLV control, meaning that more than half of the participants’ motivation had not been sufficient enough to cause a change in on-farm behavior and introduce BLV control. Consequently, the mentioned barriers and motivators were considered important and should be taken into account for the design of the BLV control program, as well as when encouraging farmers to implement BLV control on farm. While individual opinions and reasoning might differ, the uncovered factors are likely applicable to a wide range of farmers. A qualitative approach was chosen for the study, because it enabled us to gain a deep understanding of the participants’ opinion (Braun and Clarke, 2013). Qualitative methods allow the participants to elaborate freely and encourage open discussion, thereby possibly uncovering motivators and/or barriers that were not expected. While the results in this study by its nature are not generalizable to the entire Alberta dairy farmer population, it was possible to include participants from the four areas with the highest density of dairy farms in Alberta, as well as the expertise of
several veterinarians who are focused on dairy herd health. By adding the veterinarians’ feedback, the scope of information gathering was broadened as they are confronted with many different farmers and therefore personalities in their day-to-day work. Moreover, in many instances herd veterinarians introduce BLV control measures to farmers, making on-farm behavior change dependent on their support. Therefore, we considered it important to understand veterinarians’ concerns as well as to provide assistance during BLV control implementation efforts. Even though farmers and veterinarians agreed in most rankings and most rankings mirrored what was said during the conversations, there were some important discrepancies: For example, it was found that testing for BLV was generally perceived as important and useful, but the cost for it was considered prohibitive by both farmers and veterinarians. Therefore, an explanation for the disagreeing ranking scores by veterinarians and farmers could be that farmers considered testing a subset of animals easier to do than testing the entire herd, because it was a smaller investment. While BLV transmission could be controlled without individual test results, knowing the within-herd as well as within-age group prevalence could make BLV control more efficient and successful (Erskine et al., 2012b, Bartlett et al., 2014). Therefore, testing should be encouraged. Additionally, it was suggested to subsidise testing. However, free or subsidised testing in other disease control programs were not able to convince all eligible farmers to participate (Sorge et al., 2010, Wolf et al., 2014, Roche et al., 2019). Nonetheless, results from herd testing may convince farmers that BLV is present in the herd, initiating BLV control on farm. Interventions that need monetary investments, but
lead to health improvement were shown be continued by the farmer, even when subsidies were terminated (Jansen et al., 2010a).

When comparing all three suggested BLV control strategies (cull, segregate, or manage), segregating or culling positive cows were mostly considered impractical or not feasible. On average, farmers gave culling strategies a low score in the ranking exercise even though it was considered to be a very efficient BLV control strategy by both farmers and veterinarians. This discrepancy could possibly be explained by a high within-herd BLV prevalence at the time of the focus-group session on most participating farms: Culling of all test positive animals was likely not considered an option. Similarly, regrouping animals in any operation would lead to considerable, lasting management changes and could therefore be considered difficult to implement. Similarly, farmers were found to prefer financial investments over routine changes in some cases (Huijps et al., 2008).

Controlling BLV by changing on-farm management was considered the most realistic and feasible option for BLV control by all participating farmers and veterinarians. Nonetheless, there were some common barriers mentioned: Firstly, investment costs were considered an important barrier. However, for most BMP that required monetary investments, cheaper alternatives could be found. This was generally positively received by farmers and veterinarians. Secondly, some BMP were considered complicated or difficult to implement as well as consistently to adhere to. Thirdly, the inconvenience of BMP was considered an important barrier. Usually, this meant that old habits had to be overcome to implement new BMP. Finally, a need to improve knowledge about the disease, test performance, as well as effectiveness of suggested BMP was mentioned multiple times. These and other, similar barriers were found in the context of other
disease control programs (reviewed by Ritter et al., 2017). Additionally, to put these barriers into context and in an attempt to try and explain how farmer behavior could be influenced and changed, some researchers made use of behavior models (Ellis-Iversen et al., 2010, Jansen, 2010): In summary, farmers required behavioral control/self-efficacy in order to change their behavior (Janz and Becker, 1984, Ajzen, 1991). That means, farmers need sufficient knowledge and BMP have to be considered feasible before behavior change is possible. Therefore, if there is a lack of knowledge about the disease and/or its control, or if BMP are considered not feasible due to financial constraints or perceived inconvenience, basic prerequisites for change in behavior are missing. This highlights the importance of the mentioned barriers. Consequently, when introducing new BMP on a farm, emphasis should be on providing the farmer with knowledge and realistic expectations, thereby allowing the farmer to make informed decisions about the BMP. Additionally, by understanding why some BMP were considered impractical or not feasible, a mutual understanding could be generated, solutions identified, and behavior change facilitated.

Besides some important barriers, some enabling factors were also mentioned. Firstly, if BMP were perceived as easy, farmers were quickly convinced to implement them. Consequently, as mentioned above, BMP should be designed and presented in a way that is tailored to the needs of individual farmers. Secondly, when BMP were considered the norm in the industry, implementing them was considered easier. Farmers operate within a frame of reference (i.e. what they consider “normal”). Therefore, if farmers perceive themselves below this norm, they are more likely to change their behavior (Jansen et al., 2009, Ritter et al., 2016). Thus, it is important to inform farmers about industry standards.
as well as to encourage ‘above average’ behavior. Thirdly, if BMP did not interfere with convenience or were even considered to increase convenience, they were positively received (Sorge et al., 2010). Therefore, when introducing new BMP to a farm, it is important to focus on all aspects of the suggested BMP. In addition, instead of encouraging the implementation of BMP to solve one problem, it should be communicated that most BMP serve more than one purpose and could help manage other problems (Wolf et al., 2014).

Finally, there were two more notable disagreements between farmers and veterinarians in how they ranked suggested BMP: changing sleeves during pregnancy checks was rated “easy” by farmers. However, veterinarians rated it impractical and of low importance for BLV control. This could reflect some veterinarians’ aversion to changing sleeves during pregnancy examinations. It was also mentioned by veterinarians that the available research on the transmission of BLV through rectal examinations was inconclusive (Bartlett et al., 2014). Another disagreement was found for removing the calf from its dam within 1 hour after birth. During the conversations, farmers as well as veterinarians mentioned that achieving consistent removal of calves 100% of the time was difficult. The importance given to this barrier might be reflected in the given scores.

6.6 Conclusions

In conclusion, testing and managing BLV positive cows on farm was considered the most realistic option by farmers and veterinarians. Even though some suggested BMP were considered difficult to implement, most farmers could find at least one alteration of some BMP they considered feasible. By being made aware of the barriers presented here, the
BLV control program could be adjusted to focus on BMP that can be easily modified and tailored to individual farmers’ needs, instead of focusing on culling and segregation strategies. Additionally, the control program should be able to take testing results into account, allowing for the respective adjustment of action plans, while still being able to pin-point weaknesses in management without having test results available.
6.7 References

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Table 6-1. Results of rankings performed during focus groups and interviews.

(Ability = perceived ability to change behavior to suggested BMP, 1= easy to 5 = impossible; Importance = perceived importance of BMP to control BLV, 1=very important to 5 = irrelevant)

|                | Test All<sup>1</sup> | Test Some<sup>2</sup> | Cull<sup>3</sup> | Seg.<sup>4</sup> | Bull<sup>5</sup> | Calf<sup>6</sup> | Freeze Col<sup>7</sup> | Past Col<sup>8</sup> | Past Milk<sup>9</sup> | Milk rep.<sup>11</sup> | Elec. Dehorn.<sup>12</sup> | Needles<sup>13</sup> | Syringes<sup>14</sup> | Order Treat.<sup>15</sup> | Disinfect.<sup>16</sup> | Fles<sup>17</sup> | Sleeves (Farmer)<sup>18</sup> | Sleeves (Vet)<sup>19</sup> | Order Exam<sup>20</sup> |
|----------------|---------------------|----------------------|-----------------|-----------------|----------------|-------------------|---------------------|--------------------|-----------------|---------------------|----------------------|-----------------|-------------------|----------------------|-----------------|------------------------|------------------------|----------------------|
| **Veterinarians** |                     |                      |                 |                 |                |                   |                     |                    |                 |                     |                      |                 |                   |                      |                 |                        |                        |                      |
| Ability Average | 1.6                 | 1.3                  | 3.6             | 3.9             | 2.5            | 1.5               | 2.7                 | 2.2                | 1.4             | 1                   | 1.4                  | 2.5             | 2.1               | 1.8                   | 1.7             | 1                      | 2.5                      | 2.3                  |
| Range           | 1-4                 | 1-3                  | 1-5             | 2-5             | 1-5            | 1-4               | 1-2                 | 1-5                | 1-3             | 1-3                 | 1-4                  | 1-4             | 1-4               | 1-4                   | 1-4             | 1                      | 1-5                      | 1-4                  |
| Importance Average | 1.5                 | 2.2                  | 2.5             | 2.6             | 1.6            | 1.3               | 2.1                 | 1.7                | 1.7             | 2                   | 1.5                  | 1               | 3.2               | 2.1                   | 1.2             | 2.4                     | 2.2                      | 2.5                  |
| Range           | 1-2                 | 1-5                  | 1-5             | 1-3             | 1-2            | 1-5               | 1-3                 | 1-4                | 1-4             | 1                   | 1.4                  | 2-4             | 1-4               | 1-2                   | 1-3             | 1-4                     | 1-4                      | 1-4                  |
| **Farmers**     |                      |                      |                 |                 |                |                   |                     |                    |                 |                     |                      |                 |                   |                      |                 |                         |                          |                      |
| Ability Average | 2.5                 | 1.8                  | 4               | 3.8             | 3.6            | 1.7               | 1.7                 | 2.3                | N/A             | N/A                 | 1.2                  | 1.2             | 2.2               | 2.2                   | 1.6             | 1.7                     | 1.2                      | 1.7                  |
| Range           | 1-4                 | 1-4                  | 2-5             | 2-5             | 1-5            | 1-4               | 1-5                 | 1-4                | N/A             | N/A                 | 1-5                  | 1-3             | 1-5               | 1-5                   | 1-3             | 1-2                     | 1-4                      | 1-5                  |
| Importance Average | 2.5                 | 2                   | 3.4             | 3.8             | 2              | 1.6               | 1.7                 | 1.4                | N/A             | N/A                 | 1.6                  | 2               | 2.6               | 2.2                   | 2.6             | 1.8                     | 1.7                      | 2.1                  |
| Range           | 1-5                 | 1-5                  | 1-5             | 2-5             | 1-5            | 1-4               | 1-5                 | 1-3                | N/A             | N/A                 | 1-4                  | 1-4             | 1-4               | 1-4                   | 1-5             | 1-3                     | 1-3                      | 1-4                  |

<sup>1</sup>Test All = Test all female animals on farm ≥12 months

<sup>2</sup>Test some = Test a subset of animals on farm

<sup>3</sup>Cull = Cull all BLV test positive animals

<sup>4</sup>Seg. = Segregate BLV test positive from test negative animals

<sup>5</sup>Bull = Replace natural breeding with artificial insemination
| 6  | Calf = Calf management: remove calf from dam within 1 hour after birth to prevent colostrum intake |
| 7  | Freeze Col = Freeze and thaw colostrum to prevent vertical transmission of BLV |
| 8  | Past Col = Pasteurize colostrum to prevent vertical transmission of BLV |
| 9  | Past Milk = Pasteurize milk to prevent vertical transmission of BLV |
| 10 | N/A = Ranking sheet provided to farmers did not include milk management |
| 11 | Milk rep. = Use milk replacement products to prevent vertical transmission of BLV |
| 12 | Elec. Dehorn. = Use electric dehorner to disbudd calves without risk of BLV transmission |
| 13 | Needles = Single-use of needles |
| 14 | Syringes = Single-use of syringes |
| 15 | Order Treat. = Treat BLV test negative animals first, then treat BLV test positive animals |
| 16 | Disinfect = Disinfect equipment that could cause transmission of BLV between animals |
| 17 | Flies = Implement consistent and effective fly control |
| 18 | Sleeves (Farmer) = Single use of rectal examination sleeves by farmer for artificial insemination, pregnancy examinations, etc. |
| 19 | Sleeves (Vet) = Single use of rectal examination sleeves by veterinarian for pregnancy examinations |
| 20 | Order Exam = Rectal examination of BLV test negative animals before BLV test positive animals by veterinarian |
Table 6-2. Summary of important findings from focus group sessions with farmers and interviews with veterinarians concerning testing, culling, segregation, and management bovine leukemia virus (BLV) control strategies.

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<th>Veterinarians’ feedback</th>
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</thead>
<tbody>
<tr>
<td><strong>Testing</strong></td>
<td>Considered important for control; cost is prohibitive; testing some animals is more feasible than testing all animals on farm; potential for subsidization; milk sampling very feasible; veterinarians could potentially be unaware of milk testing</td>
<td>Considered important, but not necessary for control; cost is prohibitive; potential for subsidization; testing some animals is more feasible than testing all animals on farm; milk testing more feasible than serum testing</td>
</tr>
<tr>
<td><strong>Test and cull</strong></td>
<td>Very efficient for control; considered not feasible for high within-herd prevalence herds, possible if within-herd prevalence is low; dependent on milk quota</td>
<td>Considered not feasible for high within-herd prevalence herds, possible if within-herd prevalence is low; dependent on milk quota; potential for mandatory programs</td>
</tr>
<tr>
<td><strong>Test and segregate</strong></td>
<td>Efficient control possible; increased workload and pre-existing grouping of animals are prohibitive; visual segregation of animals through ear tags or similar</td>
<td>Efficient control possible; not practical due to increased workload and pre-existing grouping of animals or structural restrictions</td>
</tr>
<tr>
<td><strong>Test and manage</strong></td>
<td>Most feasible option; can be done without testing</td>
<td>Most feasible option; can be done without testing</td>
</tr>
</tbody>
</table>
Table 6-3. Summary of important findings from focus group sessions with farmers and interviews with veterinarians concerning bovine leukemia virus (BLV) best management practices.

