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Bovine *SLC11A1* 3′ UTR SSCP genotype evaluated by a macrophage *in vitro* killing assay employing a *Brucella abortus* strain

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Summary

The 3′ untranslated region (3′ UTR) of the bovine natural resistance-associated macrophage gene (*NRAMP1* or *SLC11A1*) was genotyped in Colombian Creole Blanco Orejinegro (BON) (*Bos taurus*) (n = 140) and Zebu Brahman (*Bos indicus*) (Z) (n = 20) cattle and their crosses (BON × Zebu Brahman [B × Z] [n = 10]; Zebu Brahman × BON [Z × B] [n = 10]), and in animals from a Holstein × BON (H × B) (n = 10) cross. Direct sequencing and single-strand conformation polymorphism analysis (SSCP) helped in detecting the polymorphic behaviour. The association between resistance to brucellosis infection and SSCP genotype was evaluated using a macrophage *in vitro* killing assay employing a virulent *Brucella abortus* strain. The 3′ UTR (GT) repeated polymorphism was genotyped and its association with resistance to brucellosis was evaluated. When all breeds were grouped, a high frequency in the homozygote GT_{12} (AA genotype) (0.823) and a very low frequency in the homozygote GT_{10} (BB genotype) (0.047) were detected. The BON (0.963), Z × B (0.60) and H × B (1.00) cattle showed high GT_{12} allele frequencies, unlike that seen for the B × Z and Zebu cattle (0.3002 and 0.218, respectively). The GT_{10} allele was only found in the Zebu cattle (0.391). A significant association ($p < 0.001$) was found between the *B. abortus* macrophage *in vitro* killing assay phenotypes and the bovine *SLC11A1* 3′ UTR genotypes, which suggests that the A allele may be associated with resistance. Because only nine animals had the BB genotype, the results require some confirmation in more extensive populations.

Introduction

Natural susceptibility/resistance to brucellosis has been identified in cattle by using *in vivo* and *in vitro* challenge trials (Harmon *et al.* 1985, 1989; Price *et al.* 1990). Monocyte-derived or mammary macrophages from cattle genetically resistant to *in vivo* challenge with *Brucella abortus* (strain 2308) are better able to prevent the intracellular replication of this bacterium (Harmon *et al.* 1989; Campbell & Adams 1992), as well as that of *Salmonella dublin* and *Mycobacterium bovis* BCG in mouse (Radzioch *et al.* 1991, 1994). Natural resistance to *B. abortus* (based on recovery after an induced bacterial challenge during pregnancy) is heritable. Classic breeding studies suggest that the resistant phenotype is under multigenic control. (Templeton *et al.* 1990).

Macrophage protein 1 (Nramp1) – associated with natural resistance – informally known as solute carrier 11A1 (slc11a1), has been reported to confer resistance or susceptibility to *M. bovis*, *Salmonella typhimurium* and *Leishmania donovani* in mouse (*Mus musculus*), depending on the allele inherited. Gly in codon 169 of the mouse Nramp protein is invariably associated with resistance, but its substitution for Asp produces a susceptible phenotype (Blackwell *et al.* 1991, 1995; Vidal *et al.* 1993, 1995; Govoni *et al.* 1995, 1996). However, Sathiyaseelan *et al.* (2000) found that macrophages from resistant mice did not prevent the intracellular growth of *B. abortus* strain 2308 efficiently than those from susceptible mice, either with or without interferon gamma (IFN-γ) activation or iron supplementation.

