Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2019.091

SHORT COMMUNICATION

Comparative Association of *slc11a1* (*Nramp1*) Gene with Susceptibility and Resistance to Brucellosis in Various Cattle and Buffaloes Breeds of Pakistan

Raheela Akhtar[1], Muhammad Zaheer Iqbal[1], Azmat Ullah[1], Muhammad Muddassir Ali[2*], Beenish Zahid[3], Muhammad Ijaz[4], Arif Ullah[2] and Muhammad Sulman Ali Taseer[3]

[1]Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[3]Khan Bahadar Chaudhry Mushtaq Ahamd College of Veterinary and Animal Sciences Narowal, Sub-Campus University of Veterinary and Animal Sciences, Lahore, Pakistan
[4]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan
*Corresponding author: muddassir.ali@uvas.edu.pk

ARTICLE HISTORY (19-068)

Received: February 19, 2019
Revised: June 20, 2019
Accepted: July 03, 2019
Published online: August 15, 2019

Key words:
Brucella
Genotyping
Nili Ravi
Sahiwal
Sequencing

ABSTRACT

It is observed that different *Nramp1* genotypes are linked with *Brucella* resistance or susceptibility. So, current study aimed to find the *Brucella* resistance and susceptibility of local and exotic breeds of Pakistan on the basis of *Nramp1* genotypes. Two breeds of buffaloes and five breeds of cattle were screened for brucellosis. The overall seropositivity determined by RBPT in cattle and buffaloes was 77.5% and 33.5% respectively. PCR results showed 54.5 and 25% brucella positive cases in cattle and buffaloes respectively. Nili-Ravi buffaloes pertain resistant genotype *Nramp1 BB* as compared to Kundi breeds of buffaloes possessed susceptible genotype *Nramp1 AA*. Similarly, Sahiwal breed was more resistant for brucellosis compared to cross breeds and Friesian. Phylogenetic analysis showed that *Nramp1* gene of local breed had more closeness with *B. taurus* of China. This gene resistance can be used for genomic selection of animals to reduce disease cost and economic losses due to brucellosis.

To Cite This Article: Akhtar R, Iqbal MZ, Ullah A, Ali MM, Zahid B, Ijaz M, Ullah A and Taseer MSA, 2019. Comparative association of *slc11a1* (*Nramp1*) gene with susceptibility and resistance to brucellosis in various cattle and buffaloes breeds of Pakistan. Pak Vet J, 39(4): 612-614. http://dx.doi.org/10.29261/pakvetj/2019.091

INTRODUCTION

Brucellosis is an infectious and costly disease of livestock caused by an intracellular bacterium of genus *Brucella*. It causes major economic and health losses in spite of mass vaccination, excessive treatment cost and slaughtering policy. A number of billion dollar brucellosis obliteration programs have failed and disease is still prevalent worldwide and has public health concerns (Mittal *et al*., 2018). New strategies related to natural disease resistance are now required for eradication. As genomics is playing a vital role in the genetic severance of some traits, such as resistance to infectious diseases. Therefore, by using such information we can coerce genetic selection for disease resistance as a method to control brucellosis.

The previous literature suggests that *Nramp1* gene produces resistance to *Brucella abortus* in humans. This gene was first identified in the mouse and is present in different species, including mammals and bacteria (Cassidy *et al*., 2008). The occurrence of *Nramp1* in mammals and bacteria proposes that the pathogen and host struggle for the equivalent nutrients, each attempting to take essential cations for its advantage (Gruenheid *et al*., 2000). The *Nramp1* gene has three genotypes. The individuals with type A genotypes are considered susceptible to brucellosis while the individuals with genotype type B are resistant to brucellosis and the individuals with genotype AB are neither resistant nor susceptible.

There have been few studies to determine the relation of this gene with *Brucella* resistance in bovines. However, we need to know the status of our local breeds for genetic resistance to brucellosis. The present study will assist to suggest the alternative solution for the control of brucellosis by selective breeding.