<table>
<thead>
<tr>
<th>Best management practice</th>
<th>Veterinarians</th>
<th>Farmers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bull breeding</strong></td>
<td>Cannot be eliminated within hutterites community; if bull is used, unlikely to be eliminated; testing bulls before purchase is realistic</td>
<td>Cannot be eliminated within hutterites community; if bull is used, unlikely to be eliminated; testing bulls before purchase is realistic</td>
</tr>
<tr>
<td><strong>Calf management</strong></td>
<td>Encouraged by Alberta Johne’s Disease Initiative; removal is common, but consistency is not always possible</td>
<td>Encouraged by Alberta Johne’s Disease Initiative; removal is common, but consistency is not always possible</td>
</tr>
<tr>
<td><strong>Colostrum</strong></td>
<td>Considered important for control; commonly recommended to farmers; quality of colostrum management dependent on priority of disease control on farm; cost prohibitive for implementation of pasteurization; freezing colostrum easier option; doubt in efficiency to render virus non-infectious by freezing; colostrum replacement products were considered easy to use, but expensive and potentially inadequate</td>
<td>Considered important for control; lack of good colostrum management protocols; doubt in efficiency of freezing to render virus non-infectious; freezing colostrum easier option, but thawing is complicated; freezing colostrum could potentially ignore other diseases; positive feedback toward colostrum pasteurization; cost prohibitive for implementation of pasteurization; colostrum replacement products considered easy to use, but expensive and potentially inadequate</td>
</tr>
<tr>
<td><strong>Milk</strong></td>
<td>Use of milk to feed calves is dependent on milk quota, milk replacement products are easy to feed, but expensive; milk pasteurizers more common than colostrum pasteurizers</td>
<td></td>
</tr>
<tr>
<td><strong>Dehorning</strong></td>
<td>Commonly done safely by heat disbudding; supported by industry programs</td>
<td>Commonly done safely by heat disbudding; sometimes done by gouge dehorning, can easily be changed</td>
</tr>
<tr>
<td>Item</td>
<td>Considered most important for control; most recommended best management practice by veterinarians; easy to implement; multiple reasons to change needles after every use; cost not considered prohibitive; inconsistent implementation due lack of employee compliance; more difficult in larger herds</td>
<td>Considered most important for control; easy to implement; multiple reasons to change needles after every use; cost was not considered prohibitive; important barriers: time and inconvenience; inconsistent implementation due lack of employee compliance; concern: disposal of used needles</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Needles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syringes</td>
<td>Insecurity about risk; implementation dependent on BLV control on farm; implementation on some farms; likelihood of implementation lower than for needles</td>
<td>Insecurity about risk; likelihood of implementation lower than for needles, but implementation on some farms; barriers: cost and convenience</td>
</tr>
<tr>
<td>Order of treatment</td>
<td>Suggested by some veterinarians; usually considered impractical</td>
<td></td>
</tr>
<tr>
<td>Hoof trimmer</td>
<td>Importance dependent on affected cows; farmer has to initiate change</td>
<td></td>
</tr>
<tr>
<td>Disinfection of equipment</td>
<td>Considered easy and quick; multiple reasons to encourage cleanliness</td>
<td></td>
</tr>
<tr>
<td>Fly control</td>
<td>Commonly and well implemented on most farms</td>
<td></td>
</tr>
<tr>
<td>Examination sleeves</td>
<td>Easy for artificial insemination; implementation for pregnancy examinations dependent on priority of BLV control on farm, cost is prohibitive; initiation dependent on farmer; impractical, frustrating, but implemented on some farms</td>
<td></td>
</tr>
</tbody>
</table>

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CHAPTER 7: Trial implementation of a newly developed bovine leukemia virus control program on 10 Alberta dairy farms

7.1 Abstract
In order to provide the Alberta dairy industry with a bovine leukemia virus (BLV) control program, a risk assessment as well as a comprehensive list of best management practices (BMP) aiming at the prevention of BLV transmission between animals was developed. This control program was implemented on 11 farms for approximately 1 year to evaluate the necessity of serum testing and the applicability of the risk assessment, as well as the effectiveness of BLV control based on management changes. Eleven farmers were recruited. All animals on farm that were ≥12 months old were blood sampled and serum was tested with a commercially available ELISA. Based on test and risk assessment results, the farmers were provided with suggestions for management changes aiming at preventing transmission of BLV between animals. Throughout the following year, all participating farmers were visited multiple times to provide support with barriers to implementation and to follow progress. After approximately one year of implementing on-farm BLV control, all animals ≥12 months old on farm with a negative or no previous test result were sampled again, the within-herd prevalence was measured, and seroconversions were counted. The average number of animals on farm that were 12 months or older was 285 (range 110-524). On average, 5 BMP (3-7) were suggested to each farmer. All farms implemented at least one BMP and on average 4 BMPs (1-7) were implemented. At the second sampling, the average within-herd prevalence was 36.4% (12-62%). Eight farms were able to reduce their within-herd BLV prevalence, 1 farm’s
within-herd prevalence stayed constant, 1 farm’s within-herd prevalence increased, and 1 farm terminated the trial before the second sampling. The number of seroconversions varied between 3 and 109 among farms. The risk assessment was proven to be a valuable tool to identify flaws in on-farm management but risk assessment score was unrelated to the within-herd BLV prevalence. Additionally, it appeared that the implementation of BMP aimed at the prevention of BLV transmission between animals would be able to reduce the within-herd prevalence, if done over an extended period of time, despite seroconversions.

7.2 Introduction

Infectious animal diseases have always posed a risk to food animal production. They decrease production, impact farm profitability, and have a negative effect on animal welfare (Bartlett et al., 2014, Wolf et al., 2014, Santman-Berends et al., 2015). Therefore, various disease control and pathogen eradication strategies have been applied in agriculture whereby farmers strive to enhance the health of their herds, thereby improving the well-being of animals and farms (Leach et al., 2010b, Nielsen, 2011). Because of the complexity of infectious disease control, government as well as industry initiatives assist farmers with disease eradication (Dairy Farmers of Canada, 2019, Government of Alberta, 2019). For several diseases (e.g. bovine spongiform encephalopathy, brucellosis), mandatory disease control or eradication programs were initiated by the government (Canadian Food Inspection Agency, 2018). Additionally, for some other diseases, voluntary disease control programs were established (e.g. Johne’s Disease, lameness). By providing information on the disease as well as by discussing
farm specific best management practices (BMP) that can limit pathogen transmission, farmers were enabled to control different diseases on farm. Additionally, by using different risk assessments in collaboration with the farm veterinarian, behaviors that increase the risk for diseases transmission on farm could be identified and BMP that reduce disease transmission could be tailored to specific farm situations. This approach was able to decrease disease prevalence on participating farms for Johne's Disease (Sorge et al., 2011).

Bovine Leukemia Virus (BLV) is another causative agent of a common production limiting cattle disease. BLV infection of cattle is common in North American dairy herds (LaDronka et al., 2018, Kuczewski et al., 2019). It can impair milk production, reduce cow longevity, decrease the ability to respond to vaccines, and can lead to the development of Enzootic Bovine Leukosis (EBL). Because BLV is a retrovirus, infection with this virus is lifelong (Goff, 2013). Therefore, reduction in BLV prevalence across and within herds is only possible by replacing BLV infected animals with uninfected ones. Following identification of BLV positive animals, there are three common options for BLV control: culling, segregation, or management of infected animals (Rodriguez et al., 2011, Bartlett et al., 2014). Culling or segregation programs have been used to successfully eradicate BLV from many European countries, as well as from New Zealand and the Australian dairy herd (European Commission, 2003, Voges, 2012, Dairy Australia Limited, 2015). These programs were mandatory: the entire cattle population was laboratory tested on regular bases, test positive animals were eliminated or physically segregated, and a monitoring program was initiated (EFSA Panel on Animal Health and Welfare, 2015). However, thus far, in Western Canada no mandatory or
voluntary BLV control program has been implemented. It is up to the farmer to decide to control BLV and which strategy to pursue once infected animals are identified. Nonetheless, culling or segregation strategies are oftentimes not feasible or logistically challenging (Huijps et al., 2009) as the mean within-herd prevalence is relatively high, approximately 40% in Alberta, Canada (Kuczewski et al., 2019). Therefore, Canadian farmers oftentimes prefer to implement management practices that allow for the reduction of BLV transmission: the replacement of infected with uninfected animals, is meant to result in a lower within-herd prevalence over time (Chapter 6). In order to support the Canadian dairy industry in their efforts to decrease the BLV prevalence within and across herds, a voluntary BLV control program, based on a risk assessment approach was developed. The goal of this study was to evaluate and improve a newly designed BLV risk assessment and potential on-farm BLV control measures by implementing them on a small number of Alberta dairy farms in order to identify and address potential shortcomings before they are introduced to farmers on a larger scale.

7.3 Materials and Methods

7.3.1 Purpose of trial implementation

The goals of the trial implementation were: 1) To evaluate the design and validity of a risk assessment tool as well as to determine the necessity of laboratory testing for the identification of infected animals, 2) to evaluate the effectiveness of BLV control after 1 year. In order to reach these goals, the BLV control program was implemented on a small number (11) of farms. Participating farmers received recommendations on BMP based on
the risk assessment (RA) and laboratory testing results of their herd. The implementation of BMP as well as the within-herd prevalence were monitored.

All visits and interviews were conducted by the first author. Samplings were conducted by the first author, as well as by accompanying graduate students, the farmer, and farm employees, when present.

The project was approved by the Conjoint Faculties Research Ethics Board (CFREB) of the University of Calgary (REB16-0224) and by the Veterinary Sciences Animal Care Committee of the University of Calgary (VSACC AC17-0242).

### 7.3.2 Recruitment

Farmers were recruited through voluntary subscription to this trial between March 2016 and November 2017 during various dairy farmer focused events (e.g. Western Canadian Dairy Seminar (WCDS, Red Deer, AB, Canada)) and publications (e.g. Alberta Milk Milking Times (Alberta Milk, Edmonton, AB, Canada)) in Alberta. All Alberta dairy farmers were invited to provide their contact information. The inclusion criteria for the trial were: The farm had to be located within a 4-hour driving distance from Calgary and be within Alberta. In order to recruit farmers for the trial implementation, all farmers that provided their contact information previously (42 contacts) were sent an e-mail with a short outline of the trial and the invitation to respond, if they were interested in participation. Afterwards, 11 farmers were recruited in their order of response. These farmers were called, an outline of the trial was discussed, and all potentially participating farmers were given a chance to ask questions as well as to consent to participating in the trial. All contacted farmers consented and the first meeting on-farm was scheduled.
7.3.3 Risk assessment

BLV control for Alberta was meant to be based on a risk assessment whose results would identify management practices on farm that could allow the transmission of BLV between animals and would then facilitate their mitigation. In order to develop an applicable risk assessment, literature, as well as feedback from farmers and veterinarians (Chapter 2, Chapter 6), were used. First, a list of transmission routes was established. Then, risk-promoting practices on farm were identified, listed according to animal management groups (i.e. cows, heifers, calves), and used to design questions for the risk assessment. The answers consisted of a comprehensive list of alternatives for the performance of a certain practice (e.g. different ways of dehorning). Every answer was given a weighted value (5-25, in increments of 5). An increase in value indicated a higher risk for BLV transmission. If a practice was considered to have a negligible risk for transmission it was assigned “0”. Where possible, literature was used to assign levels of risk, otherwise expert opinion was used. Literature about BLV transmission risk and control was thoroughly evaluated and findings summarized (Chapter 2). Experts were the authors as well as other veterinarians and extension officers with extensive practical and research experience in on-farm BLV control.

The risk assessment tool was designed to be used in combination with BLV laboratory test results that allowed the identification of BLV positive animals as well as within-herd prevalence estimates. Additionally, it was intended to be repeated on a regular basis (e.g. once a year) to allow for the adjustment of the BMP to management changes on farm and in BLV prevalence. By laboratory testing the entire adult herd (≥12 months of age) the
age group of animals with the highest increase in seropositive animals could be identified (Erskine et al., 2012), meaning that the management period with the highest prevalence could be determined and control efforts could be focused on the identified age group. Consequently, the risk assessment was divided into 4 sections: 1) General biosecurity measures that were applicable to the entire operation and could facilitate the introduction of BLV into the herd (e.g. the purchase of animals), 2) adult cows (>2 years), 3) heifers (<2 years), and 4) calves (animals up to 9 months). The biosecurity section consisted of 3 subcategories and the maximum score was 55. Adult cow management had 10 subcategories and a maximum score of 145. Next, was the heifer category with 11 subcategories and a maximum score of 170. The calf management category consisted of 9 subcategories and a maximum score of 155. (The risk assessment used for this trial can be found in Appendix II.)

7.3.4 Trial implementation

The trial consisted of 7 steps: 1) All farms were first visited in January and February 2018 and a risk assessment as well as an interview were conducted. The farmers’ attitude toward, and knowledge about BLV, as well as the expectations toward the trial implementation were discussed. 2) During a second visit between February and April 2018 blood samples were obtained. The laboratory test results were communicated to the farmers as soon as they were available. 3) Between February and May 2018 test results were discussed during a phone conversation with each farmer individually, and recommendations for farm specific BMPs were provided. Then, during the same phone call, another short interview was conducted to discuss the farmers’ expectations.
concerning the implementation of the BMP. 4 and 5) After approximately 3 and 6 months since the recommendation of BMPs, all participating farmers were visited again to evaluate BMP implementation, whereby reasons for (not) implementing certain BMP were discussed. Additionally, practical solutions were identified. 6) After approximately 13 months following the first sampling, a second sampling was performed, and a second risk assessment was conducted. This allowed for a thorough comparison of on-farm management practices between first and last sampling. Once laboratory test results for the second samples were available, they were communicated to the farmers. 7) Finally, a last phone interview was conducted between April and June 2019 to assess the farmers’ experience during the trial. (Figure 7-1)

7.3.4.1 Sampling and testing.

Blood samples were taken at the start and the end of the trial evaluation period (T= 0 and ~13 months) from the coccygeal vein and collected in 10 mL serum tubes (Vacutainer, BD, Franklin Lakes, NJ, USA). Within 48 hours after sampling, all samples were centrifuged (1500g, 13 minutes, 7 degrees Celsius). The serum was separated and stored at -20 degrees Celsius. All samples were tested for BLV antibodies (IDEXX Leukosis Serum X2 Ab Test; IDEXX, Westbrook, ME, USA). On sampling days, all female animals on farm of 12 months or older, as well as breeding bulls that could be safely handled were sampled. At approximately 13 months, blood samples were obtained from animals that were not previously tested (calves that were younger than 12 months during the first sampling and additions to the herd) or with a negative test result at the first sampling. Test positive animals were considered infected due to the high specificity of
the ELISA (Kuczewski et al., 2018). The age of 12 months was chosen to avoid detection of maternal antibodies, while still making a comprehensive overview of infection risk of the youngstock possible (Johnson et al., 1987, Gutierrez et al., 2011, Erskine et al., 2012).

7.3.4.2 Recommendation of best management practices.

Specific BMPs based on risk assessment and laboratory test results were discussed during a phone conversation with each farmer. Following this phone conversation, farmers were provided with a list of these recommendations, as well as an explanation and reasoning as discussed, via e-mail, on farmers’ request. The farmers were purposely offered multiple BMPs, whereby the farmer could decide if and when a specific BMP would be implemented. Moreover, farmers also had the opportunity to ask questions that arose since the previous discussion.

7.4. Results

7.4.1 Participants

In total, 11 farms participated. One farmer withdrew from the trial after the 6-month interview for personal reasons. On 8 farms, the first author communicated with a male farmer only. On 2 farms both male and female farmers were involved. Finally, on 1 farm, communications were with a father and son. All farmers were born between 1950 and 1990, whereas most (8) farmers were born between 1970 and 1990. The average number of animals on farm that were 12 months or older was 285 (range 110-524). On average, the daily milk production per animal was 38.4 kg (range 34-44). Two farms had tie-stalls for the milking herd, while 9 milking herds were housed in free stalls. Within the free stalls, three farms used automated milking systems, whereas 5 farms used conventional
milking parlours. Three farmers had not implemented any measures with the sole purpose to control BLV before the start of the trial. The remaining 8 farmers had implemented some BLV control (e.g. single use of needles). Therefore, all participating farmers could optimize their BLV control activities, implemented at least one BMP, and could provide feedback about implementing these on commercial farms.

