A novel association of *BoLA DRB3* alleles in BLV infected cattle with different proviral loads

Uma nova associação de alelos de BoLA DRB3 em bovinos infectados com BLV com diferentes cargas provirais

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Abstract

Bovine leukemia virus (BLV) is associated with the most common neoplastic disease of cattle. BLV has a silent dissemination in the herd due to infected cell exchange, thus the concentration of BLV-infected cells in blood should play a major role in the success of viral transmission. Genes from Bovine leukocyte antigen (*BoLA*), the MHC system of cattle, are associated with genetic resistance and susceptibility to a wide range of diseases, and also with production traits. Some *BoLA DRB3.2* allele polymorphisms in Holstein cattle have been associated with resistance or susceptibility to BLV-disease development, or with proviral load (PVL). This investigation studied 107 BLV-infected Argentinean Holstein dairy cows, all of them belonging to one herd. PVL was analysed by qPCR and animals were classified as high proviral load (HPVL, $N = 88$) and low proviral load (LPVL, $N = 19$), and *BoLA DRB3.2* alleles were genotyped. Alleles *BoLA DRB3.2*1501* and **1201* were significantly associated with HPVL ($p = 0.0230$ and $p = 0.0111$ respectively), while allele *BoLA DRB3.2**0201 was significantly associated with LPVL ($p = 0.0030$). The present study aims at contributing to the knowledge of the association between BoLA polymorphism and development of a BLV infection profile. Genes that best explain the PVL in this population resulted *BoLA DRB3.2*0201* (as a protection factor) and **1501* (as a risk factor). Allelic differences may play an important role in the development of effective immune responses. A better understanding of how *BoLA* polymorphism contributes to these responses and the establishment of a BLV status is desirable to schedule and evaluate control measures.

Keywords: BLV. Proviral load. *BoLA DRB3* polymorphism.

Resumo

O vírus da leucemia bovina (BLV) está associado à doença neoplásica mais comum do gado bovino. O BLV tem uma disseminação silenciosa no rebanho devido à troca de células infectadas, assim, a concentração de células BLV infectadas no sangue deve desempenhar um papel importante no sucesso da transmissão viral. Os genes do antígeno leucocitário bovino (*BoLA*), sistema MHC do gado bovino, estão associados à resistência genética e à susceptibilidade a uma ampla gama de doenças, bem como às características da produção. Alguns polimorfismos de alelos de *BoLA DRB3.2* em bovinos Holstein têm sido associados à resistência ou susceptibilidade ao desenvolvimento da doença BLV, ou com carga proviral (PVL). Esta investigação avaliou 107 vacas leiteiras da raça Holstein argentina infectadas com BLV e pertencentes a um único rebanho. A PVL foi analisada por qPCR, os animais foram classificados em alta carga proviral (HPVL, N = 88) e baixa carga proviral (LPVL, N = 19), e os alelos *BoLA DRB3.2* foram genotipados. Os alelos *BoLA DRB3.2*1501* e **1201* estavam significativamente relacionados à HPVL ($p = 0{,}0230$ e $p = 0{,}0111$, respectivamente), enquanto o alelo *BoLA DRB3.2*0201*, à LPVL ($p = 0{,}0030$). O objetivo deste estudo é contribuir para o conhecimento da associação entre o polimorfismo de *BoLA* e o desenvolvimento de infecção por BLV. Os genes que melhor explicam a PVL na população analisada resultaram em *BoLA DRB3.2*0201* (como fator de proteção) e **1501* (como fator de risco). As diferenças alélicas podem desempenhar um papel importante no desenvolvimento de respostas imunitárias eficazes. Uma melhor compreensão de como o polimorfismo *BoLA* contribui para estas respostas e o estabelecimento de um estado BLV é desejável para agendar e avaliar as medidas de controle.

Palavras-chave: BLV. Carga proviral. Polimorfismo do *BoLA DRB3*.

