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Association of Bola-DRB3.2 Alleles with Enzootic Bovine Leukosis: Profiles BLV Infection, Persistent Lymphocytosis and Antibody Production in Harton Del Valle Cattle

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Abstract

Objectives: To associate the BoLA-DRB3 alleles with the resistance and/or susceptibility to the development of the Enzootic Bovine Leukosis (EBL), in the Harton del Valle cattle. **Methods**: In 93 animals of the Harton del Valle breed, the infection with the Bovine Leukosis Virus (BLV), the development of Persistent Lymphocytosis (PL), the production of antibodies against the virus (anti-BLV) for one year were evaluated. The animals were genotyped for the BoLA-DRB3 locus. The number of alleles and their frequencies was estimated. Alleles were associated with infection with BLV (env+/-), development of PL (PL+/-) and production of anti-VL (anti-BLVhigh/low) using Odds Ratio (OR), the alleles were classified as Resistant (R), Neutral (N) or Susceptible (S) to the variables evaluated. Additionally, the sequences were explored in order to find common amino acid motifs. **Findings**: We found 27 alleles BoLA-DRB3, the most frequent alleles were *1101 (14.5%), *0902 (7%) and *2703 (7%), *20012 (6.5%), *1501 (5.9%) and *1701 (5.9%) The remaining 21 alleles had a cumulative frequency of 53.2%. The alleles *1001, *1101, *2006, *2703, *3001 and *4802 were considered as Renv infection with the virus, whereas, the alleles *0902, *1601, *1701, *20012 and *25011 were they considered Senv. Only the allele *1101 was significantly associated with the absence of PL and the alleles *25011 and *2703 were considered SPL. The alleles *1101 and *2703 were Ranti-BLV, while, the alleles *1601 and *1701 were classified as Santi-BLV. The allelic frequencies of the R alleles were higher than the S. Some common amino acid motifs were found between alleles with the same classification. Only the allele *1101 was Renv/PL/anti-BLV. The protective effect of the allele was greater in the heterozygous state (OR = 0.18, p<0.00005). **Application/Improvements:** The BoLA-DRB3 locus is highly polymorphic. The allele *1101 was considered as Renv/PL/anti-BLV. The heterozygote advantage was shown when responding against the development of the disease. The data presented here can be used in future genetic improvement programs aimed at increasing the frequency of alleles of resistance to the disease.

Keywords: Bovine Leucocyte Antigens, Colombian Creole Cattle, Genetic Diversity

1. Introduction

Enzootic Bovine Leukosis (EBL) is an infectious disease caused by the Bovine Leukosis Virus (BLV), which affects the cells of the lymphoid line[1]. It is lymphoproliferative

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and is characterized by Persistent Lymphocytosis (PL) in approximately 30% of animals, tumors (lymphosarcoma and/or malignant lymphoma) with a frequency between 1 and 5% that appear in animals older than two years and one form in which the animals have antibodies (anti-BLV) without persistent lymphocytosis or tumor lesions, a condition known as the aleukemic phase[2]. The clinical manifestation of EBL begins after two years of age, in most cases; the symptoms are nonspecific and variable as they depend on the location of the neoplastic process and the degree of involvement of the vital organs. Anemia, wasting and infertility have been observed, although the most obvious signs that allow suspecting the disease are bilateral more or less symmetric enlargement of the explorable lymph nodes, exophthalmia due to retro-ocular tissue involvement or internal structures of the eye and the presence of subcutaneous tumor masses in different locations[2,3].

The BLV belongs to the genus Delta retrovirus and as such has a reverse transcriptase responsible for the synthesis of a DNA copy from viral RNA. The formed DNA (provirus) can be conserved in the nucleus of diverse cells of the host and this property is the cause of the particular characteristics of the different infections due to retroviruses[4]. The BLV can be transmitted vertically from the cow to the brood either via the placenta or during the peripartum in colostrum and milk; horizontal or from a sick anima to a healthy one by exposure to blood or fluids containing infected lymphocytes); or through the iatrogenic route due to poor livestock practices[5]. The disease can be diagnosed by serological or molecular methods; these techniques vary in specificity and sensitivity, have comparative advantages and disadvantages, but are ultimately complementary in the correct diagnosis of viral infection and the development of the disease[6].