### 7.4.2 Trial implementation

The first interview was held in January 2018 and the last interview was completed in June 2019. The average score of the first risk assessment was 234 (155-315). At the first sampling, the average within-herd prevalence was 39% (13-66%). Based on the individual test results and risk assessments, different BMP were recommended (Table 7-3). On average, the first author suggested 5 BMPs (3-7) to each farmer. All farms implemented at least one BMP and on average 4 BMPs (1-7) were implemented. At the second sampling, the average within-herd prevalence was 36.4% (12-62%). All but two farms were able to decrease their within-herd prevalence. On one farm the within-herd prevalence remained constant, while on another farm the within herd prevalence increased within the examined timeframe. On all farms seroconversions were detected between first and second sampling. On average 20.8 animals seroconverted (3-109 animals per farm). The average score of the second risk assessment was 162 (80-305). More detailed results can be found in Tables 7-1, 7-2, and 7-3.
7.4.3 Evaluation of risk assessment and recommendation of best management practices.

Overall risk assessment score was not related to within-herd prevalence, based on visual exploration of graphs plotting risk assessment scores and within-herd prevalence (not reported). Some high prevalence herds had low total first risk assessment scores (e.g. 64% within-herd prevalence, 155 total risk assessment score), whereas some low prevalence herds had high total risk assessment scores (e.g. 21% within-herd prevalence, 295 total risk assessment score).

All farmers completed all parts of the risk assessment, facilitating evaluation of the included questions as well as the design of the risk assessment. The detailed questions were able to shed light on specific management practices, without overlooking small, but important flaws in on-farm management: For example, some farmers changed needles after every use for most applications, but did not change them for others, like the administration of local anaesthetics (e.g. dehorning), or for the administration of oxytocin in the milking parlour. Additionally, it was noticed that the section of the risk assessment aiming at the highest risk age group could not always highlight suboptimal management practices. Some farmers had already implemented some BMPs before the onset of the trial (e.g. use of milk and colostrum pasteurizer, dehorning methods, or similar) in the highest risk age group in their herd (e.g. approx. 40% heifers infected) or in general (e.g. changing needles). Similarly, financial or structural restrictions (e.g. space limitations in the milk house) did not always allow the farmer to implement the BMP in the identified highest risk age group or in the most optimal way. Then, other sections of the risk assessment were used, and/or management practices were adjusted accordingly. For
example, some farmers couldn’t purchase a colostrum pasteurizer, because of financial constraints. In order to prevent transmission of BLV from dams to calves, freezing and thawing colostrum from negative dams was considered the next-best option.

7.5 Discussion

The goal of this study was to evaluate and improve a newly designed BLV risk assessment and potential on-farm BLV control measures in order to identify and address potential shortcomings, thereby, increasing the chances of a successful implementation on a larger group of farms.

While a sample size of eleven farms was insufficient to reach statistical power, it allowed for close monitoring of all participating farms, permitting a thorough examination of the risk assessment and BMPs, making the set goals achievable. However, a selection bias might have been introduced, because of the recruitment process. Even though participating farmers might have been more interested in BLV control than non-participating farmers, the lack of, or incomplete BLV control on farm, suggests that participating farmers’ motivations were not substantially different from non-participating ones. Additionally, even though true generalizability cannot be achieved, the participating farmers were close to the Alberta average for some important characteristics: average number of milking cows in the herd: 153 (trial) vs. 131 (Alberta); average daily milk production per cow: 38 kg/day (trial) vs. 38.5kg/day (Alberta); average BLV within-herd prevalence: 39.2% (trial, first sampling) vs. 39.5% (Alberta; CanWest DHI, 2019).
The risk assessment was designed to be combined with laboratory testing to allow for the focus of recommended BMPs on the age group with the highest risk of BLV transmission. This could be achieved by testing the entire herd, or alternatively, by testing only a subset of the adult herd according to a scheme established by Erskine et al. (2012). In addition, knowing each individual animal’s BLV status is helpful for the implementation of some BMP (e.g. freezing colostrum from ELISA test-negative dams). However, farmers may be reluctant to invest in laboratory testing due to financial constrains (Leach et al., 2010a, Sorge et al., 2010), although they might value the implementation of BLV control strategies. In the latter case, specific age groups identified through laboratory testing might not be the focus of the risk assessment, but all age groups could be considered. Additionally, participating farmers were purposely offered a choice of BMPs, when possible, instead, for example, of 3 distinct recommendations as was described previously (Wolf et al., 2014). The farmer decided how many and which BMPs he/she wanted to implement and when this implementation should take place. It was considered valuable to leave this decision with the farmers in order to better understand motivators and barriers influencing farmers’ decision making processes. Moreover, by allowing the farmer to decide which BMP would be implemented and how, mutual trust could be built, encouraging openness from the farmer. Therefore, there was more confidence in the reports on implementation of BMPs and socially desirable answers were not expected (Braun and Clarke, 2013).

As mentioned above, within-herd prevalence and risk assessment score were unrelated. This was expected and could be explained by the farm’s management and the period of
time since initial BLV infection and the duration of BLV control. For example, one farm had a high within-herd BLV prevalence as well as numerous EBL cases and consequently already implemented BLV control measures approximately 6 months before the first risk assessment. Thus, the effect of these control measures was not yet reflected in the within-herd prevalence. Similarly, for herds with a low prevalence and a high risk assessment score, BLV control was not a priority at the start of the trial implementation. These herds were either closed herds or recently established herds that were founded with BLV-free youngstock. However, without effective BLV control, the within-herd prevalence would have likely increased in the following years. Alternatively, the relative weightings assigned to various risk assessment questions may not have been appropriate, which would result in a poor correlation.

It is important to note that the goal of this study was not to maximize the reduction of within-herd BLV prevalence within 1 year. It was considered more important to allow the farmers to decide when and which BMP were implemented in order to better understand which BMP were easy to implement, which BMP were met with challenges, but also to better understand farmers’ motivators and barriers (Chapter 5). Additionally, monitoring on-farm BLV control as well as the within-herd prevalence for 5 to 10 years would result in a better assessment of the effect of management optimization through BMP implementation on BLV prevalence than just 1 year. However, even after 1 year of BLV control, 8 farmers were able to reduce their herd’s prevalence, an effect that would likely be enhanced, if BLV control was adhered to consistently and for longer periods of time (Johnson et al., 1985, DiGiacomo et al., 1987). Nonetheless, one farm’s prevalence remained constant over the examined time frame. This farm had to rely on natural
breeding, making new infections likely (Benitez et al., 2019). Additionally, this farm
decreased its herd size. The number of test negative as well as test positive animals
decreased, keeping the proportions consistent and making it difficult to assess the
development of BLV prevalence. Another farm’s within-herd prevalence increased when
comparing the results of the first to the second year. This was likely caused by a delay in
implementation of some BMP. Needles were reused multiple times for multiple animals
at the beginning of the trial. Implementing the single-use needles was delayed and
convincing all employees takes time during which needles were reused, therefore likely
causing BLV transmission (Bartlett et al., 2014). Additionally, a large number of untested
animals was purchased and introduced into this herd, creating additional sources of
infection.
At least 3 animals seroconverted on all participating farms. While it is possible that
animals were infected during the trial, animals may have also already been infected
before the first sampling and seroconverted later (Klintevall et al., 1997, Nagy et al.,
2007). However, it was not possible to distinguish between the two scenarios within the
scope of this trial. Even though animals seroconverted in every participating herd, most
farms were able to decrease the within-herd prevalence, meaning that the seroconversion
rate is smaller than the rate of positive animals leaving the operation. This is consistent
with other studies, which have found that BLV positive animals are more likely to be
culled or die than laboratory test negative animals (Bartlett et al., 2013, Nekouei et al.,
2016). If new infections can be prevented or minimized, this would result in a net
reduction of infected animals. Nevertheless, because farmers knew the individual
animals’ status, it is possible that positive animals were culled more readily than test
negative animals. However, it was beyond the scope of this study to investigate farmers' culling decisions.

7.6 Conclusion

In conclusion, the risk assessment was proven to be a useful tool to identify management practices that allow the transmission of BLV. By collaborating with the participating farmers, it was possible to identify at least one BMP or a variation thereof that could be implemented and adhered to consistently throughout the trial on every farm. One year of on-farm BLV control can already reduce BLV prevalence. Therefore, even though long-term studies are needed to better understand the effect of management on BLV prevalence, managing BLV positive animals seems to be an option for successful on-farm BLV control.
7.7 References


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Table 7-1. Overview sampling and ELISA results.

<table>
<thead>
<tr>
<th>Farm ID</th>
<th>Number of animals I&lt;sup&gt;1&lt;/sup&gt;</th>
<th>No. of missing samples&lt;sup&gt;4&lt;/sup&gt; I</th>
<th>Prevalence I&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Prevalence 2+ I&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Number of animals II&lt;sup&gt;2&lt;/sup&gt;</th>
<th>No. of missing samples&lt;sup&gt;4&lt;/sup&gt; II&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Prevalence II&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Prevalence 2+ II&lt;sup&gt;2&lt;/sup&gt;</th>
<th># of seroconversions&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>517</td>
<td>2</td>
<td>26</td>
<td>34</td>
<td>543</td>
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<td>40</td>
<td>51</td>
<td>109</td>
</tr>
<tr>
<td>2</td>
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<td>1&lt;sup&gt;5&lt;/sup&gt;</td>
<td>64</td>
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<td>1</td>
<td>62</td>
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<td>4</td>
<td>110</td>
<td>0</td>
<td>45</td>
<td>53</td>
<td>101</td>
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<td>37</td>
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<td>-&lt;sup&gt;6&lt;/sup&gt;</td>
<td>-&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>66</td>
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<td>43</td>
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<td>0</td>
<td>66</td>
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<td>1</td>
<td>58</td>
<td>69</td>
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</tr>
<tr>
<td>11</td>
<td>524</td>
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<td>21</td>
<td>27</td>
<td>570</td>
<td>4</td>
<td>17</td>
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<td>Average</td>
<td>285</td>
<td>0.9</td>
<td>39</td>
<td>50</td>
<td>283</td>
<td>2.3</td>
<td>36</td>
<td>44</td>
<td>21</td>
</tr>
</tbody>
</table>

1. I = results at first sampling (number of animals present on farm, within-herd prevalence, within-herd prevalence of animals 2 years of age and older)

2. II = results at second sampling (number of animals present on farm, within-herd prevalence, within-herd prevalence of animals 2 years of age and older)

3. Number of seroconversions from first to second sampling. Only animals were included that had test results from both samplings

4. Number of animals that were missed on sampling days.

5. Animal had anatomically deformed tail and sampling from coccygeal vein was not possible. This animal was tested by milk.

6. Farmer withdrew from trial for personal reasons before second sampling.
Table 7-2. Overview risk assessment scores, number of recommendations, as well as number of implementations by farm.

<table>
<thead>
<tr>
<th>Farm ID</th>
<th>Score RA I(^1)</th>
<th># of recommendations(^2)</th>
<th># of implementations(^3)</th>
<th>Score RA II(^4)</th>
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</thead>
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<td>1</td>
<td>275</td>
<td>5</td>
<td>2</td>
<td>180</td>
</tr>
<tr>
<td>2</td>
<td>155</td>
<td>4</td>
<td>5</td>
<td>120</td>
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<tr>
<td>3</td>
<td>315</td>
<td>7</td>
<td>3</td>
<td>305</td>
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<td>245</td>
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<tr>
<td>7</td>
<td>280</td>
<td>6</td>
<td>1</td>
<td>N/A(^5)</td>
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<td>165</td>
<td>5</td>
<td>5</td>
<td>105</td>
</tr>
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<td>9</td>
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</tr>
<tr>
<td>Average</td>
<td>234</td>
<td>5.2</td>
<td>4</td>
<td>162</td>
</tr>
</tbody>
</table>
1 Total score of first risk assessment (RA)

2 Number of recommendations made by first author to control BLV on farm

3 Number of measures implemented by participating farmers to control BLV on farm

4 Total score of second risk assessment (RA)

5 Farmer withdrew from trial before risk assessment could be completed
Table 7-3. Overview of within-age group prevalence, recommended, and implemented best management practices

<table>
<thead>
<tr>
<th>ID</th>
<th>Prevalence</th>
<th>Reasoning</th>
<th>Recommendations(^1)</th>
<th>Comments</th>
<th>BMP implemented</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>By age group (in years)</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.5%</td>
<td>28.4%</td>
<td>34.7%</td>
<td>28.1%</td>
<td>48.8%</td>
</tr>
<tr>
<td>2</td>
<td>24.4%</td>
<td>50.0%</td>
<td>93.0%</td>
<td>100.0%</td>
<td>95.0%</td>
</tr>
<tr>
<td>3</td>
<td>11.3%</td>
<td>27.5%</td>
<td>50.0%</td>
<td>35.0%</td>
<td>38.9%</td>
</tr>
<tr>
<td></td>
<td>24.1%</td>
<td>37.5%</td>
<td>50.0%</td>
<td>50.0%</td>
<td>76.2%</td>
</tr>
<tr>
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<td>---</td>
</tr>
<tr>
<td>5</td>
<td>15.1%</td>
<td>2.3%</td>
<td>11.8%</td>
<td>5.9%</td>
<td>32.3%</td>
</tr>
<tr>
<td>6</td>
<td>5.6%</td>
<td>26.4%</td>
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<td>15.9%</td>
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<td>49.2%</td>
<td>58.4%</td>
</tr>
<tr>
<td>8</td>
<td>12.3%</td>
<td>42.3%</td>
<td>60.5%</td>
<td>90.0%</td>
<td>85.3%</td>
</tr>
<tr>
<td></td>
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<td>3</td>
<td>4</td>
<td>5</td>
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<td>---</td>
</tr>
<tr>
<td>9</td>
<td>7.8%</td>
<td>46.3%</td>
<td>69.2%</td>
<td>81.8%</td>
<td>95.0%</td>
</tr>
<tr>
<td>10</td>
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<td>70.4%</td>
<td>79.2%</td>
<td>85.4%</td>
</tr>
<tr>
<td>11</td>
<td>6.1%</td>
<td>14.0%</td>
<td>11.6%</td>
<td>33.3%</td>
<td>47.0%</td>
</tr>
</tbody>
</table>

1Explanation of recommended and implemented BMP: test additions: Laboratory test animals before purchase and introduction into the herd, purchase only test negative animals; reduce overstocking: Reduce overstocking in pens; needles (LA, Oxy, ET, I.V.): Do not reuse needles (sometimes needles were not reused except for local anaesthetics during dehorning (LA)/Oxytocin in the milking
parlour (Oxy)/i.v. treatments (i.v.)/embryo transfer (ET)); syringes: Do not reuse syringes; disinfection: Disinfect commonly used equipment (e.g. drench guns, calving chains, pill guns); hoof trimmer: ask hoof trimmer to disinfect hand knives between animals; gloves vet: Ask veterinarian to change gloves between animals, or to examine laboratory test negative animals before test positive ones; gloves AI: Do not reuse gloves for AI; bull: Purchase only laboratory test negative bulls, try to breed test positive cows with different bull(s) than test negative cows; tag: Use coloured ear tags to identify laboratory test positive animals in the herd; pasteurizer: Pasteurize colostrum/milk before feeding it to calves; colostrum: Implement safe colostrum management (i.e. pasteurizer/freeze colostrum from negative dams/use powdered colostrum); dehorner: Implement safe dehorning methods (i.e. hot dehorning or caustic paste); bull calves: Implement BMP for bull calves as well, if they will be sold as breeding bulls
Figure 7-1. Overview and timeline for interviews and samplings.

