Incidence of bovine leukocyte adhesion deficiency, complex vertebral malformation, and deficiency of uridine-5-monophosphate synthase carriers in Brazilian Girolando cattle

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ABSTRACT. Among the various hereditary diseases that have been widely studied in dairy cattle, bovine leukocyte adhesion deficiency (BLAD), deficiency of uridine-5-monophosphate synthase (DUMPS), and complex vertebral malformation (CVM) are noteworthy because of their high impact on overall herd productivity as a consequence of increased calf mortality. The aim of this study was to verify the frequency of carriers of BLAD, CVM, and DUMPS mutant alleles in cows and bulls from the National Girolando Progeny Test carried out in

Brazil by using polymerase chain reaction (PCR)-restriction fragment length polymorphism and allele-specific PCR assays. A total of 777 animals were genotyped for BLAD, 783 for CVM, and 122 for DUMPS. The frequencies of carriers for BLAD and CVM were 0.77 and 1.53%, respectively, whereas no carriers of DUMPS were observed.

Key words: *CD18*; Progeny test; *SLC35A3*; *UMPS*

INTRODUCTION

The incidence of hereditary diseases causes not only direct economic losses to live-stock breeders but also leads to reductions in the genetic diversity of animal populations as a result of extensive culling of disease carriers (Kunieda, 2005). The identification of carriers with the use of molecular diagnostic tests is an important step to reduce the frequency of detrimental alleles and consequently to lower the incidence of hereditary diseases in the herd. This is particularly important when considering bulls inserted into progeny testing programs because they can potentially sire thousands of progeny before the incidence of affected progeny can be associated with a particular animal.

Among the hereditary diseases most studied in livestock, bovine leukocyte adhesion deficiency (BLAD) is caused by a point mutation in the gene encoding the CD18 subunit of beta-2 integrin (*CD18*) (Shuster et al., 1992; Tammen et al., 1996). This mutation causes a deficiency in a family of glycoproteins normally present on the surface of leukocytes, which normally allows them to move in the bloodstream. Leukocytes from affected individuals are unable to perform their biological functions; therefore, cattle with BLAD die at an early age due to complications from simple infections, pneumonia, tooth loss, and general impairment of the immune system (Nagahata, 2004; Nasreen et al., 2009; Riojas-Valdéz et al., 2009).

Another important hereditary disease in cattle is deficiency of uridine-5-monophosphate synthase (DUMPS), which is caused by a nonsense mutation (C to T) at codon 405 of the uridine monophosphate synthase (*UMPS*) gene, resulting in a total loss of function of the enzyme (Schwenger et al., 1993; Ghanem et al., 2006). This gene catalyzes the conversion of orotic acid to uridine monophosphate, the precursor of all pyrimidine nucleotides (Shanks and Robinson, 1989). Because pyrimidines are heavily required for nucleic acid synthesis during embryonic development, embryos that are homozygous for the recessive mutant allele die before day 40 of gestation (Schwenger et al., 1994; Ghanem et al., 2006). Heterozygotes are phenotypically normal but show half of the normal activity of the *UMPS* enzyme, which in turn causes elevated levels of orotic acid in their milk and urine (Shanks and Robinson, 1989; Shanks et al., 1992).

Complex vertebral malformation (CVM) is a lethal hereditary condition characterized by growth retardation, vertebral malformation, and bilateral symmetrical arthrogryposis, leading to the premature death of calves (Duncan Jr. et al., 2001; Agerholm et al., 2004; Kanae et al., 2005). CVM is caused by a point mutation (G to T) at nucleotide position 559 of the solute carrier family 35 member 3, UDP-N-acetylglucosamine transporter (*SLC35A3*) gene on BTA3 (Thomsen et al., 2006). This gene encodes a nucleotide-sugar transporter that plays an essential role in mechanisms controlling the formation of vertebrae from the unsegmented paraxial mesoderm. As the transporter molecule is defective in affected animals, this condition

leads to vertebral malformations (Thomsen et al., 2006; Agerholm, 2007). A pedigree analysis traced the origin of the disease-causing allele to 2 common ancestors, Carlin-M Ivanhoe Bell and Penstate Ivanhoe Star, which are also BLAD carriers. These bulls were used worldwide in dairy cattle breeding due to the superior lactation performance of their daughters. Thus, both allele-causing diseases were widely disseminated throughout the world (Thomsen et al., 2006; Schütz et al., 2008).