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Introduction

Bovine leukemia virus (BLV) shares the genomic organization structure with T-cell tropic viruses from primates (human and simian), and constitute a unique subgroup within the *Retroviridae* family, characterized by distinct genetic content, genomic organization, and strategy for gene expression (COFFIN, 1996). BLV is associated with enzootic bovine leucosis (EBL), which is the most common neoplastic disease of cattle. Most infected cattle remain asymptomatic. By contrast, approximately 30% of cattle develop persistent lymphocytosis (PL), which is characterized as the polyclonal expansion of non-neoplastic CD5+ B-lymphocytes. PL often precedes tumorigenesis but is usually regarded as clinically healthy. After a latency period of 1-8 years, less than 10% of infected cattle develop EBL, which is a malignant $CD5^+$ B-cell lymphoma (FERRER, 1980; GILLET et al., 2007). BLV has a silent dissemination in the herd due to infected cell exchange (HOPKINS; DIGIACOMO, 1997), thus the concentration of BLV-infected cells in blood should play a major role in the success of viral transmission. Even though PL cattle are considered the most risky for transmission, asymptomatic animals may also play a role depending on their proviral load (JULIARENA et al., 2007).

The major histocompatibility complex (MHC) molecules are responsible for presentation of processed antigen peptides to T cells, and thereby an adaptive immune response to specific pathogens is trigged. The high degree of polymorphism of the MHC molecules enables the presentation of a wide array of peptides that differ in their length and sequence. Thus, the nature and strength of the immune responses depend on the peptide and the MHC molecules, and may influence disease progression (AMILLS et al., 1998). Bovine leukocyte antigen (*BoLA*), the MHC system of cattle, is highly polymorphic. Functionally, the *BoLA class II* gene is classified into two groups, *DR* and *DQ*. The *DRA* and *DRB3* gene products form the *DR* molecule, as *DRB3* is the only functional *DRB* gene within *BoLA* (TAKESHIMA; AIDA, 2006).

Genes from BoLA are remarkably attractive to animal breeders and veterinary geneticist, and polymorphism in *BoLA* genes has been relatively well studied. These genes are associated with genetic resistance and susceptibility to a wide range of diseases, including mastitis (DIETZ et al., 1997; RUPP et al., 2007; YOSHIDA et al., 2009; YOSHIDA et al., 2012), dermatophilosis (MAILLARD et al., 2002), tick-borne diseases (DUANGJINDA et al., 2013), infection with *Neospora caninum* (SCHWAB et al., 2009), African trypanosome species (KARIMURIBO et al., 2011), and *Theileria annulata* (GLASS et al., 2012b). Moreover, *BoLA* appears to influence other traits such as milk yield, growth and reproduction, and variations in immune responses to antigens (TAKESHIMA; AIDA, 2006) and even vaccine peptides (GARCIA-BRIONES et al., 2000; GLASS et al., 2012a).

The greatest amount of evidence linking *BoLA* gene polymorphisms and disease outcome is given by BLV infection. First evidences revealed an association between resistance or susceptibility to PL and *BoLA A class I* alleles (LEWIN; BERNOCO, 1986); however, these associations were relatively weak at population levels and different *BoLA A* alleles had significant effects in different breeds (LEWIN et al., 1988; STEAR et al., 1988). Subsequent studies showed that resistance and susceptibility to PL map more closely to MHC class II *BoLA DRB3.2* gene than to *BoLA A* locus (ESTEBAN et al., 2009). Thus, most studies have focused on *BoLA DRB3.2* allele polymorphisms in Holstein cattle, and their relationship with disease. Some alleles were associated with resistance to PL or to BLV-induced lymphosarcoma, or the capability of controlling the number of BLV-infected cells, while other alleles were associated with susceptibility to PL (XU et al., 1993; ZANOTTI et al., 1996; MIRSKY et al., 1998; PANEI et al., 2009; NIKBAKHT BRUJENI et al., 2016). When BLV-infected animals were characterized into two profiles of infection – high and low proviral load (HPVL and LPVL respectively) (JULIARENA et al., 2007), *BoLA DRB3.2 *0902* allele was the strongest associated with LPVL profile, while **1501 or 03* allele showed a significant association with HPVL in carrier animals (JULIARENA et al., 2008). Miyasaka et al. (2013) also analyzed the effect of *BoLA DRB3.2* alleles on proviral load in Japanese cattle and found that **0902* and **1101* alleles were associated with LPVL and **1601* allele with HPVL and identified *BoLA class II* haplotypes that correlated with different PVL status.