- **Interview I and Risk Assessment I**
  - January - February 2018

- **Sampling I**
  - January - April 2018

- **Recommendation of BMP and Interview II**
  - February - May 2018

- **Interview III**
  - June - August 2018

- **Interview IV**
  - October - December 2018

- **Sampling II and Risk Assessment II**
  - February - May 2019

- **Final Interview/Interview V**
  - April - June 2019
CHAPTER 8: Summarizing Discussion:
Experiences with and lessons learned from the development of a bovine leukemia virus control program

Faced with an increasing herd and within-herd prevalence and therefore with increasing negative impacts of a Bovine leukemia virus (BLV) infection, the Alberta dairy farming community voiced the need for a BLV control program. In collaboration with Alberta dairy farmers and veterinarians, a research team at the University of Calgary accepted the task and developed a voluntary, risk assessment based, on-farm BLV control program. By choosing a holistic approach, a practical and applicable solution was meant to be found: First, virus transmission and options for the control of BLV on farm were studied and important gaps in knowledge identified (Chapter 2). Second, the identification of BLV infected animals was investigated by comparing different commercially available ELISA (Chapter 3). Third, the economic impact of BLV infection and control were examined (Chapter 4). Fourth, dairy farmers’ and veterinarians’ feedback was sought toward BLV control, as well as to understand important motivators and barriers for on-farm BLV control. This was done by conducting focus groups with farmers, interviews with veterinarians, as well as interviews with farmers during the BLV control program trial implementation (Chapter 5 and 6). Fifth, a risk assessment for the identification of less than optimal management practices was designed and implemented on a small subset of farms in order to identify positive and improvement-worthy aspects of the designed risk assessment and potential best management practices (BMP) aimed at the control of
BLV (Chapter 7). Here, the findings of the mentioned steps will be summarized and discussed.

8.1 Bovine leukemia virus, its most important characteristics, transmission, and options for control

Bovine Leukemia Virus belongs to the family of *retroviridae*. Therefore, causing a lifelong infection of infected animals (Goff, 2013). The virus’ impact results in lymphocytosis for roughly 30% of infected animals and enzootic bovine leukosis (EBL, fatal tumors) for approximately 5% of infected animals (Schwartz and Levy, 1994).

BLV transmission between animals is dependent on the transmission of infected B-Lymphocytes. Consequently, transmission can happen horizontally through blood and possibly animal-to-animal contact, as well as vertically *in-utero*, *peripartum*, through colostrum and milk (Hopkins and DiGiacomo, 1997; list of possible transmission routes can be found in Table 8-1). Animals with lymphocytosis or EBL were found to be more likely to transmit BLV to uninfected animals than aleukemic animals (Kettmann et al., 1980). Additionally, it has been discovered that infected animals harbor different amounts of integrated virus DNA (provirus) in their B-Lymphocytes (proviral load) (Esteban, 2009, Jimba et al., 2010). Animals with a high proviral load were found to be more likely to develop lymphocytosis and/or EBL than animals with a low proviral load (Nishiike et al., 2016, Kobayashi et al., 2019). Moreover, it was hypothesized that animals with a high proviral load were more likely to transmit the virus, whereas animals with a low proviral load seemed to have a low risk for virus transmission (Juliarena et al.,
2016, Mekata et al., 2018). However, detailed knowledge on the importance of the identified transmission routes with and without taking the proviral load into account, as well as the importance of different transmission routes compared to each other is still missing. Nonetheless, important transmission routes were found to be of iatrogenic nature through blood contaminated equipment and instruments, like needles, dehorners, and rectal examination sleeves, for example (Hopkins and DiGiacomo, 1997).

Because there is no cure or vaccination for the infection with BLV available to date, the reduction of BLV infection rates is dependent on the replacement of BLV infected with uninfected animals (Barez et al., 2015). This can be achieved by identifying and culling all BLV positive animals; by identifying and physically separating test positive from test negative animals; by the implementation of different BMP that prevent the transmission of BLV from infected to uninfected animals, or by combinations thereof (DiGiacomo et al., 1987, Shettigara et al., 1989, Ruggiero and Bartlett, 2019; Chapter 2). Eradication of BLV seemed to be possible independently of the within-herd prevalence (Ferrer, 1982, Deren et al., 2003). However, the effectiveness of these control strategies seemed to be directly dependent on how vigorously these strategies were pursued and how regularly BLV positive animals were identified (e.g. every 6 months), based on the review of available literature (Meszaros et al., 1994, Lojkic et al., 2013, Ruggiero and Bartlett, 2019; Chapter 2). Moreover, it seemed that eradication of BLV from a herd could only be achieved by using culling and/or segregation approaches in combination with regular testing schedules: the sole implementation of BMP has not yet been shown to result in the eradication of BLV from a herd (Chapter 2). However, the implementation of BMP was
reported to result in a decrease in within-herd prevalence and no studies’ reported time frame was sufficient to follow farms with implemented BMP until BLV eradication was achieved (Ruppanner et al., 1983, Sprecher et al., 1991). Additionally, taking the proviral load of infected animals into account, and eliminating or segregating high proviral load animals could further enhance BLV control by decreasing the risk for BLV transmission between animals in a herd. Some preliminary studies have been conducted to understand the implications of proviral loads for BLV control, but no definitive recommendations can be made at this time (Juliarena et al., 2016, Mekata et al., 2018, Ruggiero et al., 2019). Consequently, large scale, long-term studies are needed to shed light on the role of BMP and proviral load in BLV transmission and eradication.

Finally, the common high within-herd prevalence in North American dairy herds oftentimes prohibits the implementation of culling or segregation strategies. Therefore, a potential BLV control approach in high within-herd prevalence herds, for individuals or large-scale programs, would be the reduction of the within-herd prevalence through the implementation of BMP and/or the culling of high proviral load animals. Once a lower within-herd prevalence is reached, segregation and culling strategies could be implemented to achieve complete eradication of BLV from a herd. Depending on how (voluntary/mandatory) these programs could be established, control and eradication of BLV would necessitate a long-term commitment by government, industry, and farmers. Based on the high herd and within-herd prevalence it is difficult to estimate a realistic timeframe to eradication, as former, successful eradication programs were targeted at
populations with low BLV herd and within-herd prevalence (Burki, 1982, Acaite et al., 2007).

8.2 The identification of bovine leukemia virus infected animals

Before the identification of BLV and anti-BLV antibodies, infected animals were identified by evaluating white blood cell (WBC) counts. A key was developed that categorized animals according to their WBC counts and animals with leukocytosis were considered test positive (Bendixen, 1963). When serological tests were developed, it became apparent that more animals than identified by WBC count were infected with BLV and WBC counts were largely abandoned as identifying tests (Flensburg, 1976). Agar gel immunodiffusion tests (AGIDT) became the standard diagnostic test until the development and establishment of ELISA, which are more sensitive than AGIDT (Deren et al., 2003). In order to evaluate the test performance of commercially available ELISA in Canada, 5 ELISA kits were compared and evaluated by using 160 serum samples from Canadian dairy and beef herds. Sensitivity and specificity for these ELISA were found to be between 95-100%. The agreement between tests was found to be almost perfect to perfect with Cohen’s Kappa values between $\kappa=0.91$ and $\kappa=1$ (Kuczewski et al., 2018; Chapter 3). Additionally, other studies found milk ELISA results to agree $\geq$90% with serum ELISA results (Erskine et al., 2012b, Evermann et al., 2019). PCR was found to be able to identify infected animals earlier than ELISA (Klintevall et al., 1994). Additionally, quantitative PCR methods could be used to quantify proviral loads of BLV ELISA positive animals (Jimba et al., 2010, Petersen et al., 2018).
In order to measure the severity of a herd’s infection, to accelerate BLV eradication, as well as to monitor progress and success of control efforts, the BLV within-herd prevalence should be investigated on regular bases. Testing the entire herd would result in the most accurate estimate of the within-herd prevalence. Additionally, it would be possible to compare prevalence between age groups. Thus, age groups with the highest increase in prevalence and therefore the highest risk for BLV transmission could be identified. Then, BLV control could be focused on these age groups, increasing the effectiveness of BLV control (Erskine et al., 2012b, Bartlett et al., 2014). Nonetheless, farmers were found to be reluctant to invest in testing (Leach et al., 2010, Sorge et al., 2010; Chapter 6). However, different options to decrease cost for testing exist. Generally, using milk samples was found to be less expensive than using serum samples (cost/sample tested; communication with laboratories). Even though the use of milk samples restricts testing to milking animals, an accurate estimate of within-herd and within-age group prevalence could be made. As an alternative to testing the entire adult herd, Erskine et al. (2012b) found that, by testing a specific subset of the herd, the within-herd prevalence could be estimated and the age group with the highest increase in prevalence identified. Additionally, in order to obtain an initial estimate of the within-herd prevalence, bulk-milk samples could be tested and used to calculate an approximation of the within-herd prevalence (Nekouei et al., 2015a).

After the identification of infected animals, their proviral load could be investigated. The measurement of proviral loads has been dependent on relatively expensive quantitative PCR to this date (Petersen et al., 2018). Nonetheless, p24 specific ELISA OD results
seem to correlate with the proviral load (Gutierrez et al., 2012). Additionally, OD values for other ELISA seem to correlate with the clinical severity of BLV infection and therefore with proviral loads (Norby et al., 2016). Consequently, inexpensive alternatives to quantitative PCR might be available in the near future.

8.3 Bovine leukemia virus’ impact on the dairy industry, economical losses, and benefits of bovine leukemia virus control

The BLV herd prevalence in North America was recently found to be approximately 90% for many provinces in Canada as well as the United States of America (LaDronka et al., 2018, Kuczewski et al., 2019; Chapter 4). Therefore, BLV’s negative impact on the dairy industry should be considered substantial: As mentioned before, BLV infected animals can develop lymphocytosis and/or EBL. Additionally, due to the virus targeting cattle’s immune cells, BLV infected animals were found to suffer from an impaired immune system (Frie and Coussens, 2015). Hence, BLV infection was found to have production limiting effects on the dairy industry: Infected animals were reported to have less efficient immune responses to vaccines and therefore likely less efficient defense mechanisms toward pathogens (Erskine et al., 2011, Frie et al., 2016). Additionally, BLV infection was found to cause decreased milk production, as well as decreased cow longevity (Bartlett et al., 2013, Nekouei et al., 2016, Norby et al., 2016). Even though some studies could not find a connection between BLV infection and production decrease, the development of EBL results in a negative financial impact by causing the premature death of infected animals, loss of potential offspring and slaughter price, as
well as by potentially incurring cost for veterinary visits and treatments (Rhodes et al., 2003, Tiwari et al., 2007). Moreover, BLV has been eradicated in many European countries, as well as in the dairy herds of New Zealand and Australia, resulting in export restrictions to these countries and further economic damage (Voges, 2012, European Commission, 2015, Queensland Government Department of Agriculture and Fisheries, 2016,). In addition to production limiting effects, the infection with BLV can cause animal welfare issues, especially for animals with EBL (Bartlett et al., 2014). Moreover, recently, a potential link between human breast cancer and BLV was claimed (Buehring et al., 2015, Buehring et al., 2017). Even though no definitive conclusion about the causative nature of BLV has been established, this could result in consumer concerns and potential detrimental effects for the dairy industry.

When considering on-farm BLV control, losses because of BLV infection should be considered and compared to potential cost for control measures to evaluate potential financial benefits of BLV control. Depending on what kind of strategy would be chosen, different cost would be incurred (e.g. cost for needles, dehorner, gates to segregate animals). Therefore, in order to better understand specific losses due to BLV infection as well as potential cost for different BLV control strategies, an economic analysis was conducted (Kuczewski et al., 2019; Chapter 4). Infected animals were found to have an annual mean partial net revenue of Can$ 7,641, whereas the mean partial net revenue of an uninfected cow was found to be Can$ 8,276, when EBL, reduced milk production, as well as reduced cow longevity were taken into account. Implementation of BLV control was modelled for a 149-animal herd, with a 40% initial within-herd prevalence over 10
years for 5 different control strategies. The yearly mean net benefit per cow in a 149-animal herd was found to be between Can$ 785 and Can$ 1,594, if BLV control was implemented, compared to not implementing any BLV control on farm (Kuczewski et al., 2019/Chapter 4). Another economic evaluation also found that controlling BLV on farm resulted in a net benefit over time (Rhodes et al., 2003). Even though a thorough economic evaluation of different control strategies and their potential cost was conducted, it was found during the trial implementation of the developed control program (Chapter 7) that the implementation of on-farm BLV control could cause unexpected cost. For example, the purchase of a colostrum pasteurizer did not only comprise the cost for the pasteurizer, but also cost for the installment of new plumbing as well as ongoing cost for water usage and colostrum bags. Similarly, calculated benefits did not consider all benefits identified by farmers in various conversations. For example, farmers mentioned the increase in calf health, because of the use of a colostrum pasteurizer, resulting in less economic losses and less time spent tending to sick animals. Similarly, an improvement in animal health due to the lack of BLV’s negative impact on the immune system should result in less cost because of sick animals. However, it is important to mention that the assumed reduction in prevalence in the economic model might not reflect reality for all farms. This reduction in prevalence was assumed for an initial 40% within-herd prevalence and the implementation of all available BMP in a perfect manner. When comparing this assumption to the results of the trial implementation, the following aspects should be considered. Firstly, participating farmers in the trial implementation were not required to implement all or some BMP in a specified manner in a certain time
frame, as the goal was a critical evaluation of risk assessment, available BMP, as well as a better understanding of farmer motivation. Therefore, the assumed perfect implementation of all BMP in the economic analysis could not be guaranteed. Secondly, the model considered the adult herd of a farm only. Therefore, only the results for animals 2 years and older in the trial implementation should be considered for a comparison. Thirdly, participating herds’ prevalence varied greatly from 13 to 80%. The assumed reduction in prevalence might not be applicable to extremes in within-herd prevalence. By applying the last two aspects to the results of the trial implementation, only 3 farms’ prevalence was close to 40% (farm 3, 6, 11; 37, 36, 27% respectively). When applying the estimated reduction in prevalence of 25% for the implementation of all management strategies, the predicted within-herd prevalence after the first year of on-farm BLV control should be 27.75, 27, and 20.25% respectively, compared 25, 28, and 21% measured in the trial. Therefore, the assumed reduction in prevalence seems to be reasonable, when applied to farms with the mentioned prerequisites. Nonetheless, the reduction in within-herd prevalence was different for herds with higher or lower within-herd prevalence (e.g. farm 2, 8; 80, 64% respectively), making more applicable estimates necessary. Even though modelled economic evaluations could not reflect reality, it appeared that benefits outweigh cost for on-farm BLV control, further highlighted by participating farmers’ feedback. Based on these findings, well thought-through and detailed economic analyses can aid in the decision-making process of whether to implement disease-control programs or not. While it is impossible to fully reflect reality, a well-informed estimate can provide valuable information on the potential positive or
negative financial benefit of disease control and can help shape expectations. In order to
evaluate the true financial impact of BLV infection and control, detailed, long-term
studies in collaboration with farmers should be conducted and economic information as
well as within-herd BLV prevalence and on-farm management should be closely
monitored.