Girolando is a synthetic breed created by crossing Holstein and Gyr cattle in a final proportion of 5:8, respectively. According to the Brazilian Association of Girolando Breeders (2005), this breed is currently responsible for 80% of the milk produced in Brazil. The Brazilian Program for the Genetic Evaluation and Breeding of Girolando Cattle has been underway for the past 15 years as a result of a collaboration between the breeder association and the Embrapa Dairy Cattle Research Unit. This program is focused on improving production traits and is close to reaching a total of 131 bulls under progeny testing. The main objective of this study was to identify hereditary disease carrier Girolando bulls that are under progeny testing and to estimate the frequency of disease-causing alleles in the breed.

MATERIAL AND METHODS

Blood or semen samples were collected from Girolando bulls under progeny testing (N = 122 for BLAD and DUMPS, and N = 120 for CVM) and sent to the laboratory for processing. In addition, blood samples from bull daughters (N = 655 for BLAD and N = 663 for CVM) were also collected. DNA was extracted from the semen or blood samples using the DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA, USA), following the manufacturer protocol. Previously described primers and polymerase chain reaction (PCR) conditions were used to amplify the respective regions of interest from *CD18*, *UMPS*, and *SLC35A3* (Table 1). All reactions were conducted in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA), and agarose gel images were recorded using the Eagle Eye II software (Stratagene, La Jolla, CA, USA).
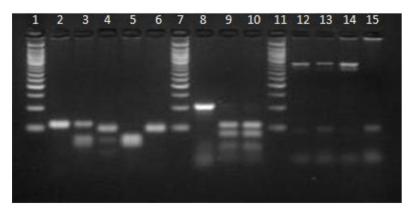
Table 1. Primer sequences used for genotyping *CD18*, *UMPS*, and *SLC35A3* mutations.

Gene	Target	Primers (5'→3')	Annealing temperature (°C)	Restriction enzymes	Reference
CD18	BLAD	TCCGGAGGGCCAAGGGCTA GAGTAGGAGAGGTCCATCAGGTAGTACAGG	65	*Taq*I or *Hae*III	Shuster et al., 1992
UMPS	DUMPS	GCAAATGGCTGAAGAACATTCTG GCTTCTAACTGAACTCCTCGAGT	57	*Ava*I	Schwenger et al., 1993
SLC35A3	CVM	Allele G: CACAATTTGTAGGTCTCATGGCAG Allele T: CACAATTTGTAGGTCTCATGGCAT Reverse: GTTATACTACAGGAGTCACCTCT	62	-	Ghanem et al., 2008

For target abbreviations, see legend to Figure 1.

For BLAD genotyping, the PCR products were digested with 4 U *Taq*I or *Hae*III (Promega, Madison, WI, USA) in 2 separate reactions. The digested products were visualized on 3% agarose gels stained with a 0.001% ethidium bromide solution. Genotype calls were based on the observed restriction patterns: carriers produce fragments of 58, 32, and 26 bp with *Taq*I and 49, 30, 19, and 9 bp with *Hae*III. Non-carriers produce fragments of 32 and 26 bp

with *Taq*I and 49 and 9 bp with *Hae*III (Figure 1). For *UMPS* genotyping, PCR products were digested with 5 U *Ava*I (Promega). The digested products were visualized on 3% agarose gels stained with a 0.001% ethidium bromide solution. Genotype calls were based on the observed restriction patterns: carriers produced fragments of 89, 53, and 36 bp, while non-carriers produced fragments of 53 and 36 bp (Figure 1). *SLC35A3* genotyping was performed by allele-specific-PCR (AS-PCR), as described previously (Table 1). The PCR products were visualized on 3% agarose gels stained with a 0.001% ethidium bromide solution. Genotype calls were based on the presence/absence of each AS fragment (Figure 1). Allelic and genotypic frequencies were estimated using Genepop web version 3.4 (Raymond and Rousset, 1995).