Some genetic variations, especially those related to Bovine Leukocyte Antigens (BoLA) class II, in the DRB3 gene (BoLA-DRB3), have been associated with resistance and/or susceptibility to infection with BLV, development of PL, development of antibodies against the virus and the pro-viral load[7-11]. This gene has an approximate length of 11.4 Kpb, with five introns and six exons and codes for functional restriction elements, exon two is the most polymorphic, has an approximate size of 270 bp and codes for the peptide binding site which one wants to present[10].

The objective of the present investigation was to associate the BoLA-DRB3 alleles with the resistance and/or susceptibility to the development of the EBL, by evaluating the probability of infection with the BLV, the development of PL, the production of antibodies against the virus in Harton del Valle cattle.

2. Materials and Methods

2.1 Animals and Sample Processing

A follow-up of 93 Harton del Valle (HV) animals was done during one year, collecting blood samples every three months. In each bleed the serum was separated by centrifugation at 3000 rpm for 13 minutes and stored at -80°C until its use and peripheral blood lymphocytes were extracted following the methodology of[9] from which DNA was extracted using the Wizard® Genomic DNA Purification Kit from Promega, following the manufacturer's instructions.

2.2 Presence of BLV by PCR (env)

A highly conserved region of the viral env gene was amplified by nested PCR. In the first reaction 25 ng of DNA, 10mM of each forward primer env5032 5'-TCTGTGCCAAGTCTCCCAGATA-3' and reverse env5608r 5'-AACAACAACCTCTGGGAAGGGT-3', 0.2mM of each dNTP, 1X of PCR buffer were used, 2.5 mM $MgCl_2$ and 1U Taq DNA Polymerase. In the second reaction, 1µl of the amplification product of the first reaction were used as template, the same concentrations of the other reagents and the internal forward primers env5099 5'-CCCACAAGGGCGGCGCCGGTTT-3' and reverse

env5521r 5'-. GCGAGGCCGGGTCCAGAGCTGG-3', both reactions were performed at a final volume of 15µl[12]. The thermal profile of the first reaction included an initial denaturation at 94°C for 5 minutes, followed by 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds and 72°C C for 1 minute, to finish with an extension final at 72°C for 5 minutes. The thermocycling conditions of the second reaction were the same, except for the hybridization temperature which was increased to 6°C. The amplified ones were observed in 1.2% agarose gels stained with ethidium bromide, the product of both reactions is a fragment of 444 bp in the animals carrying BLV (env^+), the animals without infection of the virus were considered env^-. DNA from a positive animal and a negative one was used as PCR controls. To ensure that there was no PCR inhibitor in the DNA samples, each sample was tested by amplifying the Glyceraldehyde 3 Phosphate Dehydrogenase (GAPDH) gene[13].

2.3 Development of Persistent Lymphocytosis (PL)

The development of PL was evaluated using the peripheral blood plate spreading methodology. Using the pushing technique, 20µl of peripheral blood were extended, fixed with 96% methanol for 5 minutes, stained with 1% Methylene Blue 100x DNA Stain, (AMRESCO®) for 3 minutes, the reaction was stopped with a buffer solution (10 mM Na_2HPO_4, 2 mM KH_2PO_4)[14]. The plates were observed with an optical microscope at an increase of 1000x and the percentage of lymphocytes was calculated. The evaluated animal was considered to have lymphocytosis when the percentage of lymphocytes was equal to or greater than the mean plus twice the standard deviation for the sex and age group. An animal was considered to have PL when in two consecutive samplings it presented lymphocytosis[2]. Thus, the animals were classified as those that developed Persistent Lymphocytosis (PL^+) and those that did not (PL^-).

2.4 Quantification of the Development of Antibodies against the Virus (Anti-BLV)

Antibodies against BLV (anti-BLV) were quantified using the ELISA test (Svanova, SVANOVIR® BLV gp 51-Ab) following the manufacturer's instructions. The plates were read with a microplate photometer (BioTeK® ELx808™). The optical density values were interpreted with the program xChek 3.3. The animals were classified according to the Antibody Titers (AT) obtained, AT^- <15 were considered negative and AT^+ ≥15 were considered positive, the positive animals were further classified as having low AT ($anti\text{-}BLV^{low}$) or high AT ($anti\text{-}BLV^{high}$), the critical value used for the classification of the animals was the median (95.43) of the AT value.

