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Short Communication

A microsatellite study of bovine solute carrier family 11 a1 *(Slc11a1*) gene diversity in Mexico in relation to bovine tuberculosis

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Abstract

Bovine tuberculosis, caused by *Mycobacterium bovis*, is a disease of socio-economic and public health importance and of significance to international trade regulation. Allelic variants of several genes have been implicated in the genetic susceptibility to tuberculosis in some human populations, but little is known in cattle. We surveyed 34 European, 18 Asian, 20 Creole and 23 hybrid bovines for polymorphisms of the bovine solute carrier family 11 a1*(Slc11a1*) gene, formerly known as natural resistance associated macrophage protein (*Nramp1*), gene by typing the cattle using two microsatellite loci closely linked to this gene. The microsatellites used were 311-22, located at the 3' untranslated region (3' UTR) of the *Slc11a1* gene, and ARO28 situated about 0.6 cM upstream of the same gene Based on allele size in base pairs (bp) we determined five 311-223 locus variants (221, 223, 225, 227 and 229 bp) and 12 ARO28 loci. There was marked diversity and a very high level of heterozygosity in most of the cattle surveyed except the Europeans bovines and especially Holsteins in relation to the 3' UTR microsatellite locus.

Key words: bovine lineages, *Mycobacterium bovis*, microsatellite loci, *Nramp1*, *Slc11a1*, 3' UTR.

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Bovine tuberculosis (BTB), caused by *Mycobacterium bovis*, is a disease of socio-economic and public health importance and of significance to international trade regulation. Because *M. bovis* infects cattle and other domesticated and wild animals world-wide this disease is difficult to control or eradicate (Cousins 2001) and remains a major problem to the livestock industry since vaccination is not used for the control of the disease or as therapy for infected animals. Resistance to infectious diseases is often influenced by the genetics of the host and identifying the genes involved can lead to marker-assisted selection strategies which can contribute to controlling the spread of *M. bovis* infection by increasing the frequency of desirable genes. Molecular genetics studies have focused on understanding host susceptibility and resistance factors such as innate, specific and regulatory immune response-related genes and their relationship to infectious pathogens. Allelic variants of several genes have been implicated in the genetic susceptibility to tuberculosis in some human populations, but little is known in cattle (Estrada-Chavez *et al*. 2001).

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The solute carrier family 11 a1 gene *(Slc11a1*), formerly known as natural resistance associated macrophage protein (*Nramp1*), has been positionally cloned in mice and it has been established that mutations of this gene are responsible for susceptibility to a number of intracellular microorganisms, including *M. bovis*/BCG, *Salmonella typhimurium* and *Leishmania donovani* (Govoni *et al*. 1996). The *Slc11a1/Nramp1* gene encodes a pH-dependent proton/divalent cation antiporter localized in mature endosome or lysosome membranes, this protein specifically transports iron (Fe) from the cytosol into these acidic compartments and contributes to the generation of antimicrobial reactive oxygen intermediates (ROI) by the mononuclear phagocytic cell system via the Fenton and Haber-Weiss reaction (Goswami *et al*. 2001). Genetic variation in the *Slc11a1* locus affects susceptibility to tuberculosis in West African, Guinea-Conakry, Koreans, and Japa-

nese patients (Bellamy *et al*. 1998; Cervino *et al*. 2000; Ryu *et al*. 2000; Gao *et al*. 2000). Microsatellite polymorphism located in the 5' untranscribed promoter region, with at least 10 identified alleles, has been found to regulate different levels of *Slc11a1* expression by the juxtaposition of *trans*-acting elements which appear to differentially affect the functionality of this gene (Searle *et al*. 1999).

The bovine *Slc11a1* gene has been isolated as the homologue of the mouse and human genes (Feng *et al*. 1993; Horín *et al*. 1999), but the influence of this gene on bovine tuberculosis remains undetermined due to insufficient studies (Barthel *et al*. 2000). In order to study the association between *Slc11a1* and susceptibility to disease information must be obtained on the number and frequency of *Slc11a1* alleles in different lineages of bovines, such studies being best carried out using the microsatellite approach.

