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Genetic analysis of the 3′ untranslated region of the bovine *SLC11A1* gene reveals novel polymorphisms

F. C. Hasenauer · M. E. Caffaro · C. Czibener · D. Comerci · M. A. Poli · C. A. Rossetti

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Abstract Polymorphisms in microsatellites at the 3′ untranslated region (3′UTR) of the *SLC11A1* (solute carrier family 11 member A1) gene have been associated with natural resistance to *Brucella abortus* and *Mycobacterium bovis* infection in livestock species. Here, we carried out an individual genetic analysis of the two microsatellites present at the 3′UTR *SLC11A1* gene in 254 *Bos taurus* purebred, 125 *B. indicus* purebred and 54 *B. taurus* × *B. indicus* crossbred cattle. The genotyping by capillary electrophoresis showed the presence of four alleles (157, 159, 161 and 163) for the first microsatellite (MS1) and six alleles (175, 177, 179, 181, 183 and 185) for the second microsatellite (MS2). The alleles 159 and 175 were the most frequent in all breeds analyzed. *B. taurus* showed the most homogeneous haplotype and genotype for both microsatellites, whereas *B. indicus* showed the most heterogeneous haplotype and genotype. Two novel variants (alleles 161 and 163) within the MS1 are reported as well as novel variants in MS2 in Holstein breed. The knowledge of the polymorphisms distribution in both microsatellites at the 3′UTR of the *SLC11A1* gene in cattle breeds is useful for future experimental design to evaluate the association between reported genotypes and natural resistance to pathogens infection.

Keywords *SLC11A1* gene · Cattle · Polymorphisms · Microsatellites · Genetic resistance · *NRAMP1* gene

F. C. Hasenauer · C. A. Rossetti (✉)
Instituto de Patobiología, CICVyA-CNIA, INTA, Nicolás Repetto y de Los Reseros s/n, Hurlingham, Buenos Aires B1686, Argentina
e-mail: crossetti@cnia.inta.gov.ar

F. C. Hasenauer · C. Czibener · D. Comerci
CONICET, Buenos Aires, Argentina

M. E. Caffaro · M. A. Poli
Instituto de Genética "Ewald A. Favret", CICVyA-CNIA, INTA, Nicolás Repetto Y de Los Reseros s/n, Hurlingham, Buenos Aires B1686, Argentina

C. Czibener · D. Comerci
IIB-INTECH, Universidad de San Martín, San Martín, Buenos Aires, Argentina

Introduction

Genetically controlled resistance to infectious diseases is a polygenic trait; however, it has been observed that single genes have a major effect on immune mediated resistance in mice in a wide range of infectious disease [1]. The solute carrier family 11 member A1 (*SLC11A1*) gene, formerly known as natural resistance-associated macrophage protein 1 (*NRAMP1*) gene, has been identified as the candidate gene controlling innate resistance to intracellular pathogens in mice. A single, non-conserved glycine-to-aspartic acid substitution at position 169 has been associated with a susceptible phenotype to several intracellular pathogens such as *Mycobacterium bovis*, *Leishmania donovani*, *Salmonella typhimurium*, and several atypical mycobacteriae [2]. Homologues of murine *SLC11A1* gene has been identified in genetically distant organisms, such as livestock species, human, insects, worms, plants, yeasts and bacteria [3]. Among livestock species, polymorphisms in microsatellites of 3′ untranslated region (3′UTR) of the gene were associated with resistance to *Brucella abortus* [4–7] and *M. bovis* [8] infection. *Brucella* sp. and *Mycobacterium* sp. are etiological agents of brucellosis and tuberculosis respectively, worldwide zoonotic infectious diseases that have a significant economic impact on animal production and human public health.

The *SLC11A1* gene is expressed in phagocytic cells and encodes for a protein localized to the membrane of late phagolysosomes, particularly in macrophages [9]. The Slc11a1 protein works as a transporter for protons, iron and other divalent cations [10]; however, the direction of iron transport is still in debate. It has been reported that Slc11a1 protein transports iron into phagolysosomes, leading to enhanced formation of reactive oxygen intermediates through the Fenton reaction [11]. Contrary, several studies suggest that Slc11a1 protein transports iron out of phagolysosomes and even out of the cells, thus preventing acquisition of iron by intracellular pathogens [12, 13].