MATERIALS AND METHODS

The study was conducted on 400 bovines, including five breeds of cattle (Sahiwal, Jersy, Frisian, Sahiwal cross Jersy, Sahiwal cross Fresian) and two breeds of

buffaloes (Nilli-Ravi and Kundi) from different farms of Punjab (Rakh Dera chal farm, military dairy Okara, Bedian road private farm), Pakistan. Permission was obtained from ethical review committee of UVAS before conducting this research. Serum samples were collected from animals having the history of abortion and retained placenta. The samples were screened for brucellosis by Rose Bengal Plate Test (RBPT). RBPT positive samples were subjected to DNA extraction by Genome all kit method. Following forward primer 5-TGGCTCGGTTGCCAATATCAA-3 and reverse primer 5-CGCGCTTGCCTTTCAGGTCTG-3 were used for the confirmation of *Brucella* in selected animals targeting IS*711* gene with amplicon size of 498bp (Fig 2.) and accession No. CP023309.1.

PCR was performed with a total volume of 20µL by adding 2µL of each forward and reverse primer, 4µL of PCR grade water, 2µL of extracted DNA, 10µL of master mix. The thermocycler (Bio-Rad, CFX ™, Singapore) was set with the conditions for the total of 35 cycles of PCR. Denaturation of DNA samples were performed at 94°C for 5minutes. Annealing was performed at 63°C for 30 second. Then, initial extension of DNA samples was conducted at 72°C for 30 second. Finally, extension was performed at 72°C for 5 minutes.

Determination of *Nramp1* genotype: The PCR confirmed positive and negative animals were divided into infected (positive) and non-infected (negative) groups and the *Nramp1* genotypes were compared by employing another PCR in these groups. The 3′ untranslated region of Nramp1 gene was amplified using the following primers (5-GATCAGGAGAAGG GGAGGA-3; 5-CAGCTTCCAGAACTCCCTGT-3**)** with product size 174bp.

The sequenced gene fragment was submitted to NCBI GenBank aligned and analyzed in software MEGA7 (Molecular Evolutionary Genetics Analysis). The nucleotide sequence analysis of *Nramp1* gene *SLC11A1* was carried out along with other published sequences of bovine breeds to find phylogenetic association.

RESULTS AND DISCUSSION

The overall seropositivity determined by RBPT in cattle and buffaloes was 77.5 and 33.5% respectively. PCR results showed 54.5 and 25% brucella positive cases in cattle and buffaloes respectively. Sahiwal cattle showed least susceptibility by both RBPT and PCR. Frisian cross Sahiwal (FXS) was found more susceptible to brucellosis than Jersy cross Sahiwal (JXS) (Table 1). Similarly, more positive cases of brucellosis were found in Kundi breed of buffaloes by RBPT and PCR compared to Nili-Ravi breed. At present, control of different infectious diseases including brucellosis is carried out through the serology and slaughtering of infected (seropositive) animals. However, carrier animals, vaccine failure and prolonged incubation periods of the disease decreases the success of this approach. So, selective breeding could be an alternative solution for the control of brucellosis. Nowadays, search for polymorphisms yielded a high throughput reports on the association of allelic variants with diseases (Mittal *et al*., 2018). In current study, the genetic resistance and susceptibility against brucellosis targeting *Nramp1* gene was determined among the local and exotic breeds of Pakistan. Nilli-Ravi buffaloes showed a significant number of animals that had genotype *Nramp1 BB* and were resistant to brucellosis. While in Kundi breed, a significant number of animals had genotype *NrampI AB* that could be resistant or susceptible at the same time (Table 2). More incidence of brucellosis in cattle was observed compared to buffaloes. Nili Ravi and Kundi showed more resistant genotype *Nramp1 BB* as compare to cattle.

This gene produces an *Nramp1* protein that controls the function of macrophages, handling the infectious organisms. As Brucella resides within the macrophages, this might be possible that Brucella resistant species produce more amount of this protein (Gruenheid *et al*., 2000). Polymorphisms in *Nramp1* gene already have been observed in cattle, zebu, and the water buffalo (Kumar *et al*., 2005; Paixao *et al*., 2006). However, few discrepancies have been reported with respect to the current data.