The purpose of the work described in this paper was to assess the *Slc11a1* polymorphism by microsatellites typing. We investigated frozen semen samples from the following 52 bovines: 34 European (*Bos taurus*) sires of 10 different breeds (16 Holstein-Frisian, 5 Simmental, 3 American Swiss, 2 European Swiss, 2 Jersey, 2 Charolais, 1 Red Angus, 1 Limousin, 1 Belgian Blue and 1 Blond d'Aquitaine); and 18 Asian (*Bos indicus*) sires (6 Brahman, 5 Gyr, 5 Guzerat, and 2 Nellore). Samples were obtained from the Mexican National Council for Animal improvement, Genetics and Reproduction (Consejo Nacional para el Mejoramiento Genetico y Reproduccion Animal (CONAMEGRA), Mexico City, Mexico). Blood samples were also collected from 20 pure Creole dairy cattle from 4 different herds of a geographically isolated population in the Mexican state of Nayarit and from 23 cross-breed hybrids (eight 1/2 Guzerat x 1/2 Creole and fifteen 3/4 Holstein-Frisian x 1/4 Brahman).

DNA was obtained from semen or blood samples according to previously described methods (Williams, 1997; Miller, 1988) and DNA was quantified by fluorimetry and the concentration adjusted to 20 ng/μL and its quality evaluated using ethidium bromide-stained agarose gels. To genotype the cattle we used two microsatellite loci closely-linked to the *Slc11a1* gene, the 311-22 microsatellite located at the 3' untranslated region (3' UTR) of the *Slc11a1* gene and the ARO28 microsatellite about 0.6 cM upstream of, but closely-linked to, the same gene (Kappes 1997). Primers were designed to amplify a 223 bp fragment containing the 311-22 microsatellite (GenBank U12862) (5' GGA ATG AGT GGG CAC AGT GGC 3'; 5' CCT TCC AGA ACT CCC TCT CCG 3'). A segment located between 256 and 276 bp containing the ARO28 microsatellite was amplified using previously described primers (GenBank L06143) (5' GAT TTC TCT AGT GAG TAA CA 3'; 5' TCT TGC CCA GAT GTT CTT AG 3').

The PCR mix (20 μL) consisted of 10 mM of Tris-HCl (pH 8.3), 50 mM KCl, 0.4 μM each primer, 200 μM each dNTPs, 1.25 mM of $MgCl_2$ for 311-22 and 2.0 mM of $MgCl_2$ for ARO28, 0.25 units of Taq-DNA-polymerase (Biogenica, Mexico City) and 40 ng of DNA. Amplification conditions for the 311-22 locus were 30 cycles of 94 °C for 30 s, 62 °C for 30 s and 72 °C for 30 s. Same conditions were used for the ARO28 microsatellite, with 56 °C at the annealing step.

The PCR products were verified using ethidium bromide-stained agarose gels and then 1 μL of each product was mixed with 0.5 μL of the TAMRA 350 molecular marker (Applied Biosystem) and 30 μL of deionized formamide. Microsatellite sizing was carried out with the ABIprism 310 automatic sequencer and fragments analyzed with the GeneScan 2.1 and Genotyper 2.0 (Applied Biosystem) using at least duplicate samples. Allele identification was based on size.

Gene frequencies and individual genotypes were determined by direct allele counting and observed and expected heterozygosity was calculated and a Chi-squared (χ^2) test with one degree of freedom (df) was performed to assess Hardy-Weinberg equilibrium (HWE) using the computer program GENEPOP 1.2 software (Rymond and Rousset 1995).

There have been no previous reports on *Slc11a1* (or the *Nramp1* locus as it was previously known) polymorphism frequencies in bovines from different lineages or breeds. Based on allele size in base pairs (bp) we determined a total of five 311-22 locus alleles and twelve ARO28 alleles, of which three 311-22 alleles (225, 227 and 229) and three ARO28 alleles (246, 280 and 288) have not, to the best of our knowledge, been previously described. The number of alleles found indicates that both loci were polymorphic.

