Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

Molecular characterization of the NRAMP1 gene in buffalo

Giovanna De Matteis, Francesco Napolitano, Maria Carmela Scatà, Alessandra Crisà, Gennaro Catillo, Federica Signorelli, Corrado Pacelli & Michel Moioli

To cite this article: Giovanna De Matteis, Francesco Napolitano, Maria Carmela Scatà, Alessandra Crisà, Gennaro Catillo, Federica Signorelli, Corrado Pacelli & Michel Moioli (2009) Molecular characterization of the NRAMP1 gene in buffalo, Italian Journal of Animal Science, 8:sup2, 72-74, DOI: 10.4081/ijas.2009.s2.72

To link to this article: https://doi.org/10.4081/ijas.2009.s2.72

Copyright 2009 Taylor & Francis Group LLC

Published online: 07 Mar 2016.

Submit your article to this journal

Article views: 22

View related articles

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=tjas20

Molecular characterization of the NRAMP1 gene in buffalo

Giovanna De Matteis[1], Francesco Napolitano[1],
Maria Carmela Scatà[1], Alessandra Crisà[1], Gennaro Catillo[1],
Federica Signorelli[1], Corrado Pacelli[2], Bianca Moioli[1]

[1]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Monterotondo, Italy

[2]Dipartimento di Scienze delle Produzioni Animali, Università della Basilicata, Italy

Corresponding author: Bianca Moioli. Consiglio per la Ricerca e la Sperimentazione in Agricoltura. Via Salaria 31, 00015 Monterotondo (RM), Italy - Tel. +39 06 90090213 - Fax: +39 06 9061541 - Email: bianca.moioli@entecra.it

ABSTRACT - NRAMP1 (natural-resistance-associated macrophage protein) gene influences the initial phase of bacterial cellular infections, regulating macrophage activation. Recent literature on buffalo has attempted to associate the genotypes at the polymorphic microsatellite, that is located in the 3'-UTR of the gene, with either susceptibility to brucellosis or improved macrophage function. However, contradictory results were reported. In the present work, we have sequenced the whole coding region, as well as part of the introns and UTRs, of the NRAMP1 gene in 49 Mediterranean buffaloes, including both serologically positive and negative animals to *Brucella abortus* test. We have detected 12 mutations. Nineteen haplotypes were built from the detected variant alleles, so demonstrating the high variability of this gene in buffalo, but no significant differences in haplotype frequencies were found between serologically positive/negative animals.

Key words: NRAMP1 gene, Buffalo, Mutation detection, Haplotypes.

Introduction - NRAMP1 (natural-resistance-associated macrophage protein) gene influences the initial phase of bacterial cellular infections, regulating macrophage activation (Blackwell *et al.*, 2000). NRAMP1 belongs to a well-conserved group of genes in various mammalian species (Vidal *et al.*, 1993). A Gly to Asp substitution at position 169 of the mouse NRAMP1 protein is invariably associated with the resistant/susceptible phenotypes to Mycobacterium bovis, Salmonella typhimurium, and Leishmania donovani (Ables *et al.*, 2002). The transcript of the gene was sequenced by Feng *et al.* (1996) in cattle (Acc. no. U12862) and in water buffalo (Acc. no. U27105); by Matthews and Crawford (1998) in sheep and deer (Acc. no. AF005380 and AF005379). A $(GT)_n$ microsatellite repeat was located in the 3'-UTR, 60 nucleotides downstream from the TGA stop codon, in all four species. Ables *et al.* (2002) have sequenced the NRAMP1 gene in various breeds of cattle and buffalo but did not find any polymorphism at the position which corresponds to the same amino acid of the murine NRAMP1-resistant phenotype. Ganguly *et al.* (2008) evaluated the association of the NRAMP1 polymorphisms of the 3'-UTR with the macrophage function, demonstrating that the $(GT)_{13}$ allelic variant is significantly associated with the improved macrophage function in buffalo. Capparelli *et al.* (2007) demonstrated that the $(GT)_{16}$ carrier buffaloes are inherently resistant to brucellosis. On the contrary, Kumar *et al.* (2005) found that, in the Indian zebu and crossbred cattle, the microsatellite genotype is not associated with resistance to brucellosis. In the present work, we sequenced the whole coding region, as well as part of the introns and UTRs, of the NRAMP1 gene in 49 Mediterranean buffaloes with the purpose of detecting mutations.

