Lack of polymorphism in NRAMP1 gene exon V-VII region in Murrah buffaloes as explored by PCR-RFLP

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ABSTRACT

The present study was conducted on 50 Murrah buffaloes (20 were seropositive for brucellosis). DNA from blood samples was isolated using Sambrook and Russel method. An attempt was made to study the polymorphic pattern of NRAMP1(Natural resistance associated macrophage protein) gene with 959 bp region of exon V-VII through PCR RFLP using AluI, HaeIII, RsaI, StyI and TaqI restriction enzymes. All restriction enzymes showed monomorphic pattern viz. xx, aa, bb, cc, and dd respectively which revealed that there was no polymorphism in this region in Murrah buffaloes. It is inferred that there was no selective advantage of Exon V-VII part of NRAMP1 gene in respect of disease resistance.

Key Words: NRAMP1, PCR-RFLP, Brucellosis, Polymorphism

INTRODUCTION

Brucellosis, one of the most important infectious diseases, caused by *Brucella abortus*, is responsible for severe and acute or chronic disease in cattle and buffalo, causing huge economic losses to the livestock industry. NRAMP1 (Natural resistance associated macrophage Protien) gene confer resistance against several intracellular organisms (Vidal et al., 1993). It encodes integral trans-membrane protein being particularly expressed on phagosomal membrane of macrophages. It encodes a divalent cation transporter located in the phagolysosome membrane of macrophages. NRAMP1 functions as a pH dependent transporter of divalent cations such as Fe++ and Mn++ through the phagolysosomal membrane, to the cytosol, hence prevent acquisition of these cations by intracellular pathogens (Zwilling et al., 1999). In an in vitro macrophage challenge study, Barthel et al. (2001) showed that (GT) 13 allele restricted the intracellular replication of Brucella organisms. On the contrary, Kumar et al. (2005) and more recently Paixao et al. (2007) demonstrated a lack of association of 3′ UTR polymorphisms with the resistance against bovine brucellosis. On the other hand, Capparelli et al. (2007a, 2007b) reported a significant association of polymorphisms at 3′ UTR of NRAMP1 gene with resistance/susceptibility to brucellosis in buffalo. Nevertheless, polymorphisms in other regions of NRAMP1 gene can serve as a potential marker (Liu et al., 1995; Abel et al., 1998; Bellamy et al., 1998). Hence considering the potential role of NRAMP1 in determining genetic resistance against brucellosis, the present study was carried out to study polymorphism in Exon V-VII of NRAMP1 gene of Murrah buffalo and its association with brucellosis.

MATERIALS AND METHODS

Genomic DNA was isolated from blood samples from fifty unrelated Murrah buffaloes (including 20 animals were suffering from brucellosis i.e. declared RBPT positive) as described by Sambrook and Russel (2001) with minor modifications. The samples having OD ratio between 1.7-1.9 were considered having acceptable purity and used in future experiments. The concentration of DNA was calculated using the following formula

DNA concentration (µg DNA/ml) = OD260 x 50 x dilution factor.

Following primers were used to amplify exon V - VII of NRAMP1 gene in buffalo in present study.

Forward Primer: 5′ TCC GAC ATG CAG GAA GTC ATC G 3′

Reverse Primer: 5′ GCC AAA GGT CAA GGC CAT AAT GG 3′

The PCR amplification was carried out with initial denaturation for 3min at 95°C followed by 35 cycles of 45 sec at 94°C, 58°C for 1 min, 72°C for 90 sec, with final extension for 7min at 72°C. The PCR products were analyzed by standard gel electrophoresis with 1kb ladder DNA marker using 1.8% agarose gel stained with

ethidium bromide for confirmation of amplified PCR products 15 µl of the amplified products were digested with 10 units of either AluI, HaeIII, RsaI, StyI or TaqI restriction endonucleases [New England Biolabs] in a final volume of 20 µl at 37°C or 60oC (TaqI) overnight. After the digestion, heat inactivation of the enzymes was done at 80°C (HaeIII and TaqI) or 65°C (AluI and StyI) for 20 min and the RE products were stored at 4°C for further analysis. The restriction fragments were resolved by electrophoresis on 4.5 % agarose gels (Sigma, USA) in 1X TAE buffer at 80 V for 3 hrs. As a DNA size marker, low molecular weight ladder (New England Biolabs) and 50 bp ladder (MBI, Fermentas) were used. The fragments were visualized by UV transilluminator and documented by photography.