The bovine *SLC11A1* gene has been mapped to chromosome BTA 2 [14]. The bovine *SLC11A1* product is a 548 amino acid protein containing 12 putative transmembrane domains, an extracellular glycosylation site, four putative phosphorylation sites and a SH3 binding motif. Comparison of murine, human and bovine Slc11a1 protein sequences has demonstrated a remarkable degree of homology, i.e., 86.9 % identical residues between murine and bovine; 88.6 % identical residues between human and bovine [14].

There are two polymorphic microsatellites in the 3′UTR end of the bovine *SLC11A1* gene as a result of a variation in the number of GTn repeats. The complex pattern of the first microsatellite (MS1) polymorphism, nucleotide positions 1,781–1,804, was originally reported by Horin et al. [15], who differentiated two alleles: *NRAMP1.1* that correlates with 12 GTs repeats, and *NRAMP1.2* that correlates with 10 GTs repeats. The variation in the length of the microsatellite is due not only to the number of GTn but also due to the presence of a T versus G transversion at position 1,782 at the 5′ end of the microsatellite. Later, Martinez et al. [7] genotyping 192 Colombian cattle breeds by single-strand conformation polymorphism analysis (SSCP) found the same two alleles described by Horin et al. [15].

The second microsatellite (MS2), nucleotide positions 1,908–1,933, was first identified by Feng et al. [14], who reported the allele 175 corresponding with 13 GT pairs. Later, Gonzalez et al. [16] and Paixao et al. [17] detected other alleles such as 177, 179, 181, 183, 185 and 189 in several Colombian and Brazilian cattle breeds genotyped by SSCP. When a fragment of the 3′UTR *SLC11A1* bovine gene containing both microsatellites (i.e., from nucleotide positions 1,745 to 1,945) was genotyped, polymorphisms were found only in one microsatellite [7, 16].

The goal of this study was to detect allelic variants in MS1 and MS2 at the 3‘UTR region of the *SLC11A1* gene in cattle breeds present in Argentina as an initial step for a rational experimental design to evaluate the role that each allele/genotype/haplotype has in resistance or susceptibility to intracellular pathogens infection such as *Brucella* sp. or *Mycobacterium* sp.

Materials and methods

Bovine population and DNA isolation

A 254 *Bos taurus* purebred, 125 *B. indicus* purebred and 54 *B. taurus* × *B. indicus* crossbred cattle (n total = 433) with known genealogy were selected for this study with special care to avoid selecting closely related animals (Table 1).

Genomic DNA was isolated from blood samples or hair follicles. DNA from blood was isolated using standard phenol:chloroform:isoamyl alcohol extraction method as previously described [18], whereas DNA from hair follicles was isolated based on a protocol published by Iglesias et al. [19] and adapted for our experiments. Briefly, four to six 70 % ethanol-cleaned hair follicles were collected into a 0.2 ml microtube containing 100 µl of SNET lysis buffer (20 mM Tris HCl, pH 8.0; 5 mM EDTA; 400 mM NaCl; 1 % w/v SDS) and 2 µl of proteinase K (19.6 mg/ml, Fermentas). Samples were incubated for 30 min at 55 °C followed by 15 min at 95 °C. The solution was transferred to a clean 1.5 ml tube where 100 µl of phenol:chloroform:isoamyl alcohol (Invitrogen) was added and mixed by

Table 1 Cattle population surveyed

Specie	Breed	Source [country (province)]	Sample size
B. taurus	Creole	Argentina (Buenos Aires, Santa Fe, Tucumán, Córdoba, Formosa, Santa Cruz)	41
	Angus	Argentina (Buenos Aires) and Paraguay	47
	Hereford	Argentina (Buenos Aires, Santa Fe, Corrientes)	57
	Holstein	Argentina (Buenos Aires, Santa Fe)	109
B. indicus	Brahman	Argentina (Corrientes)	90
	Nelore	Argentina (Tucumán) and Paraguay	35
B. taurus × *B. indicus*	Braford	Argentina (Corrientes, Tucumán) and Paraguay	32
	Brangus	Paraguay	22
Total			433