The 311-22 and ARO28 frequencies and observed and expected heterozygosities (HS), and fixation indices (Fis) for each bovine population are shown in Table 1. The size of the 311-22 alleles ranged from 221 to 229 bp, of which the 223 allele was the most common in all groups at an average frequency of 57.4% and a range of from 37% to 65%, followed by the 227 and the 225 alleles with a frequency of about 16% for each. The 311-22 microsatellite 229 allele was present in Asian cattle and its hybrids (3/4 Holstein x 1/4 Brahman) but not in pure European or Creole cattle, while the 221 allele was absent from Creole cattle and the 227 allele was not found in Holsteins. The most frequent ARO28 alleles in almost all populations were alleles 260 (36.3%), 262 (21.6%) and 254 (13.6%), with the 246 and 256 alleles being found only in Asiatic cattle (Guzerat), the 270 allele only Holsteins and the 288 allele only in Jersey cattle.

Heterozygosity at the 311-22 locus exceeded 60% for Asian, Creole or Creole hybrid cattle but only about 20% for European cattle. Similarly, ARO28 heterozygosity ranged from 85% to 100% in Asian and Creole and Creole hybrid cattle but from only 31% to 35% for European cattle

Table 1 - Allele frequencies for ARO28 and 311-22 microsatellites within six bovines populations.

Locus size (bp)	European breeds[†]	Holstein	Asians breeds[‡]	Creole cattle	Guz x Cre[§]	Hol x Bra[¶]
ARO 28						
246	0	0	0.028	0	0	0
254	0	0	0.306	0.200	0.063	0.167
256	0	0	0.028	0	0	0
258	0.028	0	0.139	0.050	0.250	0.100
260	0.472	0.188	0.250	0.475	0.313	0.433
262	0.111	0.594	0.222	0.125	0.125	0.100
264	0.222	0.156	0.028	0.050	0.188	0.133
266	0.056	0.031	0	0.025	0	0.067
268	0.056	0	0	0.025	0	0
270	0	0.031	0	0	0	0
280	0	0	0	0.050	0.063	0
288	0.056	0	0	0	0	0
Ho[|]	0.389	0.313	0.889	0.850	1.000	0.400
Hs[††]	0.705	0.586	0.773	0.710	0.781	0.742
Fis[‡‡]	0.449	0.467	-0.150	-0.197	-0.280	0.461
n[§§]	18	16	18	20	8	15
X^{2}[¶¶]	3.629	3.489	0.405	0.776	0.627	3.188
311-22						
221	0.111	0.188	0.028	0	0.125	0
223	0.611	0.656	0.444	0.600	0.375	0.667
225	0.250	0.156	0.111	0.175	0.063	0.033
227	0.028	0	0.389	0.225	0.250	0.200
229	0	0	0.028	0	0.188	0.100
Ho	0.222	0.188	0.667	0.700	0.625	0.400
Hs	0.551	0.510	0.637	0.559	0.742	0.504
Fis	0.597	0.632	-0.046	-0.253	0.158	0.207
n	18	16	18	20	8	15
X^{2}	6.415*	6.391*	0.038	1.280	0.200	0.643

[†]European breeds: 5 Simmental, 3 American Swiss, 2 European Swiss, 2 Jersey, 2 Charolais, 1 Angus red variety, 1 Limousin, 1 Belgian Blue and 1 Blond d'Aquitaine; [‡]Asian breeds: 6 Brahman, 5 Gyr, 5 Guzerat, and 2 Nellore; [§]Guz x Cre: Guzerat x Creols; [¶]Hol x ZBra: Holstein Frisian x Brahman; [|]Ho:Heterozigosis observed; [††]Hs: Heterozigosis expected; [‡‡]Fis: Fixation index; [§§]n: bovines studied; [¶¶]X^{2}:Chi-squared test; *: Populations and locus deviated from HWE (1 df; $p < 0.05$).

and their hybrids (3/4 Holstein x 1/4 Brahman) (Table 1). Since the sample size was small no significant inferences could be made regarding genetic equilibrium. However, analysis showed conformity to HWE expectations ($X^{2}_{(1df)} = 3.84$; $p < 05$) in almost the entire population studied except for European cattle ($X^{2}_{(1df)} = 6.41$), including Holstein-Frisian ($X^{2}_{(1df)} = 6.39$) for the 311-22 *locus* (Table 1).