Material and methods - The serological tests for brucellosis were carried out by rapid serum agglutination and complement fixation tests (Alton *et al.*, 1975). DNA from two groups of buffaloes of the same farm (23 serologically positive and 26 negative to *Brucella abortus* tests) was extracted from fresh blood samples and was amplified with primers designed on cattle and buffalo sequences

(DQ848779.1, DQ493965, U27105) in order to produce 7 amplicons; the PCR primers, $T_{annealing}$ and locations on buffalo gene are reported in Table1. The amplification was performed in a final volume of 20 µl, containing 100 ng genomic DNA, 0.2 mM of each dNTP, 20 pmol of each primer, and 1 U GoTaq® DNA Polymerase (Promega).

Table 1. Primers, amplicons size and location.

Forward primer	Reverse primer	T.a.	Size (bp)	Covered region in *Bubalus*
gggagaggtgcagaactca	tgctcacctgacatgagga	60°C	315	Promoter, partial exon 1
cagccactcgcacagagag	aattttaagcgccccagct	58°C	914	Partial exon1 to partial intron 2
ctccacagggtgcattcag	tttctggcccatttgaactt	58°C	290	Partial intron 2 to partial intron 3
tcctctccccagtgagtgtc	gtcacttccccaggtttcaa	62°C	443	Partial intron 3 to partial intron 4
cggccttgcgcaacttagac	gctaaggtcctctcccctgt	61°C	387	Partial intron 6 to partial intron 7
acatgtgttggccaagtgaa	gctgccttaaggatcaagga	58°C	578	Partial intron 9 to partial intron 11
gaaaatggccaggggtctac	tgtctgacaggccactatgc	60°C	572	Partial intron 13, to 3′UTR
gctgtgatgagtgggcatag	ttggccaacttccagaactc	60°C	110	3′UTR

The detection of sequence variations was performed on a ABI Prism 310 DNA sequencer by using the ABI Prism BigDye Terminator Cycle Sequencing, Ready Reaction Kits (version 1.1 - Applied Biosystems). Haplotype analysis was performed by using the Arlequin 3.0 software (Excoffier *et al.*, 2005).

Table 2. Detected haplotypes in the sampled buffaloes.

Acc.	DQ 390205			DQ 6581	AY 398760		DQ 658151	DQ 441408		AY 702720	DQ 390206	DQ 095781
	5′-utr	intr 1	intr 2	ex 3	intr 3		ex 4	intr 6	intr 7	intr 10	ex 14	3′-utr
Pos.	g86a	g306a	g973a	c275t	g6t	g31a	g422a	a201t	c319g	del 154^55	t132c	gt(n)
1	G	G	G	T	T	G	A	A	G	-	T	$GT_{(12)}$
2	G	G	G	T	G	G	A	A	G	-	T	$GT_{(12)}$
3	G	G	G	C	G	G	G	A	C	CC	T	$GT_{(16)}$
4	G	G	G	C	G	G	G	A	C	CC	T	$GT_{(12)}$
5	G	G	A	C	G	A	A	T	C	CC	C	$GT_{(9)}$
6	A	A	G	T	G	G	A	A	G	-	T	$GT_{(12)}$
7	G	G	G	C	G	G	G	A	C	-	T	$GT_{(12)}$
8	G	G	G	T	G	G	A	A	G	CC	T	$GT_{(12)}$
9	G	G	A	C	G	A	A	T	C	CC	T	$GT_{(16)}$
10	A	G	G	T	T	G	A	A	G	-	T	$GT_{(12)}$
11	A	A	G	T	G	G	A	A	G	-	T	$GT_{(12)}$
12	G	G	A	C	G	A	A	A	C	CC	C	$GT_{(9)}$
13	G	G	A	C	G	A	A	A	G	CC	C	$GT_{(9)}$
14	G	G	G	T	G	G	A	T	C	-	T	$GT_{(12)}$
15	G	G	G	C	G	G	G	A	G	CC	T	$GT_{(16)}$
16	G	G	G	T	G	G	A	A	C	-	T	$GT_{(12)}$
17	G	G	G	T	G	G	G	A	C	CC	T	$GT_{(16)}$
18	A	A	G	T	G	G	A	A	G	CC	T	$GT_{(12)}$
19	G	G	G	T	G	G	A	T	C	CC	C	$GT_{(9)}$

The significance of differences in haplotype frequency between serologically positive/negative animals was calculated with Chi-square test.