Figure 1. Agarose gel (3%) showing genotyping results for bovine leukocyte adhesion deficiency (BLAD) (*lanes 2* to *6*), deficiency of uridine-5-monophosphate synthase (DUMPS) (*lanes 8* to *10*), and complex vertebral malformation (CVM) (*lanes 12* to *15*). *Lanes 1*, *7*, and *11* = 50-bp DNA ladder. I) BLAD: non-digested PCR product shows a single fragment of 58 bp (*lane 2*). Carriers have fragments of 58, 32, and 26 bp when digested with *Taq*I (*lane 3*) and fragments of 49, 30, 19, and 9 bp when digested with *Hae*III (*lane 4*). BLAD-free animals show 2 fragments of 32 and 26 bp when digestion is carried out with *Taq*I (*lane 5*) and fragments of 49 and 9 bp when digestion is performed with *Hae*III (*lane 6*). II) DUMPS: non-digested PCR product shows a single fragment of 108 bp (*lane 8*). Non-carriers (*lanes 9* and *10*) show 2 fragments of 53 and 36 bp when digested with *Ava*I. III) CVM: CVM carriers show fragments of 395 bp to G and T alleles (*lanes 12* and *13*, respectively) while non-carriers show 1 fragment of 395 bp to G allele (*lane 14*) and absence to T allele (*lane 15*).

RESULTS

Genotyping of 777 Girolando cattle identified 6 (0.77%) BLAD carriers. Among these animals, one was a bull and the others were cows (Table 2). The frequencies of carriers and of the mutant allele were low in the cow herd (0.76 and 0.32%, respectively) and among bulls (0.82 and 0.41%, respectively). The allelic frequencies within the entire population studied differed greatly between the animals, being 0.39% for the lethal allele and 99.61% for the normal one. Furthermore, there was a low incidence of carriers (0.77%) of the *CD18* allele (Table 2).

In all, 11 cows and 1 bull, among the 783 genotyped animals (1.53%), were found to be carriers of the *SLC35A3* mutation, resulting in an estimated allele frequency of 0.76% for the mutant allele (Table 2). Among the cows, the frequencies of the mutant allele and carrier animals were 0.83 and 1.66%, while they were 0.42 and 0.83% among the bulls, respectively.

None of the affected cows were daughters of the carrier bull evaluated in the study. No animals were found with the *UMPS* mutation (Table 2).

Table 2. Allelic and genotypic frequencies in Girolando bulls and cows, only bulls, and only cows.

	Genes	Genotype	Number of animals		Frequency	
			Observed	Expected	Genotypic	Allelic
Bulls and cows	*CD18*[1]	TL	771.00	771.01	99.23	0.9961
		BL	6.00	5.98	0.77	0.0039
	UMPS[2]	TD	122.00	122.00	100.00	1.000
		DP	0.00	0.00	0.00	0.000
	SLC35A3[3]	TV	771.00	771.04	98.47	0.9924
		CV	12.00	11.91	1.53	0.0076
Bulls	*CD18*1	TL	121.00	121.00	99.18	0.9959
		BL	1.00	1.00	0.82	0.0041
	*UMPS*2	TD	122.00	122.00	100.00	1.000
		DP	0.00	0.00	0.00	0.000
	*SLC35A3*3	TV	119.00	119.00	99.17	0.9958
		CV	1.00	1.00	0.83	0.0042
Cows	*CD18*[1]	TL	650.00	650.01	99.24	0.9962
		BL	5.00	4.98	0.76	0.0032
	SLC35A3[3]	TV	652.00	652.04	98.34	0.9917
		CV	11.00	10.92	1.66	0.0083