inverting the tube. Phase separation was achieved by centrifugation at 800×*g* for 10 min. The upper phase (aqueous phase) was transferred to a clean 1.5 ml eppendorf tube where two volume of 100 % ethanol was added and mixed by inverting the tube. The DNA pellet was recovered by centrifugation at 12,000×*g* for 15 min, washed twice with 70 % ethanol and recovered by centrifugation at 12,000×*g* for 10 min. The DNA pellet was air-dried and then resuspended in 50 µl of TE buffer (10 mM Tris–HCl; 1 mM EDTA, pH 8.0). DNA samples' concentration was quantified by NanoDrop® ND-1000 (NanoDrop) and stored at −20 °C until used.

Genotyping

Multiplex PCR was carried out to amplify both microsatellites at the 3′UTR of the bovine *SLC11A1* gene (GenBank U12862). Primer pair 1 (Fw1 5′-GATCAGGAGAAGG GGAGGA-3′ and Rv1 5′-CAGCTTCCAGAACTCCCTGT-3′) was designed based on the previously published sequence [14] using Primer3 software [20] and used to amplify a region containing microsatellite 1 (nucleotide positions 1,691–1,848). Primer pair 2 (Fw2 5′-AAGGCAGCAAGACAGA-CAGG-3′ and Rv2 5′-ATGGAACTCACGTTGGCTG-3′), used to amplify the region containing microsatellite 2 (nucleotide positions 1,814–1,988), had been previously described [21] (Fig. 1). Primers Fw1 and Rv2 were 5′ labeled with the fluorescent dye NED and 6-carboxyfluorescein (6-FAM), respectively. Multiplex PCR was carried out with 20 ng of genomic DNA in a total reaction volume of 15 µl containing: reaction buffer 1× (50 mM KCl; 100 mM Tris–HCl, pH 9.0 a 25 °C; 1 % Triton X-100), 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.2666 µM of the primer Fw1 and Rv1 and 0.0533 µM of the primer Fw2 and Rv2, and 0.5 U of *Taq* DNA polymerase (Inbio-HighWay). Amplification reactions were run with the following program: an initial denaturation step at 94 °C for 5 min followed by 40 cycles of denaturation (94 °C for 45 s), annealing (59 °C for 30 s), and extension (72 °C for 45 s), with a final extension step at 72 °C for 60 min. PCR products were evaluated in a 2 % agarose gel electrophoresis stained with ethidium bromide.

Genotyping was performed by capillary electrophoresis using the Applied Biosystems ABI PRIrism 3130xl Genetic Analyzer (Applied Biosystem) equipped with a 36-cm-long capillary. The separation medium used was the POP-7 polymer (Applied Biosystems). One microlitre of the PCR reaction was added to 10.0 µl of deionized formamide and 0.1 µl of GeneScan 500 LIZ Size Standard (Applied Biosystems). Then, the samples were denatured at 94 °C for 3 min, cooled at 4 °C, and loaded on the ABI Prism 3130xl Genetic Analyzer. Electrophoresis data was acquired using ABI Prism 3130xl software (Applied Biosystems) and the size of alleles was determined using GeneMapper software (Applied Biosystems).

DNA sequencing

To confirm the length of microsatellites 1 and 2 (GT repeats), PCR products from at least three homozygous animals for the identified alleles were sequenced in both directions. The nucleotide sequence was determined using version 3.1 of the Big Dye terminator cycle sequencing kit (Applied Biosystems) and the ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems). The length of the capillary was 36 cm and the separation medium was POP-7 polymer (Applied Biosystems). The sequence data were analyzed using BioEdit [22].

Statistical analysis

Allelic and genotype frequencies, Hardy–Weinberg equilibrium and genotypic linkage disequilibrium were calculated

Fig. 1 Sequence of the 3′UTR of the bovine *SLC11A1* gene. The figure shows the sequence of the 3′UTR *SLC11A1* gene (5′–3′, position 1,681–2,040) according to Feng et al. [14]. Microsatellite 1 is highlighted in *light grey* and microsatellite 2 in *dark grey*. Primers hybridization sites are shown in *bold* (*Fw1* forward 1 and *Rv1* reverse 1) and *underlined* (*Fw2* forward 2 and *Rv2* reverse 2) on the gene sequence

using GenePop [23]. Breed haplotype frequencies were estimated using Arlequin software version 3.5 [24]. Allele count, polymorphism information content (PIC), observed heterozygosity and Nei's unbiased gene diversity were calculated using the Microsatellite toolkit version 3.1.1 [25].

