BOVINE LEUKEMIA VIRUS IN BEEF CATTLE: SEXUAL TRANSMISSION AND COW SURVIVABILITY

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ABSTRACT

BOVINE LEUKEMIA VIRUS IN BEEF CATTLE:
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The chronic lymphoproliferative disorder, bovine leukosis, is caused by the deltaretrovirus bovine leukemia virus (BLV). Surveys indicate that 39% of the US beef cow-calf operations have at least one BLV-infected animal compared with 83% of dairy herds. Most BLV infected cattle remain asymptomatic and act as carriers of the virus while less than 5% progress to lymphosarcoma. There are limited studies on the impact of BLV in beef cattle. Understanding the impact as well as identifying important route of disease transmission in beef cattle enterprises is important to better design intervention strategies. The primary goals of the studies described in this this dissertation were to 1) further understand the risk of breeding bulls in the transmission of BLV and 2) to determine the effect of BLV on the survival of cattle in beef and dairy herds. We first determined BLV prevalence in breeding beef bulls and the presence of BLV provirus DNA in genital secretions (smegma and semen). In our study population, 44.6% of beef bulls were seropositive for BLV and 48.7% of herds had at least 1 BLV-infected bull. Bovine leukemia virus provirus DNA was detected in smegma samples of 4/54 (7.4%) BLV-seropositive bulls and represent a potential risk for the transmission of BLV from infected bulls to uninfected cows during natural service breeding programs. To evaluate this risk, we exposed BLV negative heifers to a BLV positive bull during a defined 38-day breeding period. Although BLV provirus was found in the smegma and blood of the BLV positive bull prior to and after the breeding period, we detected no evidence of seroconversion or presence of BLV provirus DNA in the

blood of naïve heifers. These results suggest that BLV infected bulls that are healthy and aleukemic may not be a significant risk of BLV transmission during a defined breeding season.

We next evaluated the impact of BLV infection on beef and dairy cow' longevity within herds. The presence of BLV antibodies in blood was not associated with a change in beef cow longevity over 2 years monitoring period, but decreased survival was observed in cattle in which BLV infection had advanced clinically as indicated by a high BLV provirusload in blood. In dairy cows, we demonstrated that infected females lived significantly shorter than their negative herd mates and were at a 30% greater hazard of being culled compared with BLV negative cows.

In summary, there is high prevalence of BLV in breeding beef bulls which could serve as a source of transmission both within and between herds. Based on our study results, the risk of transmission of BLV from healthy infected bulls to naïve heifers is low when bulls are housed with heifers for a defined breeding period, but this scenario should not be considered without risk. BLV does not appear to have an impact on beef cow longevity, but in contrast, is associated with decreased longevity in dairy cows. These studies provide important information for supporting and designing risk based BLV control programs.

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KEY TO ABBREVIATIONS

AL: aleukemic

BLV: bovine leukemia virus

BoLA: bovine leukocyte antigen

BSE: breeding soundness examination

CBC: complete blood count

CI: confidence interval

CoCoMo: coordination of common motifs

CTLA-4: cytotoxic T-lymphocyte-associated protein 4

DHIA: dairy herd improvement association

EBL: enzootic bovine leukosis

EDTA: ethylenediaminetetraacetic acid

ELISA: enzyme-linked immunosorbent assay

gp51: BLV glycoprotein 51

HTLV-1: human T cell lymphotropic virus type I

IQR: interquartile range

LC: lymphocyte count

MHCII: major histocompatibility complex type II

miRNA: microRNA

OD: optical density

p24: BLV capsid protein

PCR: polymerase chain reaction

PVL: provirus load

qPCR: quantitative polymerase chain reaction

SD: standard deviation

CHAPTER 1

INTRODUCTION

Abstract

Research indicates that cattle infected with bovine leukemia virus (BLV) have seriously altered immune systems which contribute to their observed reduced milk production, shortened lifespan, and predisposition to lymphoma. Annual costs associated with BLV infection per cow are estimated at $283. While over 21 nations have eradicated BLV by culling serologic positive animals, the U.S. prevalence has grown to about 45% in dairy cattle. Unlike in dairy cattle, in beef cattle, very little is known about BLV spreading and impact on health and performance. In one of the few studies reporting BLV prevalence in beef cattle over twenty years ago, the individual prevalence was 10.3%. A more recent study reports that at least 33.6% of cull beef cattle brought into U.S. slaughterhouses are seropositive for BLV. Our group has reported that 25.7% of beef bulls in Michigan, one year of age and older, were infected. The primary route of virus transmission is iatrogenic through the transfer of blood or secretions contaminated with lymphocytes. Proviral DNA has been identified in nasal secretion, saliva, milk, colostrum, and semen; however, natural transmission of BLV through these secretions has not been clearly demonstrated. A minority of cattle with high concentrations of proviral load (PVL) are responsible for most transmission. These animals with high BLV PVL are an obvious critical control point for the many direct and indirect routes of BLV transmission. Different studies continue to identify subclinical health effects of BLV infection that result in economic losses, particularly for dairy producers. The evidence of BLV infection in beef herds and the different transmission routes, will help to develop new strategies to reduce the prevalence of BLV infection that is more crucial than ever.

Introduction

Bovine leukosis is a chronic lymphoproliferative disorder in cattle and water buffalo caused by this deltaretrovirus bovine leukemia virus (BLV). (Kettmann et al., 1994). While not a natural host, BLV has experimentally infected sheep (Gillet et al., 2007), rabbits (Wyatt et al., 1989; Dimitrov et al., 2012), rats (Dimitrov et al., 2012), chicken (Altanerova et al., 1990), pigs (Mammerickx et al., 1981), and goats (Mammerickx et al., 1981). BLV is closely related to human T-cell leukemia virus (HTLV-1); similarities between these two viruses have helped to a better understand BLV infection dynamics within the host (Fauquet et al., 2005; Gillet et al., 2007; Panei et al., 2013). Bovine leukemia virus was described by Kettmann et al. (1976) in biochemical studies that characterized the agent as an exogenous RNA virus, and its genome was sequenced by Sagata et al. in 1985. Subsequently, the genome structure of the *env* gene has been used to identify and investigate a growing number of viral genotypes that have been found throughout the world (Polat et al., 2016). Similar to other retroviruses, infection with BLV is considered to be lifelong due to provirus integration into DNA of the infected cells (Coulston et al., 1991), primarily B-lymphocytes (Paul et al., 1977). However, other cells, including T-lymphocytes (Schwartz and Levy, 1994b), granulocytes (Schwartz et al., 1994), monocytes (Heeney et al., 1992), and mammary epithelial cells (Buehring et al., 1994) have been described to be susceptible to BLV proviral integration.

Prevalence of BLV in the U.S.

In the U.S, most BLV studies have been conducted in dairy cattle. Epidemiological studies have shown that at least 84% of U.S dairy herds are infected with BLV and 23% to 47% of cows within infected herds tested positive for BLV (USDA, 2007c; LaDronka et al., 2018). In one of the few studies reporting BLV prevalence in beef cattle, individual prevalence in

sampling of cows across the U.S. was 10.3% (USDA, 2007b). A more recent study reports that 33.6% of cull female beef cattle brought into U.S. slaughterhouses were seropositive for BLV (Bauermann et al.,2017). Our group has reported that 25.7% of beef bulls in Michigan, one year of age and older, were infected by BLV (Bartlett et al., 2013). More recently, among 121 adult beef bulls (>1-year-old) from 39 different beef farms in Michigan, 44.6% were BLV seropositive (Benitez et al., 2019). The evidence of BLV infection in beef herds raise new questions about the real impact of this disease in the beef industry. More studies at the national U.S. level should be considered to fully understand the potential impact of BLV on the beef industry.

Economic Impact

Defining the real economic impact of BLV is challenging and likely underreported due to unknown costs and unrecognized subclinical effects (Pelzer, 1997). The cost of carcass condemnation due to BLV infection-associated lymphosarcoma has been estimated to be over $400 (Rhodes et al., 2003).

Recently, our group demonstrated that BLV infected dairy cattle in herds from Michigan (n=3,849) have decreased survival as compared to their uninfected herd mates (Bartlett et al., 2013). Infected cattle were 23% more likely to be culled over the 19-month monitoring period. Cows with the highest levels of BLV antibodies in milk were 40% more likely to be culled than were their negative herd mates. While previous studies showed an association between BLV and decreased longevity (Thurmond et al., 1985; Bartlett et al., 2013), others did not find this relationship (Huber et al., 1981; Rhodes et al., 2003). A Canadian study showed a trend for ELISA-positive dairy cattle older than 3.5-years-old to be culled at a higher rate than ELISA-negative cattle (Jacobs et al., 1995). Notably, most of these studies have been developed in dairy cattle, and no data is available regarding beef cattle. However, there is no reason to believe that

BLV could not have similar effects on longevity of beef cattle as it does in dairy cattle, eventually leading to productivity losses.

Others impacts

The potential for BLV to infect humans and because disease has been studied and debated. Previous studies have attempted to determine whether BLV causes disease in humans (Perzova et al., 2000; Burmeister et al., 2007). In the 1970's, when Canada and the U.S. agreed not to control BLV spreading due to the lack of evidence on its impacts on health and profitability, all available evidence suggested that BLV was not a human health hazard. Later, it was demonstrated that BLV proliferates in human tissue cultured cells (Buehring et al., 2003) and triggers the production of antibodies against BLV (Buehring et al., 2001). Additionally, genes of BLV origin have been identified in human mammary cells, although results are conflicting regarding whether those genes are found more frequently in cancerous or noncancerous tissues (Giovanna et al., 2013). More recently, a study implicated the association of BLV with human tumors when BLV DNA was found in human mammary tumors biopsies using immunohistochemistry and *in situ* PCR (Buehring et al., 2001; Schwingel et al., 2019). The consensus regarding the zoonotic potential of BLV is now less clear than it was a few decades ago, and this public health concern clearly impacts the decision regarding whether BLV transmission should be curtailed. Even though cattle carcasses with visual evidence of lymphosarcoma are condemned at FSIS inspected slaughter facilities (USDA/FSIS/EMS, 2002), early stages of disease are not evident at postmortem inspection and are missed. The effects of BLV-infected milk on human health have not been addressed and need careful consideration.

Even with the lack of evidence on a detrimental effect of BLV on human health, uncontrolled transmission of BLV in the U.S. may become a major obstacle to the future dairy

and beef exportation market. Both dairy and beef product exports are major contributors to the U.S. economy, accounting for 12% of all agriculture product exports (Joint Economic Committee, 2013). Import restrictions on U.S. cattle and cattle products are partially driven by a desire for countries without BLV to remain free of the disease, but importers concerned with public health and animal welfare concerns (both real and imagined) may prefer to purchase dairy products from one of the 21 countries that have eradicated BLV, or from countries/regions with lower BLV prevalence than what is found in the U.S. Thus, a loss of international markets must be considered as yet one more cost component contributing to the partial budget analysis of the total economic impact of BLV.

BLV infection and transmission

New BLV infections can occur by horizontal or vertical transmission through the transfer of biological fluid contaminated with lymphocytes containing incorporated BLV provirus, Fig 1.1 (Aida et al., 2013; Bartlett et al., 2014). BLV undergoes a replication cycle and produces new infections in new target cells (Aida et al., 2013; Gutiérrez et al., 2014b).

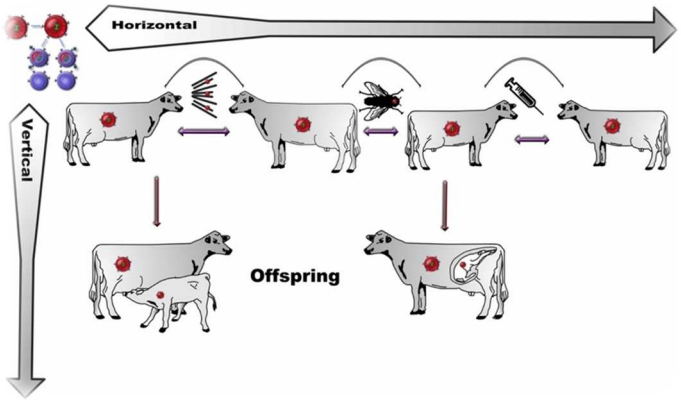

Figure 1. 1 The main transmission routes of BLV.

Bovine Leukemia Virus can spread either horizontal from one host to another, or vertical from one generation to another (dam to calf). Horizontal transmission of BLV is considered the principal route of infection in cattle.

Within 2 to 10 weeks after the initial exposure, the immune system initiates a strong response that limits the infection of new target cells (Burny et al., 1988). From here, the natural disease can progress between a couple of months to several years. Approximately 60-70 % of the BLV infected animals are mostly asymptomatic or aleukemic (AL). In these animals, no clinical symptoms are manifested, and alteration of the total lymphocyte counts does not occur (Swenson et al., 2013). Alternatively, BLV undergoes an escape phase where 30% to 40% of BLV-infected animals develop a polyclonal proliferation of B-cells, leading to a persistent lymphocytosis (PL) (Lewin, 1989; Kabeya et al., 2001). Consequently, there will be different B-cells-infected clones carrying BLV integrated provirus that increase rapidly during the PL phase (Kettmann et al., 1980). The different PL changes may disclose either a severe or transient viral condition (Swenson et al., 2013; Juliarena et al., 2016). Clinical course of BLV infection is represented in

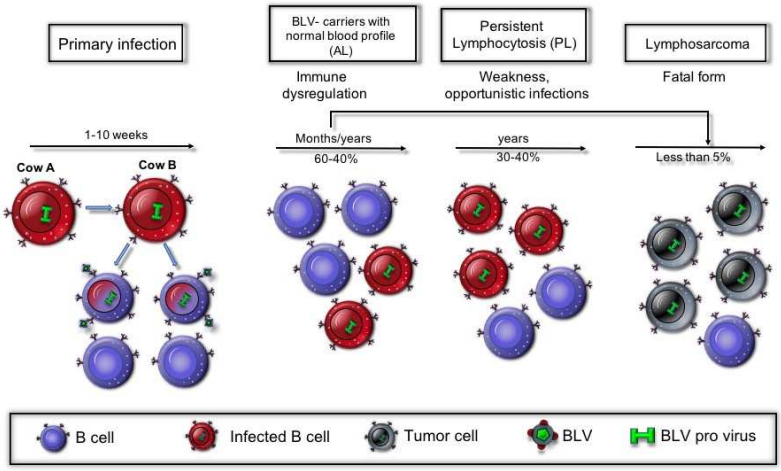

Figure 1. 2 BLV Clinical Course.