In cattle, the *SLC11A1* gene sequence was first reported by Feng *et al.* (1996). Later, Horin *et al.* (1999) identified polymorphisms because of variations in the number of GT dinucleotide repeats in the 3′ untranslated region (3′ UTR; positions 1781–1804) in Czech red pied and Czech black pied bovine breeds. Based on single-strand conformation polymorphism analysis (SSCP), these authors also found a strong correlation between the *SLC11A1* alleles and natural resistance to *B. abortus* ($p = 0.0089$). Barthel *et al.* (2001), who worked with the RAW264.7 cell line transfected with bovine *SLC11A1*, also found that polymorphism within the 3′ UTR of this gene affected its expression as well as the prevention of *B. abortus* replication *in vitro*, but not that of *S. dublin*. In addition, using DNA sequencing of different gene regions, Ables *et al.* (2002) detected polymorphism in *SLC11A1* in different cattle and buffalo breeds, identifying two nucleotide substitutions in intron 4, three in exon V and ten in intron 5. However, these polymorphisms were not associated with resistance to bacterial infection. More recently, Coussens *et al.* (2004) reported the nearly complete structure of bovine *SLC11A1*, including the size and position of 13 introns in the *SLC11A1* coding sequence, as well as the DNA sequence of intron–exon junctions.

Functional studies have shown that *SLC11A1* regulates antimicrobial activity through tumour necrosis factor-alpha (TNFα). This activity depends on the generation of intermediate nitrogen products (Gruneheid *et al.* 1997; Skamene *et al.* 1998; Ables *et al.* 2001). Barthel *et al.* (2001) reported no difference in nitric oxide production when treating the macrophage cell line (Bcg^s) RAW264.7, *SLC11A1*-resistant or -susceptible allele transfected, stimulated with lipopolysaccharide (LPS) and/or IFN-γ. These findings suggest that resistance is controlled by the action of several genes, complicating the genetic characterization of segregation phenotypes showing natural resistance/susceptibility to bovine brucellosis.

The tropical Colombian Blanco Orejinegro (BON) Creole cattle breed has been developed over the last 500 years in the mountain areas of Colombia. These cattle are of the Criollo type, characterized by a white coat and black skin. This breed is purported to be highly fertile, resistant to mites and is known for its longevity, docile nature and ability to cross with *Bos indicus*. However, no systematic study has been undertaken to determine the genetic mechanism of its natural resistance to infection, or whether this may be related to resistance to mites or other diseases.

The aim of the present study was to determine the association of the (GT)n microsatellite polymorphism in the 3′ UTR region of *SLC11A1* in BON and Zebu cattle and their crosses, with resistance to *B. abortus* using a macrophage *in vitro* killing assay.

Materials and methods

Blood and serum samples were obtained from BON (a *Bos taurus* breed) (n = 138) cattle, the nucleus of the Zebu breed (n = 23) and the BON × Zebu (n = 10), Zebu × BON (n = 10), and Holstein × BON crosses (n = 11) maintained at the El Nus Research Centre's Germplasm Bank (San Roque, Antioquia, Colombia). All animals were negative to brucellosis, evaluated by three enzyme-linked immunosorbent assay (ELISA) serological tests.

Five millilitre of peripheral blood was taken for leukocyte separation. Genomic DNA was extracted from these cells and purified according to the procedure described by Sambrook *et al.* (1989). The *SLC11A1* sequence was obtained from GenBank (accession no. U12862). A region containing the polymorphism reported by Horin *et al.* (1999) was then located in the 3′ UTR (nucleotide positions 1745–1955), consisting of a G:T transversion at nucleotides 1782–1785 at the 5′ end of the GT repetition. The oligonucleotide primer sequence was determined using the polymerase chain reaction (PCR) routine option provided in the Biowire Jellyfish (2000–2001) programme, based on the previously published sequence (Feng *et al.* 1996). The 212-bp PCR product obtained was used for detecting mutations by SSCP analysis.