Holstein (*Bos taurus taurus*) and Indian zebu (*Bos taurus indicus*) showed the 3′ UTR *Nramp1* genotype associated resistant to brucellosis (Paixao *et al*., 2006). Whereas, Indian zebu and crossbred (*Bos taurus indicus* × *Bos taurus taurus*) cattle were not found resistance to brucellosis with the same genotype in another study (Kumar *et al*., 2005). In current study, FXS and JXS were found more susceptible to brucellosis (Table 2). These controversies in findings could be the excess of homozygosity at the *Nramp1* locus points to either due to population stratification or mistyping of genotypes (Pritchard *et al*., 1999). Since, these both conditions could lead to the false conclusions, so caution must be taken to interpret the results of these studies (Kumar *et al*., 2005; Paixao *et al*,. 2006). Several *Nramp1* single-nucleotide polymorphisms have been described in bovine including water buffalo breeds. But the association between these polymorphisms and resistance to brucellosis was still lacking in that study (Ables *et al*., 2002). Water buffaloes found most resistant to brucellosis linked with *Nramp1BB* genotype, which is in agreement with the findings of Borriello *et al*. (2006) who also found the link of *Nramp1BB* genotype with *Brucella* resistance in water buffaloes. Moreover, it was also observed that the BB genotype animals are naturally resistance to *B. Arbutus* infection, even if they are exposed artificially to *B. abortus* pathogen (Capparelli *et al*., 2007). These observations gave a clear indication for connection of disease resistance/susceptibility with breed type. Although, we did not perform the polymorphic (transition, transversions etc) study of this gene of local breeds, but phylogenetic analysis showed that the association of *Nramp1* gene had the more closeness with *B. taurus* of China (Figure 1). Whereas, local gene was more distantly related to *B. taurus* natural resistance associated macrophage protein 1(*Nramp1*) gene of Czech republic. Yakubu *et al*. 2014 did the evolutionary proximity and divergence analysis of *SLC11A1* gene in ruminants and non-ruminants. They observed the novel genetic variation and polymorphism of the *SLC11A1* gene within ruminants and among species.

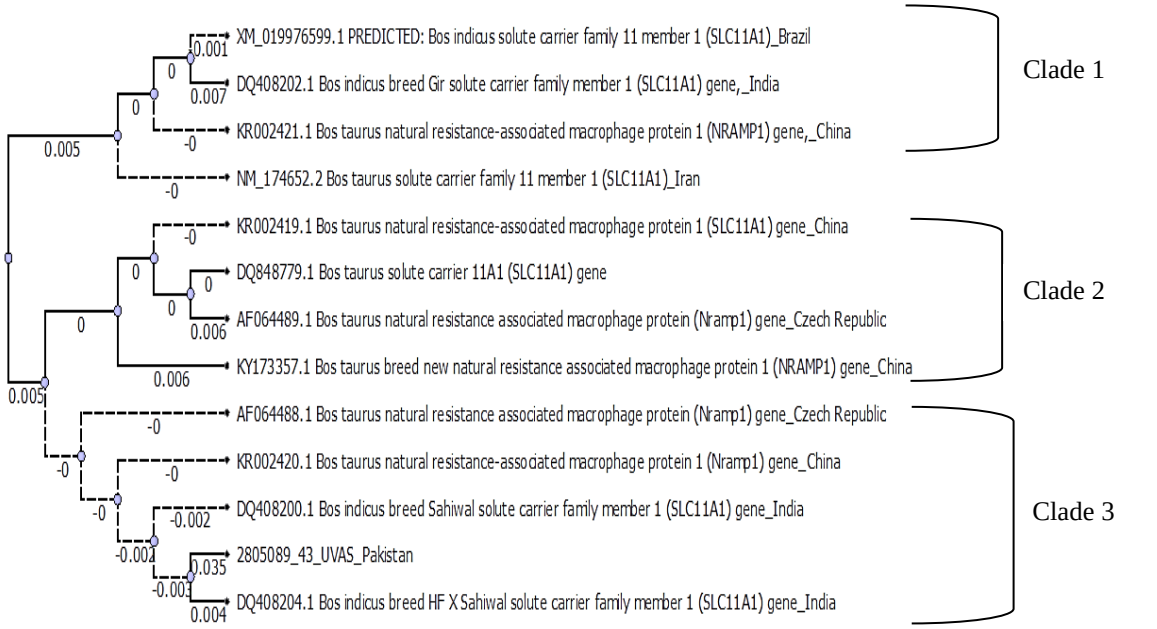

Fig. 1: The phylogenetic analysis (Maximum likelihood method) of local *Nramp1* gene with other different bovine breeds.