During primary infection an infected cell (red) with BLV integrated into the host chromosome (green provirus) is transmitted into a new animal. The BLV provirus is then expressed into viral particles (green hexagon) which further infect B-cells (purple). During persistent infection, provirus-carrying cells (red) expand mainly by mitosis because of the presence of an active immune response. This phase extends from several months to years and is characterized by an immune dysregulation that may lead to an increase in the number of infected cells (PL animals). During the PL phase, morbidity is characterized by weakness and opportunistic infections. In the tumor phase, a single infected cell undergoes genetic mutations (black) and forms a lymphoma within or outside lymph nodes, leading to the death of the animal.

Horizontal transmission

Horizontal BLV transmission has been strongly associated with iatrogenic procedures (Sprecher et al., 1991; Kobayashi et al., 2014). High infectivity was also observed when experimentally testing BLV transmission by rectal palpation using the same sleeve between a BLV infected animal and naive cows (Kohara et al., 2006). Another study reported that the presence of 2 ml of infected blood on the sleeve during palpation is the minimum volume necessary to infect animals without rectal mucosal trauma (Hopkins et al., 1988; Hopkins et al., 1991). The repetitive use of the same needle for administrating pharmaceutical products may play an important role in the iatrogenic BLV transmission (Evermann et al., 1986; Lassauzet et al., 1990). Transfer of BLV can occur through small amounts of blood with infected cells from one animal to another by vehicles that are daily used in the veterinary practice. Studies have demonstrated that 0.1µL of blood from a BLV infected animal with persistent lymphocytosis was able to successfully infect other animal independent of the inoculation route (Mammerickx et al., 1981; Evermann et al., 1986; Foil et al., 1987). A volume of BLV-infected blood of 1000 times higher was needed to induce the same response if the animal was aleukemic (Mammerickx et al., 1981). Interestingly, needles between 20G to 18G with a length 1" to 1.5" can contain volumes between 0.061 to 0.1µL in their needle body after its use (Küme et al., 2012); therefore, it implies that the risk of BLV transmission may increase from one animal to another when using the same needle for more than one animal.

Tattooing and dehorning are common management practices associated with an increase in BLV seroprevalence in beef and dairy herds (Ott et al., 2003; Erskine et al., 2012b; Kobayashi et al., 2014). During the tattooing process, the skin is punctured by the prongs of the tattooing tool and blood vessels could be ruptured in the process. In a sheep herd, 21/24 naïve animals

tattooed randomly with the same instrument used for tattooing BLV infected animals developed BLV antibodies f (Lucas et al., 1985). Similarly, during dehorning, there could be blood with BLV infected cells in the dehorning tool (Stafford and Mellor, 2011) leading to blood transfer between BLV infected and naïve animals. Previous studies have demonstrated BLV transmission between young calves during dehorning when dehorning instruments were not cleaned or disinfected between calves (DiGiacomo et al., 1985).

The role of hematophagous insects in BLV transmission is unclear and may be geographically dependent (Buxton and Schultz, 1984; Perino et al., 1990). BLV has been reported to be transmitted by insects (Foil et al., 1988; Foil et al., 1989; Manet et al., 1989). Epidemiological studies reported a high association between BLV transmission and the presence of hematophagous insects (Erskine et al., 2012; Kobayashi et al., 2014) such as ticks (Romero et al., 1984) and a wide variety of flies (Tabanid nipponicus, Tabanid trigeminus, Tabanid juscicostatus, and stable flies) (Bech-Nielsen et al., 1978; Foil et al., 1987; Foil et al., 1989). The mouthparts of many insects are similar to a small needle that can contain from 1nL to12 nL of blood (Foil et al., 1987). BLV transmission can occur with the transfer of approximately 100 to 200 nL of infected blood (Foil et al., 1987) that may contain ~2,500 lymphocytes; which is considered an appropriate intradermal infectious dose for cattle (Van der Maaten and Miller, 1977; Okada et al., 1981; Klintevall et al., 1994). The volume of infected cells transferred and the risk of BLV transmission by insects to cattle may depend on the fly size and the number of bites in the same animal (Foil et al., 1989).

Other potential sources of BLV include any fluids contaminated with infected lymphocytes (Asadpour and Jafari, 2012; Yuan et al., 2015a). Our group previously identified that the use of bulls for natural breeding was associated with a higher BLV herd prevalence in

dairy herds. (Erskine et al., 2012). Natural service is still used in approximately half of dairy operations across the U.S. (USDA, 2014) and is the most commonly used breeding method (approximately 90%) in beef cattle herds in the U.S. (USDA, 2007a). Natural service may facilitate BLV transmission from trauma to the penis, vulva and vagina during the physical act of copulation, which may lead to the transfer of blood. Preputial secretions (smegma) are also known to contain white blood cells and could serve as a source of BLV transmission during copulation (Cobo et al., 2011). Alternatively, BLV transmission may also occur via transfer of semen during copulation. The majority of studies in this topic report no evidence of BLV in semen (Monke, 1986; Santos et al., 2007); however, a single study reported the presence of BLV provirus, as determined by PCR, in semen of seropositive cattle (Asadpour and Jafari, 2012). We recently showed that BLV provirus DNA was detected in smegma of 7.4% (4/54) of BLV infected bulls. The number of BLV proviral copies in smegma ranged from 4.50 - 618.78 copies/ 10^5 cells. BLV provirus was not detected in semen samples. The identification of primary routes of BLV transmission within and between herds is crucial to formulating management decisions to reduce economic losses and improve animal welfare.

Vertical transmission

Transmission of BLV can occur in utero (Piper et al., 1979; Jacobsen et al., 1983), but it is believed to occur infrequently (Van Der Maaten et al., 1981; Thurmond et al., 1983); only 4% of calves born from naturally infected cows were BLV seropositive at birth (Jacobsen et al., 1983; Agresti et al., 1993). The risk of being BLV positive at birth appears to be greater if the calf is born from a BLV positive cow with PL or lymphosarcoma (Agresti et al., 1993). This highlights the increased risk of BLV transmission from cows that are in a more advanced stage of the disease (Gutiérrez et al., 2014a; Mekata et al., 2015). Few reports also demonstrate that

BLV could be transmitted during the parturition when infected blood and contaminated biological fluid can infect the utero (Agresti et al., 1993; Meas et al., 2002).

Both colostrum and milk may play an important role in the transmission of BLV in young calves (Sprecher et al., 1991; Meas et al., 2002). Feeding calves with colostrum have been associated with a protective role in BLV infection in experimental, as well as natural transmission, while feeding untreated milk is more frequently identified as a risk factor for BLV infection (Kanno et al., 2014). Therefore, feeding calves with milk from BLV negative cows or pasteurized colostrum from BLV-positive cows could be a preventive strategy to BLV transmission. Kanno et al. (2014) reveals that colostrum with infected lymphocytes with a 24-hour freeze period is not infectious to sensitive experimental sheep. This practice could be easily achievable as a protective strategy for small farmers instead of increasing costs with pasteurizer colostrum (Pelzer, 1997), and safe for the calves since pasteurization can denature antibodies necessaries for passive immunization (Argüello et al., 2003; McMartin et al., 2006).

New strategies for BLV control

Controlling BLV spreading by using the previously mentioned strategies have successfully decreased the prevalence of BLV in herds with high prevalence (Ruppanner et al., 1983; Sprecher et al., 1991), but have not always been successful in other cases (Kobayashi et al., 2010; Gutiérrez et al., 2011). Other studies also highlight that seasonal changes in housing, such as confinement of cattle during winter season (Sargeant et al., 1997), and an inadequate sanitization practices contribute to BLV spreading (Sargeant et al., 1997; Monti et al., 2007). Clearly, individual herd management practices, as well as geographical locations and climate factors, can influence the temporal dynamics of BLV transmission. There are still significant challenges in defining the best strategies to decrease BLV prevalence within herds since there are

no established vaccines or therapeutic tools for controlling BLV and culling of all infected animals may not be feasible (Gutiérrez et al., 2014b).

Evidence supports a correlation between the BLV level of infectioness and the likelihood of an animal to infect naive animals (BLV infectivity). Mammerickx et al., 1987 reported that as few as 200 lymphocytes from a PL BLV seropositive bovine donor are sufficient to induce BLV infection in sheep, whereas 20,000 lymphocytes from AL BLV seropositive cattle did not cause any infection after inoculated into sheep (Mammerickx et al., 1987). The same study also reported that to achieve infection, 1 ml of AL cow's blood was required to induce infection compared with only 0.1uL of blood from PL animal (Mammerickx et al., 1987). This is partially explained by the fact that 25-40% of total leucocytes of PL animals incorporate BLV proviral sequences when compared with an AL animal, where less than 5% of total leucocytes carry the provirus (Kettmann et al., 1980). In cattle, CD5+ B cells, the primary targets for BLV (Meiron et al., 1985), corresponds to approximately 90.5% of B cells from PL animals, while only 31% of B cells from uninfected cattle will typically express CD5 (Stone et al., 1995). In addition to that, PL cattle have a decreased synthesis of cytokines that are an important part of the immune responses to BLV compared with AL cattle (Trainin et al., 1996). PL cattle also increase surface receptor expression of CTLA-4 enveloped during proliferative and apoptotic capacities within infected cells, while the opposite occurs in AL cattle. (Trainin et al., 1996; Trueblood et al., 1998; Suzuki et al., 2015). These findings indicate that PL catle have a high number of infected cells compared with AL cattle, thus representing a higher risk of transmitting BLV with smaller amounts of infected fluid transfer (Johnson et al., 1985). For this reason, the culling of PL-BLV seropositive cattle is one of the potential practices to decreased BLV spreading within herds. Recent studies suggest the use of genetic selection could be used as an approach to identify

animals that are genetically predisposed to BLV infection. This approach could become a major preventive strategy for BLV in cattle (Sulimova et al., 1995; Juliarena et al., 2008). The bovine major histocompatibility complex (MHC) genes have been mapped as a target for genetic selection due to its fundamental role in the immune system response, and to its association with genetic resistance and susceptibility to a wide array of cattle diseases (Lewin et al., 1988; Dietz et al., 1997; Sharif et al., 1998; Aida, 2001). Different Bovine Lymphocyte antigens (BoLA) alleles at bovine MHC have been associated to BLV susceptibility (Fries et al., 1986; Fries et al., 1993). The BoLA-DRB3.2 gene is the most polymorphic sequence where more than 100 different alleles have been identified (Da Mota et al., 2004; Wang et al., 2008). Within the BoLA-DRB3.2 alleles, a study has reported the association of one or more alleles with susceptibility or resistance to BLV in cattle (Juliarena et al., 2008). We have described the importance of a PL animal within the potential risk for BLV transmission (Johnson et al., 1985). Interestingly, a peptide motif named ER present in BoLA-DRB3.2 alleles *11, *23 and *28 was associated with resistance to PL in BLV-infected cattle. (Xu et al., 1993). These findings provide further support to the hypothesis that BoLA-DRB3.2 alleles are associated with resistance or susceptibility to development of BLV. Further studies are required to define the key BLV epitope peptides and alleles that could be strategically used for genetic selection of animals less predisposed to BLV infections over new generations.

BLV diagnostic tests

ELISA

Cows that are infected with BLV generate antibodies to the virus envelope glycoproteins (primarily gp51) and capsid proteins (primarily p24) which can successively be detected by a variety of immunoassays for BLV infection (Reichel et al., 1998). The envelope glycoprotein

gp51 and the viral capsid protein p24 specific antibodies can be found in milk and blood about three weeks after infection (Alexander, 2008). Time to seroconversion after the infection has been shown to depend on the number of infected cells and the exposure of the animal (Nagy et al., 2007). Currently, the detection of BLV antibodies by ELISA is the standard test and the cheapest way for identifying infected animals. After infection, antibody levels usually increase over time (Kono et al., 1982), though variations may be associated with immunologic status of each cow individual (Schwartz and Levy, 1994a).

qPCR PVL

Proviral load (PVL) is a measure of the number of copies of provirus, typically reported per unit of blood, nasal secretion, semen, smegma, milk or other body fluid (Yuan et al., 2015b; Jaworski et al., 2016; Benitez et al., 2019). BLV provirus integration into the host cell genome indicates that BLV is capable of integrating at multiple sites in the genome and that multiple copies of provirus can be integrated into a single genome (Onuma et al., 1982). In addition to improved sensitivity over serologic assays (Klintevall et al., 1994; Fechner et al., 1996), several research have proposed using a quantitative polymerase chain reaction (q-PCR) to quantify PVL levels as a measure of transmission efficiency (Gutiérrez et al., 2011; Rodríguez et al., 2011). In collaboration with our Japanese colleagues, we had routinely performed the qPCR CoCoMo proviral load assay for several of our BLV research projects (Jimba et al., 2010; Takeshima et al., 2015; Benitez et al., 2018; Benitez et al., 2019). For other retroviruses, such as HTLV and HIV, it is widely accepted that viral load or PVL is associated with infectivity potential (Lee et al., 2004; Lairmore, 2014; Li et al., 2016). This tool was used to develop a field data that supports the idea that most natural BLV transmission is from high PVL cattle (Juliarena et al., 2016).

Blood lymphocyte count

Many mammals, including sheep, deer, and even humans can become infected with BLV and produce an antibody response. However, it is only those natural infected animals that can develop lymphocytosis (high lymphocyte count) that suffer from immune function interference (Frie et al., 2017). Measuring blood lymphocyte count is the easiest and most inexpensive way to screen for immune dysfunction. Experimental data has recognized that approximately 30% of BLV infected cattle will develop persistently elevated lymphocyte counts (Lewin, 1989; Kabeya et al., 2001). Up to 70% of animals experience an expansion in the B-cell population, increasing the ratio of B- to T-cells, and sometimes resulting in an inversion of the B/T-cell ratio (Lewin et al., 1988). While lymphocytosis appears to be a marker of immune compromise, in a report on 112 bovine lymphoma cases by Burton et al., (2010), lymphocytosis was detected in only 25% of the animals. However, Lewin (1989) reported that cattle with an inverted B- to T-cell ratio provided the background population for animals that developed tumors. In the last four decades, every veterinarian is familiar with using complete blood count (CBC) data for diagnosis and prognosis. Until now, this diagnostic information has never been commonly used in food animal medicine because of the cost, the need to transport blood samples to a laboratory, and the delay in obtaining the results. However, new technology is allowing for rapid and inexpensive chute side testing and this could be used in BLV control programs (von Konigslow et al., 2019).