8.4 Dairy farmers’ and veterinarians’ feedback on bovine leukemia virus control

8.4.1. Dairy farmers’ and veterinarians’ opinion toward bovine leukemia

virus control

Based on reports about conversations with farmers and veterinarians after the
implementation of other control programs, it was noted that feedback from these
stakeholders should be obtained before the introduction of the new BLV control program
to Alberta dairy farmers on a large scale (Ritter et al., 2015, Roche et al., 2019). Farmers
as well as veterinarians were asked for feedback toward the three most common BLV
control strategies (test and cull/segregate/manage of positive animals) as well as toward
specific BMP suggested for the prevention of BLV transmission (e.g. single use of
needles, colostrum management; see Table 8-1 and 8-2 for a list; Rodriguez et al., 2011,
Bartlett et al., 2014) by conducting focus groups with farmers and interviews with
veterinarians (Chapter 5 and 6). Additionally, farmers that participated in the BLV
control program trial implementation were interviewed (Chapter 5). As a result, farmers
and veterinarians agreed that culling BLV positive animals would be an efficient strategy
for BLV eradication. Nonetheless, it was oftentimes economically not feasible, as the
within-herd prevalence in Alberta dairy herds commonly exceeds 40% (Nekouei et al., 2015b, Kuczewski et al., 2019; Chapter 4). However, the milk supply management system could have a potential effect by enabling or discouraging farmers to increase their culling rate, thereby making the prioritized culling of BLV positive animals more, or less possible (Chapter 5 and 6). Segregating BLV positive from negative animals was considered a logical and efficient option, but was also considered difficult to do, as it requires space as well as management changes that were perceived as impractical (Huijps et al., 2008, Roche et al., 2019; Chapter 6). Nonetheless, these strategies could become more relevant options once the within-herd prevalence was decreased by the implementation of BMP. The implementation of BMP to prevent the transmission of BLV between animals was generally considered affordable, practical, and feasible. Additionally, it would be the only strategy that could be introduced on farm without the necessity of testing any animals, by assuming all animals on farm were infected (Chapter 6), therefore, additionally decreasing cost for farmers. Some BMP were generally considered easy to do, for example the single use of needles. Additionally, other BMP were considered as “normal” and/or convenient (e.g. fly control) and were therefore perceived as easy to implement. However, other BMP were considered difficult to implement, because they were perceived as too expensive (e.g. purchase of a colostrum pasteurizer), inconvenient (e.g. single use of syringes), or too complicated (e.g. disinfection of drenchers; Chapter 6). This was highlighted during the trial implementation, as all participating farms were able to introduce the single use of needles, but only few farms implemented the single use of syringes. Similarly, no
participating farm purchased a new colostrum pasteurizer during the trial and only some farmers started the disinfection of drenchers between animals. On the other hand, some farmers introduced the single use of syringes, alongside extensive hygiene protocols for equipment that was used on multiple animals in a row (Chapter 7). The extent to which BLV control was implemented seemed to be dependent on the priority of BLV on farm. Nonetheless, it appeared that farmers (in focus groups as well as during the trial implementation) could appreciate the importance of on-farm BLV control after they were informed about BLV and its negative effects and were inclined to implement BMP they considered feasible. Similarly, behavior models commonly used to explain farmer behavior, highlight that farmers need sufficient knowledge and have to consider a disease a threat worthwhile their attention, before the implementation of control measures is considered (Janz and Becker, 1984, Ajzen, 1991). Therefore, advisors should focus on understanding and responding to farmers’ opinions, in order to increase compliance by offering additional explanations or alternative options (Lam et al., 2011, Jansen and Lam, 2012). The consistent, continuous implementation of imperfect BLV control should be preferred over inconsistent adherence to all BMP: An “all or nothing” approach could result in less efficient BLV control than looking for an “as good as possible” solution.

8.4.2. Motivators and barriers toward bovine leukemia virus control,
identified by dairy farmers and veterinarians

Because most disease control programs rely on management changes on farm and can’t be successful without the farmers’ support and commitment, direct communication with
farmers through focus groups as well as interviews during the BLV control program trial implementation was sought. Thereby, better understanding motivators and barriers that could influence farmers’ decision process to implement BLV control measures and/or general behavior change on farm. Additionally, the veterinarians’ feedback was taken into account, as they are important stakeholders in agriculture and are known to influence farmers’ decisions (Ritter et al., 2017; Chapter 5). During the conversations it was discovered that on-farm behavior change was comprised of three consecutive steps (Chapter 5): The initiation of change and realization that a change in behavior could be beneficial, the stepwise introduction of change by farm advisors with knowledge and good standard operating procedures (SOPs), followed by the implementation of new behavior on farm while overcoming old habits and other barriers. Oftentimes, farmers motivated to control BLV on their farm suffered emotionally and financially due to BLV infection and/or EBL cases. Nonetheless, by providing farmers with knowledge about the virus and the disease it’s causing, its negative financial impact, and the benefit of controlling BLV, motivation to control BLV could be increased and the implementation of change on farm could be initiated. It appeared that different farmers’ personalities and attitudes made it easier for some farmers to change on-farm management than for others. Moreover, overcoming old habits, as well as a lack of resources (e.g. time, manpower, funds) were identified as important barriers in the implementation process (Ellis-Iversen et al., 2010, Roche et al., 2019; Chapter 5). However, by providing farmers with sufficient knowledge, realistic expectations toward BLV control, as well as SOPs for BMP that were tailored to their farm and situation, it seemed relatively easy for farmers
to make the decision to control BLV on their farm, implement, and continually adhere to 
the recommended BMP (Ritter et al., 2015; Chapter 7). Even though considered 
challenging by some farmers, it appeared to be possible to convince employees to adhere 
to the new BMP as well. However, including the hoof trimmer and/or veterinarian 
seemed to be considered more difficult (Chapter 5). During the trial implementation of 
the BLV control program it became apparent that convincing hoof trimmers to disinfect 
their tools between animals was either straightforward or extremely difficult, when hoof 
trimmers could not see the importance of their behavior. In addition, veterinarians were 
found to be important for the farmer’s opinion on BLV (Chapter 5): The veterinarian’s 
feedback could increase the priority of BLV control on farm or cause the farmer’s 
motivation to decrease, because of negative feedback from the veterinarians and/or their 
unwillingness to adjust their behavior during pregnancy examinations (single-use of 
examination gloves or examination of test negative animals before test positive ones). 
Nonetheless, regular reminders, as well as a team approach in problem solving seemed to 
encourage the long-term implementation of BLV control (Kristensen and Enevoldsen, 
2008; Chapter 5). However, during the in-depth conversations, it was found that no two 
farmers were motivated and/or hindered by the same factors. All farmers were influenced 
by an individual combination of drivers (Chapter 5). Understanding these and adjusting 
recommendations accordingly was found to be an effective way of convincing farmers to 
implement recommended practices during the trial implementation, as well as to increase 
the number of management practices that were implemented to control BLV on farm. 
Therefore, in order to motivate more farmers to implement BLV control, based on
voluntary control programs, advisors should increase their efforts to ask open questions, understand individual drivers and barriers, and find practical solutions, thereby increasing the likelihood of on-farm behavior change (Jansen, 2010, Ritter et al., 2016, Nogueira Borden et al., 2019). Moreover, if eradication of BLV from larger areas, provinces, or countries should be achieved, the implementation of a formal mandatory program would be necessary. These programs could be established by governments and implemented by local organizations like Alberta Milk (Edmonton, AB, Canada), for example. Alternatively, voluntary control programs could be established. In both scenarios, government and industry organizations should work together with local veterinarians and other industry organizations (e.g. hoof trimmer association or similar). It is important to unify advisors’ and industry’s voices in order to send clear messages and avoid conflicting information. Thereby, more farmers can be motivated and important potential shortcomings that could cause skepticism, decreased motivation, and lack of commitment could be prevented. By increasing the importance of one topic within the industry, it is seemingly given higher priority on farm (Chapter 5), potentially resulting in higher initial voluntary participation rates.

Finally, when comparing motivators and barriers encountered by farmers in the beginning of the trial implementation and at the end, no differences could be found. All farmers were planning to continue with the implemented BLV control measures regardless of the within-herd prevalence at the end of the trial. However, the observed time frame was short (approximately 13 months) and farmers’ motivation might change in the following years, for example, if control efforts don’t have the expected effect. Nonetheless, if
positive effects can be shown, the motivation to continue seems to increase and could result in successful on-farm BLV control and eradication. Long-term studies with follow-up visits could help understand change in motivation and its impact on BLV control on farm over time.

8.5 The design and evaluation of a bovine leukemia virus risk assessment

In order to identify behavior on farm that allowed for the transmission of BLV, a risk assessment tool as used in other disease control programs was considered the most appropriate approach (Sorge et al., 2011, Wolf et al., 2014). Based on the feedback of farmers and veterinarians (Chapter 6), it was decided that the risk assessment should focus on a test and manage approach for on-farm BLV control. Therefore, the risk assessment was meant to identify flaws in on-farm management that would potentially allow the transmission of BLV between animals (Chapter 7). Additionally, it was noted during the focus groups with farmers that detailed questions were necessary to identify important behavior on farm that could cause the transmission of BLV. For example, the question “Do you use an electric dehorner to dehorn calves?” oftentimes yielded the answer “Yes.”. However, producers only mentioned the common use of a gouge dehorner, when dehorning calves and the exposure of calves to a high risk of BLV transmission after more detailed questioning (DiGiacomo et al., 1985). Consequently, the risk assessment consisted of detailed questions and a variety of detailed answers about common on-farm management. Moreover, by weighing the possible management practices offered as answers in the risk assessment, it was possible to rank on-farm
management by importance for BLV transmission. For example, the pooling of colostrum for calves was assigned the highest risk, whereas the feeding of pasteurized colostrum was assigned the lowest risk for BLV transmission via colostrum (Meas et al., 2002). Similarly, the implementation of regular fly control was weighted as less important than colostrum management (Hopkins and DiGiacomo, 1997). Therefore, prioritization of BMP was possible, and recommendations could be adjusted accordingly (Chapter 7).

In order to evaluate the design and ability of the risk assessment tool to accurately capture on-farm behavior, it was used on 11 Alberta dairy farms as part of the trial implementation of a BLV control program (Chapter 7). The risk assessment was conducted with all participating farms. Additionally, all animals on farm that were ≥ 12 months old were serum tested for BLV antibodies by ELISA, allowing for the measurement of within-herd prevalence, identification of BLV positive animals, as well as identification of the age group with the highest increase in prevalence. Based on the results of the risk assessment and the test results, all participating dairy farms were recommended BMP, tailored to their farm, to prevent the transmission of BLV. After one year of implementation, the herds were tested again, and seroconversions as well as within-herd prevalence were investigated. It was found that the design of the risk assessment allowed for the successful identification of suboptimal on-farm behavior. Additionally, by doing the risk assessment with the farmer and providing him/her with additional knowledge applicable to his situation, it appeared that his/her interest as well as motivation to control BLV could be increased. Farmers oftentimes changed their behavior beyond suggested BMP and were able to overcome barriers (e.g. time to thaw
frozen colostrum) by finding useful solutions (e.g. construction of colostrum-thawing-device). Moreover, during the trial implementation, all farmers implemented at least one of the suggested BMP. After 1 year of implementation, a decrease in within-herd prevalence was found on all, but 3 farms (overall average: 39 to 36%), even though at least three (range: 3-109) seroconversions were found on each farm. One farm’s within-herd prevalence remained stable (43%), whereas one farm’s within-herd prevalence increased (26 to 40%). This was assumed to be due to a decrease in herd size combined with possible new infections due to natural breeding; and the lack of the single-use of needles in the beginning of the trial implementation, as well as the purchase of a large number of untested dairy cows, respectively. Finally, one farm terminated the trial, before the second sampling could be conducted (Chapter 7). The results of this trial showed the ability of the risk assessment to identify suboptimal on-farm behavior: possible BLV transmission routes could be identified and addressed on all farms. Additionally, the common decrease in within-herd prevalence, despite seroconversion of some animals, was a promising finding for the effectiveness of BLV control by implementing BLV control measures. It is important to note that the goal of the trial implementation was not the reduction in within-herd prevalence, but the better understanding of farmer behavior as well as obtaining farmer feedback on risk assessment and BMP and the critical evaluation thereof. Additionally, it was up to the farmer to decide when which BMP were implemented and infection might have happened before the implementation of BMP. Therefore, it was not possible to determine the time of infection based on the available samples. In order to better understand the source of seroconversions as well as the
effectiveness of on-farm BLV control by implementing BMP in the field and its capability to eradicate BLV on farm, long-term studies, monitoring multiple farms’ approaches should be conducted.

8.6 Implications for future research

Throughout the development process, it was found that the awareness of as well as knowledge about BLV and its effect on the dairy industry should be increased within the farmer as well as within the veterinary community. With that, control efforts should be prioritized and the benefit of combining BLV and other disease control should be emphasized. For example, by pasteurizing colostrum, calf health can be improved by eliminating other pathogens (Godden et al., 2012). Moreover, by establishing formal control and certification programs, additional incentives to increase BLV control could be offered (Nielsen, 2011, Ritter et al., 2017), thereby convincing more farmers to implement on-farm BLV control.