[1]Genotypes to the *CD18* gene: TL = homozygous BLAD-non-carrier; BL = heterozygous to BLAD allele (BLAD-carrier). [2]Genotypes to the *UMPS* gene: TD = homozygous DUMPS-free; DP = heterozygous to DUMPS allele (DUMPS-carrier). [3]Genotypes to the *SLC35A3* gene: TV = homozygous CVM-free; CV = heterozygous to CVM allele (CVM-carrier).

DISCUSSION

A previous study by Ribeiro et al. (2000) reported that the frequency of BLAD carriers was 5.7% among Brazilian Holstein cows and 0% in the Gyr bulls. Because the Gyr and Holstein breeds compose the Girolando, these results show that the frequency among cows is lower than our findings and the frequency among bulls is similar.

The observed frequency of BLAD carriers among cows (0.76%) may be explained by the fact that this mutation is common in Holstein bulls in different countries (Norouzy et al., 2005; Akyüz and Ertuğrul, 2006; Czarnik et al., 2007; Patel et al., 2007), and carrier bulls were most likely used as founding sires during the establishment of the breed. Three of the 5 affected cows were daughters of the carrier bull evaluated in the study.

The observed frequency of carrier bulls (0.82%) was similar to that reported by Akyüz and Ertuğrul (2006) in Turkish Holsteins (0.84%) and by Ribeiro et al. (2000) in Brazilian Gyr bulls (0%). However, it was considerably different from the frequency found in bulls by Patel et al. (2007) in Indian Holstein Friesian crossbred cattle (2.99%), Norouzy et al. (2005) in Iranian Holsteins (3.3%), Powell et al. (1996) in American Holsteins (8.2%), Czarnik et al. (2007) in the Polish Holstein-Friesian, Polish Red, and Polish Red-and-White breeds (4.3%), and Meydan et al. (2010) in Holstein cows in Turkey (4.0%). The allele frequencies estimated for bulls may not fully reflect the allele frequencies for the breed since the tested bulls were derived from the Girolando progeny test and are therefore unlikely to represent a random sample of the population because they were included in the progeny test for their genetic superiority based on parent-average predictions. However, the allele frequency estimates based on cows are more representative of the breed, considering that a large number of animals were sampled

from several herds with different production levels.

The low frequency of CVM carriers observed in the Girolando breed was also reported by Rezaee et al. (2008, 2009) in Iranian Holsteins, where no heterozygous animal was identified. This can be attributed to the low use of carrier bulls to establish the breed.

Although the frequency of CVM carriers found in this study was very low, higher frequencies of CVM carriers were reported by Meydan et al. (2010) in Turkish Holstein cows (3.4%), Ghanem et al. (2008) in Japanese Holstein cows (13%), and Chu et al. (2008) in Chinese Holstein bulls (15%). The frequency of carriers for CVM in some countries has been reported at alarming levels, e.g., Swedish Holsteins (23%) (Berglund et al., 2004) and Polish Holstein-Friesian bulls (24.79%) (Ruść and Kamiński, 2007).

The absence of DUMPS carrier animals was similar to the findings in Turkish Holstein cows (Meydan et al. 2010), Polish Holstein bulls (Kamińsk et al., 2005), Iranian Holstein bulls (Rezaee et al., 2009), and Indian Holstein cattle (Patel et al., 2006).

Although our efforts were concentrated on genotyping bulls because of their higher potential to disseminate mutant alleles by siring large numbers of progeny through artificial insemination, a larger sample size will be necessary for a better estimate of the frequency of this mutation in the Girolando breed. To prevent the increase of lethal recessive alleles, carrier animals should be eliminated from the Girolando Progeny Test since BLAD, CVM, and DUMPS are diseases with a considerable economic impact.

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