PCR was performed in 25-μl reaction volumes that included 200 mM of dNTP (Promega, San Diego, California, USA), 0.5 mM of each oligonucleotide (Gibco BRL, Rockville, Maryland, USA), 2.5 μl 10×

buffer (500 mM of KCl, 100 mM of Tris HCl, pH 9.0 and 1% X-100 Triton), 1 U of Taq polymerase (Promega), 2.5 μl of $MgCl_2$ (25 mM) (Promega) and 20 ng of genomic DNA. All amplifications were performed in a thermocycler (MJ Research, Waltham, Massachusetts, USA). The first denaturing stage was carried out at 94°C for 5 min, followed by 30 cycles at 94°C for 60 s, 60°C for 60 s, and a final extension step at 72°C for 5 min. Once the amplification reaction was complete, the samples were subjected to electrophoresis on a 2% agarose gel (Sigma, St. Louis, Missouri, USA) in TBE buffer (0.9 M Tris base, 0.09 M boric acid, 2.5 mM EDTA, pH 8.0) at 80 V for 90 min, and visualized with ethidium bromide (Sigma; 1 μg/ml).

SSCP analysis was performed with 5 μl of the denatured PCR products that were loaded onto a 6% non-denatured polyacrylamide gel plus 5% glycerol (acrylamide:Bis 19:1, 5% glycerol [Sigma], 10× TBE, 200 μl of ammonium persulphate [Sigma] and 20 μl of Temed [Bio-Rad, Mississauga, Ontario, Canada]) and run for 16 h at 4 W. The genotypes were visualized by silver staining (Wallace 1997).

To confirm the sequences of the amplification products, these were purified in Wizard micro columns (Promega) and then re-amplified sequentially using the Big Dye Terminator technique (Perkin Elmer, Norwalk, Connecticut, USA). They were then precipitated with ethanol, dried in a vacuum and sequenced in an automatic sequencer (ABI 310; Perkin Elmer). They were then edited and analysed using Chromas 1.62 software (Technelysium Pty Ltd., Helensvale, Queensland, Australia).

Cell preparation

Macrophages were cultured following the method of Price *et al.* (1990). Briefly, 400 ml of blood from each animal was collected in a blood bag (Baxter, Bloomington, Indiana, USA) containing citrate phosphate dextrose adenine as an anticoagulant. This blood was placed in 50-ml centrifuge tubes which were spun at 1500 ***g*** for 20 min to extract the white blood cells. These were then diluted in phosphate buffer saline citrate (PBSc), poured into 20 ml of Histopaque (Sigma Chemicals) (density 1.077) and centrifuged again at 1500***g*** for 20 min for cleansing.

Interface cells, containing an enriched monocyte and lymphocyte population, were suspended in 20 ml of RPMI 1640 medium supplemented with 20% heat-inactivated foetal bovine serum, 4 mM of L-glutamine, 10 μl/ml of non-essential amino acid solution, 1 mM of sodium pyruvate, 100 μg/ml of streptomycin and 100 IU/ml of penicillin in a 250-ml tissue culture flask (Corning, New York, USA). Mononuclear cells were allowed to adhere to the surface for 24 h at 37°C in a 7.5% CO_2 atmosphere. All non-adherent cells were removed. Fresh medium was then added to the flask and the cells were incubated for another 24 h. Hanks' balanced salt solution (20 ml) without calcium or magnesium was then added to the cultures for 12 min to detach the cells. They were then centrifuged at 75***g*** for 5 min and suspended at a concentration of 5×10^5 cells per millilitre in supplemented RPMI 1640 medium containing penicillin and streptomycin. One hundred microlitre of this suspension was added to each of the six wells in a flat-bottomed 96-well microdilution plate. The monocytes were allowed to mature into macrophages in culture for an additional 5 days at 37°C in a 7.5% CO_2 atmosphere, according to Price *et al.* (1990).

Bacterial strain and growth conditions

The *B. abortus* Cumbal 1 strain (isolated from a field case) from the CORPOICA germplasm bank (Colombia) was maintained at 37°C in a microanaerobiosis chamber (Oxoid, Hampshire England) for 4 days in a selective medium for *Brucella* (Oxoid) supplemented with 5% horse serum (Gibco BRL) and 5% dextrose (Sigma Chemicals).