Conclusion

Other nations that have eradicated BLV using the strategy of testing and removing infected cattle in conjunction with management practices to reduce transmission. However, implementing similar culling programs in the U.S. and other countries is unfeasible due to the high BLV prevalence. In beef cattle, despite some recent epidemiological studies regarding BLV

infection, there are no studies exploring the effects of BLV infection on health and performance, nor on it impact on beef industry profitability. We believe that identifying primary routes of BLV transmission and revealing the impact of BLV infection on survival and performance of beef cattle will help to develop strategies for preventing BLV spreading.

Overall Objective and Research Aims

The overall objective of this dissertation is to further understand the importance of BLV in beef and to identify new potential routes of virus transmission. Identifying primary routes of BLV transmission and revealing the impact of BLV infection on survival and performance of beef cattle will help to develop strategies for preventing BLV spreading. Most studies on the effect of BLV on performance have been done in dairy cattle. There are limited studies exploring the effects of BLV infection on health and performance of beef cattle, and no studies targeting the effect of BLV on longevity. The main objective of the research presented in this dissertation was to increase our knowledge about the impacts and epidemiology of BLV in beef cattle via the following specific aims:

Specific Aim 1. Assess breeding beef bulls as a potential source of BLV transmission.

Specific Aim 2. Evaluate BLV transmission during natural breeding between a BLV-infected bull and uninfected heifers.

Specific Aim 3: Determine the impact of BLV infection on cattle longevity.

> **Sub-aim 3.1:** Determine the impact of BLV infection on cow longevity in cow-calf beef operations in Michigan.
>
> **Sub-aim 3.2:** Determine the impact of BLV infection on cow longevity in dairy cattle farms at national level.

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CHAPTER 2

Breeding Bulls as a Source of Bovine Leukemia

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Abstract

Bovine leukosis is a chronic lymphoproliferative disorder caused by bovine leukemia virus (BLV). BVL infection is mainly asymptomatic and can lead to a leukemic state characterized by persistent lymphocytosis. Breeding bulls were identified as a risk factor for BLV transmission and therefore their infected genital secretions could act as a route of BLV spread. However, little is known about the current prevalence of leukosis in beef cattle and specifically in breeding beef bulls, as well as the potential risk offered by using BLV positive bulls as breeding animals. Using breeding beef bulls presented for breeding soundness exams (BSE) at Michigan State University (MSU), our objectives were to 1) determine the prevalence of BLV, 2) evaluate the presence of BLV proviral DNA in bull's smegma and semen, and 3) analyze potential associations between lymphocyte concentrations and blood PVL. Blood, smegma and semen samples were obtained from 121 beef bulls from 39 farms. BLV proviral DNA was performed in all samples through CoCoMo-qPCR, while blood samples were also analyzed for BLV antibodies by ELISA and for lymphocyte concentrations by Qscout. BLV prevalence in individual beef bulls was 45% (54/120), while 48% (19/39) of farms had at least one BLV positive bull. Lymphocyte count was significantly higher in BLV-seropositive bulls compared with seronegative bulls and was also higher in infected animals with high blood PVL compared with low or moderate PVL. The proportion of BLV seropositive bulls with lymphocytosis (16/55, 29.09%) was higher than the bulls seronegative for BLV (6/66, 9.09%). BLV proviral DNA was also detected in 4/54 smegma samples in seropositive bulls. Overall, we observed that BLV prevalence is high breeding beef bulls using for natural breeding, and smegma may be a source for BLV transmission during copulation. The presence of leukemia and high blood PVL in asymptomatic BLV positive bulls in this study underscores that these animals

may be a major reservoir of BLV and may also shed this virus in body secretions, such as genital secretions, as observed in this study in smegma samples. Future studies by our group will evaluate asymptomatic BLV positive beef bulls as a source of BLV infection during natural breeding and will identify potential BLV "super-shedders" in positive herds.

Key Words: Beef cattle, bovine leukemia virus, lymphocytosis, semen, smegma

Introduction

Bovine leukosis is a chronic lymphoproliferative disorder in cattle caused by the bovine leukemia virus (BLV) deltaretrovirus. Infection has been associated with immune dysfunction and decreased milk production and longevity in dairy cows leading to reduced profitability in the dairy industry (Schwartz and Levy, 1994; Panei et al., 2013; Bartlett et al., 2014). Little is known about the effect on productivity in beef cattle. Approximately 30-40% of BLV infected cattle develop a persistent lymphocytosis and 1-5% develop lymphosarcoma (2017). In beef cattle, the condemnation of carcasses due to cancerous form of BLV infection at slaughter is the primary reason significant economic losses in the US (USDA/FSIS/EMS, 2002; White and Moore, 2009). The National Animal Health Monitoring System (NAHMS) Beef 97 study reported that 38% of beef herds were seropositive for BLV in the US with an individual prevalence of 10.3% (USDA/NAHMS, 2010). More recent studies reported 25.7% of beef bulls in Michigan, one year of age and older, were infected (Zalucha et al., 2013).

Transmission of BLV is believed to occur most commonly by horizontal transfer through direct contact with biological fluid contaminated with lymphocytes containing incorporated BLV provirus (Bartlett et al., 2014). The major route of virus transmission is believed to be iatrogenic through routine procedures in cattle herds that permit the transfer of blood between infected and non-infected animals. For instance, the use of the same needle to inject several animals and rectal palpation of different animals using common sleeves contaminated (Wentink et al., 1993; Divers et al., 1995; Kohara et al., 2006) with blood have been identified as methods of BLV transmission in cattle herds (Bartlett et al., 2014). Other potential sources of BLV include biting flies, nasal secretions, saliva (Yuan et al., 2015), semen (Asadpour and Jafari, 2012), milk (Jaworski et al., 2016), and colostrum (Gutiérrez et al., 2011).

Our group previously identified that the use of bulls for natural breeding was associated with a higher BLV herd prevalence in dairy herds (Erskine et al., 2012). Natural service is still used in approximately half of dairy operations across the US,(USDA) and is the most commonly used breeding method (approximately 90%) in beef cattle herds in the U.S.(USDA) Natural service may facilitate BLV transmission from trauma to the penis, vulva and vagina during the physical act of copulation, which may lead to the transfer of blood. Preputial secretions (smegma) are also known to contain white blood cells and could serve as a source of BLV transmission during copulation. Alternatively, BLV transmission may also occur via transfer of semen during copulation. Most studies report no evidence of BLV in semen (Monke, 1986; Santos et al., 2007), however one study reported the presence of the BLV provirus, as determined by PCR, in semen of seropositive cattle (Asadpour and Jafari, 2012).

The identification of primary routes of BLV transmission within and between herds is crucial to formulating management decisions to reduce economic losses and improve animal welfare. The objectives of this study were to 1) determine the prevalence of BLV in beef bulls, 2) evaluate the presence of BLV proviral DNA in smegma and semen, and 3) analyze the association among differential blood counts and blood BLV provirus.

Materials and Methods

Animals and Experimental Design

All animal procedures were approved by the Michigan State University Animal Care and Use Committee. One hundred and twenty-one (121) adult beef bulls (2 – 7 years old) from 39 different Michigan beef herds presented to the Michigan State University, College of Veterinary Medicine for breeding soundness exams (BSE) over 2016, were enrolled in the study.

BLV infection status

BLV infection was determined by both antibody detection ELISA and Coordination of Common Motifs (CoCoMo) algorithm by quantitative polymerase chain reaction (qPCR). In our study, a BLV-infected bull was defined as a bull that was positive by both the antibody ELISA and CoCoMo-qPCR (Riken Genesis CO, Tokyo, Japan) in blood.

Semen and Smegma Collection, Processing and Analysis

Semen samples were collected using an electroejaculator during routine breeding soundness examination (Lane Pulsator IV, Lane Manufacturing, Denver, CO). Smegma samples were collected using a collection device (TRICHIT^{TM}, Morris Livestock Products, Delavan, WI). The device was inserted into the prepuce and then moved smoothly back and forth scraping along the mucosa of the distal penis and fornix area while applying negative pressure with an attached 10 ml syringe. The collection process was performed with care to avoid any trauma to the penile and preputial epithelium which might cause iatrogenic blood to contaminate the sample. Five to eight back-and-forth scrapings of the pipette were required to obtain an adequate volume of sample (>2 ml). Semen and smegma samples were placed in 15 mL polypropylene conical tubes and stored at -80°C until further analysis. Smegma samples were collected before the electroejaculation procedure was realized for semen samples collection.

Blood Collection and Analysis

Whole blood (EDTA) and serum samples were collected by coccygeal venipuncture. Serum samples were analyzed for BLV antibodies using a commercial ELISA kit (IDEXX Leukosis Serum Screening Ab Test, IDEXX, Laboratories, Westbrook, Maine). A differential white blood cell count was conducted on whole blood by a leukocyte differential blood test (Qscout, Advanced Animal Diagnostics, Morrisville, NC) within 12 hours after each sample

collection. Aliquots of whole blood were stored at -80 C until further analysis by qPCR for BLV provirus DNA.

BLV Provirus DNA Analysis by CoCoMo-qPCR

DNA from frozen whole blood, semen, and smegma was extracted using a commercial Genomic DNA purification kit (Wizard® Genomic DNA Purification Kit, Promega Corporatin, Madison, WI) following the manufacturer recommended protocol with some modifications. Briefly, 900 µL cell lysis solution, 300 µL nuclei lysis solution, 100 µL protein precipitation solution and 100 µL of 100% isopropanol were added to 300 µL whole blood, semen, or smegma. Dithiothreitol (1.57 mg/mL) was added only to semen samples. Extracted DNA was quantified using a microplate plate reader[f] and a 260/280 ratio was used to assess sample purity. DNA was diluted in DNA rehydration solution to a final concentration of 30 ng/µL. Bovine leukemia proviral DNA was detected and quantified (proviral load (PVL)) by CoCoMo-qPCR as previously described, with some modifications (Jimba et al., 2010b). In brief, 30 ng/µL of genomic DNA were assayed using a probe-based master mix (TaqMan Gene Expression Master Mix, Applied Biosystems Inc., Foster City, California). PVL was calculated as the ratio between the number of BLV copies and BoLA-DRA copy number which was multiplied by 10^5 cells (Jimba et al., 2012). Each value was calculated using the algorithm suggested by the manufacturer. PVL was expressed as copy number / 10^5 cells.

Statistical Analysis

All statistical analyses were perform using SAS 9.4 (SAS 9.4, SAS Institute, Cary, NC). Prevalence of BLV in our population was calculated as the percentage of infected bulls divided by the total bulls sampled and the 95% confidence interval (CI) was determined using the exact method (Clopper-Pearson). BLV infected bulls were stratified into three groups based on PVL

(copy number/10^5 cells): low (1-526); moderate (527-10,000), and high (>10,000). One-way ANOVA and Bonferroni correction was performed for all pairwise comparisons between lymphocyte counts and the different proviral load strata. Lymphocyte count between BLV infected and BLV uninfected bulls were analyzed by unpaired t-test. Chi-square was used to compare the proportion of animals with or without lymphocytosis in BLV uninfected and infected animals. Age between BLV infected and BLV uninfected bulls were also analyzed by unpaired t-test.

Results

Prevalence of BLV in Beef Bulls

All BLV results fell into one of two scenarios without exception 1) ELISA positive and CoCoMo-qPCR positive that were defined as BLV infected bulls, 2) ELISA Negative and CoCoMo-qPCR negative that were defined as BLV negative bulls. From all sampled bulls, 54 out of 121 bulls (44.6%; 95% CI: 35.6%-53.9%) were BLV infected. BLV infected bulls were significantly older than BLV uninfected bulls (4.055 ± 0.1787 vs. 2.836 ± 0.1372 years old; P= > 0.0001) (Figure 2.1). At the farm level, 19 out of 39 farms that presented bulls for BSE's (48.7%; 95% CI: 32.4%-65.2%) had at least one BLV positive bull.

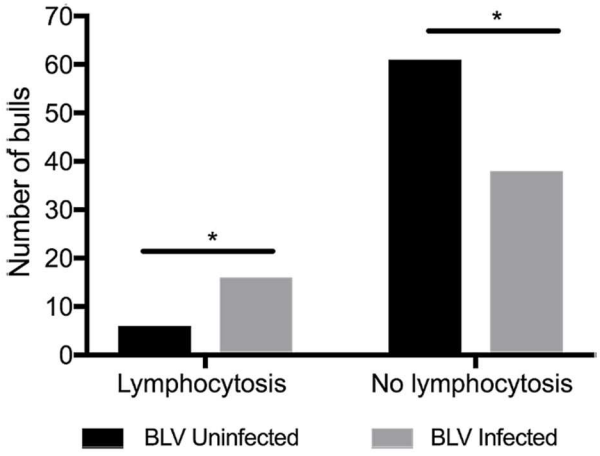

Figure 2.1 Bulls' age frequency distribution.

Age frequency distribution in 54 BLV infected bulls and 67 uninfected bulls sampled in 39 beef herds from Michigan during 2016.

BLV Provirus in Semen and Smegma

BLV provirus DNA was detected in smegma of 7.4% (4/54) of BLV infected bulls. The number of BLV proviral copies in smegma ranged from 4.50 - 618.78 copies/ 10^5 cells. BLV provirus was not detected in semen samples.

Lymphocyte Count in BLV Positive Bulls

Lymphocyte count (LC) was significantly higher in BLV infected bulls compared with BLV uninfected bulls (mean ± SD, 6.17 ± 1.77 vs. 5.38 ± 1.64 x10^3/μL; P=0.0033; Table 2.1). There were no differences in neutrophil count or neutrophil:lymphocyte ratio between the two groups. When bulls were divided into groups with high, medium and low PVL, BLV-infected bulls with high PVL had higher LC compared with the groups with low or moderate PVL (P=0.0001) (Table 2.2). Lymphocytosis, as defined by having a lymphocyte count >7.5 x 10^3 cells/uL, was found in 16/54 (29.63%; 95% CI: 19.1%-42.9%) of BLV infected bulls and was significantly higher (P=0.0045) than the proportion of BLV uninfected bulls with lymphocytosis 6/67 (8.9%; 95% CI: 3.8%-18.5%) (Figure 2.2).

