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

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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.

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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, Jersey, Frisian, Sahiwal cross Jersey, Sahiwal cross Friesian) 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'-TGGCTCCTTGCAATATC-3' and reverse primer 5'-CGCCCTTGCTTCCAGGTCGTG-3' were used for the confirmation of Brucella in selected animals targeting IS711 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 ofNramp1 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 Jersey 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 Nramp1 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 compared 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

| Breeds       | Positive by RBPT | Positive by PCR |
|--------------|------------------|-----------------|
| Cattle       | Buffaloes        |                 |
| Sahiwal      | 23               | 14              |
| Jersey       | 36               | 25              |
| Frisian      | 38               | 31              |
| Sahiwal X Jersey | 28           | 18              |
| Sahiwal X Fresian | 30           | 21              |
| Total        | 155              | 109             |

| Breeds       | Positive by RBPT | Positive by PCR |
|--------------|------------------|-----------------|
| NiliRavi     | 26               | 12              |
| Kundi        | 41               | 38              |

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        |
| Jersey                                 | 13       | 9         | 3         |
| Frisian                                | 24       | 7         | 0         |
| Sahiwal X Jersey                       | 16       | 2         | 0         |
| Sahiwal X Fresian                      | 19       | 2         | 0         |
| Buffaloes                              |          |           |           |
| NiliRavi                               | 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.

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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.

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