RESEARCH ARTICLE

Evaluation of the association of *SLC11A1* gene polymorphism with incidence of paratuberculosis in goats

ASHA ABRAHAM [1], THOMAS NAICY [2*], KUNNIYOOR CHEEMANI RAGHAVAN[3], JOSEPH SIJU [4], THAZHATHUVEETTIL ARAVINDAKSHAN[3]

[1]*Department of Animal Genetics and Breeding, CVAS, Mannuthy*
[2]*Department of Animal Genetics and Breeding, CVAS, Pookode*
[3]*Centre for Advanced Studies in Animal Genetics and Breeding*
[4]*Department of Veterinary Microbiology, CVAS, Pookode*
Kerala Veterinary and Animal Sciences University, Kerala, India

*Corresponding author:
Assistant Professor
Department of Animal Breeding &Genetics, College of Veterinary and Animal Sciences,
Pookode, Thrissur-680651, Kerala, India
Email. naicy@kvasu.ac.in

Abstract

Paratuberculosis is one of the chronic granulomatous enteritis that predominantly affects ruminants worldwide, caused by *Mycobacterium avium* subsp. *Paratuberculosis* (MAP). In ruminants, microsatellite polymorphisms of the 3' untranslated region (3' UTR) of the *solute carrier family 11 member A1* (*SLC11A1*) gene were associated with resistance to intracellular pathogen infections. This research was carried out to detect the polymorphisms in A and B region of the 3' UTR of *SLC11A1* gene and to evaluate the potential association between these polymorphisms and MAP infection in goats. MAP-specific antibodies were detected by ELISA and MAP infection was confirmed by *IS900 PCR* in 150 adult goats from different regions of Kerala, India. The polymorphism of microsatellite regions A and B at 3' UTR of the *SLC11A1* gene was analysed in goats by an automated technique, fragment analysis, using fluorescent tagged forward primers. Eight alleles of sizes ranging from 221 bp to 239 bp were found in Region A. Region B revealed two alleles, 117 bp (B_7) and 119 bp (B_8). Animals with B_8 alleles were found to have higher incidence of paratuberculosis than animals with B_7 alleles ($P<0.01$).

There is no statistically significant association was found between region A genotypes and paratuberculosis incidence. These results suggest that caprine *SLC11A1* gene has significant role in paratuberculosis resistance in goats and further studies might help in development of a PCR based genotyping test for paratuberculosis resistance and selection of superior animals for future goat breeding programmes.

Keywords: paratuberculosis; microsatellite; natural resistance; *SLC11A1* gene; goats;

Introduction

Paratuberculosis or Johne's disease is considered as one of the most serious, contagious, bacterial diseases of ruminants especially cattle, sheep and goats caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The disease is characterized by diarrhoea, rapid weight loss, reduced milk production, reproductive failure and death in farm animals (Chiodini *et al*. 1984). Infections with MAP in caprine herds result in significant economic loss, through slow progressive wasting and the subsequent death of the infected animals. A study conducted by Singh *et al.* (2008) showed that prevalence of MAP in domestic livestock in India was moderately higher and there is urgent need to control the disease at national level in order to improve per animal productivity in India. The disease is zoonotically important, since *IS900* characterization of positive cultures in stool and biopsies from confirmed cases of Crohn's disease in Northern India, proved the association between MAP and Crohn's disease (Singh *et al.* 2008). Diagnostic tests include isolation of MAP, Ziehl-Nielsen's (ZN) acid fast staining, Enzyme Linked Immunosorbent Assay (ELISA) and *IS900* PCR. Since these tests are time consuming and costly, the early detection of subclinical paratuberculosis is difficult, combined with the incapability of currently available vaccines to prevent the disease or disease shedding, necessitates the adoption of newer techniques for the prevention of MAP infection. This could be overcome by selection of disease resistant animals by appropriate selection methods like Marker Assisted Selection (MAS). Most of the recent researches regarding disease resistance suggests that limiting the spread of the disease may be possible through selective breeding of animals based on genetic markers associated with resistance or susceptibility. One of the genes that have been targeted for this purpose is the *SLC11A1* (solute carrier family 11 member A1) (Bellamy *et al*. 1998) in goats which is located in chromosome 2 (Vacca *et al.* 2011).