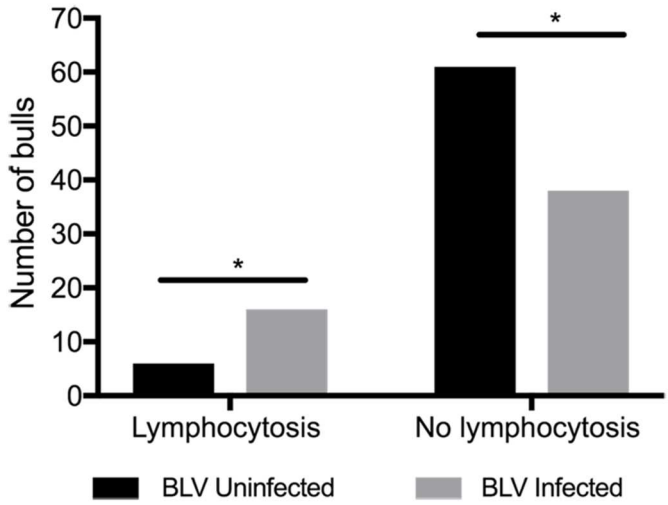

Figure 2.2 Proportion of BLV bulls by ELISA status with and without lymphocytosis.

Proportion of BLV infected (n=54) and uninfected (n=67) bulls with and without lymphocytosis. Values are expressed as percentage of animals. (*) Statistical difference by Chi-square analysis ($P<0.05$).

Discussion

In this study 44.6% of bulls were found infected with BLV. Our previous study in Michigan reported a BLV prevalence of 24.7% among beef bulls (Zalucha et al., 2013). However, the previous study included bulls less than 24 months of age whereas bulls enrolled in the current study were 24 months of age and older. When bulls less than 24 month of age were removed from the previous Michigan study, the crude prevalence was 42%, which is similar to our current results. In a study of U.S. beef herds in 1997, 38% of farms had at least one seropositive animal and 10.3% of individual cows were BLV seropositive; however, the prevalence among beef

Table 2.1 Absolute lymphocyte and neutrophil concentrations and Neutrophil-to-lymphocyte for all bulls.

Absolute lymphocyte and neutrophil concentrations and Neutrophil-to-lymphocyte for all bulls, BLV infected bulls, and BLV-uninfected bulls in 39 beef herds from Michigan (n = 39), presented for BSE during 2016.

Variable		All bulls (n=121)	BLV infected (n=54)	BLV uninfected (n=67)
Lymphocyte ($x10^3/\mu L$)	Mean ± SD	5.74 ± 1.73	6.17 ± 1.77	5.38 ± 1.64
	25th percentile	4.34	5.11	4.03
	Median	5.56	5.86	5.29
	75th percentile	7.21	7.59	6.72
Neutrophils ($x10^3/\mu L$)	Mean ± SD	3.15 ± 1.61	3.27 ± 1.65	3.06 ± 1.59
	25th percentile	1.95	1.85	1.97
	Median	2.84	2.88	2.78
	75th percentile	4.021	4.24	3.95
Neutrophils to Lymphocytes Ratio ($x10^3/\mu L$)	Mean ± SD	0.58 ± 0.31	0.57 ± 0.35	0.58 ± 0.26
	25th percentile	0.36	0.31	0.4
	Median	0.54	0.52	0.55
	75th percentile	0.75	0.81	0.73

Table 2.2 Bovine Leukosis Virus Proviral Load (PVL) concentration and absolute lymphocyte concentration data Bovine Leukosis.

Virus Proviral Load (PVL) concentration and absolute lymphocyte concentration data for all BLV-infected bulls and PVL-based groups in 39 beef herds from Michigan (n = 39), presented for BSE during 2016.

Variable		PVL Low (n=27)	PVL Moderate (n=11)	PVL High (n=16)	PVL All (n=54)
PVL (copy number/10^5)	Mean ± SD	99.82 ± 133.55	3581.81 ± 2643.81	26910 ± 13698	8224 ± 13830
	25th percentile	29.08	1247.52	10456	41.07
	Median	42.91	3237.01	15301	513.9
	75th percentile	102.44	5282.83	27472	11084
Lymphocyte Count ($x10^3/\mu L$)	Mean ± SD	5.95 ± 1.81	5.04 ± 1.17	7.42 ± 1.301	6.17 ± 1.76
	25th percentile	5.09	4.01	6.22	5.11
	Median	5.59	5.11	7.48	5.86
	75th percentile	7.66	5.84	8.44	7.59

bulls were not reported (USDA/NAHMS, 2010). Similar findings were reported in beef herds in Japan and China where 35.2% of farms and 28.7% of breeding animals were seropositive for BLV (Murakami et al., 2013, Sun et al., 2015). High BLV seroprevalence was also observed in beef herds in five different countries in South America and ranged between 27.9 % to 77.4% (Polat et al., 2016). These results demonstrate that BLV infection is high in beef cattle and it is a current problem in beef farms in the Michigan and possibly the U.S.

To our knowledge, this is the first study to report the presence of BLV proviral DNA in smegma samples of BLV infected beef bulls. Smegma is an accumulation of glandular secretions and desquamated epithelial cells within the preputial orifice of the bull. Physiologically, the preputial epithelium, where smegma is diffusely distributed, is populated by white cells such as lymphocytes and neutrophils dispersed near the basement membrane (Cobo et al., 2011). The presence of lymphocytes in smegma, which are the most common cell type infected with BLV provirus, could explain why we found BLV provirus in smegma (Jimba et al., 2010a). These results support the possibility that smegma could be a source of BLV transmission during natural breeding and using BLV infected breeding bulls may increase the risk of BLV spreading within herds.

In contrast, no BLV provirus was detected in semen samples, which is consistent with previous studies (Kaja and Olson, 1982; Monke, 1986; Santos et al., 2007). Absence of BLV provirus in semen may be explained by lack of infected lympohocytes in semen. In our study, all bulls sampled were healthy with no clinical signs of BLV infection such as testicular lymphoma, and no indication of reproductive organ inflammation, such as orchitis or seminal vesiculitis, all conditions that could be possibly associated with lymphocyte presence in semen (Kaja and Olson, 1982; Choi et al., 2002).

In addition to potential BLV transmission by smegma, bulls could serve as a source of transmission during the breeding season through trauma-induced hemorrhage during copulation. The physical act of copulation can lead to trauma to the penis, vulva and vagina resulting in the transfer of blood among the bull and the cow. Infected bulls could also potentially transmit the disease through nose-to-nose contact during the mating process.

In our study, a higher proportion of bulls with lymphocytosis was observed in BLV infected bulls compared with bulls negative on both ELISA and PCR. In general, lymphocytosis not associated with BLV is an uncommon finding in ruminants (Alvarez et al., 2013). Similar to previous studies (Sordillo et al., 1994; Swenson et al., 2013; Frie and Coussens, 2015), we observed a positive association between blood lymphocyte concentrations and BLV PVL.

Previous studies reported that 25% to 35% of circulating lymphocytes in BLV infected cows with lymphocytosis have integrated BLV proviral DNA compared with 5% in infected cows without lymphocytosis (Hopkins and DiGiacomo, 1997; Esteban et al., 2009). The association of higher BLV proviral loads with bulls that have lymphocytosis would suggest these bulls are at higher risk for BLV transmission.

The high prevalence of BLV in breeding bulls and the presence of BLV provirus in smegma provides evidence that breeding bulls may play a role in transmitting BLV especially during natural service. Control of BLV at the herd level should include identifying bulls free of BLV infection.

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CHAPTER 3

Lack of Bovine Leukemia Virus transmission during natural breeding of cattle

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Abstract

Bovine leukosis is a chronic lymphoproliferative disorder that leads to significant economic losses in the beef and dairy industries. The major route of virus transmission is believed to be iatrogenic through the transfer of blood containing infected lymphocytes. In addition, BLV proviral DNA has been identified in nasal secretions, saliva, milk, colostrum, semen and smegma; however, natural transmission of BLV through these secretions has not been clearly demonstrated. The use of bulls for natural breeding has been identified as a risk factor in BLV infected dairy herds. However, the risk of BLV-infected bulls transmitting the virus is unknown. The objective of this study was to evaluate the potential for BLV transmission during natural breeding between a BLV-infected bull and uninfected heifers. Forty healthy, BLV seronegative, and proviral-negative beef heifers were randomly assigned to one of two groups: control heifers (n=20) exposed to a BLV seronegative and proviral negative bull and challenged heifers (n=20) exposed to a BLV seropositive and proviral-positive bull. Each group was housed with the bull for a period of 38 days in a 5-acre pasture to replicate the housing of commercial beef cattle during the breeding season. Blood samples were collected from heifers at -60, -30 and 0 days prior to breeding and day 30, 60 and 90 after the breeding period ended. Blood samples were tested for BLV antibodies by ELISA and BLV proviral DNA by CoCoMo-qPCR. New infection was not detected by ELISA or CoCoMo-qPCR in any of the challenge or control heifers at any time point during the study. Based on these results, BLV infected bulls that are healthy and aleukemic may not be a significant risk of BLV transmission during a defined breeding season.

Key Words: Bovine leukemia virus, Proviral Load, Smegma

Introduction

Bovine leukosis is a chronic lymphoproliferative disorder in cattle caused by the deltaretrovirus, bovine leukemia virus (BLV). Most BLV infected animals remain asymptomatic and act as carriers of the virus; 30-40% of infected cattle have a persistent lymphocytosis, while less than 5% progress to lymphosarcoma, the fatal, clinical form of BLV infection (Panei et al., 2013; Bartlett et al., 2014). Surveys indicate that lymphoma accounts for 13.5% of beef cattle condemnations at U.S. slaughter plants (USDA/FSIS/EMS; White and Moore, 2009). Economic losses associated with BLV in the dairy industry in the U.S. estimated at $285 million (Ott et al., 2003). To prevent further economic losses and animal welfare concerns due to BLV, the identification of primary routes of BLV transmission within and among herds is crucial to optimize management decisions.

The most common means of BLV spread is horizontal transmission by direct contact with biological fluid contaminated with BLV infected lymphocytes (Bartlett et al., 2014). Virus transmission can happen through iatrogenic procedures that transfer blood between cattle (DiGiacomo et al., 1985; Kobayashi et al., 2014). Also, proviral DNA has been identified in nasal secretions (Yuan et al., 2015), saliva (Yuan et al., 2015), semen (Asadpour and Jafari, 2012) and smegma (Benitez et al., 2018) however, natural transmission through these secretions has not been clearly demonstrated. Transmission via milk and biting flies has been well documented (Foil et al., 1988; Meas et al., 2002).

Our research group has identified the use of breeding bulls in dairy herds as a risk factor for BLV infection at the herd level (Erskine et al., 2012). As part of an ongoing integrated study of chronic diseases in bulls, we collected blood from 121 beef bulls (>2 years old) from 38 beef farms in Michigan. Amongst sampled bulls, 45% (55/122) were BLV seropositive and 5.5% had

detectable BLV proviral DNA in smegma (Benitez et al., 2018). Natural service is still used in approximately half of dairy operations across the U.S. (USDA), and is the most commonly used breeding method (approximately 90%) in beef cattle herds in the U.S. (USDA). The high rate of BLV infection in beef bulls and their frequent use for natural breeding combine to create a considerable risk of BLV transmission during natural breeding via smegma, semen or from blood transfer from trauma to the penis, vulva and vagina. The objective of this study was to assess the risk of BLV transmission by breeding bulls during natural breeding.

Materials and Methods

Animals

Forty 12-month-old Angus crossbred heifers were selected from a larger group of purchased heifers. To be selected, the heifers needed to be clinically healthy, seronegative for BLV, negative for BLV provirus by Coordination of Common Motifs-Quantitative polymerase chain reaction (CoCoMo –qPCR) and determined to be cycling and not pregnant during examination of the uterus and ovaries by transrectal ultrasonography. Two bulls were acquired for this study. The first bull was a 30-month-old Angus bull that was BLV seronegative and CoCoMo-qPCR negative for BLV in blood, semen, smegma and nasal secretions. The second bull was a 36-month-old Angus bull that was BLV seropositive and CoCoMo-qPCR positive for BLV in blood, smegma and negative for BLV in semen and nasal secretions. Both bulls were found to be sound for breeding based on a breeding soundness exam conducted 30 days prior to the beginning of the study. This study was approved by the Michigan State University Animal Care and Use Committee.

Study Design

Heifers were blocked by weight and then randomly assigned to one of two groups: control (n=20) heifers exposed to the BLV-negative bull and challenge (n=20) heifers exposed to the BLV-positive bull. On day 0 of the study, the bulls were introduced into their respective breeding group for a period of 38 days to allow each cycling heifer at least one opportunity for natural service with the bull. The challenge and control groups were housed separately with no nose-to-nose or fence-line contact and were isolated on the Michigan State University Veterinary Research Farm with no other cattle within 1,000 meters. To reduce the risk of horizontal transmission by biting insects, the project was conducted between November and March when the average high temperature is between 2-7^{o} C and insect activity was negligible.

Serum and whole blood samples were collected from all heifers at days -60, -30 and 0 days prior to introduction of bulls and 30, 60 and 90 days after bulls were removed (days 68, 95 and 128 after initial exposure to bulls). Pregnancy status was determined by transrectal ultrasonography at day 30 and 60 after bulls were removed. Serum, whole blood, nasal swabs, semen and smegma samples were collected from bulls at days -60, -30, 0 and 38

Breeding management

To more closely group estrous cycles, all heifers received a single injection of 25mg dinoprost tromethamine on day 0 immediately prior to introduction of the bulls. In addition, a visual heat detection device was attached to the tail head. Heifers were visually observed for 1 hour in the morning (from 07:30 to 08:30) and afternoon (17:30 to 18:30). All signs of estrous activity were recorded. At day 38, bulls were removed while heifers were maintained in their separate groups for another 90 days.