Bacterial opsonization

The bacteria were washed once with PBS without calcium or magnesium (ICN, Costa Mesa, USA) and centrifuged at 1500***g*** for 10 min. The pellet obtained was diluted in PBS and the absorbance measured in a spectrophotometer; dilution was continued until a reading of 1110 at a wavelength of 650 nm was obtained. Twenty-five microlitre of anti-*Brucella* serum (heat-inactivated) was then added to 50 μl of the bacterial dilution (about 500 000 bacteria) and placed in a shaking water bath at 37°C for 45 min.

Macrophage infection

Seventy-five microlitre of opsonized bacterial mixture was added to each well of a 96-well plate containing 5×10^4 macrophages per well; this mixture was centrifuged at 170***g*** for 10 min and then incubated for 1 h at 37°C in a 5% CO_2 atmosphere. RPMI-streptomycin medium at a final concentration of 13.5 mg/ml was added to eliminate extracellular bacteria before a further incubation at 37°C for

30 min. The medium was later set aside from the wells and 200 μl of RPMI medium added. Ten minutes later, 100 μl of this medium was skimmed off (to extract any streptomycin residue and dead bacteria) and another 100 μl RPMI medium supplemented with 5% autologous heat-inactivated serum (from the same animal) was added.

To obtain results for time zero (T0 h), the RPMI medium was immediately extracted from the wells and 100 μl of deionized sterile cold water was added for 10 min. This solution was used to make 1/5, 1/10 and 1/50 dilutions; 100 μl of each dilution was plated in a petri dish (in triplicate) containing selective agar for *B. abortus* (Oxoid). The petri dishes were maintained at 37°C in a 5% CO_2 atmosphere. The same serial dilutions were made at T24 h and T48 h; again, 100 μl of each was plated in a petri dish (in triplicate) containing agar selective for *B. abortus* (Oxoid). Colony-forming units (CFU) were counted 4 days later and termed the 'number of bacteria at time zero' (NBT0), 'number of bacteria at time 24 h' (NBT24) and 'number of bacteria at time 48 h' (NBT48). The resistance index was estimated as the square root (necessary to obtain normal distribution of residuals and phenotypic values, which was evaluated using Proc Univariate Normal of SAS) CFU ratio percentage at 24 and 48 h (with respect to T0 h) [the survival measure may be defined by SOBi = sqrt(100*NBTi/NBT0) where I = 24 or 48]. Higher values show a poorer response to infection.

Analysis of information

The number of bacteria (*B. abortus*) was counted as Brucellosis-resistant indices at T 0 h (NBT0). Survival was measured at 24 (SOB24) and 48 h (SOB48). All samples were evaluated in triplicate. All values were log-transformed to obtain a normal distribution for further analysis.

An animal model with the additive genetic relationship matrix was employed for the association analyses between marker genotypes and phenotypic traits. The SOB24 and SOB48 traits were analysed using Proc Mixed (SAS, North Carolina, USA; 1985) with the following linear animal model:

$$Y_{ijk} = \mu + G_{Ti} + B_j + a_{ijk} + e_{ijk}.$$

The GT_i [the effect in the ith genotype (AA, AB, BB)] and B_j [the effect in the jth breed (BON, Z, Z × B, B × Z and H × B)], were regarded as fixed and random effects including the additive genetic (a) effect of the animal and a residual effect. In all populations, additive genetic effects were estimated by pairwise comparison of the two homozygous genotypes, and the dominance effects were calculated as the deviation of the heterozygote effect from the average of the two homozygous genotypes. The estimated effects were tested for significance using the t-test. The number of pedigree data was 28 sires and 139 dams, where some of them had a phenotype record or had sons with a phenotype and genotype record and belonged to the pedigree dataset with 3230 animals.