Additionally, it was found that recent data on BLV transmission routes’ applicability to modern farms is missing. For example, the reuse of hoof trimming knives and other equipment without cleaning between animals has unknown risks. Similarly, the use of breeding bulls appeared to cause BLV transmission during the trial implementation. However, available data suggests that bulls infected with low proviral loads did not transmit BLV (Benitez et al., 2018, Mekata et al., 2018), but data on bulls infected with a high proviral load is missing. Additionally, large-scale detailed data on various other transmission routes (e.g. rectal examinations), taking different animals’ proviral loads
into account is missing. Consequently, the role of proviral loads in the transmission of BLV has to be investigated further and taken into account, when examining transmission risks. By better understanding BLV transmission, advice could be improved and risk evaluation could be done more accurately, thereby improving on-farm BLV control. Additionally, during long-term studies, the financial impact of BLV infection as well as the real cost for BLV control could be measured, allowing for a true economic evaluation of BLV’s financial impact and the benefit of BLV control. Finally, the relationship between advisors and farmers should change in order to improve on-farm disease management. By adjusting communication patterns to more open-ended questions and by better understanding each other, the adjustment of on-farm management could be done more successfully and efficiently.
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herds by identifying and removing cows with the highest proviral load and

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for control of bovine leukemia virus infection: test and corrective management.


Table 8-1. Transmission routes of bovine leukemia virus (BLV) and possible alterations in management to control transmission of BLV.

<table>
<thead>
<tr>
<th>Transmission route</th>
<th>Corrective management (starting with best option)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction of BLV by purchasing animals</td>
<td>Do not purchase animals (male and female)</td>
</tr>
<tr>
<td></td>
<td>Only purchase BLV test negative animals</td>
</tr>
<tr>
<td>Contact to other herds</td>
<td>Avoid contact with other herds (over the fence, cattle shows, similar)</td>
</tr>
<tr>
<td>Farm visitors</td>
<td>Make the use of freshly laundered coveralls and clean rubber boots mandatory</td>
</tr>
<tr>
<td>Stocking density</td>
<td>Do not overstock</td>
</tr>
<tr>
<td>Housing of youngstock</td>
<td>Do not use a heifer rearer</td>
</tr>
<tr>
<td></td>
<td>Do not house heifers together with adult animals</td>
</tr>
<tr>
<td>Needles</td>
<td>Do not reuse needles between animals</td>
</tr>
<tr>
<td>Syringes</td>
<td>Do not reuse syringes between animals</td>
</tr>
<tr>
<td></td>
<td>Clean and disinfect syringes between reuses</td>
</tr>
<tr>
<td>Equipment (tattoo pliers, ear tagger, ear notcher, removal of supernumerary tests, hoof knives, pill gun, stomach tubes, i.v. tubes, dehorning wire, obstetrical straps/chains)</td>
<td>Clean and disinfect between animals</td>
</tr>
<tr>
<td>Hoof trimmer (hand knives, electric tools)</td>
<td>Clean and disinfect equipment between animals</td>
</tr>
<tr>
<td></td>
<td>Trim test negative animals before test positive animals</td>
</tr>
<tr>
<td>Fly control</td>
<td>Follow strict and regular fly control protocols</td>
</tr>
<tr>
<td>Artificial insemination</td>
<td>Do not reuse examination sleeves between animals</td>
</tr>
<tr>
<td></td>
<td>Do not split straws between animals</td>
</tr>
<tr>
<td></td>
<td>Inseminate test negative animals before test positive animals</td>
</tr>
<tr>
<td>Natural breeding (bull)</td>
<td>Do not use natural breeding</td>
</tr>
<tr>
<td></td>
<td>Only purchase test negative bulls, use separate bulls for test positive and test negative cows</td>
</tr>
<tr>
<td>Rectal pregnancy examinations</td>
<td>Do not reuse rectal examination sleeves between animals</td>
</tr>
</tbody>
</table>
|                             | Clean and disinfect rectal examination sleeve between animals  
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Examine test negative animals before test positive animals</td>
</tr>
</tbody>
</table>
| Calving (dam)               | Have separate calving areas for test positive and test negative dams  
|                             | Have separate calving areas for heifers                       |
| Calving (calf)              | Do not let calf suckle dam                                    |
| Colostrum                   | Feed pasteurized colostrum                                   
|                             | Feed colostrum replacer                                      
|                             | Feed frozen colostrum                                        
|                             | Feed colostrum from negative dams only                       
|                             | Do not pool colostrum                                        |
| Milk                        | Feed pasteurized milk                                         
|                             | Feed milk replacer                                            
|                             | Feed milk from negative dams only                            |
Table 8-2. Possible bovine leukemia virus (BLV) control strategies

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Possible management practices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test and cull</td>
<td>Test animals on regular bases (every 4-12 months) and cull test positive animals immediately or determine proviral load of test positive animals and eliminate high proviral load animals first.</td>
</tr>
<tr>
<td>Test and segregate</td>
<td>Test animals on regular basis (every 2-4 months initially, then every 6 – 12 months) and physically segregate test positive animals from test negative ones. Either in a separate barn, in a separate pen within the same barn without contact to each other, or in the same barn with a foil wall to separate positive and negative animals. Segregate heifers from dry cows. Have separate calving pens for test positive and test negative animals.</td>
</tr>
<tr>
<td>(Test and) manage</td>
<td>If possible: test animals on regular basis (e.g. once a year before dry off); implement as many best management practices from table 1 as possible.</td>
</tr>
</tbody>
</table>
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APPENDICES

Appendix A

Interview and focus group guides used in Chapter 5 and 6

1. Focus group guide for focus groups with farmers.

Focus group guide: Improvement of and opinions about a BLV control program

Schedule

Survey: Demographics + Risk Assessment;

Greeting, short introduction and outline of the day (10:00 AM)

I would like to start with a short introduction round and it would be great, if you could answer the following questions:

• What’s your name? Where do you farm? Why are you here today?

Bovine Leukemia Virus (BLV) and Leukosis (10:30 AM)

Open Discussion:

We will start with a very broad and open discussion about the dairy industry and we will then work our way to more detailed topics, especially Bovine Leukemia Virus/Leukosis.

• What, in your opinion, are the 5 most important issues you are concerned about on your farm? (Any topic! Not restricted to diseases)
  => write on cards

  o How would you rank these issues compared to each other, based on their importance to you?
    => pile sorting (most important on top), number cards according to order (1 = most important)
  o How would you rank these issues compared to each other, based on your power to change them?
    => pile sorting (most power on top), do not shuffle!

• What, in your opinion, are the 5 most important issues for the dairy industry? (Any topic! Not restricted to diseases.)
  => write on cards
How would you rank these issues compared to each other, based on their importance to you?
=> pile sorting (most important on top)
How would you rank these issues compared to each other, based on your power to change them?
=> pile sorting (most power on top)

- What do you know about Leukosis? (For example: How is it transmitted? What do you know about the disease?)
=> Write results on a white board.

- Do you think BLV/Leukosis is a problem for the dairy industry and/or your farm?
- What makes you think it is important/not important?

<table>
<thead>
<tr>
<th>Goals:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Importance of BLV for the industry, compared to other problems</td>
</tr>
<tr>
<td>- Importance of BLV on farm, compared to other problems</td>
</tr>
</tbody>
</table>

Combine ideas that came up during discussion with details about BLV, we want to make sure everybody is aware of. (Poster, 5-10 mins)

Goal: Pre-existing knowledge of BLV

**Control (11:15 AM)**

Open Discussion:
- If you hear us say “control program”, what do you think we are talking about?
- What do you expect from a control program? (Effectiveness, labor-intensiveness, time, ..)
- What aspects of a control program do you think are important?

- Combine thoughts of Leukosis and control program: How would you control Leukosis on your farm? (Which management strategies/activities on your farm, do you think, are important in the transmission of the virus? How can you change these activities to prevent transmission?)

<table>
<thead>
<tr>
<th>Goals</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Control Program: Expectations, Important Aspects</td>
</tr>
<tr>
<td>- BLV control on Farm: Important factors on farm, possibilities for prevention of transmission</td>
</tr>
</tbody>
</table>
12.00 Break (Lunch)

12:45 PM

- Sum up ideas from before lunch
- Did you have any new ideas over the lunch break?

=> Probing for control strategies that haven’t come up yet and evaluating them.

- E.g. What about needles? Do you think they are a problem?; Repeat probing until all important strategies are discussed.
- Goal of discussion:
  - Which strategies do you think are practicable? What makes you consider them practicable?
  - Which strategies do you think are not practicable? What makes you consider them not practicable?
  - Which strategies need a high compliance of the farmer? What makes you think of these strategies in this way (=barriers)?
  - How can we remove those barriers?

<table>
<thead>
<tr>
<th>Goals</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Important control strategies</td>
</tr>
<tr>
<td>- Practicality + WHY</td>
</tr>
<tr>
<td>- Barriers</td>
</tr>
<tr>
<td>- Solutions/Tweaks to make control easier</td>
</tr>
</tbody>
</table>

Short Summary of findings regarding BLV and its control

Action:

Hand out list of management strategies for ranking:

- Could you please rank the suggested changes according to how hard they are to implement in your opinion?!
- Could you please rank the suggested changes according to how important they are to combat BLV in your opinion?!

Motivation (1:45 PM)

- What motivates you to make changes on your farm?
• Why have you made changes before? (Recent changes in protocol, bedding, milking, etc. and why)
• What do you think motivates other producers to think about, implement and continuously use a BLV control program on their farm?
• What impact does the action of your peers have on your action?
• What impact does your veterinarian have on your action?

Goals
-Motivation
-Personally
-Others
-Peers
-Vet

Short Summary of findings regarding Motivation (2:30 PM)

Summary of other topics, any additional ideas??

• Any other ideas: more discussion after: phone, email, on-farm; pictures, etc.

“Thank you and Goodbye” (2:50 PM)

Ultimate Goals:
• importance of BLV for present farmers
• initial insight in general knowledge of farmers about BLV
• set of ranked, feasible management strategies
• details about the risk assessment (length, format)
• initial overview of involved motivational factors
  o Which strategies are “popular”? Why? (Affected by knowledge/priority? Consent over ranking and reasoning?)
  o What are producers willing to change, even when a strategy isn’t “easy”? What incentive is needed?
  o Why do producers change their behavior?
2. Interview guide for interviews with veterinarians.

Interview guide: Improvement of and opinions about a BLV control program

Schedule

Survey: Demographics + Risk Assessment:

Greeting, short introduction and outline of the interview

*Bovine Leukemia Virus (BLV) and Leukosis*

- “Icebreaker”: How would you describe your role in disease control on farm? (Pro-active, only when farmer asks for help, strong herd health approach, etc.)
- How will your role or the role of the veterinary profession change in the future? (long pause after!)
- How important do you think Leukosis is for the dairy industry in general?
- How big do you consider the impact of Leukosis for individual producers?
- Have you ever encouraged or recommended farmers to control BLV on their farm?
- Which management strategies have you recommended to those farmers?
- Which of those strategies did the farmers implement?
- How would you describe your level of knowledge of BLV?
- Would you mind elaborating further on BLV, its clinical signs, transmission, and diagnosis?
- If you could design a continuing education workshop for veterinarians, what would you like to see?

If necessary: Short discussion about Leukosis

<table>
<thead>
<tr>
<th>Goals:</th>
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<tbody>
<tr>
<td>-Importance of BLV for the industry, compared to other problems</td>
</tr>
<tr>
<td>-Importance of BLV on farm, compared to other problems</td>
</tr>
<tr>
<td>-BLV in herd health</td>
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<tr>
<td>-Pre-existing knowledge of BLV</td>
</tr>
</tbody>
</table>

*Control*

- What do you expect from a control program?
- If you could design your perfect on-farm Leukosis control program, what would it look like?
• What would you identify as risk factors in transmission of BLV? How important do you think they are?
  -> comparison to risk factors on list

• Could you identify other disease control measures on farm that are beneficial in BLV control?
• *Because we realize that this could become a problem and because we don’t want the veterinarians to be the bottle neck in BLV control:*
• How important do you think examination sleeves are for the transmission of BLV? Why?
• Are you changing examination sleeves between animals?
• What is holding you back from changing examination sleeves during pregnancy checks?
• What, do you think, would motivate you and other veterinarians to change examination sleeves during pregnancy checks on farm?

(List of control strategies, evidence for transmission of BLV with sleeves (study underway in Michigan)

<table>
<thead>
<tr>
<th>Goals</th>
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</thead>
<tbody>
<tr>
<td>-Control Program: Expectations, Important Aspects</td>
</tr>
<tr>
<td>-Perceived relative importance of transmission routes</td>
</tr>
<tr>
<td>-BLV control within herd health</td>
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<tr>
<td>-Opinion about “sleeve BMP”</td>
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</tbody>
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<thead>
<tr>
<th>Action:</th>
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<tbody>
<tr>
<td>Hand out list of management strategies for rating:</td>
</tr>
<tr>
<td>• Could you please rate the suggested changes according to how hard they are to implement in your opinion!?</td>
</tr>
<tr>
<td>• Could you please rate the suggested changes according to how important they are to combat BLV in your opinion!?</td>
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</tbody>
</table>

<table>
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<tr>
<th>Experience with producers</th>
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<tbody>
<tr>
<td>• Which strategies need a high compliance of the producer? Why (=barriers)?</td>
</tr>
<tr>
<td>• What could be a solution to these barriers?</td>
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<tr>
<td>• How do you motivate “your” producers to make changes in their on-farm management?</td>
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<tr>
<td>• What do you think motivates producers to think about, implement and continuously use a BLV control program on their farm?</td>
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<tr>
<td>• How do you reach “hard to reach farmers”?</td>
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<tr>
<td>Goals</td>
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<tr>
<td>-------</td>
</tr>
<tr>
<td>- Compliance for control</td>
</tr>
<tr>
<td>- Practicality of BMP and why</td>
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<tr>
<td>- Barriers</td>
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<tr>
<td>- Solutions/Tweaks to make control easier</td>
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<tr>
<td>- Motivation for change and/or BLV control</td>
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**Ultimate Goals:**
- Understand what veterinarians know about BLV and its control
- Understand if veterinarians consider BLV a problem and why they do/don’t (yes/no)
- Understand how important veterinarians consider BLV to be (to which degree)
- Understand motivation of veterinarians to implement control (especially sleeves)
- Understand how to motivate them to adopt control strategies (especially sleeves)
- What do veterinarians think farmers think?
  - How important do farmers consider BLV?
  - How high is the motivation of farmers to do something against BLV?
  - How can we generate motivation amongst farmers?
3. **Rankings used during focus groups and interviews.**

Rankings were identical for both events with the exception of “pasteurize milk” and “feed milk replacer”. These two items were only evaluated by the veterinarians.