2.5 Amplification and Sequencing (PCR-SBT) of the Bola-DRB3 Gene

Genotyping of the alleles BoLA-DRB3.2 was performed using the PCR-SBT methodology, with the primers DRB3FEW (5'-CGCTCCTGTGACCAGATCTATCC-3') and DRB3REV (5'-GGTGAGCGCGGGGGTG-3')[15] at a concentration of 10 mM, 25 ng of DNA, 0.2 mM of each dNTP, 1X of PCR buffer, 2.5 mM of $MgCl_2$ and 1U of Taq DNA Polymerase. The thermal profile of the reaction included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds 62°C for 30 seconds and 72°C for 1 minute, to finish with a final extension of 72°C for 5 minutes. The PCR product was a fragment of approximately 281 bp that was sequenced in the MACROGEN Company.

2.6 Statistical Analysis

The percentages of individuals who developed persistent lymphocytosis (PL^+) and those who did not (PL^-) was calculated; the percentage of animals infected with the virus (env^+) and not infected (env^-) and the percentage of animals TA^+ and $anti\text{-}BLV^{low/high}$.

The sequences were edited using the GENEIOUS 6.1 program (Biomatters development team, USA) and

aligned using the MEGA 6 software[16]. Genotypes were determined using HAPLOFINDER software (www.bioinfortmatics.roslin.ac.uk/haplofinder/haplofinder.py). The number of Alleles and their frequencies was estimated[17].

Chi-square independence tests were performed between the variables (PL, *env*, and anti-BLV) and the BoLA-DRB3 alleles. The alleles BoLA-DRB3 were associated with the presence/absence ($env^{+/-}$) of the BLV through the Odds Ratio (OR) with a confidence interval of 95%, this same association was made between the animals $env^{+}/PL^{+/-}$ and env^{+}/anti- $BLV^{low/high}$. The OR indicates the relative frequency of exposure between cases (env^{+}, env^{+}/PL^{+}, env^{+}/anti-BLV^{high}) and controls (env^{+}, env^{+}/PL^{-}, env^{+}/anti-BLV^{low}). OR values greater than 1 indicate that these animals are at high risk and were considered Susceptible (S) (negative association), OR values less than 1 indicate that these animals are at low risk and were considered Resistant (R) (positive association), OR values close to 1 were considered as Neutral (N)[7]. An exact Fischer test was performed to determine the statistical significance of OR values using SAS software version 9.1.

The cumulative allele frequencies of the alleles with R, S and N categories were calculated; the animals were classified according to the category of the alleles of their genotype in N/N when their two alleles were neutral, N/R when they had a neutral one and the other was resistant, N/S when they had a neutral allele and another susceptible one, R/R when they had two resistant alleles, R/S when they had one resistant and the other was susceptible, and S/S when their two alleles were susceptible.

The sequences of the alleles with some significant association were translated and aligned using the pro program MEGA 6[16] to look for common amino acid motifs among the alleles.

3. Results and Discussion

The average of animals positive to the env^{+} was of 38.3%, this value was lower than the one reported by[18] in this same racial group (83%); in other Colombian criollo breeds the percentages of infection that are reported are 26.7% in Casanareno, 23.3% in Costeno with Cuernos, 60% in Chino Santandereano and 16.7% in Caqueteno race[18-19]. In Holstein cows the infection values vary from 44%[20] to 83.3%[18]. In Brahmin animals present in Colombia the infection is lower (6.7%)[18]. The differences in the percentages of infection can be attributed to the effects of the sample size and the origin of the animals evaluated.

The percentage of animals that developed PL^{+} was 15%, while the AT^{+} animals were 30.3%. Of the animals classified as AT^{+} the average of the antibody titers was 96.7 in the animals classified with anti-BLV^{high} and of 25.6 in the animals with anti-BLV^{low}. This is the first quantification report of the development of PL and AT in the Harton del Valle breed. Some authors indicate that between 30% and 70% of animals infected with BLV develop Persistent Lymphocytosis (PL^{+})[1,2,21]. In race HV the animal's env^{+}/PL^{+} were 32.7%. Additionally, it is reported that 66% of the animal's env^{+}/PL^{+} present a pathological lymphocyte count[21].

We found 27 alleles BoLA-DRB3 shown in Table 1, allele *1101 had the highest frequency (14.5%), alleles *0902 and *2703 followed in frequency, the fourth most frequent allele was *20012 followed by alleles *1501 and *1701. The remaining 21 alleles had a cumulative frequency of 53.2%.