Association of *SLC11A1* gene polymorphism with caprine paratuberculosis

The *SLC11A1* is a potential candidate gene that confers innate resistance against various intracellular pathogens including MAP. The *SLC11A1* gene, previously known as *natural resistance-associated macrophage protein 1* (*NRAMP1*) gene is a member of large family of metal ion-transport proteins. *SLC11A1* gene encodes SLC11A1 protein which is a member of large family of metal ion-transport proteins linked to infectious disease susceptibility in mouse (Vidal *et al.* 1993), functions as a pH dependent transporter that prevents the acquisition of divalent cations like Fe^{2+} and Mn^{2+} towards the cytosol through the phagosome membranes and thus it favours bacterial killing (Forbes and Gros, 2003). *SLC11A1* gene delivers bivalent metal cations from the cytosol into acidic endosomal and lysosomal compartments under normal physiological conditions, where the Fenton and Haber-Weiss reaction generate toxic antimicrobial radicals for direct antimicrobial activity against phagocytosed microorganisms (Goswami *et al.* 2001). This gene has pleiotropic effects on macrophage function, that include increased keratinocyte chemoattractant (chemokine KC), tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), inducible nitric oxide synthase and Major Histo Compatability (MHC) class II expression; all are important in resistance to intracellular pathogens (Awomoyi, 2007).

In ruminants microsatellite polymorphisms of 3'untranslated region (3'UTR) of the *SLC11A1* gene were associated with resistance to *Brucella abortus*, *Mycobacterium bovis* and *Mycobacterium avium* subsp. *paratuberculosis* (Barthel *et al.* 2001; Borriello *et al.* 2006; Capparelli *et al.* 2007a; Kadarmideen *et al.* 2011; Korou *et al.* 2010; Martinez *et al.* 2008; Pinedo *et al.* 2009; Reddacliff *et al.* 2005; Taka *et al.* 2013; Taka e*t al.* 2015). There are two polymorphic microsatellites in the 3' UTR of the caprine *SLC11A1* gene with a variation in the number of guanine thymine repeats (GT)n (Vacca *et al.* 2011). The region A was found to be more polymorphic than the region B, where only two alleles were reported for region B in goats (Korou *et al.* 2010; Vacca *et al.* 2011). Thomas *et al.* (2012) reviewed the role of *SLC11A1* gene in diseases resistance especially for intracellular pathogens and opined that disease is the most important constraint in the animal production system and the selection of animals for increased genetic resistance to diseases will lead to the production of a healthy and productive stock. The present study was designed to detect the polymorphisms in A and B region of the 3' UTR of *SLC11A1* gene and to evaluate the potential association between these polymorphisms and the presence or absence of MAP infection in goats of Kerala, South India.

Materials and methods

Association of *SLC11A1* gene polymorphism with caprine paratuberculosis

Sample collection

All experimental procedures were performed according to the guidelines of the Institutional Animal Ethics Committee of Kerala Veterinary and Animal Sciences University. Blood, serum and faecal samples were collected from one hundred and fifty adult goats comprising 50 Malabari, 47 Attappady Black and 53 Malabari crossbreds from Thrissur and Malappuram Districts of Kerala, India. Animals included in the present study were maintained at similar environmental conditions and not vaccinated for paratuberculosis. Blood samples were used for the isolation of genomic DNA for microsatellite genotyping of the goats under study, where as serum and faecal samples were used for the detection of MAP antibodies by ELISA and MAP antigens by *IS900* PCR.

Genomic DNA isolation and genotyping

Genomic DNA was extracted from whole blood using the standard phenol-chloroform extraction procedure. DNA concentration of samples was quantified by NanoDrop (NanoDrop, ThermoScientific, USA) and stored at −20°C until used. PCR was carried out to amplify both A and B region at the 3' UTR of the caprine *SLC11A1* gene. Primer pairs for A region (Ex15F1: 5'-GTCTGGACCTGTCTCATCACC-3' and Ex15R1: 5'-ACTCCCTCTCCATCTTGCTG -3'), and B region (Ex15F2: 5'- GGAGTTCACGGGTGGGA -3' and Ex15R2: 5'-GGGTCTCTATGTCGTGGGGG -3'), were designed on the basis of the goat genomic sequence (GenBank Accession no. GU440577) using Primer3 software. To amplify PCR products of approximately 233 bp and 117 bp. Primers Ex15F1 and Ex15F2 were 5' labeled with the fluorescent dye 6-FAM (6-carboxyfluorescein). PCR was carried out with 50 ng of genomic DNA in a total reaction volume of 25µL containing 10× PCR buffer, 1.5 mM $MgCl_2$, 200µM dNTPs, 10 pM of forward and reverse primers and 0.5 U of Taq DNA polymerase (Sigma Aldrich). Amplification reactions were performed with an initial denaturation step of 5 min at 94°C, followed by 35 cycles of 30 seconds 94°C, 30 seconds X°C (X was 59 for region A and 62.2 for B region) and 25 seconds 72°C with a final extension step of 5 min at 72°C. PCR products were screened by 2 % agarose gel electrophoresis stained with ethidium bromide and visualized in a Gel Documentation System (Biorad, USA).