BLV ELISA, CoCoMo PCR and Leukocyte Count

Serum samples were analyzed for BLV antibodies using a commercial ELISA kit. DNA from frozen whole blood, semen, and smegma was extracted using a commercial Genomic DNA purification kit[e] following the manufacturer recommended protocol with some modifications. Briefly, 900 µL cell lysis solution, 300 µL nuclei lysis solution, 100 µL protein precipitation solution and 100 µL of 100% isopropanol were added to 300 µL whole blood, semen, or smegma. Dithiothreitol (1.57 mg/mL) was added only to semen samples. Extracted DNA was quantified using a microplate plate reader[f] and a 260/280 ratio was used to assess sample purity. DNA was diluted in DNA rehydration solution to a final concentration of 30 ng/µL. Bovine leukemia proviral DNA was detected and quantified (proviral load (PVL) by CoCoMo-qPCR as previously described, with some modifications (Jimba et al., 2010). In brief, 30 ng/µL of genomic DNA were assayed using a probe-based master mix. Proviral load (PVL) was calculated as the ratio between the number of BLV copies and BoLA-DRA copy number which was multiplied by 10^5 cells (Jimba et al., 2012). Each value was calculated using the algorithm suggested by the manufacturer. PVL was expressed as copy number / 10^5 cells. To establish lymphocyte counts in the bulls, a differential white blood cell count was conducted on whole blood using an automated commercial leukocyte count system.

Data Analysis

The main outcome variables were seroconversion to BLV and detection of BLV provirus in blood samples. A dichotomous outcome of BLV-positive or negative was used. The Fishers Exact Test was used to test the hypothesis that proportions are the same in different groups. All statistical analyses were performed using SAS 9.4.

Results

Characteristics and BLV diagnostics and of the bulls prior to and during the study are shown in Table 1. In the BLV challenge group, estrus activity was observed in 100% of the heifers and all were mounted by the BLV positive bull at least once during the study. Pregnancy percentage for the BLV challenge group was 85% (17/20). Among the 17 pregnant heifers, 70.5% (n=12/17) became pregnant in the first two weeks of the breeding period (Figure 3.1). In the control group, estrus activity was observed in 90% (18/20) of the heifers and 65% (n=13/20) were confirmed pregnant. Within the pregnant heifers, 84.6% (n=11/13) became pregnant in the first two week of the breeding period. Seroconversion to BLV or BLV provirus was not detected in any of the challenge or control heifers at any time point during the study.

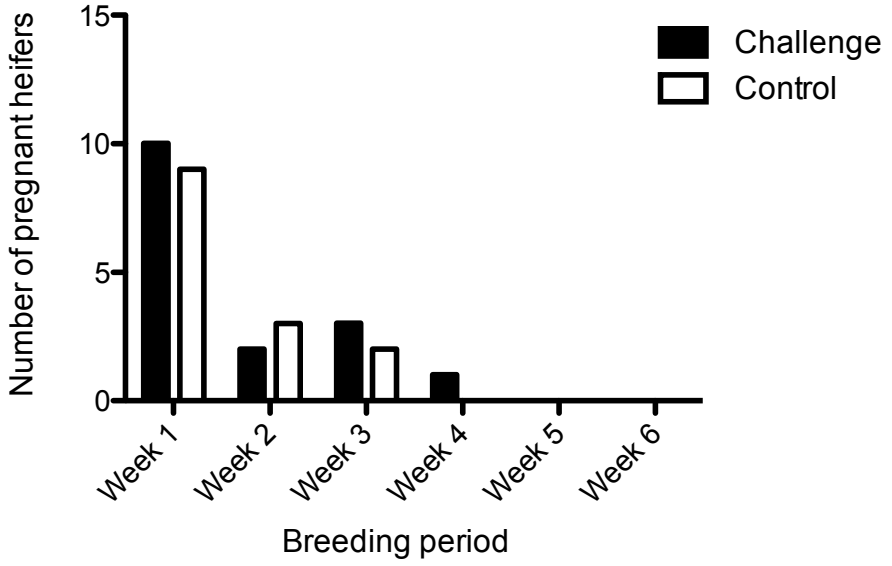

Figure 3.1 Distribution of pregnant heifers by BLV status.

Number of pregnant heifers by week during (38 days) breeding period as determined by transrectal ultrasonography.

Discussion

To our knowledge, this is the first study evaluating the possibility of BLV transmission though natural breeding in cattle. In this study, we were unable to detect any BLV transmission from a BLV-infected bull to BLV-negative heifers during a 38-day breeding period. Although BLV provirus was found in the smegma and blood of the BLV positive bull and there was direct contact during copulation with all the heifers at least once (as determined by observation and pregnancy status), no evidence of infection in the heifers could be detected by either ELISA seroconversion or detection of BLV provirus. A likely reason for this finding is that insufficient virus was transmitted during contact between the BLV infected bull and heifers. We determined the BLV infected bull had a PVL of 175.90 copies /10^5 cells in the smegma sample collected at the end of the breeding season (Table 3.1). This translates into an estimate of 175 infected cells per 10,000 cells within the collected smegma sample. The total lymphocyte count and volume of smegma in bulls, and how much of this secretion is transferred to the female during the sexual act have not been reported before; thus, it is difficult to estimate the total number of BLV-infected cells potentially transmitted from the bull to the heifers. Additionally, to our knowledge, there are no reports evaluating the infectivity of provirus in smegma in cattle. Given this information, the risk of BLV transmission during natural breeding may have been low, especially because the bull was healthy, was not lymphocytotic and had a relatively low PVL. Other sources of BLV spread could have occurred through blood transfer during copulation and nose-to-nose contact. It has been estimated that approximately 2,500 (~0.1 to 0.2 µL of blood from a Persistent Lymphocytic (PL) animal) -to 20,000 (~1ml of blood from Aleukemic (AL) animal) BLV-infected lymphocytes are needed to establish an infection (Foil et al., 1987) (Mammerickx et al., 1987). The fact that the bull used in this study had a moderately elevated lymphocyte

Table 3.1 Bovine Leukosis virus and lymphocyte characteristics of bulls.

BLV Proviral Load (PVL (copy number/10^5)) in whole blood, semen and smegma; BLV antibody ELISA (OD) and Lymphocyte count (LC ($x10^3/\mu L$)).

	BLV ELISA		Lymphocytes $x10^3/\mu L$	BLV PVL copy #/10^5 cells			
Bull	**OD**	**Interpretation**		**Blood**	**Smegma**	**Semen**	**Nasal Swab**
90 Days Prior to Breeding Period							
+ Bull	2.616	Positive	5,634	10,795.54	0	0	0
- Bull	0.104	Negative	2,381	0	0	0	0
60 Days Prior to Breeding Period							
+ Bull	2.315	Positive	4,583	16,731.26	0	0	0
- Bull	0.089	Negative	2,421	0	0	0	0
30 Days Prior to Breeding Period							
+ Bull	3.114	Positive	4,186	20,865.85	47.61	0	0
- Bull	0.706	Negative	2,647	0	0	0	0
Start of Breeding Period							
+ Bull	2.820	Positive	4,876	20,449.10	119.18	0	0
- Bull	0.106	Negative	2,223	0	0	0	0
End of Breeding Period							
+ Bull	2.656	Positive	3,589	18,405.16	175.90	0	0
- Bull	0.104	Negative	2,453	0	0	0	0

count and a moderate PVL would have made it likely that a significant amount of blood would need to be transferred to cause infection. From this study, we cannot determine what might have happened during natural breeding if the BLV positive bull was lymphocytotic and/or had a higher PVL, or if significant blood transfer had occurred, for instance, following penile trauma induced during breeding.

Another factor that could have led to the lack of BLV-transmission during breeding was the natural immunological barriers in the vaginal and uterus epithelium of females which play a key role in reducing the risk of infectious agents entering the body via this route (Sheldon et al., 2009). Effective defense against reproductive tract invasion by infectious agents is mediated by anatomical and functional barriers as well as nonspecific and specific immune responses. The capability of the immune system is also influenced by steroid hormones that increase the activity of the antigen-presenting cells in the vagina and uterus (Corbeil et al., 1974; Bondurant, 1999). In situations where these barriers may be compromised, the risk of infections may increase.

In the present study, it may be that BLV transmission occurred during the exposure period, however, because of the limited follow-up period, we were unable to detect BLV provirus or seroconversion. In our study, we assessed BLV seroconversion and provirus presence in blood out to 17 weeks after the bulls were first exposed to the heifers, and 12 weeks after the bulls were removed from the same paddock as heifers. We also know that the majority of the heifers were mounted and bred within he first two weeks of exposure (Figures 3.1 and 3.2) therefore allowing up to 15 weeks for virus replication and seroconversion to occur. Our study design was based on previous reports in which seroconversion to BLV occurred between the 3rd and 14th weeks following exposure (Evermann et al., 1986). Other studies have reported even shorter period of time for detection of BLV provirus in blood, ranging from 2-4 weeks after

experimental exposure to the virus (Kelly et al., 1993; Klintevall et al., 1994). Given this information and the fact that no evidence of infection could be detected in any of the heifers despite direct exposure to the bull through nose-to-nose contact and copulation, we are confident that infection did not occur.

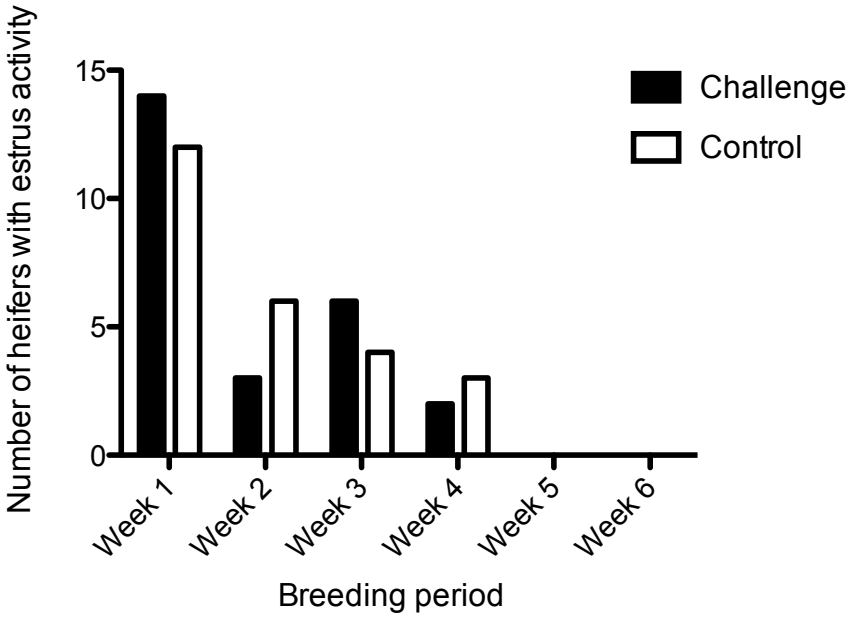

Figure 3.2 Distribution of heifers with estrus activity during the study.

Number of heifers mounted by week during (38 days) breeding period as determined by observation.

Based on the findings of this study, ELISA-positive bulls that are healthy and aleukemic may not be a significant risk of BLV transmission during a defined breeding season. Regardless, veterinarians and producers should be aware that the risk of BLV transmission will likely increase as the length of exposure to infected bulls increases and if bulls progress to develop lymphocytosis with higher BLV proviral loads in blood and smegma. Future studies to assess the ability of bulls with high PVL and lymphocytosis are needed to further assess the ability of bulls to transmit BLV during natural service.

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CHAPTER 4

Impact of bovine leukemia virus infection on beef cow longevity

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Authors who contributed to this study were:
Benitez, O. J., J. N., Norby, B., Bartlett, P. C., Maeroff, J. E., & Grooms, D. L.

Abstract

Bovine leukosis is a chronic lymphoproliferative disorder caused by bovine leukemia virus (BLV). Previous studies estimate that 38% of cow-calf beef herds and 10.3% of individual beef cows in the U.S. are BLV seropositive. About 70% of BLV infected animals are asymptomatic carriers of the virus, while less than 5% develop lymphosarcoma, the leading reason for U.S. carcass condemnation at slaughter. Studies provide evidence that BLV infection leads to decreased immune function making animals more vulnerable to other diseases, which could shorten their productive lifespan and increase economic losses in the cattle industry. BLV seropositive dairy cows are reportedly more likely to be culled sooner compared with their uninfected herd mates. Beyond simple prevalence studies, little is known about the impact of BLV infection in beef cattle production or specifically on beef cow longevity. Our objective was to determine the association between BLV infection and cow longevity in beef cow-calf operations. Twenty-seven cow-calf herds from the Upper Midwest volunteered to participate in this study. Female beef cattle (n=3,146) were tested for serum BLV antibodies by ELISA. A subsample of 648 cows were also tested for circulating BLV proviral load. Culling data was collected for the following 24 months. Twenty-one herds (77.7%) had at least one BLV-infected animal, and 29.2% (930/3146) of tested animals were BLV seropositive. 33.7% (318/943) of culled cows were BLV-positive compared with 32.1% (541/1682) BLV seronegative cows. BLV status did not affect cows' longevity within herds ($P>0.05$). However, cows with high BLV proviral load had decreased survival within the herd compared with ELISA- negative cows ($P = 0.01$). Overall, infection with BLV did not impact beef cow longevity unless the disease had progressed to a point of high BLV proviral load.

Key Words: Bovine leukemia virus, Beef cattle, Survival Analysis

Introduction

Bovine leukosis is a chronic lymphoproliferative disorder in cattle caused by the deltaretrovirus, bovine leukemia virus (BLV). Most BLV-infected animals remain asymptomatic and act as carriers of the virus; ~30-40% of infected cattle can develop a persistent lymphocytosis, while less than 5% progress to lymphosarcoma, the fatal, clinical form of BLV infection (Tajima et al., 1998; USDA, 2007; Bartlett et al., 2013). Surveys indicate that lymphoma accounts for 13.5% of beef cattle carcass condemnation in U.S. slaughterhouses leading to significant economic loss (USDA/FSIS/EMS, 2002; White and Moore, 2009); however, the impact of BLV on beef cattle productive longevity and survival rates is currently unknown.