Seven of 27 alleles presented frequencies greater than 5%, similar results are reported by[22] in this breed where they found[23-24] alleles of which six had a frequency greater than 5% and by[23] who reported 37 alleles in 66 samples and only four alleles with high frequency. In these reports concluded that **1101* allele had the highest frequency. The number of alleles and the most frequent alleles in races around the world is presented in Table 2.

The Chi-square test showed high dependence ($p<0.0001$) between the infection with the virus (env^{+}), the development of PL^{+} in the env^{+} animals and the anti-BLV^{high} with the BoLA-DRB3 alleles. The values of OR,

Table 1. Allele frequencies at the BoLA-DRB3 locus in the study population

BoLA-DRB3 allele	Frequency (%)	BoLA-DRB3 allele	Frequency (%)	BoLA-DRB3 allele	Frequency (%)
*0101	3,2%	*1501	5,9%	*2801	1,1%
*0501	4,3%	*1601	2,2%	*2802	2,7%
*0701	1,1%	*1701	5,9%	*2902	2,2%
***0902**	**7,0%**	*20012	6,5%	*3001	3,2%
*1001	2,7%	*2006	3,8%	*3501	0,5%
*1002	4,3%	*2201	1,6%	*3601	4,8%
***1101**	**14,5%**	*25011	3,2%	*3901	2,2%
*1104	4,8%	*25012	1,1%	*4401	1,1%
*14011	1,6%	***2703**	**7,0%**	*4802	1,6%

the confidence interval, the value of significance and their classification as N, R or S for each of the variables studied are presented in Table 3.

The alleles **1001*, **1101*, **2006*, **2703*, **3001* and **4802* presented low values of OR ($p<0.05$) and were considered as R to infection with the virus (R^{env}), whereas, the alleles *0902, *1601, *1701, *20012 and *25011 were classified as S^{env}. The cumulative frequency of the neutral alleles to infection (N^{env}) was 49.4%, followed by the accumulated frequencies of the R^{env} (32.8%) and S^{env} (24.8%) alleles. Only the allele *1101 was significantly associated with the absence of PL in the animals in env^+ (R^{PL}) and the alleles *25011 and *2703 were considered S^{PL}, the cumulative frequency of these two alleles was 8.1%, whereas, 19 alleles were N^{PL}. Nine alleles BoLA-DRB3 were considered $N^{anti\text{-}BLV}$, the alleles *1101 and *2703 with the accumulated frequency of 21.5% were classified as $R^{anti\text{-}BLV}$, while, the alleles *1601 and *1701 with the accumulated frequency of 8.1% were classified as $S^{anti\text{-}BLV}$ shown in Table 3).

Some alleles of the DRB3 gene have been associated with a high risk of infection with BLV including *1002 and *25011 instead, the alleles *1101, *2709 and *20012 have been associated with a low risk of infection[8]. In the present study the alleles *1002 were classified N^{env} and the *25011 as S^{env}. The allele *1101 presented the same classification given by[8], while the alleles *2709 and *20012 are reported here as N^{env}. The alleles *1101, *1501 and *2703 have also been reported in the races Yacumeno, Harton del Valle, Holstein, Japanese Shorthorn, Japanese Black, Jersey, and Hanwoo shown in Table 2. The allele *2006 (R^{env}) has only been found in HV and the allele *1701 of (S^{env}) has only been reported in races Yacumeno, Harton del Valle[8,22]. No common amino acid motifs were found between the R^{env} alleles.

Table 2. Allelic frequencies in the BoLA-DRB3 locus in breed of the world using PCR-SBT