Results

DNA samples used in this experiments were of good to excellent quality ($OD_{260/280} \geq 1.75$, $OD_{260/230} > 1.85$). Capillary electrophoresis analysis of 433 samples from different animals detected 4 alleles for the MS1 and 6 for the MS2 at the 3′UTR *SLC11A1* gene.

Alleles 157, 159, 161 and 163 were detected for the MS1. Allele 159 and genotype 159/159 were the most frequent in all breeds analyzed (Tables 2 and 3, respectively). Sequence of PCR products was performed to confirm the number of GTs dinucleotide repeat in homozygous animals, i.e. 157, 159 and 161, which correlate with 10, 12 and 13 GTs repeats, respectively (Table 4). Allele 163 was not sequenced because no homozygous animal was found.

Six alleles (175, 177, 179, 181, 183 and 185) were detected for the MS2. Allele 175 was the most frequent in all breeds analyzed (Table 5). The genotype 175/175 was the most frequent in *B. taurus* breeds and crossbreds analyzed, whereas the genotype 175/181 was the most frequent in *B. indicus* breeds (Table 6). Sequence analysis confirmed that alleles 175 and 181 correlate to GT13 and GT16 repeats, respectively.

Genotypic linkage disequilibrium analysis was performed across all breeds to assess whether the genotype frequencies at microsatellite 1 were independent of the genotype frequencies at microsatellite 2. The X^2 obtained was infinity and the *P* value was highly sign, which indicate that the two microsatellites were in linkage disequilibrium.

Fourteen haplotypes were estimated when all breeds were grouped. The higher number of haplotypes was

Table 2 Allelic frequencies at the 3′UTR *SLC11A1* microsatellite 1 in breeds of cattle

	Brahman $n = 90$	Nelore $n = 35$	Creole $n = 41$	Holstein $n = 109$	Hereford $n = 57$	Angus $n = 47$	Braford $n = 32$	Brangus $n = 22$	Overall population $n = 433$
157	0.306	0.329	0.049	0.206	0.132	0.053	0.188	0.273	0.197
159	0.478	0.629	0.951	0.794	0.798	0.947	0.734	0.727	0.739
161	0.200	0.043	–	–	0.070	–	0.078	–	0.060
163	0.017	–	–	–	–	–	–	–	0.003

Table 3 Genotype frequencies at the 3′UTR *SLC11A1* microsatellite 1 in breeds of cattle

	Brahman $n = 90$	Nelore $n = 35$	Creole $n = 41$	Holstein $n = 109$	Hereford $n = 57$	Angus $n = 47$	Braford $n = 32$	Brangus $n = 22$	Overall population $n = 433$
157/157	0.122	0.086	0.024	0.064	0.035	–	0.063	0.091	0.065
157/159	0.244	0.457	0.049	0.285	0.175	0.106	0.187	0.364	0.231
157/161	0.100	0.029	–	–	0.018	–	0.063	–	0.030
157/163	0.022	–	–	–	–	–	–	–	0.005
159/159	0.256	0.371	0.927	0.651	0.649	0.894	0.625	0.545	0.591
159/161	0.189	0.057	–	–	0.123	–	0.031	–	0.062
159/163	0.011	–	–	–	–	–	–	–	0.002
161/161	0.056	–	–	–	–	–	0.031	–	0.014

Table 4 Sequence of the microsatellite 1 (nucleotide position 1,781–1,804 for *NRAMP1.1*) at the 3′UTR of the bovine *SLC11A1* gene