The genotyping of microsatellite markers in the region A and B was performed by an automated fragment analysis technique. (Scigenom Pvt. Ltd., Ernakulam). The fluorescent 5' end-labeled PCR products (with fluorescent dye, 6-FAM) were run on 3730 XL ABI PRISM

automated genetic analyzer (Applied Bio-systems, Germany) and analysed. Microsatellite fragment sizing was performed by the Gene Mapper™ software version 4.0. Allele calling was performed with the software and were checked manually to avoid any false calling of alleles. Sequencing of representative samples from each pattern, obtained by genotype analysis, confirmed that the only nucleotide differences among the PCR products were in the number of GT repeats.

Detection of MAP-specific antibodies by ELISA

Blood samples were collected by jugular puncture; following centrifugation (2500 rpm for 10 minutes), the sera were separated and stored at −20°C until use. Serum samples (n = 150) were screened for detection of MAP specific antibodies by ELISA kit (ID vet innovative diagnostics, France). Optical Density (OD) values measured at 450 nm. Positive and negative sera were included as control. As per Manufacturers instruction, serum samples with corrected sample/positive control ratio above 60% were considered as positive for paratuberculosis.

Detection of MAP by IS900 PCR

Faecal samples were collected by rectal pinch method. DNA was isolated from faecal sample as per Braunstein *et al.* (2002). *IS900* PCR was performed as per the protocols of Halldorsdottir et al. (2002). The primer pair used was 5'GGCCGTCGCTTAGGCTTCGA 3' and: 5' CGTCGT TAATAACCATGCAG 3' to amplify a 279 bp PCR product. The PCR mixture (50 µL total volume) comprised 5 µL of DNA, 10 X PCR Buffer, 10 pM primers, 1.5 mM for $MgCl_2$, 0.2 mM dNTPs and 0.5 U of Taq DNA polymerase. The cycling protocol was an initial denaturation at 94°C for 3 min followed by 35 cycles of 1 min denaturation at 94 °C, 25 seconds primer annealing at 55°C, and extension at 72 °C for 1 min. The *IS900* PCR products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide and visualised by Gel documentation system (Biorad, USA).

Statistical analysis

The allelic and genotype frequencies of A and B microsatellite loci at 3' UTR of *SLC11A1* gene in each genetic group were calculated by direct counting method. Association of the different genotypes in the A and B microsatellite regions at the 3' UTR of *SLC11A1* gene

with the presence of MAP-specific antibodies in the serum and MAP DNA in the faeces in goats were assessed by Chi-square test and the Fisher's exact test by SPSS V.21.

Results and Discussions

The number of GT repeats found in region A was ranged from 10 to 19, being eight alleles identified (A_{10}-GT10, A_{12}-GT12, A_{14}-GT14, A_{15}-GT15, A_{16}-GT16, A_{17}-GT17, A_{18}-GT18 and A_{19}-GT19) with size range of 221-239 bp and 12 genotypes were observed. The 233 bp allele was the most abundant in goat population (0.660). Only two alleles (B_7-GT7) and B_8-GT8) with three genotypes were present in region B. The direct count heterozygosity, unbiased heterozygosity and PIC value for microsatellite A region of *SLC11A1* gene were 0.6833, 0.6995 and 0.5474 and for microsatellite B region were 0.4985, 0.5036 and 0.2485 in goats, respectively (Table 1). Liandris *et al.* (2009) detected two microsatellite regions with different GT(dinucleotide) repeat number and different sequence motif in native Greek goats named region A and region B at the 3' UTR of *SLC11A1* gene and detected four alleles (GT14, GT15, GT16 or GT18) in region A and two alleles at region B (GT7 and GT8). In addition to this four other alleles (GT11, GT12, GT17 and GT19) at region A were recognized in Sarda goats by Piras *et al.* (2011). Korou *et al.* (2010) detected six alleles (GT13, GT14, GT15, GT16, GT17 and GT18) in microsatellite region A at the 3' UTR of *SLC11A1* gene in Greece goats, and two alleles at region B (B_7 and B_8). The allele frequency in region B of the 3'UTR of the *SLC11A1* gene was slightly different to that observed by Korou *et al.* (2010) (53 % allele B_7 and 47% allele B_8 in this study vs. 45 and 55%; Table 1); but, the percentage of goats with B_7/B_7genotype found among animals evaluated by us was similar to those reported in other goat breeds (17% in this study vs. 26% and 16%) (Korou *et al*. 2010; Iacoboni *et al.* 2014). The percentage of goats with B_7/B_8 and B_8/B_8 genotype, which was found to be in risk of paratuberculosis incidence was higher than those reported in other goat breeds (83% in this study vs. 75%b reported by Korou *et al.* 2010).