Results and conclusions - The buffalo sequences not previously described were deposited into NCBI with Acc. no. DQ390205, DQ441408, DQ390206. A total of 12 allelic variants were identified and the SNP positions, relative to the available sequences, are reported in Table 2. Using these polymorphisms, a total of 19 haplotypes were inferred occurring at variable frequency (from 1% to 35%) (Table 2).

Three allelic variants (respectively, $GT_{(9)}$, $GT_{(12)}$, and $GT_{(16)}$) at the polymorphic microsatellite in the 3'-UTR were identified, with $GT_{(12)}$ allele occurring most frequently (67%), followed by $GT_{(16)}$ allele (18%) and $GT_{(9)}$ (14%). No significant differences in the frequency of $GT_{(n)}$ variant between serologically positive/negative animals were detected.

Table 3. Haplotype frequency in serologically positive/negative animals.

Haplotype	Positive		Negative	
	n.	freq. %	n.	freq. %
1	3	6.5	3	5.8
2	21	45.6	13	25.0
3	8	17.4	5	9.6
5	7	15.2	3	5.8
6	5	10.9	6	11.3
8	1	2.2	5	9.6

In the analysed sample, only six haplotypes showed a frequency higher than 6% and for them we reported the differences in haplotype frequency between serologically positive/negative animals (Table 3). The remaining 13 rare haplotypes occurred in only 2.2% of serologically positive animals but in 32.9% of the negative ones.

Chi-square analysis did not show significant differences in haplotype frequencies between serologically positive/negative animals.

In this work we showed, for the first time, the complexity of NRAMP1 gene, evident from the high number of haplotypes, despite the low sample size of the analysed animals. In order to assess the influence of the different genotypes on disease resistance, it is therefore necessary to perform deeper investigation with wider buffalo populations.

This study is part of the "GENZOOT" research programme, funded by the Italian Ministry of Agriculture.

REFERENCES - **Ables,** G.P., Nishibori, M., Kanemaki, M., Watanabe, T., 2002. Sequence analysis of the NRAMP1 genes from different bovine and buffalo breeds, J. Vet. Med. Sci. 64:1081-1083. **Alton,** G.G., Jones, W.M., Pietz, D.E., 1975. Laboratory techniques in brucellosis, pp. 64-124, WHO monograph series 55. World Health Organization, Geneva. Switzerland. **Blackwell,** J.M., Searle, S., Goswami, T., Miller, E.N., 2000. Understanding the multiple functions of Nramp1. Microbes. Infect. 2:317. **Capparelli,** R., Alfano, F., Amoroso, M.G., Borriello, G., Fenizia, D., Bianco, A., Roperto, S., Roperto, F., Iannelli, D., 2007. Protective effect of the NrampI BB genotype against Brucella abortus in the Water Buffalo. Inf. Immun. J. 75:988-996. **Excoffier,** L., Laval, G., Schneider, S., 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinf. Online 1:47-50. **Feng,** J., Li, Y., Hashad, M., Schurr, E., Gros, P., Adams, L.G., Templeton , J.W., 1996. Bovine natural resistance associated macrophage protein 1 (Nramp1) gene. Genome Res. 6:956-964. **Ganguly,** I., Sharmam, A., Singh, R., Sitnangshu, M.D., Singh, D.K., Mitra, A., 2008. Association of microsatellite $(GT)_n$ polymorphism at 3'UTR of NRAMP1 with the macrophage function following challenge with Brucella LPS in buffalo (Bubalus bubalis). Vet. Microbiol. 129:188-196. **Kumar,** N., Mitra, A., Ganguly, I., Singh, R., Deb, S.M., Srivastava, S.K., Sharma, A., 2005. Lack of association of brucellosis resistance with (GT)13 microsatellite allele at 3' UTR of *Nramp1* gene in Indian zebu (*Bos indicus*) and crossbred (*Bos indicus _ Bos taurus*) cattle. Vet. Microbiol. 111:139-143. **Matthews,** G.D., Crawford, A.M., 1998. Cloning, sequencing and linkage mapping of the NRAMP1 gene of sheep and deer. Anim. Genet. 29:1-6. Vidal, S.M., Malo, D., Vogan, K., Skamene, E., Gros, P., 1993. Natural resistance to infection with intracellular parasites: isolation of a candidate for *Bcg*. Cell. **73:**469-485.