Breed	N	Na	Frequent alleles	References
Harton del Valle	93	27	*1101 (0.145), *0902 (0.070), *2703 (0.070), *20012 (0.065), *1701 (0.059), *1501 (0.059)	Present investigation
Argentine Holstein	107	18	*1001 (0.172), *1501 (0.163), *1101 (0.149), *0101 (0.121), *1201 (0.112), *0201 (0.065)	[24]
Holstein, Holsteinx Jersey	809	25	*0101 (0.174), *1101 (0.172), *1501 (0.132), *1201 (0.116), *2703 (0.083)	[7]
Girolando	99	39	*1101 (0.123), *2101 (0.061)	[38]
Holstein of Brazil	46	39	*1101 (0.151), *0101 (0.113)	
Iranian Holstein	190	37	*0101 (0.181), *1501 (0.115), *1101 (0.092), *1201 (0.084), *0902 (0.071)	[39]
Argentine Holstein	424	33	*0101 (0.177), *1501 (0.147), *1101 (0.121), *2703 (0.075), *1201 (0.071), *0902 (0.064)	[40]
Bolivian Holstein	159	23	*1501 (0.182), *0902 (0.154), *1101 (0.113), *0601 (0.104), *0101 (0.0570)	
Peruvian Holstein	127	26	*1501 (0.177), *0101 (0.177), *1101 (0.165), *0902 (0.071), *0201 (0.067), *1001 (0.063)	
Peruvian Holstein	133	20	*1501 (0.214), *1101 (0.169), *0101 (0.117), *1201 (0.098), *14011 (0.083), *0902 (0.056)	
Chilean Holstein	113	21	*1501 (0.217), *0101 (0.124), *1101 (0.119), *14011 (0.093), *1001 (0.088), *0902 (0.080)	

Table 2 Continued

Chilean Wagyu	81	27	*1001 (0.160), *1302 (0.160), *1601 (0.105)	41
Brahman	236	58	*3001 (0.138), *3601 (0.087), *1201 (0.072), *2201 (0.063), *0201 (0.057)	42
Holstein x Sahiwal	249	46	*1201 (0.136), *0101 (0.135), *1501 (0.062), *0201 (0.058), *0902 (0.052), *1101 (0.052)	
Native to the Philippines	482	71	*0301 (0.117), *0201 (0.082), *1501 (0.059), *1501 (0.052), *4101 (0.051)	
Native to the Philippines x Brahman	132	57	*3001 (0.087), *0201 (0.079), *3601 (0.064)	
Yacumeno	112	35	*0701(0.106), *0902 (0.084), *1801 (0.084), *0201 (0.070), *14011 (0.066)	
Harton del Valle	99	24	*1101 (0.121), *2703 (0.098), *2006 (0.098), *2801 (0.075), *1501 (0.075), *3601 (0.075)	22
Yacumeno	113	36	*0701 (0.106), *0902 (0.084), *1801 (0.084), *0201 (0.078)	
Hanwoo	359	39	*4301 (0.216), *1002 (0.155), *0701 (0.095), *1601 (0.093), *1501 (0.069), *0902 (0.052)	43
Harton del Valle	66	37	*1101 (0.204),*20012 (0.122), *2006 (0.071), *2801 (0.071)	23
Japanese Black	507	22	*1601 (0.318), *1501 (0.129), *0503 (0.128), *1001 (0.096), *1101 (0.07), *0201 (0.054)	44
Holstein	143	17	*1501 (0.273), *0101 (0.203), *1101 (0.112), *1001 (0.096), *1201 (0.067), *2703 (0.061)	

N: number of animals. Na: number of alleles

Table 3. BoLA-DRB3 alleles associated with env^{+}, env^{+}PL$^{+/-}$ and anti- BLV$^{low/high}$ in HV.

BoLA-DRB3 allele	env^{+}				env^{+}PL$^{+/-}$				env^{+}/anti- BLV$^{low/high}$			
	OR	IC (95%)	Fischer P-value	C	OR	IC (95%)	Fischer P-value	C	OR	IC (95%)	Fischer P-value	C
*0101	0.48	0.17-1.32	0.125	N	1.07	0.10-11.00	0.664	N				
*0501	0.72	0.29-1.72	0.306	N	0.66	0.09-5.52	0.563	N				
*0701	1.06	0.19-5.99	0.621	N	11.06	0.76-160.90	0.140	N				
*0902	2.60	1.29-5.23	0.007	S	0.42	0.14-1.27	0.099	N	2.36	0.87-6.38	0.074	N
*1001	0.22	0.07-0.66	0.003	R								
*1002	1.83	0.76-4.37	0.109	N	2.99	0.50-17.65	0.213	N	1.56	0.32-7.49	0.529	N
*1101	0.63	0.14-0.97	0.049	R	0.26	0.10-0.66	0.003	R	0.35	0.22-0.67	0.020	R
*1104	0.52	0.22-1.20	0.092	N	0.81	0.10-6.52	0.660	N				
*14011	0.37	0.09-1.55	0.166	N	0.26	0.01-27.07	0.763	N				
*1501	0.79	0.37-1.69	0.352	N	0.91	0.19-4.42	0.637	N	1	0.24-4.19	0.622	N
*1601	7.56	5.22-9.79	0.002	S	0.80	0.18-3.60	0.563	N	2.90	1.69-10.04	0.013	S
*1701	9.69	6.67-12.10	0.0001	S	1.96	0.25-3.62	0.629	N	5.91	2.45-9.28	0.014	S
*20012	2.28	1.25-4.17	0.006	S	0.64	0.20-2.03	0.340	N	0.76	0.27-2.15	0.412	N