In the 95 cattle studied we found a total of 55 different genotypes, which we designated using the ARO28 allele first and the 311-22 allele second keeping the order in the genome (Table 2). Most of the genotypes had frequencies ranging from 1 to 4% but the homozygous $ARO28^{260/260}$-$311\text{-}22^{223/223}$ and $ARO28^{262/262}$-$311\text{-}22^{223/223}$ genotypes were over-represented in European (38%) and Holstein-Frisian (50%) cattle, although these genotypes were almost absent from the other lineages. In contrast to European and Holstein-Frisian cattle, but as expected, we found that Asian, Creole and Creole/Asian hybrid cattle were mainly heterozygous for any genotypes demonstrated for them. The low *Slc11a1* diversity may be a consequence of intense selection reduced effective population number and inbreeding, which has been previously reported for dairy cattle (Lindhe *et al*. 1998).

Taking into account of previously reported sequences and proposed designations for bovine *Slc11a1* (*Nramp1*) alleles the 311-22 microsatellite 221 allele detected by us corresponds to dinucleotide GT_{10} (*Slc11a1* or *Nramp1.1*) originally found in the European Czech Red and Black Pied breeds (Horin *et al*. 1999). Likewise, our

Table 2 - Genotypes identified in the entire population.

Genotype	*f*[†]	Linage: breed	Genotype	*f*	Linage: breed
he/he[‡]			ho/ho[¶]		
254/256-223/227	1	Asian: Guzerat	254-223	1	Asian: Nellore
254/258-223/227	2	Asian: Gyr, Brahman	254-227	1	Hybrid: Hol x Bra
254/260-221/223	1	Hybrid: Hol x Bra[††]	260-223	9*	European: Blond d'Aquitane, Simmental (2), Jersey, Holstein (2); Hybrid: HolxBra (2); Creol
254/260-223/225	1	Creol	260-229	1	Hybrid: Hol x Bra
254/260-223/227	4	Asian:Brahman; Creol (3)	262-221	2	European: Holstein (2)
254/260-225/229	1	Asian:Brahman	262-223	7*	European: Holstein (6), American Swiss
254/262-221/223	1	Asian: Gyr	264-223	1	Hybrid: HolxBra
254/262-223/227	3	Asian: Gyr; Creol (2)	264-225	3	European: American Swiss, Limousin
254/264-223/227	2	Asian: Nelore; Hybrid: Hol x Bra	288-221	1	European: Jersey
258/260-221/225	1	Hybrid: Guz x Cre[‡‡]			
258/260-223/225	1	Creol	he/ho[|]		
258/260-223/227	2	Asian:Brahman; Hybrid: Guz x Cre			
258/262-223/227	1	Asian:Brahman	246/260-223	1	Asian:Guzerat
260/262-221/223	1	European: Holstein	254/260-223	4	Creol (2); Hybrid: Hol x Bra (2)
260/262-223/225	1	Creol	254/262-225	1	Asian:Gyr
260/262-223/227	2	Creol; Asian: Brahman	258/260-223	2	Creol; Hybrid: Guz x Cre
260/262-223/229	1	Hybrid: Guz x Cre	258/262-223	2	Asian: Gyr; Hybrid: Hol x Bra
260/264-221/223	1	European: Simmental	258/264-223	1	European: Charolais
260/280-223/225	1	Creol	258/264-229	1	Hybrid: Guz x Cre
262/264-221/223	1	European: Holstein	260/262-223	4	Creol; European: Charolais; Holstein; Hybrid: Hol x Bra
262/264-223/227	2	Hybrid: Guz x Cre; Hol x Bra	260/262-227	2	Asian:Guzerat (2)
264/280-225/227	1	Creol	260/264-223	2	Creol; European:Simmental
266/268-223/227	1	Creol	262/268-223	1	European: Belgian Blue
			264/266-225	2	European: European Swiss, Holstein
ho/he[§]			264/270-225	1	European: Holstein
			264/280-227	1	Hybrid: Guz x Cre
258-223/227	1	Hybrid: Hol x Bra	266/268-225	1	European: Red Angus
260-221/223	1	European: Simmental			
260-223/225	3	European: European Swiss; Hybrid: Hol x Bra; Creol			
260-223/227	1	European: American Swiss			
260-223/229	1	Hybrid: Hol x Bra			
260-225/227	2	Creol; Asian: Guzerat			
264-223/225	1	European: Holstein			
266-223/227	1	Hybrid: Hol x Bra			