In the present investigation we analysed whether HPVL or LPVL status in the BLV-infected animals from the herd

under study were associated with specific *BoLA DRB3.2* alleles. This study aims at contributing to the knowledge of the association between BoLA polymorphism and development of a BLV infection profile.

Material and Methods

Sample collection

Blood samples were obtained from 107 BLV-infected Argentinean Holstein (Holando-Argentino) dairy cows belonging to a herd from the Tandil region (Provincia de Buenos Aires, Argentina). All animals were healthy and milking (between second and third lactation period) and although the pedigree information was not complete for 3% of them, they came from at least 51 and 87 different bulls and cows, respectively. Serological BLV status was determined by testing plasma for anti-gp51 antibodies by ELISA 108 (GUTIERREZ et al., 2001). For peripheral blood mononuclear cells (PBMCs) separation, 10 mL blood samples were collected in heparinized syringes by jugular venipuncture. Blood samples were transferred to 15 mL tubes and centrifuged for 15 min at 3800 rpm at 4°C. Buffy coat was mixed with 11 mL of cold ammonium chloride buffer (150 mM NH_4Cl, 8 mM Na_2CO_3, and 6mM EDTA) for red blood cells lysis. PBMCs were obtained by centrifugation at 3000 rpm for 10min at 4°C. Cells were washed with PBS and centrifuged at 2500 rpm for 7 min at 4°C. PBMCs pellets were stored at -20°C for DNA extraction.

DNA extraction

DNA from PBMCs was extracted using Qiagen columns (QIAamp DNA Mini Kit) according to the manufacturer's protocol. DNA was eluted in 50 ml of water. The concentration and purity of DNA were determined by the OD value at 260nm and 260/280 coefficient respectively in a NanoDrop 2000 Spectrophotometer (Thermo Scientific). The purified DNAs were stored at -20°C until use.

Proviral load determination by real-time PCR (qPCR)

The absolute quantification method by qPCR used for proviral load (PVL) determination was the one described by Farias et al. (2016). The standard curve was constructed using a plasmid carrying one copy of the entire BLV genome under control of its own promoter LTR (pBLV), with six 10-fold serial dilutions of pBLV, containing 1 million to 10 BLV copies. The qPCR conditions for *pol* amplification were previously described (FARIAS et al., 2016). Each reaction contained 30 ng of DNA from BLV-infected animals. The standard curve and non-template control were included in each run. Experiments were always performed in triplicate. BLV-positive animals were classified as high proviral load (HPVL) (> 1000 BLV copies/reaction) and low proviral load (LPVL) (< 100 BLV copies/reaction). Before classifying the animals into each group, the proviral load was evaluated twice at 6-months intervals, in order to confirm their PVL. Eighty-eight out of 107 animals were HPVL, while the remaining 19 proved to be LPVL.