Table 3 Continued

*2006	0.27	0.10-0.69	0.003	R	0.26	0.01-27.07	0.763	N				N
*2201	1.06	0.26-4.38	0.588	N	4.39	0.42-44.93	0.238	N				
*25011	4.01	1.46-10.05	0.009	S	0.72	0.17-3.02	0.492	N	1.80	0.49-6.61	0.278	N
*25012	1.06	0.19-5.99	0.628	N	7.41	4.91-14.61	0.012	S				
*2703	0.64	0.32-0.79	0.042	R	6.34	1.31-10.61	0.035	S	0.71	0.41-0.90	0.034	R
*2801	0.22	0.04-1.27	0.090	N								
*2802	1.07	0.35-3.21	0.551	N	4.47	0.66-30.19	0.145	N	0.22	0.03-1.51	0.129	N
*2902	1.06	0.31-3.64	0.561	N	4.39	0.42-44.93	0.238	N	0.21	0.02-1.76	0.196	N
*3001	0.21	0.08-0.60	0.001	R								
*3501	1.06	0.09-12.16	0.685	N	0.26	0.01-27.07	0.763	N	0.23	0.01-17.26	0.668	N
*3601	0.43	0.19-1.00	0.573	N	0.26	0.01-3.86	0.441	N				
*3901	1.58	0.46-5.39	0.324	N	2.51	0.31-20.25	0.339	N	0.222	0.01-4.25	0.445	N
*4401	0.22	0.04-1.27	0.091	N								
*4802	0.22	0.05-0.92	0.038	R								

OR: Odds ratio value. IC: 95% confidence interval. C: Classification of the allele, resistant (R), neutral (N) or susceptible (S).

Table 4. Genotypes N/N, N/R, N/S, R/R, R/S and S/S for the BoLA-DRB3 gene in HV

Itmen	Percentage of individuals in each genotype					
	N/N	N/R	N/S	R/R	R/S	S/S
env	37,6	45,2	5,4	6,5	4,3	1,1
LP	47,3	31,2	5,4	10,8	4,3	1,1
anti-VLB	28,1	8,7	21,9	9,4	0	4,7
Average	37,67	28,37	10,90	8,90	2,87	2,30

Previous studies with BLV infection revealed that the presence of the amino acids Glu-Arg (ER) at position 70-71 of the BoLA-DRβ chain was associated with resistance to PL development, the alleles that encode the ER motif are DRB3 *11 (*0901, * 0902, *1201 by PCR-SBT), *23 (*2701, *2702, *2703, *2705, *2706, *2707 by PCR-SBT) and *28 (*0701)[11,25-28]. On the contrary, it has been associated with the alleles DRB3 *8 (*1201 by PCR-SBT), *16 (*1501, *1502 by PCR-SBT), *22 (*1101 by PCR-SBT) and *24 (*0101, *0102 by PCR-SBT) with susceptibility to PL[27-29]. Contrary to what was reported by these authors, the allele classified as R^{PL} (*1101) presented the RR motive (Arg-Arg) in these positions as well as the alleles classified as S^{PL} (*25012 and *2703). The differences between these reports and what is presented here can be explained by the genotyping method used (PCR-RFLP versus PCR-SBT).

No associations were found between the production of neutralizing antibodies against Bovine Viral Diarrhea Virus (BVDV), Bovine Herpes Virus type 1 (BHV-1), Foot-and-Mouth Disease Virus (FMDV) and Bovine Leukosis Virus (BLV) and DRB3 gene polymorphisms[30]. However, they associated alleles *0902 and *1701 with low antibody titers against BLV. Similar results were found for the allele *1701, but the allele *0902 that here, presented a $N^{anti\text{-}BLV}$ association. Alleles *1101 and *2703 associated with high titers of antibodies against BLV ($R^{anti\text{-}BLV}$) produced 0.04 and 0.14 ($p<0.05$) times more antibodies than the $S^{anti\text{-}BLV}$ allele. These results together with that reported by[30] suggest that the implementation of genetic improvement systems aimed at increasing the frequency of the alleles of interest would have no effect on the susceptibility to other viruses of economic importance.