Results and discussion

For this experiment 192 animals, which were evaluated initially using a macrophage *in vitro* killing assay employing a virulent *B. abortus* strain, were randomized and then genotyped for the Slc11A1 3′ UTR marker. However, the allelic frequency for this marker has been found to be in disequilibrium of Hardy–Weinberg in *Bo. taurus* and *Bo. indicus*. Similar results have been to reported by Kumar *et al.* (2005) and Horin *et al.* (1999). Paixao *et al.* (2005) also has reported a marked difference in the allele frequency between the Zebu and Holstein breeds, where Holsteins had a homogeneous genotype, with 100% of individuals having the same genotype, similar to our results; in contrast, the Zebu breed had the most heterogeneous genotype, with all allele combinations. Conversely, Kumar *et al.* (2005), genotyped a total of 100 samples (comprising 50 random samples of each breed) and they revealed that all animals had the same genotype. These results indicate strong disequilibrium of the allele frequencies among breeds, and for this reason it was not possible to select a population with a similar size for each genotype in the two breeds and crosses, which must also be phenotypically evaluated by *in vitro* test. This is the reason for insufficient information for a robust comparison of the genotypes, but our results suggest aspects that could be the subject of future analysis.

Only two alleles $(GT)_{12}$ or A and $(GT)_{10}$ or B and three genotypes AA, AB and BB were detected (Table 1). Figure 1 shows the *SLC11A1* 3′ UTR sequence for the homozygous genotypes AA and BB, and for the heterozygotes AB. When all breeds were grouped, a high frequency was observed for AA (0.823) and a very low frequency for BB (0.047). Marked differences were detected between breeds: the BON breed had a high AA frequency (0.963), similar to the Zebu × BON (0.60) and Holstein × BON crosses (1.00). However, a low AA frequency was observed in the BON × Zebu and Zebu

Table 1 Genotype and allele frequencies of *SLC11A1* 3′ untranslated region polymorphism in Colombian Blanco Orejinegro Creole cattle (BON), Zebu cattle and their crosses (numbers in parentheses)

	Genotype frequencies			Allele frequencies	
Breed/ cross	AA	AB	BB	A	B
Bon × Zebu	0.300 (3)	0.700 (7)	0 (0)	0.65	0.35
BON	0.963 (133)	0.037 (5)	0 (0)	0.9815	0.0185
Zebu × Bon	0.600 (6)	0.400 (4)	0 (0)	0.80	0.20
Zebu	0.218 (5)	0.391 (6)	0.391 (9)	0.4135	0.5865
Holstein × Bon	1.00 (11)	0 (0)	0 (0)	1.0	0
Total	0.823 (158)	0.130 (25)	0.470 (9)	0.888	0.112

populations (0.30 and 0.218, respectively). In contrast, the BB genotype was only found at a relatively high frequency in the Zebu population (0.391) (Table 1). This difference between observed frequencies for the A allele in Z × B and B × Z cross-breds may be because of low sample size, which in spite of being random may have individuals (sires) with low frequencies in this allele. Similar results have been reported by other authors. Horin *et al.* (1999) found the $(GT)_{12}$ sequence reported by Feng *et al.* (1996) to show a high frequency (0.72) in *B. taurus*. However, the sequence corresponding to allele B of the present study was identified in 28% for that population – far above that found in the study reported by Horin *et al.* (1999). Paixao *et al.* (2005) also reported a high frequency (100%) for the $(GT)_{12}$ allele in Holstein cattle.

SLC11A1 has been presented as one of the major candidate loci for natural resistance to bovine brucellosis (Feng *et al.* 1996; Horin *et al.* 1999; Barthel *et al.* 2001). However, no report has really proved the effect of gene variants on the bacterial survival *in vivo* or *in vitro*. In our dataset, the number of individuals with BB genotype may seem insufficient; this may provide a less reliable comparison between AA, AB and BB genotypes (because of random sampling, this population displayed a low frequency of allele B). Nevertheless, these results could suggest a significant effect of the SLC11A1 3′ UTR genotype on phagocytosis and bacterial survival within macrophages following *in vitro* challenge with *B. abortus*. Both the AA and AB genotypes conferred similar resistance ($p > 0.05$); but, the resistance conferred by AA (19.00 ± 1.66 and 19.77 ± 1.87 for SOB24 and SOB48, respectively) and AB (18.91 ± 4.39 and 18.88 ± 4.50 for SOB24 and SOB48, respectively) genotypes was over 10 times greater than that conferred by the BB genotype (78.07 ± 33.86 and 65.52 ± 28.64 for SOB24 and SOB48, respectively) ($p > 0.01$) (Table 2.). It may be necessary to explore a more extensive population to find a uniform distribution of genotypes to confirm our results, but it may also be important to search for other gene variants within coding regions with functional mutations.