In the U.S, most BLV research has targeted dairy cattle (Ott et al., 2003; Erskine et al., 2012; LaDronka et al., 2018). We recently demonstrated that dairy cattle with BLV antibodies were 23% more likely to be culled over the 19-month monitoring period (Bartlett et al., 2013). Those cows with the highest levels of BLV antibodies in milk were 40% more likely to be culled. Previous studies had also showed an association between BLV infection and decreased dairy cattle longevity (Thurmond et al., 1985). A Canadian study showed a trend for ELISA-positive dairy cattle older than 3.5-years-old to be culled at a higher rate than ELISA-negative cattle (Jacobs et al., 1995).

Much less is known about BLV in U.S. beef cattle. Over 20 years ago, the individual-cow BLV prevalence in beef cows was estimated at10.3% (USDA/NAHMS, 2010). By 2017, Bauermann et al. (2017) were reporting that 33.6% of beef cull cattle brought into the U.S. slaughterhouses were seropositive for BLV. In Michigan in 2013, we reported a BLV prevalence of 25.7% among beef bulls ages 1 to 10 years that were presented for breeding soundness exams

(Zalucha et al., 2013). More recently we found BLV antibodies in 44.6% of 121 Michigan adult beef bulls from 39 different farms that were ages 2 to 14 years-old (Benitez et al., 2019). The objectives of this study were to 1) determine the impact of BLV infection on longevity of beef cows, and 2) determine the association between cow longevity and measures of BVL disease progression: using blood BLV proviral load (PVL).

Materials and Methods

Study Design and study population

Twenty-seven cow-calf operations with a total of 3,146 cows were enrolled in this study. 19 herds and 2,118 cows (67.32%) were from Michigan and the rest were from Indiana, Iowa and Ohio. Criteria for being selected were a history of BLV being diagnosed or suspected in the herd, individual-animal identification, and individual animal records that included dates and reasons for leaving the herd by either death or culling. Blood samples were collected by coccygeal venipuncture for serum and plasma extraction. All serum samples were analyzed for the presence of BLV-specific antibodies. BLV provirus load (PVL) were determined in a subset of randomly selected seropositive and seronegative cows (648 cows from 9 different Michigan herds).

To determine cow survival in the herds, records were reviewed at the end of the first and second years. The reasons why each animal died or was culled were recorded (Table 4.1). Producers were provided with the overall BLV prevalence within their herd but were blinded as to the individual-cow ELISA results to avoid influencing culling decisions.

Table 4. 1 Reasons that bovine leukemia virus (BLV) ELISA positive and negative cows died or were culled.

Reason for Leaving the Herd	BLV Status		
	Negative	**Positive**	**Total**
Failure to get pregnant	221 (25.73%)	117 (13.62%)	338 (39.35%)
Early fetal loss	8 (0.93%)	3 (0.35%)	11 (1.28%)
Abortion	19 (2.21%)	22 (2.56%)	41 (4.77%)
Early calf born death	42 (4.89%)	14 (1.63%)	56 (6.52%)
Calving associated injury	3 (0.35%)	4 (0.47%)	7 (0.81%)
Uterine or vaginal problem	1 (0.12%)	0 (0.00%)	1 (0.12%)
Cancer	2 (0.23%)	8 (0.93%)	10 (1.16%)
Johne's	32 (3.73%)	0 (0.00%)	32 (3.73%)
Bloat	2 (0.23%)	1 (0.12%)	3 (0.35%)
Mastitis	3 (0.35%)	3 (0.35%)	6 (0.70%)
Lameness	3 (0.35%)	8 (0.93%)	11 (1.28%)
Thin cow	8 (0.93%)	2 (0.23%)	10 (1.16%)
Bad mother	6 (0.70%)	1 (0.12%)	7 (0.81%)
Low calf weight	3 (0.35%)	6 (0.70%)	9 (1.05%)
Poor udder	28 (3.26%)	17 (1.98)	45 (5.24%)
Bad feet/leg structure	20 (2.33%)	15 (1.98%)	35 (4.07%)
Age	17 (1.98%)	22 (2.56%)	39 (4.54%)
Injury	14 (1.63%)	9 (1.05%)	23 (2.68%)
Unknown	14 (1.63%)	8 (0.93%)	22 (2.56%)
Temperament	36 (4.19%)	16 (1.86%)	52 (6.05%)
Total	**541**	**318**	**859**
Culled	541 (32.16%)	318 (33.72)	859 (32.72%)
Still within the herd	1,141 (67.84%)	625 (66.28%)	1,766 (67.28%)
Total	**1,682**	**943**	**2,625**

Blood samples processing and analysis

Serum samples were submitted to the Michigan State University College of Veterinary Medicine Veterinary Diagnostic Laboratory and analyzed for BLV antibodies using a commercial ELISA kit (IDEXX Leukosis Serum Screening Ab Test, IDEXX, Laboratories, Westbrook, Maine). For BLV PVL quantification, DNA was extracted from frozen whole blood using a commercial Genomic DNA purification kit (Wizard® Genomic DNA Purification Kit, Promega Corporatin, Madison, WI) following the manufacturer's recommended protocol. Briefly, 900 µL cell lysis solution, 300 µL nuclei lysis solution, 100 µL protein precipitation solution and 100 µL of 100% isopropanol were added to 300 µL whole blood. Extracted DNA was quantified using a microplate plate reader (Infinite m200 PRO TECAN, Tecan Austria GmbH, Grödig, Austria) and a 260/280 ratio was used to assess sample purity. DNA was diluted in DNA rehydration solution to a final concentration of 30 ng/µL. BLV proviral DNA (PVL) was detected and quantified by CoCoMo-qPCR as previously described (Benitez et al., 2019). In brief, 30 ng/µL of genomic DNA were assayed using a probe-based master mix (TaqMan Gene Expression Master Mix, Applied Biosystems Inc., Foster City, California). Proviral load (PVL) was calculated as the ratio between the number of BLV copies and BoLA-DRA copy number which was multiplied by 10^5 cells (Jimba et al., 2010). Each value was calculated using the algorithm suggested by the manufacturer. PVL was expressed as copy number / 10^5 cells. The PVL was further categorized as: High (≥60,000 copies), Low (<60,000 copies), and Negative (0 copies)

Data Analysis

Survival analysis was conducted using Stata software (version 15.1; StataCorp LP, College Station, TX) using nonparametric Kaplan-Meier survival graphs (sts graph procedure) and semi-parametric Cox proportional hazard models (stcox procedure) handling ties with the Breslow method. Kaplan-Meier graphs were used to visually assess the survival curves. A total of three Cox proportional hazard models were used to analyze our data base: *Model #1* used BLV ELISA (negative: OD <0.1; positive: ≥0.1); *Model #2* used BLV status determined by both antibody detection ELISA and CoCoMo algorithm by qPCR analysis; *Model #3*; used ELISA-positive cows that were stratified into three groups based on PVL (copy number/10^5 cells): Negative, low (≤ 60,000), and High (>60,000). Shared frailty (random effect) of the herd was included in all models. Results were considered statistically significant at $P < 0.05$. The shared frailty model using multiplicative gamma-distributed random effects on the hazard scale was used to model within-herd correlation among cows. Model diagnostics for Cox models were based on Cox-Snell residuals, Schoenfeld residuals, as well as interaction terms between log-transformed time to event and all or each of the predictor variables (Cleves et al., 2010).

Results

Prevalence of BLV

Twenty-one of the twenty-seven herds 77.7% (95% CI: 69.5 -85.4) had at least one BLV seropositive cow in it. The prevalence of individual BLV seropositive cows was 29.2% (95% CI: 27.6-30.8). Within herd-BLV prevalence displayed 45.15% (IQR = 29.85 - 71.84).

Survival analysis

A total of 7 herds were excluded from the analysis due to inadequate follow-up records (n=1) or absence of any BLV positive cows (n=6). After the exclusion, 20 herds and 2,625 cows

remained in the study with complete records. A total of 943 (35.9%; 95% CI: 34.1 - 37.7) cows tested positive for BLV antibodies and 1,682 tested negatives. During the monitoring period, 32.7% (95% CI: 30.9 -34.5) of the cows were culled or died, and 67.2% (95% CI: 65.4 - 69) remained in the herd. Of the ELISA-positive cows, 33.7% (95% CI: 30.7 – 36.7) left the herd during the study period as compared to 32.1% (95% CI: 29.8 – 34.3) BLV seronegative cows. Reasons for cows leaving the herd shown in Table 4.1. There was no statistically significant difference in the survival of the cows based on ELISA status (P > 0.10), as shown in Table 4.2 and Figure 4.1.

Table 4.2 Survival of beef cows over a two-year period following bovine leukemia virus (BLV) testing (negative or positive) for serum ELISA antibodies.

[1]Z = Z statistic (β/β SE).

Effect	Hazard ratio	SE	Z	*P*-value	95% CI
BLV ELISA -	-	-	-	-	-
BLV ELISA +	1.18	1.188	1.87	0.062	0.99-1.42

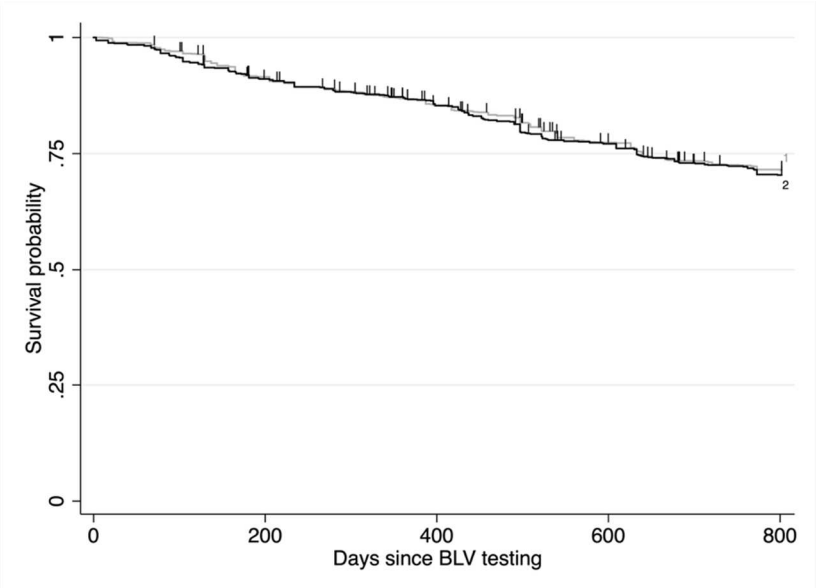

Figure 4.1 Survival of cattle following bovine leukemia virus (BLV) testing.

Proportion of cattle surviving (y-axis) and days since BLV testing (x-axis). [1] Negative cows, [2] Positive cows.

Association of survival with BLV antibodies and PVL

BLV PVL were measured in a total of 648 cows from 9 different Michigan herds. Results had 100% agreement between ELISA and CoCoMo-qPCR. Of these, 181 cows (28.8%) tested positive for BLV ELISA antibodies and CoCoMo-qPCR, and 461 (71.1%) tested negative. During the monitoring period, 29% (95% CI: 25.5 – 32.5) of the cows died or were culled, and 80% (95% CI: 76.9 – 83.1) remained in the herd. Among this subset, cull rates between cows testing positive or negative for BLV antibodies were not different: 30% (95% CI: 23.4 – 36.6) of BLV positive cows were culled, compared with 28.6% (95% CI: 24.5 – 32.7) of the BLV negative cows. Of the 59 cows with high PVL, there were 35 (59.2%) remaining after the two-year follow-up period, compared with 75% with low PVL cows and 76.5% ELISA-negative cows. Our analysis showed that cows with high-PVL had an 84% greater hazard of leaving the herd, while cows with low-PVL faced 0.9% greater hazard than their uninfected cows (P = 0.01) (Table 4.3 and Figure 4.2), using the Cox proportional hazard model # 3.

Table 4.3 Survival of beef cows over a two-year period by ELISA status and PVL levels.

Survival of beef cows over a two-year period following bovine leukemia virus (BLV) testing (negative or positive) for serum ELISA antibodies and CoCoMo-qPCR to determine proviral load (PVL).

Effect	Hazard ratio	SE	Z	*P*-value	95% CI
BLV ELISA -	-	-	-	-	-
BLV ELISA +	0.48	0.21	-1.61	0.107	0.20-1.16
PVL Negative	-	-	-	-	-
PVL Low	1.09	0.24	0.42	0.675	0.71-1.68
PVL High	1.84	0.43	2.58	0.01	1.15-2.92

[1]Z = Z statistic (β/β SE).
PVL (copy number/105 cells): low (≤ 30,000), High (>30,000-<60,000) and Very high PVL (>60,000)
*PVL (copy number/10^5 cells)

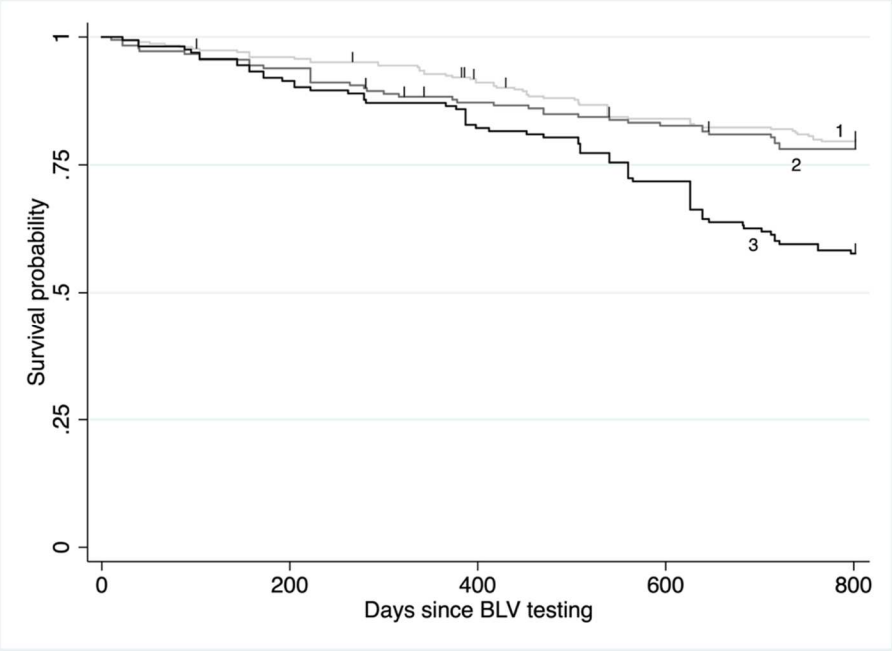

Figure 4.2 Survival of 648 cows following bovine leukemia virus (BLV) testing by ELISA and CoCoMo-qPCR.