The alleles classified $R^{anti\text{-}BLV}$ in HV shared several amino acids including Glu-Arg-Val (ERV) in positions 58-70-77 of the BoLA-DRβ chain, highlighting among them the presence of the amino acid Arginine in position 70, this is located right in the groove of binding to the peptide in the BoLA-DRβ chain. The $S^{anti\text{-}BLV}$ alleles in these same positions carry the KEY motif for the allele *1701 and KKY for the allele *1601.

On average, 37.67% of the animals evaluated were classified as N/N, 8.90% were R/R and only 2.30% were S/S. On the other hand, 28.37%, 10.90%, and 2.87% were classified as N/R, N/S, and R/S respectively. The details of the classification of the animals according to the category of their alleles are presented in Table 4.

The first work on genetic resistance to leukosis revealed that the characteristic had a heritability coefficient of 0.24 and suggests that using selection methods the frequency of the characteristic could be increased[31]. This work proposes as the main determinant of genetic resistance to leukosis, the alleles of the DRB3 gene, in this sense, only the allele **1101* was considered as R^{env}, R^{PL} and $R^{anti\text{-}BLV}$, which means that the animals carrying

Table 5. BoLA-DRB3 *1101 genotype associated with resistance to EBL

BoLA-DRB3 Genotype	OR	IC (95%)	Fischer P-value
*1101/*1101	0.56	0.25-0.75	0.0004
*1101/*otro	0.18	0.05-0.35	0.00005
*otro/*otro	0.98	0.80-1.99	0.145

OR: Odds ratio value. IC: 95% confidence interval.

this allele they become infected less frequently with BLV, animals that become infected do not develop PL and also maintain high titers of antibodies against the virus. On the other hand, no allele was considered as S^{env}, S^{PL}, and $S^{anti\text{-}BLV}$, although the alleles **1601* and **1701* were for two of the three evaluated characteristics (S^{env} and $S^{anti\text{-}BLV}$).

The homozygous genotype *1101/*1101 had a frequency of 1.07%, while in the heterozygous *1101/*other its frequency was 26.8%. The association of this genotype with the development of bovine leukosis is presented in Table 5. The OR value of the homozygous genotype was lower than that of the heterozygote, but both highly significant. The OR of the genotypes does not carry the allele *1101 showed an OR value close to one. These results suggest an advantage of the heterozygote. It has been proposed that heterozygous individuals are able to recognize some broad-spectrum antigens, thus increasing the efficiency of these individuals compared to homozygous individuals[32]. Several studies have shown that heterozygosity confers selective advantages against infectious diseases, for example, human heterozygotes at HLA class II loci showed resistance against the hepatitis B virus and the Human Immunodeficiency Virus (HIV)[33-34]. In Holstein cattle with mastitis caused by Escherichia or Streptococcus it was found that in heterozygous animals in the class II locus DQA1, the progression of the disease was slower[35-36]. A similar result of heterozygous advantage over proviral load control is also demonstrated and proposed by[10].

Finally, the data presented here indicate and those discussed in other races[9,10], indicate that the development of bovine leukosis presents particularities according to breed since some alleles with the same classification differ. As has been demonstrated in others in other retroviruses[36-37], the low infection rate, the low lymphocytosis development, and the high antibody titers are good predictors of disease progression, this suggests that the data presented here can be used in future plans to control the disease.

4. Conclusions

The BoLA-DRB3 locus is highly polymorphic; the high-frequency alleles coincide with other reports of the breed and others in the world. For all the characteristics evaluated, alleles with significant associations were found, however, only the allele *1101 was considered as resistant to all. For all the characteristics, a higher percentage of animals with genotypes N/N, N/R and R/R were found. There was an advantage of the heterozygote when responding against the development of the disease. Some amino acids motifs present in the alleles of interest and absent in the susceptibility alleles were found. The data presented here can be used in future genetic improvement programs aimed at increasing the frequency of alleles of resistance to the disease.

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