The BB genotype showed the highest phagocyte efficiency at T0 h, but the AA and AB genotypes were over three times more successful in preventing

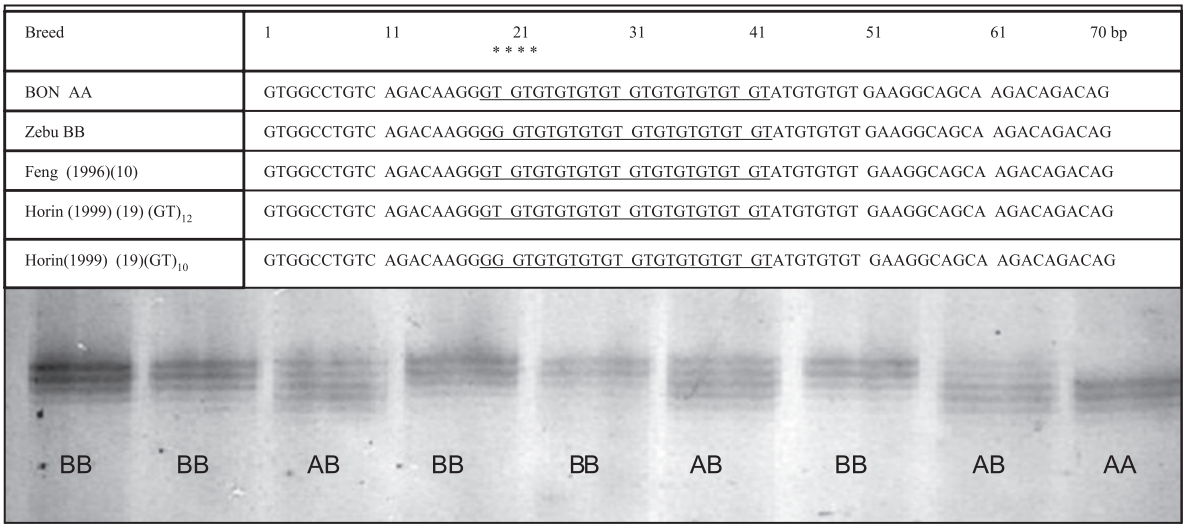

Figure 1 Sequence of the 3′UTR fragment (70bp). The AA genotype is indicated by the GT_{12} repetition. The BB genotype is indicated by the GT_{10} repetition. Also sequence reported by Feng et al., (1996) and Horin., (1999) and down representative results of SSCP analysis showing each genotype.

Table 2 Genotype effect on the variables survival was measured at 24 (SOB24) and 48 h (SOB48)

		SOB24					SOB48				
Genotype	n	Mean	SEM	SD	Min	Max	Mean	SEM	SD	Min	Max
AA	163	19.00 b	1.66	21.19	0.11	88.07	19.77 b	1.87	23.87	0.33	101.2
AB	25	18.91 b	4.39	21.95	5.09	57.33	18.88 b	4.50	22.50	1.08	69.21
BB	9	78.07 a	33.90	101.58	10.00	208.75	65.52 a	28.64	85.92	5.83	189.00

Values of means with different letters are significantly different ($p < 0.05$ for values transformed by the square root of the value) (SEM, standard error of mean; SD, standard deviation; Min, minimum; Max, maximum).

bacterial growth, both at 24 and 48 h. A direct relationship therefore exists between genotypes and the possession of macrophages that can kill the bacteria after entering the phagosome.