BoLA DRB3.2 genotyping

PCR reactions were performed in a final volume of 15 L, where the volume mixture contained: ng of genomic DNA, 0.03 X PCR buffer (Mg^{2+} plus), 0.3 M of each primer (HL030; 5'-ATCCTCTCTCTGCAGCACATTTCC-3', and HL031; 5'-TTTAAATTCGCGCTCACCTCGCCGCT-3'), 0.2 mM of dNTP and 0.75U Paq5000® DNA Polymerase (Agilent Technologies, Stratagene) and water up to a total volume of 15 µL. Conditions for amplification were 94°C for 3 min, followed by 35 cycles of 20 s at 94°C, 20 s at 60°C (annealing temperature), and 1min at 72°C. Reactions were finished with a 72°C, 5-min extension. PCR reactions were performed in a Mastercycler (Eppendorf®) thermocycler. Amplified fragments of DNA were visualized in a 1% agarose gel and sequenced using BigDye® chemistry on an ABI3130xl sequencer (Applied Biosystems) following the manufacturer's protocol. Sequencing reactions were performed in both senses using the same primers as for PCR reactions, in a total volume of 10 L containing a 1:10 dilution of the PCR product as template and 0.1M of primer, in addition to the mix and buffer 5X of BigDye® Terminator kit. The sequence analysis and obtaining genotypes was performed using Haplofinder (BAXTER et al., 2008).

Statistics analysis

Association between different BoLA DRB3.2 alleles and proviral load was estimated by Chi^2 test or exact Fisher test, as appropriate, implemented by PROC FREQ (SAS v9.3, Institute Inc., Cary, NC, USA). With genes that were significant to the bivariate analysis logistic regression was performed in order to identify genes that best explain the CPV, using the PROC LOGISTIC (SAS v9.3).

Results

Analysis of BoLA DRB3.2 genotyping

After genotyping, a total of 18 *BoLA DRB3.2* alleles defined according to the ISAG BoLA Nomenclature Committee were identified in these Argentinean Holstein cattle. The five most frequently alleles were *BoLA DRB3.2*1001* (17.29%), **1501* (16.36%), **1101* (14.95%), **0101* (12.15%), and **1201* (11.21%) (Table 1). We found 47 genotypes, where *BoLA DRB3.2*1001* + *BoLA-DRB3.2*1501* (9 animals, 18.75%), *BoLA DRB3.2*1101* + *BoLA DRB3.2*1501* (8 animals, 16.67%), and *BoLA DRB3.2*1001* + *BoLA DRB3.2*1201* (6 animals, 12.5%) were the most frequent in the population (data not shown).

Table 1 – *BoLA DRB3.2* allele frequency in the studied population

Alelle	No.	%
**1001*	37	17.29
**1501*	35	16.36
**1101*	32	14.95
**0101*	26	12.15
**1201*	24	11.21
**0201*	14	6.54
**0902*	9	4.21
**0601*	7	3.27
**1801*	6	2.80
**2703*	5	2.34
**14011*	5	2.34
**3103*	4	1.87
**1001/1002*	3	1.40
**1402*	2	0.93
**2707*	2	0.93
**0701*	1	0.47
**1701*	1	0.47
**2003*	1	0.47

Association between BoLA DRB3.2 alleles and proviral load (PVL)

BLV-positive cattle ($N = 107$) were categorized into two groups according to their PVL by qPCR: 88 had HPVL and 19 had LPVL. The presence or absence of each BoLA DRB3.2 allele in this cattle population was analysed in association with PVL (Table 2). No significant association was observed between PVL and alleles having frequency lower than 10%. Amongst alleles with higher frequency in the population, **1501* and **1201* were significantly associated with HPVL ($p = 0.0230$ and $p = 0.0111$ respectively), while allele *0201 was significantly associated with LPVL ($p = 0.0030$) (Table 2).

Table 2 – Association between *BoLA DRB3.2* alleles and proviral load (PVL)