RESULTS AND DISCUSSION

The isolated genomic DNA which was showing single intact distinct band and showing absorbance ratio (260/280nm) between 1.7 to 1.9 which was indicating of acceptable quality of genomic DNA. The PCR product was estimated to be 959bp by comparing with DNA size markers. On digestion of PCR product with different restriction enzymes, different sizes of RE fragments were obtained as shown in table 1. Although we predicted the approximate fragment size by Insilco restriction digestion of cloned PCR product of Exon V-VII of NRAMP1 gene:

When the PCR product of healthy and brucella infected animals digested with above said restriction enzymes revealed same genotype in all animals with respect to restriction enzymes (Fig.1-7). No polymorphism within this gene locus could be detected with these restriction endonucleases. Kumar et al. (1999) observed polymorphism in NRAMP1 gene in a study conducted to verify polymorphism in bovine NRAMP1 genomic sequences from tuberculosis infected and uninfected Holstein Fresian cattle using PCR-RFLP. TaqI and AluI restriction enzymes exhibited polymorphism and revealed two and three allelic patterns respectively, AluI was the most informative as it resolved three different genotypic patterns within the group.

In our study digestion of 959 bp PCR product of Exon V-VII of NRAMP1 gene using HaeIII, AluI, RsaI, StyI, and TaqI resulted in single restriction pattern. It detected single genotype in all animals under study viz. xx, aa, bb, cc, and dd respectively (Fig. 1-7). This study reveals that there is no polymorphism in Exon V-VII of NRAMP1 gene in Murrah buffalo. Borriello et al. (2006) worked on 3'UTR of NRAMP1

Table.1 Different Fragments and RE pattern obtained after digestion with different restriction enzymes

S.N	Enzyme	Fragment size in bp	RE pattern
1	HaeIII	476,231,80,50	Monomorphic
2	AluI	546, 255,79,50	Monomorphic
3	RsaI	564, 334,61	Monomorphic
4	StyI	893, 66	Monomorphic
5	TaqI	547,413	Monomorphic

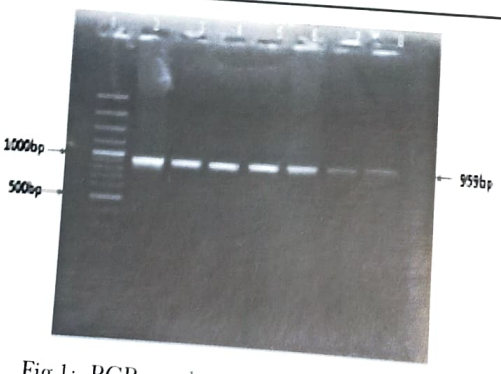

Fig.1: PCR product of exon V-VII of Nramp1

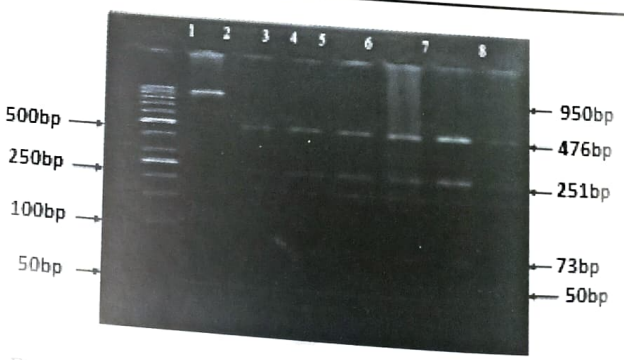

Fig.2: Restriction pattern of exon V-VII of gene (959 bp) NRAMP1 gene with HaeIII: Lane 1: 50 bp ladder, Lane 2: uncut PCR product (959 bp), Lane 3-8: xx pattern (fragment size: 476bp, 231bp, 73bp, 70bp and 50bp

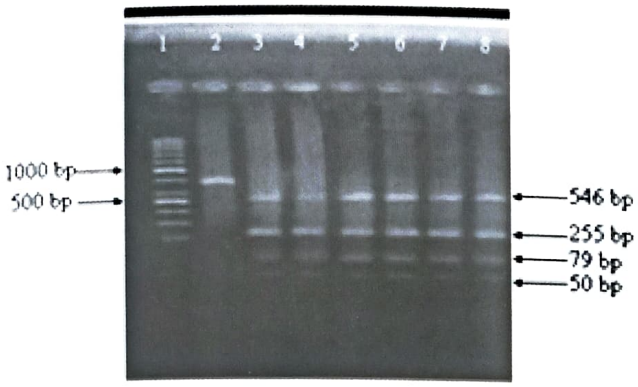

Fig. 3: Restriction pattern of exon V-VII of NRAMP1 gene with AluI: Lane 1= 100bp ladder, Lane 2= uncut PCR product (959 bp), Lane 3-8: pattern aa (fragment size: 546bp, 255bp, 79bp and 50bp).