A significant difference between breeds was also found. The Zebu × BON crosses always showed lower SOB24 and SOB48 values (9.15 ± 0.64 and 8.16 ± 1.20, respectively) compared with all other breeds ($p < 0.05$), except the BON breed (17.87 ± 1.62 and 17.71 ± 1.79, respectively) ($p < 0.05$), BON × Zebu (34.15 ± 5.48 and 40.34 ± 7.39) ($p < 0.001$) and Zebu (39.61 ± 12.78 and 46.57 ± 14.48) for SOB24 and SOB48, respectively ($p < 0.001$) (Table 3). No significant interaction between the two sources of variation (genotype and breed) ($p > 0.05$) was seen. In the BON × Zebu, the homozygous AA genotype had higher SOB24 and SOB48 values (47.10 ± 6.99 and 59.54 ± 8.96, respectively) than the heterozygous AB genotype (28.60 ± 5.96 and 32.10 ± 7.72, respectively).

With respect to the AA genotype, the BON animals showed significantly lower ($p < 0.05$) SOB24 and SOB48 values (17.82 ± 1.58 and 18.37 ± 1.74, respectively) than the Zebu breed (46.15 ± 19.58 and 39.23 ± 24.92, respectively). Furthermore, the BON heterozygotes showed significantly ($p < 0.05$) lower SOB24 and SOB48 values (5.63 ± 0.34 and 4.19 ± 2.11, respectively) than the Zebu heterozygotes (23.09 ± 10.03 and 20.74 ± 8.10, respectively). The BB genotype was found only in the Zebu breed, and showed significantly ($p < 0.01$) higher NBT0, SOB24 and SOB48 values (650.11 ± 207.2, 78.07 ± 29.87 and 65.52 ± 25.216, respectively) than the other genotypes. The animal random effect was significant ($p < 0.001$) and the residual variance was close to 20% of total variance and, if due to the unbalanced sample, it could suggest that the animal genetic background would affect trait variation to a great extent.

Some experimental issues of the present study were based on that by Price *et al.* (1990), who compared *in vitro* killing assay and *in vivo* challenge: in the first case reported data were a survival percentage and a 100% cut-off point was used in bacterial survival to define restrictive phenotypes (resistance individuals). With this cut-off, the *in vitro* assignment of monocyte-derived macrophage function showed a strong correlation ($r = 0.82$) with the postchallenge phenotype classification. However, Qureshi *et al.* (1996) used a 70% cut-off value. This correlated perfectly with the number of animals designated to categories by *in vivo* challenge. The *in vitro* bactericidal assay can therefore be used to establish the phenotype of naturally resistant or susceptible cattle, and the assay supports variation as a result of genetic background (Qureshi *et al.* 1996).

The significantly ($p < 0.05$) higher values for all traits in crosses in which the dam was a Zebu compared with those in which the sire was a Zebu could indicate an imprinting effect (an epigenetic

Table 3 Breed effect on the variables survival was measured at 24 (SOB24) and 48 h (SOB48)

		SOB24					SOB48				
Breed/cross	n	Mean	SEM	SD	Min	Max	Mean	SEM	SD	Min	Max
BON × Zebu	10	34.15 a	5.48	17.33	7.54	61.10	40.34 a	7.39	23.37	3.60	77.44
BON	138	17.87 b	1.62	19.03	0.11	88.27	17.71 c	1.79	21.03	0.33	101.2
Zebu × BON	10	9.15 c	0.64	2.02	6.40	12.44	8.16 d	1.20	3.79	5.09	16.34
Zebu	23	46.57 a	14.50	69.44	3.74	208.75	39.61 a	12.78	61.29	0.58	189.00
Holstein × BON	11	18.91 b	4.00	13.27	2.44	47.66	22.36 b	4.57	15.16	7.93	62.75

Values of means with different letters are significantly different ($p < 0.05$ for values transformed by the square root of the value) (SEM, standard error of mean; SD, standard deviation; Min, minimum; Max, maximum).