Evaluated allele	PVL	Presence (No.)	Absence (No.)	OR (CI 95%)	*p* value
**1001*	HPVL LPVL	33 3	56 16		0.0830[a]
****1501***	HPVL LPVL	33 2	55 17	**5.10 (1,27 – 20,53)**	**0.0230**[a]
**1101*	HPVL LPVL	24 5	64 14		0.9322[a]
**0101*	HPVL LPVL	20 6	68 13		0.3947[b]
****1201***	HPVL LPVL	23 0	65 19	-------	**0.0111**[b]
****0201***	HPVL LPVL	7 7	81 12	**0.15 (0.05 – 0.48)**	**0.0030**[b]
**0902*	HPVL LPVL	7 2	81 17		0.6594[b]
**0601*	HPVL LPVL	5 2	83 17		0.6051[b]
**1801*	HPVL LPVL	5 1	83 18		1[b]
**2703*	HPVL LPVL	4 1	84 18		1[b]
**3103*	HPVL LPVL	4 0	84 19		1[b]
**14011*	HPVL LPVL	1 2	87 17		0.0807[b]
**1001/*1002*	HPVL LPVL	1 2	87 17		0.0807[b]
**1402*	HPVL LPVL	2 0	86 19		1[b]
**2707*	HPVL LPVL	1 1	87 18		0.3250[b]
**0701*	HPVL LPVL	1 0	87 19		1[b]
**1701*	HPVL LPVL	0 1	88 18		0.1776[b]
**2003*	HPVL LPVL	1 0	87 19		1[b]

Values in bold are statistically significant – odds ratios with $p < 0.05$
[a] Chi^2 test
[b] Exact Fisher test

Distribution of BoLA DRB3.2 alleles significantly associated with PVL

*BoLA DRB3.2*1501*, **1201,* and **0201* were significantly associated with PVL. Contrasts were estimated in order to compare absence and presence of each one in homozygous and heterozygous form.

*BoLA DRB3.2*1501* allele is present in heterozygosis in 94% of HPVL animals; animals that carry it are about 5 times more likely to have HPLP (Chi² test: $p = 0.0230$) (Table 3). *BoLA DRB3.2*1201* allele is present only in HPVL animals (Fisher exact test: $p = 0.0187$) (Table 4). *BoLA DRB3.2*0201* allele is present in heterozygous in 50% of HPVL animals and 50% of LPVL animals; among animals that do not possess this allele, 87% have HPVL (Fisher exact test: $p = 0.0030$) (Table 5). Thus, animals that have **0201* allele would be protected to develop HPVL.

Table 3 – Distribution of *BoLA DRB3.2*1501*

Genotype \ PVL	HPVL	LPVL	Total
Homozygote	0	0	0
Heterozygote	33	2	35
Absence	55	17	72
Total	88	19	107

Chi² test: $p = 0.0230$; OR 5.10 (CI 1.27 – 20.53)

Table 4 – Distribution of *BoLA DRB3.2*1201*

Genotype \ PVL	HPVL	LPVL	Total
Homozygote	1	0	1
Heterozygote	22	0	22
Absence	65	19	84
Total	88	19	107

Fisher exact test: $p = 0.0187$; OR between groups cannot be estimated

Table 5 – Distribution of *BoLA DRB3.2*0201*

Genotype \ PVL	HPVL	LPVL	Total
Homozygote	0	0	0
Heterozygote	7	7	14
Absence	81	12	93
Total	88	19	107

Fisher exact test: $p = 0.0030$; OR 0.15 (CI 0.05 – 0.48)

A logistic regression model was run with the *BoLA DRB3.2*0201* and **1501* alleles as the independent variables, and the HPVL profile as the dependent variable. This analysis indicated that **0201* allele is a protective factor ($p = 0.0024$), while **1501* allele is a risk factor ($p = 0.0341$) for HPVL profile (Table 6). Thus, genes that best explain the PVL are *BoLA DRB3.2*0201* (as a protection factor) and **1501* (as a risk factor), with no significant interaction estimated between them ($p = 0.957$).