[†]*f*: Frequency of animals with this genotype; [‡]he/he: Heterozygous for both *loci*; [§]ho/he: Homozygous for ARO28 *locus* and Heterozygous for 311-22 *locus*; [¶]ho/ho: Homozygous for both *loci*; |he/ho: Heterozygous for ARO28 *locus* and homozygous for 311-22 *locus*; [††]Hol x Bra: Holstein Frisian x Brahman; [‡‡]Guz x Cre:Guzerat x Creols; *: The most frequents genotypes /: Divide alleles; -: Divide *loci*.

311-22 microsatellite 223 allele corresponds to the GT_{11} dinucleotide (*Slc11a1* or *Nramp1.2*) previously reported in a bovine hybrid obtained by crossing *B. taurus* (Angus, Jersey and Hereford) and with 1/8th *B. indicus* American Brahman breeds (Feng *et al*. 1993). The 311-22 microsatellite 223 allele and the 225 allele (GT_{12}: *Slc11a1* or *Nramp1.3*) were over-represented in all the cattle studied by us, while the 311-22 microsatellite 221 allele was not found in Creole cattle. Similarly, the 311-22 microsatellite 227 allele (GT_{13}: *Slc11a1* or *Nramp1.4*) and the 229 allele (GT_{14}: *Slc11a1* or *Nramp1.5*) were not found by us in Holstein-Frisian cattle.

Barthel *et al* (2001) have shown that transfected murine macrophages can be used to measure the functionality of two bovine *Slc11a1* alleles, one of which correlates with enhanced expression and the *in vitro* control of *Brucella abortus* replication but not with *Salmonella enterica*. Searle *et al*. (1999) have reported that polymorphism of the human *Slc11a1* promoter (which encodes a Z-DNA forming di-nucleotide repeat) affects the level of gene expression and correlates with autoimmune versus infectious disease susceptibility in different human populations. Similarly, Beaumont *et al*. (2003) have shown that a polymorphic microsatellite located close to the 3' untranslated region of the *Slc11a1* gene is a useful tool in co-segregation studies aimed at elucidating the involvement of this gene in the control of sheep and fowl resistance to *Salmonella* infection.

Most of the cattle studied by us are sires used by one of the major semen suppliers in Mexico (CONAMEGRA) and our work shows that polymorphism in Mexican cattle populations has not been sufficiently studied. Microsatellite polymorphisms at the *Slc11a1* (*Nramp1*) locus could be very useful in association studies to test the relationship of this gene with resistance to Bovine tuberculosis.

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References

Barthel R, Feng J, Piedrahita JA, McMurray DN, Templeton JW and Adams LG (2001) Stable transfection of the bovine NRAMP1 gene into murine RAW264.7 cells: Effect on *Brucella abortus* survival. Infection and Immunity 69:3110-3119.

Barthel R, Piedrahita JA, McMurray DN, Payeur J, Baca D, Suarez Guemes F, Perumaalla VS, Ficht TA, Templeton JW and Adams LG (2000) Pathologic findings and association of *Mycobacterium bovis* infection with the bovine NRAMP1 gene in cattle from herds with naturally occurring tuberculosis. American Journal of Veterinary Research 61:1140-1144.