Table 4 Effects of *SLC11A1* 3′ untranslated region polymorphisms on survival was measured at 24 (SOB24) and 48 h (SOB48)

Genotype	BB	AB	AA	*a*	p(*a* = 0)	*d*	p(*d* = 0)
SOB24	0.00	−42.14	−36.62	−18.31	0.001	−23.83	0.14
SOB48	0.00	−73.24	−66.33	−33.16	0.001	−40.075	0.09
n	9	25	158				

Deviation of the least square means from the *BB* genotype least squares mean.
a, additive effect value; *d*, dominance effect value; p, probability value.

phenomenon by which the expression of a gene is determined by its parental origin; Giacobino & Chaillet 2004). The latter has been described in the callipyge phenotype in sheep, which is manifested as an inherited muscular hypertrophy affecting only heterozygous individuals receiving the Callipype (CLPG) mutation from their father (Georges *et al.* 2003). However, more studies with a larger population are required to test this effect.

When evaluating each genotype effect within the breeds, the SOB24 and SOB48 values were greater for AA than for AB genotype, suggesting an overdominance effect. The within-breed SOB24 and SOB48 values for heterozygous individuals did not generally exceed 50% of the homozygous AA value, except for Zebu × BON animals. Additive and dominance effects could not be estimated with contrasts because only two genotypes (AA and AB) occurred in BON and their crosses and only the BB genotype was found in the Zebu breed. After pooling all the populations, there was a significant additive effect of the *Slc11A1* allele *A*. The favourable allelic substitution effect was –18.31 for SOB24 ($p = 0.001$) and –33.16 for SOB48 ($p = 0.001$) and the dominance deviation was not significant in either trait (–32.31 and –51.88, respectively, with $p > 0.05$) (Table 4).

This indicates that a significant association exists between *SLC11A1* 3′ UTR genotype and bacterial survival, confirming that reported by Feng *et al.* (1996) and Horin *et al.* (1999). These authors identified a significant ($p = 0.0089$) association of *SLC11A1* with resistant phenotypes based on SSCP analysis and a report by Barthel *et al.* (2001) who used murine RAW264.7 cells transfected with the bovine *SLC11A1* gene.

Although the polymorphism studied was limited to a single microsatellite in the 3′ UTR, it has been shown that this critically affects *SLC11A1* gene expression (Barthel *et al.* 2001); this therefore affects *B. abortus* intraphagosome replication but not that of the *S. dublin* enteric serovar. However, as indicated by Barthel *et al.* (2001), this is not the only gene controlling susceptibility or resistance to bovine brucellosis. For instance, the major histocompatibility complex *BoLA* gene is also a candidate for contributing to phenotype variation or interactions with TNFα. The lipopolysaccharide (LPS) gene has been described as exerting a pleiotropic effect with *SLC11A1* in mice. In addition, a new locus with a greater effect on tuberculosis susceptibility has been mapped on mouse chromosome 1, at an area of 10–19 centimorgans from *SLC11A1* (Kramnik *et al.* 2000). Moreover, Seabury *et al.* (2005) have found evidence of a potential association between nucleotide variation within *PRNP* exon 3 and the presence of *Brucella* spp. antibodies in the American bison (*Bison bison*), implicating PrP^C as having a role in the natural resistance of this species to brucellosis infection.

Although *SLC11A1* is the main candidate for controlling intraphagosome survival of some bacteria, it seems to have no control over *M. bovis* infection under field conditions (Barthel *et al.* 2000). In addition, Guilloteau *et al.* (2003) have shown that *SLC11A1* is not the main determinant controlling *Brucella mellitensis* infection in mice.

In conclusion, a significant effect was found between *SLC11A1* 3′ UTR genotype and the intraphagosomal survival of *B. abortus*, indicating an association between the former and natural resistance to brucellosis. This should be confirmed in a larger population study and other breeds and, additionally, by selecting individuals assessed as resistant by the *in vitro* assay and performing *in vivo* challenges. This association of bovine *SLC11A1* polymorphism with natural resistance to *B. abortus* may open up the possibility of undertaking selection programmes to obtain cattle populations naturally resistant to brucellosis and other infectious agents with the same pathogenic mechanism.

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