Table 6 – Logistic regression for *BoLA DRB3.2*0201* or **1501* alleles and HPVL profile

Independent variables	OR (CI 95%)	*p* value
*BoLA DRB3.2*0201*	0.13 (0.04 – 0.49)	0.0024
*BoLA DRB3.2 *1501*	5.65 (1.14 – 28.01)	0.0341

Discussion

This study analysed the distribution of *BoLA DRB3.2* alleles in an Argentinean Holstein herd and its association with the BLV proviral load. In this group of animals ($N = 107$), we identified 18 alleles and *BoLA DRB3.2*1001* had the highest allelic frequency (17.29%). There is not too much information about the allelic diversity of *BoLA DRB3 class II* gene in different cattle breeds. As far as it is known, only one study described this diversity in South American Holstein populations (TAKESHIMA et al., 2015). These authors found that *BoLA DRB3*1501* appeared to be widely spread in the cattle of countries of South America: 18.2% in Bolivia, 17.7% in Paraguay, 21.4% in Peru, 21,7% in Chile, and 14.7% in Argentina. In the present investigation we found a slightly higher frequency of this allele in our population (16.36%). *BoLA DRB3*0101* and **1101* were also in high frequency in these Holstein population of South American countries. Even though *BoLA DRB3*0101* was the most frequently found in Argentina (17.7%), its presence was lower by one third in our population (12.15%). The greatest differences in allelic frequency between Takeshima's and in the present study were in *BoLA DRB3*1201* (7.3% vs 11.21%), **1001* (5% vs 17.29%), and **0201* (3.2% vs 6.54%). The highest frequency of the analysed alleles found in this research could be a result of the selection for trait parameters applied in our herd. And this fact might have implications

on susceptibility or resistance to diseases, immunological and production trails, and vaccine responses.

Related to the infection with BLV in Holstein breed, several studies have shown that the susceptibility to PL was associated with the presence of *BoLA DRB3*0101*, **1501 or 03*, **1101 or 02,* and **1201* (XU et al., 1993; ZANOTTI et al., 1996; MIRSKY et al., 1998; JULIARENA et al., 2008; PANEI et al., 2009; NIKBAKHT BRUJENI et al., 2016). In the present study, *BoLA DRB3*1201* was the most significantly associated with HPVL ($p = 0.0111$), and this might be due to the fact that its frequency in the investigated population was about one third higher than in other Argentinean Holstein populations (11.21% vs 7.3%, respectively) (TAKESHIMA et al., 2015). In this investigation we also found a strong association between HPVL and the presence of *BoLA DRB3*1501* ($p = 0.0230$). These two alleles are the most frequently associated with susceptibility to PL or HPVL in BLV-infected animals from different cattle breeds (XU et al., 1993; SULIMOVA et al., 1995; ZANOTTI et al., 1996; UDINA et al., 2003; JULIARENA et al., 2008). *BoLA DRB3*1001* had the highest frequency in the investigated herd, and in the literature it was referred as a susceptible (XU et al., 1993; UDINA et al., 2003) or a neutral allele (XU et al., 1993) for PL development. However, no association with BLV status could be established in *BoLA DRB3*1001* carrier animals from the present study. In Japanese cattle, Miyasaka et al. (2013) found that **1601* allele was associated with HPVL, but in this investigation no animals were identified carrying this allele.

Resistance to PL or an LPVL profile were associated with alleles that are generally less distributed in cattle population. In the present investigation we found a strong association between *DRB3.2*0201* and LPVL ($p = 0.030$). It is possible to speculate that the absence of this allele could be an indicative of a putative development of HPVL (Table 5). Panei et al. (2009) studied a herd of 81 Argentinean Holstein animals by PCR-RFLP and mentioned *BoLA DRB3.2*11*, **23*, **28*, **25,* and **40* (ISAG **0902*, **2703*, **0701* or *03*, and non-classified respectively) as associated with PL resistance; but in the present investigation we did not find the same results. Even though several studies cited **1101* linked to PL susceptibility in different cattle breeds (XU et al., 1993; SULIMOVA et al., 1995; UDINA et al., 2003; PANEI et al., 2009; NIKBAKHT BRUJENI et al., 2016), it was associated with LPVL in Japanese cattle (MIYASAKA et al., 2013). In the present investigation we found a significant number of animals carrying this allele in our herd, compared to the population of Holstein in Argentina (TAKESHIMA et al., 2015); however, no association could be established between allele *BoLA DRB3.2*1101* and BLV PVL. In the literature *BoLA DRB3.2*0902* (or **11* determined by PCR-RFLP) allele was most strongly associated with PL resistance or LPVL profile in Holstein cattle (XU et al., 1993; ZANOTTI et al., 1996; MIRSKY et al., 1998; JULIARENA et al., 2008; PANEI et al., 2009; MIYASAKA et al., 2013). In the present investigation we did not find this association, which could be due to a lower frequency of presentation of **0902* allele in this herd in comparison with the Argentinean Holstein populations (4.21% vs 6.4%, respectively) (TAKESHIMA et al., 2015).