<table>
<thead>
<tr>
<th>Please mark an X to rank the following control strategies with regards to ability to implement</th>
<th>Easy to implement</th>
<th>Worth a try</th>
<th>I don’t know</th>
<th>Hard to implement</th>
<th>Impossible to implement</th>
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<tr>
<td>Single use of needles</td>
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<td>Single use of syringes</td>
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<td>Treat uninfected animals first, then infected animals</td>
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<tr>
<td>Single-use of examination sleeves (producer)</td>
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<tr>
<td>Single-use of examination sleeves (veterinarian)</td>
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<tr>
<td>Examine uninfected animals first, then infected animals</td>
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<td>Replace bull with A.I.</td>
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<td>Remove calf from dam before it can suck for the first time</td>
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<td>Freeze colostrum</td>
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<td>Use electric dehorner</td>
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<td>Disinfect equipment between uses (e.g. ear-tagging, hoof trimming)</td>
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<tr>
<td>Increase fly control</td>
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<td>Testing all animals in the herd</td>
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<td>Test and cull</td>
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<td>Test and segregate</td>
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<tr>
<td>Control Strategies</td>
<td>Not Important</td>
<td>Somewhat Important</td>
<td>I don’t know</td>
<td>Important</td>
<td>Very important</td>
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4. Interview guides used for interviews during trial implementation of bovine leukemia virus control program

Pre- and post-Implementation Opinions on an on-Farm Bovine Leukemia Virus Control Program

You agreed on being part of the trial implementation of an on-farm Bovine Leukemia Virus (BLV) control program. This includes the following:

We will sample all animals on your farm, 12 months and older and test their blood for BLV. Additionally, we will conduct a risk assessment for your farm and suggest a tailored action plan, consisting of at least one best management practice that is meant to reduce the number of Bovine Leukemia virus infected animals on your farm. We would also like to ask you some questions before and after the risk assessment and every three months during the following year after you implemented the control program on your farm (5 questionnaires in total).

These questionnaires are intended to help us understand critical points in the control program, practicability of some management changes as well as possibilities for improvement. On top of this we want to understand what is important for you in order to be interested in this virus and the disease, to consider and implement a control program on your farm, as well as find reasons that make it worth for you to continue the control program over an extended period of time. By better understanding these topics, we want to motivate more producers to control BLV on farm.

If you feel uncomfortable answering any of the questions, please feel free to not answer them.

Survey ID # (for office use only)

Date

Name of Producer

Farm Name
Questions Pre-Implementation

BLV-specific Questions

1. Do you think BLV is a problem for the dairy industry?
   - Yes
   - No
   1.1. Why?

2. Do you think BLV is a problem on your farm?
   - Yes
   - No
   2.1. Why?

3. How important is BLV compared to four other animal diseases issues on your farm?
   (please rank them 1-5)

4. Are you currently controlling BLV on your farm?
   - Yes
   - No
   4.1. If yes, please list them below.
   4.1.1. If yes, why did you stop?
   4.1.2. If no, why not?
Before the Risk Assessment

Before we start with your risk assessment, we would like to ask you a few questions that aim on learning about the knowledge you already have, your expectations for BLV control and motivational factors that are important.

1) Why do you want to participate in this trial?

2) What do you know about Bovine Leukosis? (E.g. cause, transmission, disease, prevention)

3) Where did you learn what you know?

4) What do you expect from this control program?

5) What do you think are the best management practices suggested in this control program?

6) How important do you think these best management are for your farm? Please rank them from least to most important.

7) Do you want to implement this control program?
   - Yes
   - No
   7.1) Depending on you answer above: Why do you want to implement the control program? / Why do you not want to implement the control program?

8) How much extra time per day, week, and month would you be willing to invest into a control program on your farm?

9) Does any of the following persons play an important role in motivating you to control BLV on your farm?
   - Veterinarian
   - Neighboring farmer
   - Hoof trimmer
   - Feed specialist
   - Milking parlor specialist
   - Other, please specify: __________________

10) How did your peers affect your decision to start controlling BLV on your farm?

11) What do you think will make other producers interested in a BLV control program?
After the Risk Assessment

Now that you know your personal action plan, we would like to get your opinion about risk assessment and action plan.

1) Do you think we missed something when we introduced the control program to you/in the risk assessment/in the best management practices we proposed to you or any other part?
   o Yes
   o No
   1.1) If yes, what did we miss?

2) What do you think will be easy about the implementation of the suggested best management practices?

3) Which difficulties can you identify, when you think about the implementation of the best management practices?

4) How long, do you think, are you going to take to get used to the new best management practices?

5) How long do you expect to adhere to your on-farm control measures until you will notice a difference in the number of infected animals on your farm?

6) How are you going to ensure that all employees and/or temporarily hired hands on your farm will adhere to the implemented best management practices?
Post-Implementation (3 months)

Since you have been working with your personal control program for some time now, we would like to learn about your opinion about and your experience with it. We would also like to know how your opinion towards the control program has changed. In order to see if your perception changes over time and to make sure that we don’t miss anything, we will interview you multiple times during the next 12 months. Please keep in mind that you are not forced to answer any of these questions. Your decision to continuously implement any best management strategies or to not implement them, has no consequences for your participation in this trial.

1) Which best management practices did you implement and for how long?

2) Do you still follow the best management practices the way they were suggested after your initial risk assessment?
   - Yes
   - No
   2.1) If no, what are you doing differently from the initial suggestions?
   2.2) If no, why did decide to implement different best management practices?
   2.3) If yes, why did you decide to implement the best management practices the way they were suggested?

3) How did you implement the best management practice(s) you committed yourself to? (E.g. containers for used needles, testing of bulls, pasteurizing colostrum)

4) Which best management practices were easy to introduce to your farm?
   4.1) What made it easy?

5) Which best management practices were hard to introduce to your farm?
   5.1) What made it hard?

6) How long did it take you to get used to the new best management practices?

7) Have you seen results yet?
   - Yes
   - No
   7.1) If your answer is yes: what are the results you have seen?

8) What were some of the challenges that you experienced, when you introduced the new best management practices to your employees?

9) How did you ensure the compliance of all employees?

364
10) How much extra time (per day/week/month) did you spend on this control program?

11) Did you receive help from other parties with the implementation of the recommended best management strategies? (E.g. veterinarian, hoof trimmers, ...)
   - Yes
   - No

   11.1) If yes, what did the help include?

12) How did other involved parties (e.g. veterinarian, hoof trimmer) react to your suggestions?

13) Would you sign up for the trial implementation of the control program again?
   - Yes
   - No

   13.1) If no, why not?
   13.2) If yes, why?

14) Which suggestions for the improvement of the control program and the best management strategies in general do you have?

15) What tips do you have for other farmers (e.g. handy implementation strategies)?

16) What were your expectations for this control program?

17) Did the control program meet your expectations so far?
   - Yes
   - No

   17.1) If you answer was no: What is different from what you expected?

18) What would you have liked to know before committing to the control program?

19) What are your expectations for the control program and its effects on your farm as you are moving forward?

20) Did you talk to anybody else about your participation in the trial of the control program? Who?

21) What would you like to know about Bovine Leukemia Virus?

22) How would you like to learn?
Post-Implementation (6 months)

1) Which best management practices did you implement and for how long?

2) Do you still follow the best management practices the way they were suggested after your initial risk assessment?
   ○ Yes
   ○ No
   2.1) If no, what are you doing differently from the initial suggestions?
   2.2) If no, why did decide to implement different best management practices?
   2.3) If yes, why did you decide to implement the best management practices the way they were suggested?

3. How did you implement the best management practice(s) you committed yourself to? (E.g. containers for used needles, testing of bulls, pasteurizing colostrum)

4. Which best management practices were easy to introduce to your farm?
   4.1) What made it easy?

5. Which best management practices were hard to introduce to your farm?
   5.1) What made it hard?

6. How long did it take you to get used to the new best management practices?

7. Have you seen results yet?
   ○ Yes
   ○ No
   7.1) If your answer is yes: what are the results you have seen?

8. What were some of the challenges that you experienced, when you introduced the new best management practices to your employees?

9. How did you ensure the compliance of all employees?

10. How much extra time (per day/week/month) did you spend on this control program?

11. Did you receive help from other parties with the implementation of the recommended best management strategies? (E.g. veterinarian, hoof trimmers, ...)
   ○ Yes
   ○ No
   11.1) If yes, what did the help include?

12. How did other involved parties (e.g. veterinarian, hoof trimmer) react to your suggestions?
13. Why were you interested in BLV control in the first place?

14. Why does BLV matter to you?

15. How likely are you to continue with this control program after our study is done?
   15.1) What are your reasons for continuing/not continuing?

16. How likely are you to continue testing for BLV after this study is done?
   16.1) What are your reasons for continuing/not continuing?

17. Would you pay your veterinarian to do the risk assessment with you?
   17.1) What are your reasons for wanting to pay him/her/not wanting to pay him/her?

18. What would have made your life easier in the process of this control program?

19. What 2 things made the control program manageable for you so far?

20. What 2-3 things made the control program difficult for you?

21. Since my last visit, did you ever think about stopping the control program? If so, why?

22. Going forward, which 2-3 things would best help/support you to continue with the control program? (e.g. producer meetings, pamphlets, your herd veterinarian)

23. How do you feel, when externals come to your place and disregard your best management practices?

24. How can we help you to ensure the compliance of others with your personal best management practices?

25. Which role does the veterinarian play in disease control on your farm? (initiator, mentor, …)

26. Which role would you like the veterinarian to play in disease control on your farm?
Post-Implementation (12 months)

Now that you have the final test results and had one year to gain experience with Bovine Leukemia Virus (BLV)/Leukosis and its control, I have some final questions for you.

1) What do you think about the test results? (possible follow-up questions: Are you satisfied? Are you disappointed? or where you expecting these results?)

2) How did the test results affect your motivation to continue with BLV control?
   2.2) Why?

3) Which best management practices for BLV control where suggested to you?

4) Looking back at the last year, which of the listed control measures were easy for you to implement?
   4.1) What made it easy?

5) Which of the listed control measures didn't you implement?
   5.1) Why didn't you implement them?

6) What is your overall impression of the control program? Can you illustrate what you thought were good or bad components.

7) If you could do it again, what would you do differently?

8) Do you think the blood testing is valuable? Why?

9) How did you use the results?

10) If you could, how would you change the sampling strategy?

11) Would you prefer milk sampling for the milking animals?
    11.1) Is that easy to implement on your farm?

12) Would you be interested in future sampling of your herd?
    12.1) Under what conditions?
Appendix B

Risk assessment used for the trial implementation of a bovine leukemia virus control program.

On-farm BLV Risk Assessment

Farm Name:
Owner’s Name:
Legal Land Description: Section Township Range Meridian
Or GPS Coordinates: Latitude Longitude
Veterinarian:
Veterinary Phone: Veterinary Email:
Date of risk assessment (yy-mm-dd):
General Farm Information

Date of last BLV risk assessment (leave blank for first RA) (yy-mm-dd)

Type of housing for milking cows

Type of milking system

Number of: Lactating cows:  Dry cows:  Breeding bulls:

% of herd in 3+ Lactation:

Is this herd on DHI?  If yes: herd DHI number:
BLV-Specific Information
Do you export cattle?
-Yes
-No
If yes: Is the BLV status important for the countries you export to?
-Yes
-No
How many animals in your herd have you tested for BLV before?
-The entire adult herd, or more than 80%
-A specific subset of animals: __________
-Only some animals
-No animals
If you have tested the entire adult herd:
-When?
-How high was the prevalence in total?
-Do you test animals on regular basis (at least once every two years)?
-no
-heifers
-entire milking herd
-subgroup of animals (Please specify________)
-only negative animals
-bulk tank milk
Have you had a clinical case of Enzootic Bovine Leukosis during the past 5 years?
-No
-Yes
If yes: How many were confirmed via PM and/or ELISA?
__________
How do you segregate BLV positive from BLV negative cattle?
-BLV positive and negative cattle are housed in separate barns
-BLV positive and negative cattle are housed in the same barn, but in separate pens
-We do not segregate BLV positive from negative cattle
Is BLV a factor for culling animals?
-Yes
-No
If yes: How important is the BLV status for culling?
-Test-positive animals are culled as soon as possible
-Test-positive animals are culled if other clinical signs appear
-BLV is taken into account when a decision between multiple animals has to be made
-Animals that are likely to have tumors are culled as soon as possible
Do you manage calves from dams known to be BLV+ differently from other calves?
-Yes, we_________________________
-No
Based on your current herd test, could you please record the percentage of infected animals in each age group?
- Heifers
  - 1st lactation
  - 2nd lactation
  - 3rd lactation
  - 4th and older than 4th lactation
### Results of Risk Assessment

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Biosecurity
Cattle Purchase
In the past 5 years, have external cattle been brought onto your farm?
- No
- Yes, from 1 source
- Yes, from multiple sources

If external cattle have been brought onto your farm, were the source herds free of BLV?
- Yes
- No or I don’t know

If external cattle have been brought onto your farm, were the introduced animals free of BLV?
- Yes
- No or I don’t know

If external cattle have been brought onto your farm, do you have special measures before you introduce new animals into your herd?
- Quarantine and testing for BLV
- Testing for BLV
- None

Does the cattle purchase procedure avoid the introduction of BLV?
- Yes (0)
- No (25)

Contact to other herds
Does your herd have close contact to other herds?
- No
- Yes: adjacent pastures
- Yes: shared pastures and/or shared barn

Do you take your animals to cattle shows?
- No
- Yes

If yes: Do you have special measures before you reintroduce the animals into your herd?
- Quarantine and testing for BLV
- Testing for BLV
- None

Are measures in place to avoid the contact with other herds?
- Yes (0)
- No (20)

Farm Visitors (including veterinarians, hoof trimmers, AI personnel, etc.)
Do you require farm visitors to wear freshly laundered, disposable, or farm-supplied coveralls?
- Yes
- No
Do you require farm visitors to disinfect their footwear when they arrive on your farm or to wear disposable or farm-supplied footwear?
  - Yes
  - No

Is it possible for farm visitors to introduce BLV into the herd?
  - Yes (10)
  - No (0)
Cows

Housing
Do you maintain separation of dry cows from lactating cows?
- Yes (0)
- No (10)

Stocking Density
How many stalls are available to your lactating cows, based on the group with the highest stocking density?
- More than one usable stall per animal or more than 120 ft² per mature cow in bedded-pack pens
- One usable stall per animal or 120 ft² per mature cow in bedded-pack pen or tie-stall
- Less than one usable stall per animal or less than 120 ft² per mature cow in bedded-pack pen

How many stalls are available to your dry cows?
- More than one usable stall per animal or more than 120 ft² per mature cow in bedded-pack pens
- One usable stall per animal or 120 ft² per mature cow in bedded-pack pen or tie-stall
- Less than one usable stall per animal or less than 120 ft² per mature cow in bedded-pack pen

How much linear feed bunk space is available to each lactating cow?
- More than 30 inches/2.5 feet per animal
- 24-30 inches/2.5-2 feet per animal
- Less than 24 inches/2 feet per animal

How much linear feed bunk space is available to each dry cow?
- More than 30 inches/2.5 feet per animal
- 24-30 inches/2-2.5 feet per animal
- Less than 24 inches/2 feet per animal

Is the stocking density within the range of recommendations for all animals?
- Yes (0)
- No (10)

Needles
Do you reuse hypodermic needles between animals for any of the following?
- Routine vaccinations:
  - No
  - Yes
  - N/A
- Estrous synchronization:
  - No
  - Yes
  - N/A
- Supplemental vitamins or similar (e.g. selenium):
- No
- Yes
- N/A

-Oxytocin:
- No
- Yes
- N/A

-Treatment of sick animals:
- No
- Yes

-Administration of i.v. fluids:
- No
- Yes

-Other (e.g. embryo transfer): ________________
- No
- Yes
- N/A

Are any needles reused between animals?
- Yes (25)
- No (0)

**Syringes**
Do you reuse syringes for any of the following?
- Routine vaccinations (protocol):
  - No
  - Yes
  - N/A

- Estrous synchronization:
  - No
  - Yes, same animal same syringe
  - Yes
  - N/A

- Supplemental vitamins (e.g. selenium):
  - No
  - Yes
  - N/A

- Oxytocin:
  - No
  - Yes
  - N/A

- Treatment of sick cows:
  - No
  - Yes, same animal same syringe
  - Yes

- Other: ________________
  - No
Yes, same animal same syringe
Yes
N/A

Do you discard syringes with visible blood contamination?
Yes
No
N/A

Are any syringes reused between animals?
Yes (10)
No (0)

Equipment
If you use any of the following items, do you clean and/or disinfect equipment between animals?