Allele	Nucleotide sequence (5′–3′)	Number of GTs	Source
157	TCAGACAAGG **GG**GTGTGTGT GTGTGTGTGT GT****ATGTGT	10	This study
NRAMP1.2	TCAGACAAGG **GG**GTGTGTGT GTGTGTGTGT GT****ATGTGT	10	Horin et al. [15]
159	TCAGACAAGG GTGTGTGTGT GTGTGTGTGT GTGT**ATGTGT	12	This study
NRAMP1.1	TCAGACAAGG GTGTGTGTGT GTGTGTGTGT GTGT**ATGTGT	12	Horin et al. [15]
161	TCAGACAAGG GTGTGTGTGT GTGTGTGTGT GTGTGTATGT	13	This study

** Indicates absent GT dinucleotide repeats

Table 5 Allelic frequencies at the 3′UTR *SLC11A1* microsatellite 2 in breeds of cattle

	Brahman $n = 90$	Nelore $n = 35$	Creole $n = 41$	Holstein $n = 109$	Hereford $n = 57$	Angus $n = 47$	Braford $n = 32$	Brangus $n = 22$	Overall population $n = 433$
175	0.478	0.600	0.976	0.982	0.974	0.957	0.766	0.705	0.812
177	0.078	0.057	0.024	0.018	–	0.043	0.063	0.023	0.038
179	0.033	–	–	–	0.009	–	0.016	–	0.009
181	0.322	0.243	–	–	0.018	–	0.156	0.250	0.113
183	0.089	–	–	–	–	–	–	0.023	0.020
185	–	0.100	–	–	–	–	–	–	0.008

Table 6 Genotype frequencies at the 3′UTR *SLC11A1* microsatellite 2 in breeds of cattle

	Brahman $n = 90$	Nelore $n = 35$	Creole $n = 41$	Holstein $n = 109$	Hereford $n = 57$	Angus $n = 47$	Braford $n = 32$	Brangus $n = 22$	Overall population $n = 433$
175/175	0.211	0.286	0.976	0.963	0.947	0.915	0.531	0.500	0.691
175/177	0.067	0.114	–	0.037	–	0.085	0.125	0.045	0.053
175/179	0.011	–	–	–	0.018	–	0.031	–	0.007
175/181	0.344	0.343	–	–	0.035	–	0.313	0.319	0.143
175/183	0.111	–	–	–	–	–	–	0.045	0.025
175/185	–	0.171	–	–	–	–	–	–	0.014
177/177	–	–	0.024	–	–	–	–	–	0.002
177/181	0.067	–	–	–	–	–	–	–	0.014
177/183	0.022	–	–	–	–	–	–	–	0.005
179/181	0.056	–	–	–	–	–	–	–	0.012
181/181	0.067	0.057	–	–	–	–	–	0.091	0.023
181/183	0.044	–	–	–	–	–	–	–	0.009
181/185	–	0.029	–	–	–	–	–	–	0.002

observed in *B. indicus* breeds, whereas the least number of haplotypes was observed in *B. taurus* breeds. Haplotype 159–175 was the most frequent haplotype in all breeds analyzed. This haplotype correlates with 12 GTs repeats for the MS1 and 13 GTs repeats for the MS2. This haplotype and the haplotype 157–175 were the two present in all breeds analyzed. The haplotype 157–181 (i.e. 10 GTs for MS1 and 16 GTs for MS2) was the second most frequent haplotype in *B. indicus* breeds and Brangus, whereas the haplotype 157–175 (10 GTs for MS1 and 13 GTs for MS2) was the second most frequent haplotype in *B. taurus* breeds and Braford (Table 7).

At the GTn dinucleotide repeat, Brahman was the breed that presents the most number of alleles for both MS, whereas Creole, Holstein and Angus presented the least number of alleles for MS1 and MS2, as shown in Table 8. Brangus also presented fewer numbers of alleles in MS1. PIC ranged from 0.088 (Creole) to 0.57 (Brahman) for MS1, and 0.035 (Holstein) to 0.595 (Brahman) for MS2. The *SLC11A1* microsatellite 1 analyzed for each breed was found to be in H–W equilibrium in all breeds, except in Braford. The *SLC11A1* microsatellite 2 analyzed for each breed was found to be in H–W equilibrium in all breeds analyzed, except in Creole. Ho ranged from 0.049 and 0.000 (Creole) to 0.567 and 0.722 (Brahman) for MS1 and MS2, respectively, and He ranged from 0.094 (Creole) to 0.642 (Brahman) for MS1, and 0.036 (Holstein) to 0.656 (Brahman) for MS2 (Table 8).