Beaumont C, Protais J, Pitel F, Leveque G, Malo D, Lantier F, Plisson-Petit F, Colin P, Protais M, Le Roy P, Elsen JM, Milan D, Lantier I, Neau A, Salvat G and Vignal A (2003) Effect of two candidate genes on the Salmonella carrier state in fowl. Poultry Science 82:721-726.

Bellamy R, Ruwende C, Corrah T, McAdam KP, Whittle HC and Hill AV (1998) Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. New England Journal of Medicine 338:640-644.

Cervino AC, Lakiss S, Sow O and Hill AV (2000) Allelic association between the NRAMP1 gene and susceptibility to tuberculosis in Guinea-Conakry. Annals of Human Genetics 64:507-512.

Cousins DV (2001) *Mycobacterium bovis* infection and control in domestic livestock. Revue Scientifique et Technique 20:71-85.

Estrada-Chavez C, Pereira-Suarez AL, Meraz MA, Arriaga C, Garcia-Carranca A, Sanchez-Rodriguez C and Mancilla R (2001) High-level expression of NRAMP1 in peripheral blood cells and tuberculous granulomas from *Mycobacterium bovis*-infected bovines. Infection and Immunity 69:7165-7168.

Feng J, Li Y, Hashad M, Schurr E, Gros P, Adams LG and Templeton JW (1996) Bovine natural resistance associated macrophage protein 1 (NRAMP1) gene. Genome Research 6:956-964.

Gao PS, Fujishima S, Mao XQ, Remus N, Kanda M, Enomoto T, Dake Y, Bottini N, Tabuchi M, Hasegawa N, Yamaguchi K, Tiemessen C, Hopkin JM, Shirakawa T and Kishi F (2000) Genetic variants of NRAMP1 and active tuberculosis in Japanese populations. International Tuberculosis Genetics Team. Clinical Genetics 58:74-76.

Goswami T, Bhattacharjee A, Babal P, Searle S, Moore E, Li M and Blackwell JM (2001) Natural-resistance-associated macrophage protein 1 is an H+/bivalent cation antiporter. Biochem Journal 354:511-519.

Govoni G, Vidal S, Gauthier S, Skamene E, Malo D and Gros P (1996) The Bcg/Ity/Lsh *locus*: Genetic transfer of resistance to infections in C57BL/6J mice transgenic for the NRAMP1 Gly169 allele. Infection and Immunity 64:2923-2929.

Horin P, Rychlik I, Templeton JW and Adams LG (1999) A complex pattern of microsatellite polymorphism within the bovine NRAMP1 gene. European Journal of Immunogenetics 26:311-313.

Kappes SM, Keele JW, Stone RT, McGraw RA, Sonstegard TS, Smith TP, Lopez-Corrales NL and Beattie CW (1997) A second-generation linkage map of the bovine genome. Genome Research 7:235-249.

Lindhe B and Philipsson J (1998) Conventional breeding programmes and genetic resistance to animal diseases. Revue Scientifique et Technique 17:291-301.

Miller SA, Dykes DD and Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 6:1215.

Ryu S, Park YK, Bai GH, Kim SJ, Park SN and Kang S (2000) 3' UTR polymorphisms in the NRAMP1 gene are associated with susceptibility to tuberculosis in Koreans. The International Journal of Tuberculosis and Lung Disease 4:577-580.

Searle S and Blackwell JM (1999) Evidence for a functional repeat polymorphism in the promoter of the human NRAMP1 gene that correlates with autoimmune versus infectious disease susceptibility. Journal of Medical Genetics 36:295-299.

Williams JL, Usha AP, Urquhart GD and Kilroy M (1997) Verification of the identity of bovine semen using DNA microsatellite markers. The Veterinary Record 140:446-449.

Internet Resources

Raymond M and Rousset F (1995) GENEPOP (VERSION 1.2) population genetics software for exact test and ecumenism. The Journal of Heredity 86:248-9. Available in: http://wbiomed.curtin.edu.au/genepop/.

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