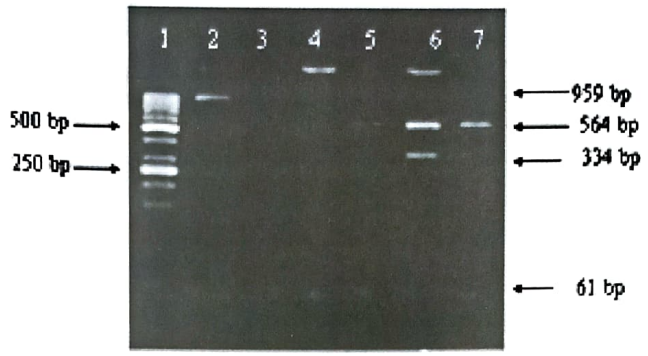

Fig4: Restriction pattern of exon V-VII of NRAMP1 with RsaI: Lane 1: 100bp ladder, Lane 2: uncut PCR product (959 bp), Lane 3-7 pattern bb (fragment size: 546bp, 255bp, 79bp and 50bp)

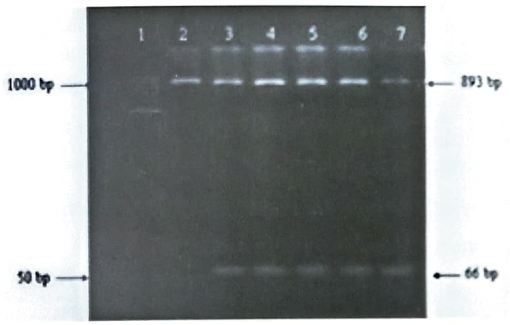

Fig.5 Restriction pattern of exon V-VII NRAMP1 StyI. Lane 1: 50 bp ladder, Lane 2: uncut product (959 bp), Lane 3-7: cc pattern (fragment size: 893bp and 66bp).

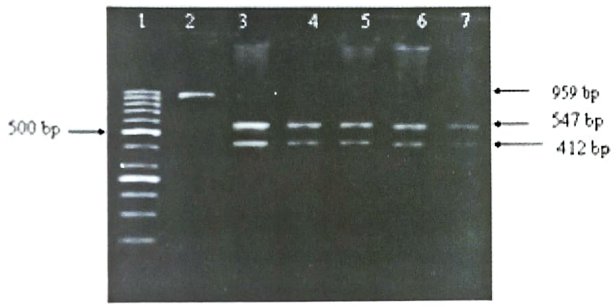

Fig.6: Restriction pattern of exon V-VII NRAMP1 gene with TaqI: Lane 1: 50 bp PCR ladder, Lane 2 : uncut PCR product (959 bp), Lane 3-7: dd pattern (fragment size: 547 bp and 412 bp).

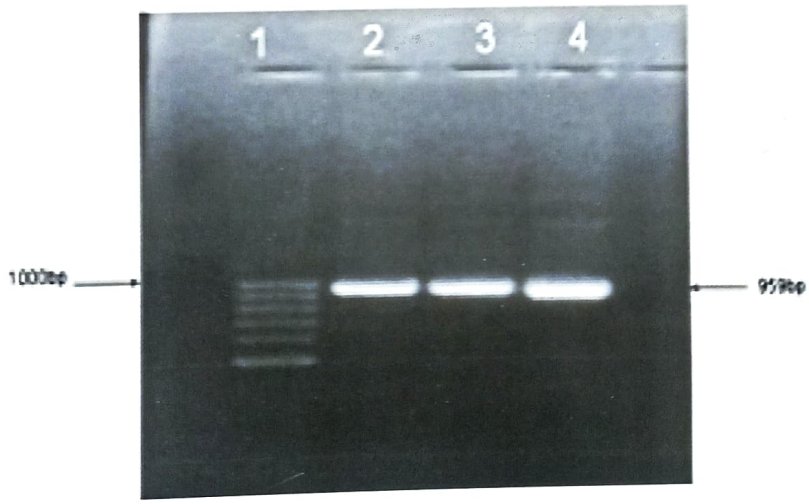

Fig.7 Agarose gel electrophoresis of colony touch PCR. Lane1: 100 bp ladder, Lane: 2-4 Recombinant clones

gene and showed association between polymorphism of 3'UTR of NRAMP1 gene and disease resistance to brucellosis in buffalo. However Paixo et al. (2007) reported no association polymorphism in 3'UTR of NRAMP1 gene with disease resistance to brucellosis in cattle. Hence such a study needs to be extended in more number of animals to draw clear picture about the association of the NRAMP1 gene with brucellosis.

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