Discussion

The *SLC11A1* gene has been a subject of study since it was identified as the major candidate gene controlling innate resistance to intracellular pathogens infection such as *M. bovis*, *L. donovani*, *S. typhimurium*, and several atypical mycobacteriae in mice [2]. Later, experimental evidences indicated that particular alleles were associated with a decreased intracellular replication of *B. abortus* and *M. bovis* in cattle [6, 7, 26, 27] and water buffalo [4, 5] macrophages. According to those reports, the origin of the resistance is related with polymorphisms in microsatellites at the 3′UTR region, where two microsatellites, named by us microsatellite 1 (MS1) and microsatellite 2 (MS2), are present.

In this study, the genotyping of 433 cattle by capillary electrophoresis showed the presence of four alleles and

Table 7 Haplotype frequencies at the 3′UTR *SLC11A1* in breeds of cattle analyzed

Haplotype		Brahman	Nelore	Creole	Holstein	Hereford	Angus	Braford	Brangus	Overall population
Definition (MS1–MS2)	Number of GTn[a] (MS1–MS2)	$n = 90$	$n = 35$	$n = 41$	$n = 109$	$n = 57$	$n = 47$	$n = 32$	$n = 22$	$n = 433$
157 175	10–13	0.011	0.043	0.024	0.206	0.132	0.053	0.109	0.068	0.095
157 177	10–14	0.006	–	0.024	–	–	–	–	–	0.003
157 181	10–16	0.200	0.186	–	–	–	–	0.078	0.182	0.072
157 183	10–17	0.089	–	–	–	–	–	–	0.023	0.020
157 185	10–18	–	0.100	–	–	–	–	–	–	0.008
159 175	12–13	0.339	0.543	0.951	0.775	0.772	0.904	0.594	0.636	0.676
159 177	12–14	0.006	0.029	–	0.018	–	0.043	0.063	0.023	0.018
159 179	12–15	0.033	–	–	–	0.009	–	–	–	0.008
159 181	12–16	0.100	0.057	–	–	0.018	–	0.078	0.068	0.037
161 175	13–13	0.128	0.014	–	–	0.070	–	0.063	–	0.042
161 177	13–14	0.050	0.029	–	–	–	–	–	–	0.013
161 179	13–15	–	–	–	–	–	–	0.016	–	0.001
161 181	13–16	0.022	–	–	–	–	–	–	–	0.005
163 177	14–14	0.017	–	–	–	–	–	–	–	0.003

[a] Number of estimated GTn dinucleotide repeat

Table 8 PIC, observed and expected heterozygosity and Hardy–Weinberg equilibrium for the 3′UTR *SLC11A1* microsatellites 1 and 2 in breeds of cattle

Locus	Brahman $n = 90$	Nelore $n = 35$	Creole $n = 41$	Holstein $n = 109$	Hereford $n = 57$	Angus $n = 47$	Braford $n = 32$	Brangus $n = 22$	Overall population $n = 433$
Microsatellite 1									
Number of alleles	4,000	3,000	2,000	2,000	3,000	2,000	3,000	2,000	4,000
PIC values	0.570	0.408	0.088	0.274	0.312	0.096	0.375	0.318	0.364
P-HW	0.442	0.874	0.073	0.234	0.464	1.000	0.018	0.624	–
HO	0.567	0.543	0.049	0.284	0.316	0.106	0.281	0.364	0.330
HE	0.642	0.502	0.094	0.329	0.344	0.102	0.426	0.406	0.412
Microsatellite 2									
Number of alleles	5,000	4,000	2,000	2,000	3,000	2,000	4,000	4,000	5,000
PIC values	0.595	0.514	0.046	0.035	0.051	0.078	0.352	0.377	0.307
P-HW	0.406	0.722	0.013	1,000	1,000	1,000	0.606	0.778	–
HO	0.722	0.657	0.000	0.037	0.053	0.085	0.469	0.409	0.284
HE	0.656	0.576	0.048	0.036	0.052	0.082	0.391	0.450	0.327