Table 1: Breed wise positive cases of brucellosis by the RBPT and PCR

Cattle (n=200)			Buffaloes (n=200)		
Breeds	Positive by RBPT	Positive by PCR	Breeds	Positive by RBPT	Positive by PCR
Sahiwal	23	14	NiliRavi	26	12
Jersy	36	25			
Frisian	38	31			
Sahiwal X Jersy	28	18	Kundi	41	38
Sahiwal X Fresian	30	21			
Total	155	109		67	50

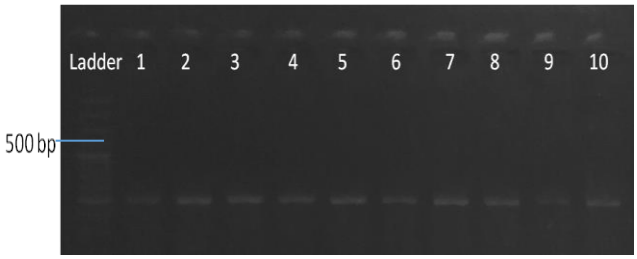

Fig. 2: Gel image of PCR amplified *Nramp1 gene* of *Brucella abortus* of amplicon size 498 bp.

Table 2: Genotype association of *NRAMP1* gene among different bovine breeds

	Resistant or sensitivity to brucellosis		
	NRAMP AA	NRAMP1 AB	NRAMP1 BB
Cattle			
Sahiwal	0	4	10
Jersy	13	9	3
Fresian	24	7	0
Sahiwal X Jersy	16	2	0
Sahiwal X Fresian	19	2	0
Buffaloes			
Nili Ravi	1	3	8
Kundi	6	29	3

Conclusions: The local breeds of either cattle (Sahiwal) or buffaloes (Nili-Ravi) investigated in the present study were more resistant to brucellosis compared to exotic breeds.

Acknowledgements: This study was financially supported project "Brucellosis research, diagnosis and PCR commercialization at UVAS, Lahore" funded by Al-Abrish Dairy Farm Islamabad.

Authors contribution: RA, MZI designed and perceived the experiments. AZ, MMA, BZ, MSAT executed the experiments and sample collection. MI, AU and MMA helped in data analysis and article write-up.

REFERENCES

Ables GP, Nishibori M, Kanemaki M, *et al*., 2002. Sequence analysis of the NRAMP1 genes from different bovine and buffalo breeds. J Vet Med Sci 64:1081-3.

Borriello G, Capparelli R, Bianco M, *et al*., 2006. Genetic Resistance to Brucella abortus in the Water Buffalo (Bubalus bubalis). Infec Immun 74:2115-20.

Capparelli R, Alfano F, Grazia M, *et al*., 2007. Protective effect of the nramp1 bb genotype against brucella abortus in the water buffalo (Bubalus bubalis). Infec Immun 75:988-96.

Cassidy JP and Martineau AR, 2014. Innate resistance to tuberculosis in man, cattle and laboratory animal models: nipping disease in the bud? J Compar Pathol 151:291-308.

Gruenheid S and Gros P, 2000. Genetic susceptibility to intracellular infections: Nramp1, macrophage function and divalent cations transport. Current Opin Microbiol 3:43-8.

Kumar N, Mitra A, Ganguly I, *et al*., 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 111:139-43.

Mittal M, Sharma V, Nehra K, *et al*., 2018. Abortions in an organized dairy farm from North India reveal the possibility of breed susceptibility to bovine brucellosis. One Health 5:1-5.

Paixao TA, Ferreira, Borges CAM, *et al*., 2006. Frequency of bovine Nramp1 (Slc11a1) alleles in Holstein and zebu breeds. Vet Immunopathol 109:37-42.

Pritchard JK and Rosenberg NA, 1999. Use of unlinked genetic markers to detect population stratification in association studies. Am J Hum Genet 65:220-8.

Yakubu A, Alade E and Dim NI, 2014. Molecular analysis of solute carrier family 11 member A1 (SLC11A1) gene in ruminants and non-ruminants using computational method. Genetika 46:925-34.