Genetic selection for *BoLA DRB3.2*0902* allele has been proposed to breed cattle that are resistant to HPVL development (JULIARENA et al., 2016). Evidences from the literature reveal that the polymorphism in the *BoLA class II* gene influences immune response by peptide binding, antigen presentation, T-cell repertoire, and cytokine networks (TAKESHIMA; AIDA, 2006). Thus, allelic differences in BLV-infected animals may play an important role in the development of effective immune responses. However, BLV-resistance might not be only related to a single allele of the *DRB3.2* gene. Indeed, about 19% of cattle carrying the *BoLA DRB3.2*0902* allele develop HPVL (JULIARENA et al., 2008). It must be considered that expression of BoLA *DRB3* might be influenced by polymorphisms in other surrounding genes. An important gene of the immune system that has been linked to the *BoLA* region is the promoter region of tumor necrosis factor-alpha (TNF-α): both genes are located in the q22 arm of the bovine chromosome 23 and in linkage disequilibrium (LENDEZ et al., 2015). Concerning BLV infection, *TNF-α* is closely related with the immune response against viral dissemination, stimulating the elimination of infected lymphocytes (KABEYA et al., 1999). Konnai et al. (2006) described that genotype G/G in position -824 of the promoter region of *TNF-α* was associated with low transcriptional activity of the promoter region. The frequency of this genotype G/G was higher in individuals with BLV-induced lymphoma than in asymptomatic carrier individuals. Moreover, a tendency toward an increased BLV provirus load in cattle with the TNF-α –824 G/G homozygote was observed when compared to the A/A homozygote or genotype A/G.

Lendez et al. (2015), analysing a large population of cattle with HPVL or LPVL, revealed a significant association between LPVL and a low frequency of the G/G genotype at position -824. That means that an animal carrying this polymorphism most probably belongs to the HPVL group. In the present study we also analysed the polymorphism in the promoter region of *TNF-α*; however, no statistical differences were found between animals with HPVL or LPVL (data not shown), probably due to the small number of animals with LPVL. Taking together, it seems that several polymorphisms in different genes may contribute to develop a resistance profile in BLV-infected cattle.

On the other hand, other issues like production traits improvement, resistance to mastitis pathogens, somatic cell counts and milk yield have also been related to specific *BoLA* alleles (DIETZ et al., 1997; RUPP et al., 2007; YOSHIDA et al., 2009; BALTIAN et al., 2012; YOSHIDA et al., 2012). However, some controversies exist between different studies. Abdalla et al. (2016) communicated a genome-wide association mapping in order to analyse BLV incidence in US Holstein cattle population, together with milk yield and somatic cell score, combining pedigree and molecular marker information, for the detection of genomic regions and gene pathways associated with the disease. The study revealed BLV incidence as a complex trait, possibly modulated by several genes of small effects.

Conclusions

The association between BoLA polymorphism and development of a BLV infection profile was investigated. Genes that best explain the PVL in this population resulted *BoLA DRB3.2*0201* (as a protection factor) and **1501* (as a risk factor). Allelic differences may play an important role in the development of effective immune responses, against BLV and other pathogens. Thus, genetic selection based on specific *BoLA* alleles should be implemented with great caution considering the variable effects of these genes on a wide range of diseases and production traits. A better understanding of how *BoLA* polymorphism contributes to the development of effective immune response and the establishment of a BLV status is desirable to schedule and evaluate control measures.

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