PIC polymorphism information content, *P-HW* probability-value for Hardy–Weinberg equilibrium, *HO*observed heterozygosity, *HE* Nei's heterozygosity

eight genotypes for the MS1. In concordance with Martinez et al. [7], the allele 159 was the most frequent in all breeds analyzed. This allele, named *NRAMP1.1* by Horin et al. [15] and A by Martinez et al. [7], has been associated with the resistance to *B. abortus* in cattle, whereas the other alleles have been associated with susceptibility [6, 7]. Similarly with that study, the genotype 159/159 showed the highest frequency when all breeds were grouped. In contrary, this study reports the genotype 157/157 not only in *B. indicus* breeds but also in almost all breeds analyzed. Additionally, we found novel variants for the MS1 within the 3′UTR of the *SLC11A1* gene: the alleles 161 and 163 and five up to date unreported genotypes (157/161, 157/163, 159/161, 159/163 and 161/161). Interestingly, the novel genotype 159/161 showed higher frequency in Brahman and Hereford breeds than the genotype 157/157.

Furthermore, this study found six alleles and thirteen genotypes for the MS2. The allele 175 that correlates with 13 GTs repeats was the most frequent in all breeds analyzed, which is coincident with previous reports [16, 17, 28]. The homozygous genotype GT13 has been associated with natural resistance to *B. abortus* infection in cattle [29].

The identification of the allele 177 (14 GTs) in four (3.7 %) Argentinean Holstein animals was unexpected since previous studies had found an homogeneous genotype GT13 in a 100 % of the Holstein animals analyzed [16, 17]. This result may be attributed to the higher sensitivity and reproducibility of the capillary electrophoresis than the SSCP, and the larger number of Holstein cattle analyzed ($n = 109$) than previous studies. On the other hand, the *B. indicus* breeds and their crossbreds showed the highest variation in allelic, genotype and haplotype frequencies. As a consequence, the allele 181 was the second most frequent observed in *B. indicus* breeds and their crosses in agreement with Gonzalez et al. [16], although this high frequency is not reflected in homozygous animals. Curiously, Kumar et al. [28] detected the homogeneous genotype 175 fixed in *B. indicus* and *B. indicus* × *B. taurus* crossbred animals genotyped by SSCP.

The high frequency of the allele 175 in *B. taurus* breeds, particularly in Holstein breed, may be as a result of inbreeding, genetic drift and/or prolonged selective breeding as an effect of husbandry. Also, it may be as a result of the effect of positive natural selection since polymorphisms at the 3′UTR of the *SLC11A1* gene has been associated with the rate of transcription or the stability of the *SLC11A1* mRNA in cattle [27] and in water buffalo [4, 5]. On the other hand, the most heterogeneous haplotype and genotype observed in *B. indicus* breeds, as well for MS1 and MS2, may be as a result of the insignificant selective pressure that *B. indicus* breeds have had in India, where cattle have been bred almost completely randomly.

In conclusion, we carried out an individual genetic analysis of both microsatellites at the 3′UTR of the bovine *SLC11A1* gene in eight different cattle breeds mostly present in Argentina. A clear difference in the polymorphisms distribution, as well for MS1 and MS2, was observed between *B. taurus* (most homogeneous haplotype and genotype) and *B. indicus* breeds (most heterogeneous haplotype and genotype). This information let us design rational experiments to associate reported genotypes with resistant or susceptible phenotype. To date, bovine resistance or susceptibility to intracellular bacterial pathogens has been associated with the genotype of only one microsatellite present at the 3′UTR of the *SLC11A1* gene. Unfortunately, the results were not conclusive and also contradictory [6, 7, 21, 26–30]. Perhaps, if other elements were considered in the analysis (i.e., either microsatellites together or a genotype of the complete fragment of the 3′UTR *SLC11A1* gene containing both microsatellites), it would be possibly easier to establish a more robust association between cattle phenotype (resistant or susceptible to intracellular bacterial pathogens) with a particular genotype. These concepts were described in recently published papers that have found strong association between 3′UTR *SLC11A1* polymorphisms in water buffalos and Zebu cattle and resistance or susceptibility to *Brucella* or *Mycobacterium* infection [5, 8].

The association between genotypes and resistance or susceptibility to intracellular pathogens infection would be useful to carry out selection programs to improve natural resistance in cattle populations.

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