Proportion of cattle surviving (y-axis) and days after enrollment (x-axis). [1] Negative cows by ELISA and CoCoMo-qPCR, [2] Cows with low PVL (<60,000 copies), and [3]Cows with high PVL (>60,000 copies).

Discussion and Concluding Comments

This study is the first to look at the effect of BLV on beef cow longevity. Our study shows that the survival of beef cows seropositive for BLV is not different from BLV seronegative cows over a period of two years subsequent to testing. While we did not observe differences in survival, our data show that seropositive cows face 18% greater hazard of being culled compared with seronegative cows and imply a trend of BLV infection on the beef cow productive life. Previous studies similarly show a lack of association between BLV infection effect and longevity in dairy cows (Huber et al., 1981; Rhodes et al., 2003; Tiwari et al., 2005),

while others show the contrary (Thurmond et al., 1985; Jacobs et al., 1995; Bartlett et al., 2013). We consider that differences between previous studies in dairy cows and our results studying beef herds are at least partially explained by intrinsic variations between the two productive systems and, potentially, with different breed-associated traits. For example, it is known that the dramatic genetic selection of dairy cows for improving production traits leads to metabolic challenges that negatively affect immune responses (Ingvartsen et al., 2003), thus also increasing the susceptibility of animals to viral infections and secondary health problems. A negative association between genetic selection for increased milk yield and incidence of disease (ketosis, ovarian cyst, mastitis, and lameness), survival, and behavioral changes has been previously reported and indicate that continued genetic selection predispose dairy cows to infectious disease and decrease their productive lifespan within herds (Ingvartsen et al., 2003; Broom and Fraser, 2015). There is also an inherent variability in the resistance and susceptibility to pathogens between beef and dairy cattle breeds (O'brien et al., 2014), which may influence the association between BLV infection and animal survival. In fact, some studies point out the use of genetic approaches as a tool to identify animals that are genetically predisposed or resistant to BLV infection (Sulimova et al., 1995; Sharif et al., 1998; Aida, 2001; Juliarena et al., 2008).

Our finding of a lack of effect of BLV on beef cow longevity may also be related to the overall reduced culling pressure seen in beef cattle herds when compared to dairy herds. Based on USDA National Animal Health Monitoring System data, dairy herd cull rate is approximately 27.5% (USDA 2007b) compared with 13.5% in beef cattle herds (USDA 2007a). Because culling pressure is lower, the impact of BLV may take longer to manifest itself. In this study, we used a study design similar to one previously used by our group in dairy cattle (Bartlett et all.,2013). A study looking at survival over a longer period of time may have demonstrated an impact of BLV in a system where culling pressures are normally much lowered.

Other factors related to our experimental design, such as the inability to include disease progression or time of infection as a covariate in the analysis, might have influenced our results. BLV records were not available prior to starting the study; therefore, new cases of BLV infection and the impact of disease progression were likely underestimated for the time frame of the analysis.

In an effort to quantitatively assess the association between the virus concentration (PVL) and its consequences survival, we assessed the number of copies of BLV provirus by qPCR in a subset of our blood samples (Yuan et al., 2015; Benitez et al., 2019). Our results revealed that cows with high-BLV PVL had decreased longevity compared with their herdmates (Table 3 and Figure 2). In addition to being more infectious to their herdmates (Juliarena et al., 2016), these high-PVL cattle are likely in a more advanced stage of disease progression and have a marked immune disruption (Frie et al., 2017). A previous study demonstrated that the average PVL in cows with persistent lymphocytosis are higher than those of healthy BLV antibody-positive cattle (Ohno et al., 2015). In cattle brought to slaughter houses, Somura et al. reported that the PVL of animals diagnosed with lymphosarcoma was higher than those of asymptomatic BLV-infected cattle (Somura et al., 2014). Proviral Load analysis could potentially be used in cattle to evaluate infection progression and an increasing risk for leaving the herd. Additionally, in herds with high level of BLV infection, these animals could be preferentially culled as part of a BLV control strategy (Juliarena et al., 2016).

In summary, the presence of BLV antibodies in blood was not associated with beef cow longevity over the subsequent two-year monitoring period, but decreased survival was observed in cattle in which BLV infection advanced as indicated by a high PVL.

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CHAPTER 5

The Impact of bovine leukemia virus on dairy longevity

This chapter represents a manuscript submitted for review by the *Journal of Dairy Science.*

Authors who contributed to this study were: Benitez, O. J., LaDronka, R. M., Norby, B., Bartlett, P. C., & Grooms, D. L.

Abstract

Bovine leukemia virus (BLV) is a retrovirus of cattle that is found in approximately 46% of all U.S. dairy cattle, with about 90% of U.S. dairy herds having at least one infected animal. A prospective study of 113 dairy herds in Michigan showed decreased cow longevity/lifespan and reduced milk production in BLV antibody-positive cows as compared to their negative herd mates. Our objective was to determine the association between BLV infection and cow longevity in dairy cows' operations across the U.S. A total of ninety-one dairy herds from 9 U.S. states were selected to participate in this study. Female dairy cattle (n=3,611) were tested for BLV antibodies with a milk ELISA test. Culling data was collected for an average of 32 months. The main effects were estimated after controlling for lactation number (1, 2, 3, and >4). From all tested animals 47.1% (1,701/3,611) were BLV seropositive. BLV-positive cows were 30% more likely that their BLV-negative herds mates to die or be culled during the monitoring period. These results provide further evidence that BLV has a negative impact on cow survival which has economic consequences. This outcome could be a motivator for dairy producers to adjust their perspective on the importance of bovine leukemia virus and implement practices to control BLV in their herds.

***Key Words*:** enzootic bovine leukosis, survival analysis

Introduction

Bovine leukemia virus (BLV) prevalence has been slowly increasing in U.S. dairy cattle. In the 1970's, about 10% of national dairy cows were seropositive for BLV compared to 46% of individual dairy cows. In 2018 about 95% of dairy herds had at least one positive cow (LaDronka et al., 2018). Most BLV-infected animals remain asymptomatic and act as carriers of the virus, while 30% to 40% of infected cattle develop a persistent lymphocytosis. Less than 5% progress to the fatal, clinical form of BLV infection, lymphosarcoma (Tajima et al., 1998; USDA, 2007b; Bartlett et al., 2013). BLV infection alters cattle's immune function by disruption of appropriate immune cell signaling molecules and cytokine production, irregularity of immune cell proliferation and apoptosis and typical lymphocyte ratios, and self-destruction of activated, infected cells (Della Libera et al., 2015; Ronald J. Erskine, Corl, Gandy, & Sordillo, 2011; Frie & Coussens, 2015; Spinola et al., 2013; Swenson, Erskine, & Bartlett, 2013). As a result, BLV-infected cattle are more susceptible to a variety of infectious diseases which negatively impacts their production performance and lifespan (Frie and Coussens, 2015; Blagitz et al., 2017). The U.S. cull rate in dairy cattle was estimated at 27.5% (USDA, 2007a). Udder health problems/mastitis and reproductive issues account for 27% and 26.5% of total national cull rate, respectively. An additional, 19.3% of culling rates in dairy cattle is due to poor milk production (USDA, 2014), which has been directly associated with BLV infection in previous studies by our group (Norby et al., 2016). Our group recently demonstrated that dairy cattle in Michigan herds with anti-BLV antibodies were 23% more likely to be culled over the 19-month monitoring period (Bartlett et al., 2013). Although small epidemiology studies in dairy cattle have successfully demonstrated an association between BLV infection and decreased productive lifespan within herds (Thurmond et al., 1985; Bartlett et al., 2013; Nekouei et al., 2016), others

have failed in showing similar results (Huber et al., 1981; Rhodes et al., 2003; Tiwari et al., 2005). The objective this current study was to expand our previous work to include herds from dairy producing states in all regions of the U.S., thus generating results that could be extrapolated to the larger population of U.S. dairy herds.

Materials and methods

Study Design and study population

The use of animals in this study was approved by the Michigan State University Animal Care and Use Committee. The data used in this study were collected as part of a larger study previously reported (LaDronka et al., 2018). Criteria for being selected from LaDronka et al., 2018 data set were a history of BLV being diagnosed or suspected in the herd, individual-animal identification, and individual animal records that included dates and reasons for leaving the herd by either death or culling. In brief, in the LaDronka et al., 2018 cross-sectional study design herds were selected based on partnerships established with DHI organizations and/or university extension agents in Wisconsin (WI), New York (NY), Pennsylvania (PA), Texas (TX), Minnesota (MN), Michigan (MI), Ohio (OH), Vermont (VT), and North Carolina (NC). In each state, herds were invited to participate from 3 herd size categories: small-sized herds (70 to 199), medium-sized herds (200 to 999), and larger herds (>1000 cows). Based on the proportions of cows in each state, herds in each category were randomly selected and established. Within each herd, ten cows in the 1st, 2nd, 3rd, and ≥4th lactations were targeted for milk samples and records collection, for a total of 40 cows per herd. The 10 targeted cows in each lactation group were selected based on their calving date (most recent); all cows had ten or more days after calving. Herds were monitored for an average 872 d (range 825 to 1128). Cows that left herds for dairy purposes (sold to another herd) were excluded from the analyses because they were still

going to be used as a dairy animal. All other cows were either dead or culled for non-dairy purposes during the follow-up period or remained in the herd until the end date of the study (censored data).

Milk samples processing and analysis

Milk samples from the selected cows were collected via routine DHI milk sampling protocols. Samples were collected in individual vials with preservative (bronopol/natamycin) and shipped from their respective local DHI to a central laboratory for analysis (CentralStar Michigan Lab, CentralStar Cooperative, Grand Ledge, MI). Samples were stored at room temperature and ELISA testing was completed within 18 days from the date of sample collection. BLV antibody detection in milk was performed by a commercially available ELISA kit (IDEXX Laboratories, Westbrook, ME). The ELISA optical density (OD) results were corrected by subtraction of the average of the negative controls, and values >0.1 were considered positive for previous BLV exposure. Using a 0.1 cutoff, this assay has nearly perfect agreement (K = 0.86) with the serum ELISA which has sensitivity and specificity of 99.8% and 100%, respectively, using AGID as the reference test (Simard et al., 2000; Walsh et al., 2013).

Data Analysis

Survival analysis was conducted using Stata software (version 15.1; StataCorp LP, College Station, TX) using nonparametric Kaplan-Meier survival graphs (sts graph procedure) and semi-parametric Cox proportional hazard models (stcox procedure) handling ties with the Breslow method. Kaplan-Meier graphs were used to visual representations that assisted in interpretation of the data. One Cox proportional hazard model was used to analyze models' fit for overall culling (regardless of reason) and for reason-specific culling (based on culling reasons reported by the producers) using ELISA-detected BLV antibody in milk (negative: OD <0.1;

positive: ≥0.1). Shared frailty (random effect) of the herd was included in all models. Results were considered statistically significant at $P < 0.05$. Days at risk for culling were calculated by subtracting the date of milk sampling from the date of culling or censoring. The main effects were estimated after controlling for lactation number (1, 2, 3, and >4). Additionally, the first-order interactions between BLV OD status and lactation were examined. The shared frailty model using multiplicative gamma-distributed random effects on the hazard scale was used to model within-herd correlation among cows. Model diagnostics for Cox models were based on Cox-Snell residuals, Schoenfeld residuals, as well as interaction terms between log-transformed time to the event and all or each of the predictor variables (Cleves et al., 2010).

Results

After the selected sample of herds that fit the inclusion criteria and the goals for the survival analysis. A total of 3,611 cows, with an average of 40 cows (range:25-48) per herd in each of 91 herds, were enrolled in this study. Holstein cows made up 94% of the tested animals; the remainder of the cows were crossbred (4%), Jersey (2%), and Brown Swiss and Guernsey (each less than <1%). Herds were located in 9 different states; 34 herds in the East (New York Pennsylvania, Vermont, and North Carolina), 55 herds in the Midwest (Minnesota, Wisconsin, Michigan, and Ohio), and 2 herds in the West (Texas).

ELISA Results

The prevalence of individual BLV seropositive cows was 47.1% (95% CI: 45.5-48.73%). A total of 1,701 (49.8%; 95% CI 48.1%-51.4%) cows tested positive for BLV antibodies and 1,910 tested negative.

Table 5.1 Reasons that bovine leukemia virus (BLV) ELISA positive and negative cows died or were culled.

Reason for Leaving the Herd	BLV Status		
	Negative	**Positive**	**Total**
Bad feet/leg structure	132 (11.1%)	116 (10.3%)	248 (10.7%)
Low milk production	188 (15.8%)	185 (16.4%)	373 (16.1%)
Reproduction associated problem	194 (16.3%)	177 (15.7%)	371 (16.1%)
Lameness/Injury	248 (20.8%)	207 (18.4%)	455 (19.6%)
Udder health and mastitis	220 (18.4%)	196 (17.4%)	416 (17.9%)
Disease	36 (3.8%)	31 (2.7%)	67 (2.9%)
Died	127 (10.6%)	154 (13.7%)	281 (12.1%)
Unknown	46 (3.8%)	58 (5.1%)	104 (4.5%)
Total	**1,191**	**1,124**	**2,315**
Culled	1,191 (62.3%)	1,124 (66.1%)	2,315 (64.1%)
Still within the herd	719 (37.6%)	577 (33.9%)	1,296 (35.9%)
Total	**1,910**	**1,701**	**3,611**

Table 5.2 Survival of cattle over a two-year period following bovine leukemia virus (BLV) testing (negative or positive) for milk ELISA antibodies.

Effect	Hazard ratio	SE	Z	*P*-value	95% CI
OD <0.1[2]	-	-	-	-	-
OD >0.1[3]	1.30	0.06	5.69	0.000	1.19-1.43
Lactation 1	-	-	-	-	-
Lactation 2	1.51	0.10	6.29	0.000	1.33-1.73
Lactation 3	1.95	0.13	10.08	0.000	1.71-2.23
Lactation 4+	2.90	0.19	16.04	0.000	2.55-3.31

[1] Z = Z statistic (β/β SE).