- Tattoo pliers:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A

- Ear tagger (without needle cover):
  - Disinfect (and clean)
  - Clean
  - No
  - N/A

- Ear notcher:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A

- Removal of supernumerary teats:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A

- Hoof knives:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A

- Pill gun:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A

- Drencher/stomach tubes/esophageal tubes:
  - Disinfect (and clean)
-Clean
-No
-N/A

-I.v. tubes:
-Disinfect (and clean)
-Clean
-No
-N/A

-Dehorning wire:
-Disinfect (and clean)
-Clean
-No
-N/A

-Obstetrical straps/chains
-Disinfect (and clean)
-Clean
-No
-N/A

-Other: __________________________
-Disinfect (and clean)
-Clean
-No
-N/A

Is all equipment disinfected between animals?
-Yes, disinfected (0)
-No, but everything is at least cleaned (5)
-No (15)

Hoof Trimmer
Does your hoof trimmer clean his chute before entering your premises?
-Yes
-No
-N/A

Does your hoof trimmer clean his tools before entering your premises?
-Yes
-No
-N/A

Are hand knives cleaned and/or disinfected between individual animals?
-Disinfected and cleaned
-Cleaned
-No

Are trimming tools cleaned and disinfected after trimming each animal?
-Disinfected and cleaned
Cleaned
-No

Does the hoof trimmer avoid the transmission of BLV between farms and between cows?
-Yes (0)
-No, but he takes some precautions (e.g. disinfecting hand knives) (10)
-No (15)

Fly Control
What does the fly control like?
-Regularly during fly season
-As soon as flies become a problem
-Sporadically during fly season
-None

What does the fly control consist of?
-Pour-on
-Fly bait, sticky-tape or similar
-Other: _______________________________
-N/A

Do the fly control protocols avoid the transmission of BLV between animals?
-Yes (0)
-No (10)

Artificial Insemination
Do you use artificial insemination (AI)?
-Yes, we use AI exclusively
-Yes, but we also use a clean-up bull
-No, we use a bull exclusively

Does the person responsible for the AI change examination sleeves between every animal?
-Yes
-No
-N/A

Does the AI management avoid the transmission of BLV between animals?
-Yes (0)
-No (15)

Bull
Do you test for BLV before introducing the bull into the herd?
-Yes
-No
-N/A

If the Bull tests positive, do you introduce him into the herd?
-Yes, to all animals

Yes, to known BLV positive animals  
-No  
-N/A  

Do you retest your bull after you introduced him into the herd?  
-Yes  
-No  
-N/A  

Does the bull management allow the introduction of BLV to the herd?  
-Yes (20)  
-No (0)  

**Rectal Pregnancy Examinations**  
How often do you examine your cows for pregnancy?  
- On average 1/pregnancy  
- On average 2/pregnancy  
- On average 3/pregnancy  

Does the person responsible for the pregnancy examination change gloves between animals?  
- Always  
- For test-negative animals  
- Between test-negative and test-positive animals  
- We examine test negative animals first, then test positive animals  
- When blood is visible  
- Never  

Does the person responsible for the pregnancy examination clean/disinfect the ultrasound probe between animals?  
- We do not use an ultrasound probe  
- Always  
- For test-negative animals  
- Between test-negative and test-positive animals  
- We examine test negative animals first, then test positive animals  
- When blood is visible  
- Never  

How well does the pregnancy exam protocol avoid the transmission of BLV between animals?  
- Very well (0)  
- Well (2 or less exams/pregnancy; examine negative animals first, then positive animals) (5)  
- Somewhat (3 or more exams/pregnancy; examine negative animals first, then positive animals) (10)  
- Not at all (15)
**Heifers (pre-calving)**

**Custom heifer rearing**

Do you send your heifers to a custom rearing-site?
- No, they are raised on-farm (0)
- Yes, but our heifers are by themselves (10)
- Yes, together with heifers from other farms (25)

**Stocking Density**

How many stalls are available for use to your heifers?
- More than one usable stall per animal or more than 120 ft\(^2\) per animal in bedded-pack pens
- One usable stall per animal or 120 ft\(^2\) per animal in bedded-pack pen or tie-stall
- Less than one usable stall per animal or less than 120 ft\(^2\) per animal in bedded-pack pen

How much linear feed bunk space is available to each heifer?
- More than 24 inches/2 feet per animal
- 22-24 inches/1.8-2 feet per animal
- Less than 22 inches/1.8 feet per animal

Is the stocking density within the range of recommendations for all animals?
- Yes (0)
- No (10)

**Housing Heifers**

Do you have separate housing for heifers?
- Yes, a separate barn (0)
- Yes, a separate pen outside (0)
- Yes, a separate pen in the milking barn (5)
- Yes, but they are housed with the dry cows shortly before calving (10)
- No, they are housed with the dry cows (10)

**Needles**

Do you reuse hypodermic needles between animals for any of the following?
- Routine vaccinations (protocol):
  - No
  - Yes
  - N/A
- Estrous synchronization:
  - No
  - Yes
  - N/A
- Supplemental vitamins or similar (e.g. selenium):
  - No
  - Yes
  - N/A
- Treatment of sick animals:
  - No
Administration of i.v. fluids:
  - No
  - Yes

Other:
  - No
  - Yes, same animal, same needle
  - Yes
  - N/A

Are any needles reused between animals?
  - Yes (25)
  - No (0)

Syringes
Do you reuse syringes for any of the following?
  - Routine vaccinations (protocol):
    - No
    - Yes
    - N/A
  - Estrous synchronization:
    - No
    - Yes, same animal same syringe
    - Yes
    - N/A
  - Supplemental vitamins (e.g. selenium):
    - No
    - Yes
    - N/A
  - Treatment of sick animals:
    - No
    - Yes, same animal same syringe
    - Yes
  - Other:
    - No
    - Yes, same animal same syringe
    - Yes
    - N/A

Do you discard syringes with visible blood contamination?
  - Yes
  - No
  - N/A

Are any syringes reused between animals?
  - Yes (10)
  - No (0)
Equipment
If you use any of the following items, do you clean and/or disinfect equipment between animals?
- Tattoo pliers:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Ear taggers (without needle cover):
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Ear notcher:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Removal of supernumerary teats:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Hoof knives:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Pill gun:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Drencher/stomach tubes/esophageal tubes:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- I.v. tubes:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Dehorning wire:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Obstetrical straps/chains
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
  - Other: __________________________
    - Disinfect (and clean)
    - Clean
    - No
    - N/A

Is all equipment disinfected between animals?
- Yes, disinfected (0)
- No, but everything is at least cleaned (5)
- No (15)

**Hoof Trimmer**

Does your hoof trimmer clean his chute before entering your premises?
- Yes
- No
- N/A

Does your hoof trimmer clean his tools before entering your premises?
- Yes
- No
- N/A

Are hand knives cleaned and/or disinfected between individual animals?
- Disinfected and cleaned
- Cleaned
- No
- N/A

Are trimming tools cleaned and disinfected after trimming each animal?
- Disinfected and cleaned
- Cleaned
- No
- N/A

Does the hoof trimmer avoid the transmission of BLV between farms and between cows?
- Yes (0)
- No, but he takes some precautions (e.g. disinfecting hand knives) (10)
- No (15)

**Fly Control**

What does the fly control like?
- Regularly during fly season

386
As soon as flies become a problem
-Sporadically during fly season
-None

What does the fly control consist of?
-Pour-on
-Fly bait, sticky-tape or similar
-Other: ______________________
-N/A

Do the fly control protocols avoid the transmission of BLV between animals?
-Yes (0)
-No (10)

**Artificial Insemination**
Do you use artificial insemination (AI)?
-Yes, we use AI exclusively
-Yes, but we also use a clean-up bull
-No, we use a bull exclusively

Does the person responsible for the AI change examination sleeves between every animal?
-Yes
-No
-N/A

Does the AI management avoid the transmission of BLV between animals?
-Yes (0)
-No (15)

**Bull**
Do you test for BLV before introducing the bull into the herd?
-Yes
-No
-N/A

If the Bull tests positive, do you introduce him into the herd?
-Yes, to all animals
-Yes, to known BLV positive animals
-No
-N/A

Do you retest your bull after you introduced him into the herd?
-Yes
-No
-N/A

Does the bull management allow the introduction of BLV to the herd?
-Yes (20)
-No (0)
Rectal Pregnancy Examinations
How often do you examine your heifers for pregnancy?
- On average 1/pregnancy
- On average 2/pregnancy
- On average 3/pregnancy

Does the person responsible for the pregnancy examination change gloves between animals?
- Always
- For test-negative animals
- Between test-negative and test-positive animals
- We examine test negative animals first, then test positive animals
- When blood is visible
- Never

Does the person responsible for the pregnancy examination clean/disinfect the ultrasound probe between animals?
- We do not use an ultrasound probe
- Always
- For test-negative animals
- Between test-negative and test-positive animals
- We examine test negative animals first, then test positive animals
- When blood is visible
- Never

How well does the pregnancy exam protocol avoid the transmission of BLV between animals?
- Very well (0)
- Well (2 or less exams/pregnancy; examine negative animals first, then positive animals) (5)
- Somewhat (3 or more exams/pregnancy & examine negative animals first, then positive animals; change gloves, when bloody) (10)
- Not at all (15)
**Calves**

**Housing Calves**
Do you maintain separation of pre-weaned calves from older animals?
- Yes
- No

Do you maintain separation of weaned calves from lactating cows?
- Yes
- No

Where is your calf-housing located?
- Further away from adult animals (>20 m)
- Close to adult animals (<20 m)
- In the same barn as adult animals (heifers, dry cows, cows, etc.)

Does the calf housing avoid the transmission of BLV from older animals to calves?
- Yes (0)
- No (10)

**Needles**
Do you reuse hypodermic needles between animals for any of the following?
- Routine vaccinations (protocol):
  - No
  - Yes
  - N/A
- Supplemental vitamins or similar (e.g. selenium):
  - No
  - Yes
  - N/A
- Treatment of sick animals:
  - No
  - Yes
- Administration of i.v. fluids:
  - No
  - Yes
- Pain medication
  - No
  - Yes
  - N/A
- Local Anesthetics
  - No
  - Yes
  - N/A
- Other:________________________
  - No
  - Yes
  - N/A

Are any needles reused between animals?
-Yes (25)
-No (0)

**Syringes**
Do you reuse syringes for any of the following?
-Routine vaccinations (protocol):
  -No
  -Yes
  -N/A
-Supplemental vitamins (e.g. selenium):
  -No
  -Yes
  -N/A
-Treatment of sick animals:
  -No
  -Yes, same animal same syringe
  -Yes
-Pain medication
  -No
  -Yes
  -N/A
-Local Anesthetics
  -No
  -Yes
  -N/A
-Other, please specify: ________________
  -No
  -Yes, same animal same syringe
  -Yes
  -N/A

Do you discard syringes with visible blood contamination?
-Yes
-No
-N/A

Are any syringes reused between animals?
-Yes (10)
-No (0)

**Equipment**
If you use any of the following items, do you clean and/or disinfect equipment between animals?
-Tattoo pliers:
  -Disinfect (and clean)
  -Clean
  -No
  -N/A
- Ear taggers (without needle cover):
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Ear notcher:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Removal of supernumerary teats:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Pill gun:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Drencher/stomach tubes/esophageal tubes:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- I.v. tubes:
  - Disinfect (and clean)
  - Clean
  - No
  - N/A
- Other: __________________________
  - Disinfect (and clean)
  - Clean
  - No
  - N/A

Is all equipment disinfected between animals?
  - Yes, disinfected (0)
  - No, but everything is at least cleaned (5)
  - No (15)

**Dehorning**

Do you dehorn your calves?
  - No (0)
  - Yes: Electric dehorner (0)
  - Yes: Acid (0)
  - Yes: Gouge/scoop dehorner, with cleaning/disinfection between animals (10)
- Yes: Gouge dehorner and electric dehorner, with cleaning/disinfection between animals (10)
- Yes: Gouge/scoop dehorner, without cleaning/disinfection between animals (25)
- Yes: Gouge dehorner and electric dehorner, without cleaning/disinfection between animals (25)

**Fly Control**
What does the fly control like?
- Regularly during fly season
- As soon as flies become a problem
- Sporadically during fly season
- None

What does the fly control consist of?
- Pour-on
- Fly bait, sticky-tape or similar
- Other: __________________________________________
- N/A

Do the fly control protocols avoid the transmission of BLV between animals?
- Yes (0)
- No (10)

**Calving**
How long is the calf in the calving pen with its dam?
- We prevent nursing by putting the calf in a tub/cuddling box/similar before the calf can nurse for the first time (10)
- Before it can nurse for the first time (0)
- Until after it nursed for the first time (20)
- It depends (15)

**Colostrum**
How do you feed colostrum to your calves?
- Pasteurized colostrum (0)
- Colostrum replacer (0)
- Previously frozen colostrum (0)
- Only colostrum from BLV negative dams (5)
- Colostrum from dam (15)
- Pooled colostrum (20)

**Milk**
How do you feed milk to your calves?
- Milk replacer (0)
- Pasteurized milk (0)
- Only milk from BLV negative dams (5)
- Pooled milk (20)
- Non-saleable milk (20)
Appendix C

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Title: Economic evaluation of 4 bovine leukemia virus control strategies for Alberta dairy farms
Author: Alesz Kuczewski, Henk Hoogeveen, Karin Orsel, Robert Wolf, Jada Thompson, Eldon Spackman, Frank van der Meer
Publication: Journal of Dairy Science
Publisher: Elsevier
Date: March 2019

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Title: Short communication: Evaluation of 5 different ELISA for the detection of bovine leukemia virus antibodies
Author: Alesz Kuczewski, Karin Orsel, Herman W. Barkema, David F. Kelton, Wendy A. Hutchins, Frank J. U.M. van der Meer
Publication: Journal of Dairy Science
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1. Kuczewski A., Adams C., van der Meer F., Motivation and Hurdles to control bovine leukemia virus on Farm, in preparation


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