[2] Bovine leukemia virus negative [optical density (OD) <0.1].

[3] Bovine leukemia virus positive [optical density (OD) >0.1].

Survival analysis

During the monitoring period, 64.1% (95% CI: 61.8-65.1) of the cows were culled or died and 35.8% (95% CI: 34.8-38.1) remained in the herd (Table 5.1). Of the cows that tested positive by the milk-ELISA, 1,124 (66.1%; 95% CI: 53.9- 68.4%) left the herd during the study period compared to 1,191 cows (62.3%; 95% CI: 52.3- 65.6%) leaving the herd that tested negative for BLV antibodies in milk. The reasons for culling decisions reported by the farmers are shown in Table 5.1. There was a statistically significant difference in the survival of the cows based on milk-ELISA status ($P < 0.00001$; Table 5.2; Figure 5.1). Positive cows faced a 30% greater hazard of being culled than their BLV-negative herd mates.

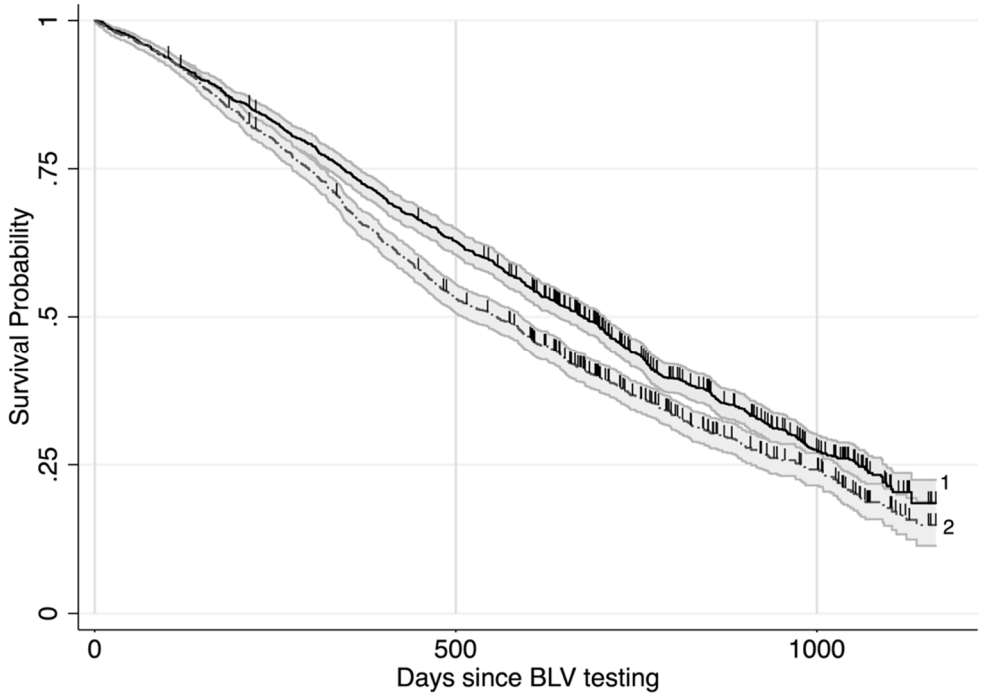

Figure 5.1 Survival of cattle following bovine leukemia virus (BLV) testing.

Proportion of cattle surviving (y-axis) and days since BLV testing (x-axis). [1] Negative cows, [2] Positive cows.

Discussion

This is the first study examining the impact of BLV infection on survival of dairy cattle at the U.S. national level. With a total of 3,611 cows from 91 herds of nine dairy producing states of the U.S., we demonstrated that BLV-infected cows had a 30% greater hazard of being culled compared to their negative herd mates in any lactation group. While previous studies have found no significant association between BLV infection and decreased longevity in dairy cows (Huber et al., 1981; Rhodes et al., 2003; Tiwari et al., 2005), studies in the U.S. (Thurmond et al., 1985; Bartlett et al., 2013), Canada (Jacobs et al., 1995) and Europe (Emanuelson et al., 1992) report results similar to ours. We previously reported a negative effect of BLV infection on longevity of dairy cows from 112 herds in Michigan in which BLV-infected cows were 23% more likely to be culled or die compared with their BLV negative herd mates (Bartlett et al., 2013). In beef cattle, we recently reported that BLV-infected cows with high-BLV proviral load in blood are 84% more likely to be culled or die compared with their BLV negative herd mates (Benitez, 2019). Altogether, these studies support the associated negative impact of BLV infection on dairy cows lifespan. The reasons underlying the detrimental effect of BLV on cows' longevity are still under investigation, but it seems probable that immune abnormalities associated with BLV infection may lead to cows being culled for a wide variety of reasons. This would be similar to how other retroviruses are known to increase morbidity and mortally, e.g. HIV/AIDS in humans (Rodríguez et al., 2011). For example, it has been shown that dysfunctional immune function from BLV infection is associated with other infections such as mastitis (VanLeeuwen et al., 2010; Frie and Coussens, 2015).

In our study, the leading reported reason for culling cows was lameness/injury, Udder/mastitis problem, and poor milk production, accounting for 19%, 18%, and 16%, of the total cull rate, respectively. In the U.S., issues related to udder health and mastitis are the most frequent reason for dairy cattle being culled, accounting for 27% of total cull rate (USDA, 2014). Additionally, poor milk production accounts for 19.3% of why dairy cows are culled (USDA, 2014), a reason that has been directly associated with BLV infection in previous studies (Norby et al., 2016; Yang et al., 2016). Further studies are necessary to understand the physiological and pathological reasons underlying the differences in longevity between BLV infected and uninfected cattle.

In the present study, we believe that there was an underestimation of the impact of BLV infection on cows' survival due to the lack of measures for estimating BLV infection progression throughout the study. BLV records were not available prior to starting the study. As a result, we were unable to include a measure of disease progression or time of infection as a covariate in the analysis. Therefore, new cases of BLV infection and the impact of the disease progression were likely underrated for the time frame of the analysis. For instance, some cows that tested negative could have become infected shortly afterward. Thus, many of the cows in the BLV-negative group could have become infected before the end of the monitoring period. Therefore, one group was known BLV positive while the comparison group included mostly negative cows but also many cows that were recently infected before the end of the study. This bias would act to diminish the difference in cow longevity between the two groups.

Previous studies have attempted to analyze BLV infection progression using different measurements. The analysis of persistent lymphocytosis, which affects 30% of the BLV-infected cattle, has been used as a measurement of disease/BLV-infection progression by Schwartz and Levy(1994) and Pollari et al. (1992), and show that, at the individual level, lymphocytotic BLV-

positive cows were culled at a younger age, thus suggesting that the effects of BLV infection on survival were dependent on the progression of the disease. Da et al.,1993 also demonstrated a strong association between the development of persistent lymphocytosis and/or lymphoma with reduced performance and survival of infected cows (Pollari et al., 1992; Da et al., 1993). Nonetheless, lymphoma, the clinical, fatal stage of BLV infection, is rarely seen in animals less than 2 years of age and is most common during lactations 2–6 (Smith, 2009). Also, most of the infected cows remain in the preclinical disease stage for years, often for their complete productive lifetime without any apparent reduction in performance; however, lymphoma eventually appears in a proportion of these cows (in <5% of the infected cows). Our study only addressed the effects of the subclinical/preclinical form of BLV.

We consider that if producers were testing for BLV and using a cow's BLV status as a factor in making culling decisions, the impact of BLV on longevity would be inflated as this scenario would bias toward the alternative hypothesis. However, producers were not informed on the individual cow's BLV status. Additionally, we determined that 88% of the farms included in our analysis had a low level of awareness about BLV in their farms and did not practice any type of eradication program for BLV specifically (LaDronka et al., 2018).

In conclusion, the presence of BLV antibodies in milk was negatively associated with dairy cow longevity (lifespan) within an average of 32 months follow-up period. This information can be used in extension and education efforts to make dairy producers aware of the potential impact of BLV in their herds. As such, it could be a strong motivator for dairy producers to adjust their perspective on the importance of bovine leukemia virus and implement practices to control BLV in their herds.

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CHAPTER 6

Conclusion and future directions

The main objective of the research presented in this dissertation was to increase our knowledge about the impacts and epidemiology of BLV in beef cattle. Our interest in this particular topic was based on previous work done at Michigan State University looking at the impact of BLV in dairy cattle and further elevated by the detection of BLV in a high proportion (45%) of adult breeding beef bulls from 39 beef herds in Michigan (Benitez et al.,2019). Based on this work, we aimed to examine the potential for BLV transmission during natural breeding and also to explore the potential impact of BLV infection on cow longevity in beef cattle herds. We first evaluated the potential presence of BLV in genital secretions of BLV-seropositive bulls. For the first time, we reported BLV provirus DNA in the smegma samples of 7.4% of BLV-infected bulls, but not in semen samples. Smegma is an accumulation of glandular secretions and desquamated epithelial cells within the preputial sheath. It is diffusely distributed along the epithelium of the preputial sheath and contains white blood cells, such as lymphocytes and neutrophils dispersed near the basement membrane (Cobo et al., 2007). In this study, we did not quantify the number of immune cells, specifically B-lymphocyte, which are the most common cell type infected with BLV provirus (Jimba et al., 2010). A measurement of BLV provirus number standardized by the concentration of lymphocytes in smegma could have provided a more quantitative estimation of smegma infectivity compared with other secretions and blood. As a part of the evaluation of BLV infected smegma samples infectivity, we also could have evaluated viability of infected cells and proliferation of BLV in an in vitro model. However, the detection of BLV provirus DNA in smegma of breeding bulls was a clinically relevant finding because it suggested that smegma could have a role in the transmission of BLV from infected

bulls to uninfected cows during copulation, thereby increasing the risk for BLV transmission within herds that use natural service breeding programs.

To further address whether BLV-infected bulls could be a risk for BLV transmission during natural breeding, we designed an in vivo study exposing naïve beef heifers to a BLV infected breeding bull. Through qPCR of genital secretions, we confirmed that the BLV-infected bull had BLV provirus DNA in smegma samples throughout serial sampling over time. Similar to our previous study, semen samples did not contain BLV provirus. All heifers had direct contact with the infected bull at least once during copulation (as determined by observation and pregnancy status) as well as further opportunity for direct contact by the fact that they were housed together in the same oasture. However, we detected no evidence of BLV infection as determined by both ELISA seroconversion and detection of BLV provirus in blood. We are aware that: 1) A longer housing period may have increased risk of transmission and 2) a longer follow-up period may have allowed for seroconversion to occur. We also believe that the fact that the BLV infected bull was not lymphocytotic and had a relatively low PVL could have limited the numbers of virus copies transmitted during copulation between the BLV infected bull and heifers. Additionally, we also think that natural immunological barriers in the vaginal and uterus epithelium of females could play an essential role in clearing infected cells, avoiding transmission when the number of infected cells is small. Overall, based on the findings of this study, ELISA-positive bulls that are healthy and aleukemic may not be a significant risk of BLV transmission during a defined breeding season. Regardless, veterinarians and producers should be aware that the risk of BLV transmission will likely increase as the length of exposure to infected bulls increases and if bulls progress to develop lymphocytosis with higher BLV proviral loads in blood and smegma. Future studies to assess the ability of bulls with high PVL and lymphocytosis to transmit BLV during breeding would be valuable. Also, a longer exposure time

may also add important information on the risk of BLV positive bulls in the transmission of the virus.

Next, we were interested in determining how BLV infection impacts the survival of cows in beef herds. This study was based on previous work conducted by our research groups demonstrating that BLV infected dairy cattle in Michigan leave the herd earlier and were 23% more likely to be culled as compared to their uninfected herd mates in a 2-year monitoring period. (Bartlett et al., 2013). In the present study, we were also interested in determining the main reasons why BLV infected cows leave the herd. We hypothesized that BLV infected beef cows leave the herd earlier than BLV seronegative cows due to health and production issues. In contrast to our hypothesis, BLV infection did not result in a significant difference in beef cows' longevity within herds. However, our data showed a trend where seropositive cows face an 18% greater hazard of being culled compared with seronegative cows. From our point of view, we consider that this slight, but not significant difference, has a practical economic impact that should be considered. A strength of our study was the analysis of BLV PVL in blood of a subset of cattle, which provided valuable information on BLV disease progression and cow survival. It is more common to detect high-PVL in cattle that are in a more advanced stage of disease and have a marked immune disruption (Frie et al., 2017). This is in line with our results demonstrating that beef cows with high BLV proviral load in blood had decreased survival compared with non-infected cows. A better understanding of the impact of BLV on cattle health and production may need to incorporate disease progression measurements, such as PVL analysis. At the same time, PVL analysis could potentially be used in cattle to evaluate infection progression and an increasing risk for leaving the herd.

Our final study extended previous work on the effect of BLV on dairy cow survival by evaluating the impact of BLV infection on dairy cows' survival in 91 dairy herds from nine

states across the U.S. Aligned with our findings, our results showed that the presence of BLV antibodies in milk was negatively associated with dairy cow longevity (lifespan). Additionally, BLV infected cows faced a 30% greater hazard of being culled than their BLV-negative herd mates.

We consider that differences on the impact of BLV infection in beef and dairy cattle may be partially explained by intrinsic variations between the two productive systems and, potentially, with different breed-associated traits. Dramatic genetic selection of dairy cows leads to metabolic challenges that negatively affect immune responses (Ingvartsen et al., 2003) and favor the development of diseases and/or culling decisions. There is also an inherent variability in the resistance and susceptibility to pathogens between beef and dairy cattle breeds (O'brien et al., 2014), which may influence the association between BLV infection and animal survival. We believe that these differences between survival of BLV infected animals in beef and dairy systems should be further explored as a way to determine whether cows from different breeds should be managed differently than beef cows regarding to BLV prevention. Additionally, genetic factors favoring or preventing BLV infection and disease progression may be an alternative for BLV control, as pointed out in previous studies (Sulimova et al., 1995; Sharif et al., 1998; Aida, 2001; Juliarena et al., 2008).

Even though we were able to clearly demonstrate a detrimental effect of BLV infection in beef and dairy cattle survival, we could not conclusively affirm or estimate a monetary value of its impact. Further evaluation of the potential effect of BLV on cow-calf needs to be performed in order to evaluate the real economic impact of BLV infection.

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