The incidence of paratuberculosis in Malabari, Attapady Black and crossbred goat were tested by ELISA and *IS900* PCR. The prevalence of paratuberculosis was between 12 and 34%. Lowest incidence was noticed in the native breeds (Attappady Black and Malabari) and highest in crossbreds. Genotype wise results of MAP infection were presented in Table 2 and 3. The association analysis between diagnostic tests results and polymorphisms in region A and B of the 3'UTR of the caprine *SLC11A1* gene indicated that genotypes of A region had no significant

effects on paratuberculosis resistance/incidence in goats, whereas region B showed a significant association ($p<0.01$) with paratuberculosis incidence (Table 4). Of the three different genotypes in the B region (117/117 (B_7B_7), 117/119 (B_7B_8) and 119/119 (B_8B_8),), those genotypes with 119 bp (B_8) alleles (both in homozygous and heterozygous condition) showed significant association with paratuberculosis incidence by both diagnostic methods ($p<0.01$). The details of the association between different genotypic variants of region B and paratuberculosis incidence in goats were given in Table 5. Korou *et al.* (2010) reported that the presence of B_7 allele was significantly associated with absence of MAP specific antibodies in goats, but they did not find association between absence/ presence of MAP antibodies with polymorphisms in region A, as in the present study. Similar associations of *SLC11A1* gene polymorphisms with susceptibility of humans and bovines to *Mycobacterium* spp. and *Brucella* spp. were reported (Bellamy, 1998; Barthel *et al.* 2001; Capparelli *et al.* 2007). The homozygous B_7/B_7 genotype was reported to be associated with increased expression of the *SLC11A1* and *IL-1α* genes indicating increased *in vitro* responsiveness and therefore resistance of mononuclear derived macrophages to MAP infection (Taka *et al.,* 2013).

The results of the present study will augment the information available for the role of *SLC11A1* gene in disease resistance/ susceptibility and will be useful in further studies to determine the markers for selection of paratuberculosis resistant animals. Further investigations are necessary to unravel regulation of *SLC11A1* gene expression based on the genetic variants after intracellular pathogen infection in native goats. So the physiological and biochemical functions, together with the results obtained in the current research, indicate that the *SLC11A1* gene might play a crucial role in disease resistance in goats. The results obtained in the research open a promising opportunity to use these markers as one of the tool in a selective breeding program to control paratuberculosis in goats.

Conflicts of interest

The authors report no conflicts of interest to disclose.

Acknowledgement

The authors acknowledge Kerala Veterinary and Animal Sciences University, Kerala, India for providing the financial support and laboratory facilities for the successful completion of this work.

References

Awomoyi A. A., 2007 The human solute carrier family 11 member 1 protein (SLC11A1): linking infections, autoimmunity and cancer. *FEMS Immunol. Med. Microbiol.* **49**, 324-329.

Barthel R., Feng J., Piedrathia J. A., Mcmurray D. N., Templeton J. W. and Adams L.G. 2001 Stable transfection of the bovine NRAMP1 gene into murine RAW 264.7 cells: Effect on Brucella abortus survival. *Infect. Immun.* **69**, 3110- 3119.

Bellamy R., Ruwende C., Corrah T., McAdam K. P. W. J., Whittle H. C. and Hill A. V. S. 1998 Variation in the NRAMP1 gene is associated with susceptibility to tuberculosis in West Africans. *N. Engl. J. Med.* **338**, 640–644.

Borriello G., Capparelli R., Bianco M., Fenizia D., Alfano F., Capuano F., Ercolini D., Parisi A., Roperto S. and Iannelli D. 2006 Genetic resistance to Brucella abortus in the water buffalo (Bubalus bubalis). *Infect. Immun.* **74**, 2115–2120.

Braunstein M., Bardarov S. S. and Jacobs Jr W. R. 2002 Genetic methods for deciphering virulence determinants of Mycobacterium tuberculosis. *Meth. Enzymol.* **358**, 67-99.

Capparelli R., Alfano F., Amoroso M. G., Borriello G., Fenizia D., Bianco A., Roperto S., Roperto F. and Iannelli D. 2007 Protective effect of the Nramp1 BB genotype against Brucelia abortus in water buffalo (Bubalus bubalis). *Infect. Immun.* **75**, 988-996.

Chiodini R. J., Kruininjen H. J. and Merkal R. S. 1984 Ruminant paratuberculosis (Johne's disease): the current status and future prospects. *Cornell Vet.* **74**, 218-262.

Forbes J. R. and Gros P. 2003 Iron, manganese, and cobalt transport by Nramp1 (Slc11a1) and Nramp2 (Slc11a2) expressed at the plasma membrane. *Blood,* **102**, 1884–1892.

Goswami T., Bhattacharjee A., Babal P., Searle S., Moore E., Li M. and Blackwell J. M. 2001 Natural-resistance-associated macrophage protein 1 is an H+/bivalent cation antiporter. *Biochem. J.* **354**, 511–519.

Iacoboni P. A., Hasenauer F. C., Caffaro M. E., Gaido A., Rossetto C., Neumann R. D., Salatin A., Bertoni E., Poli M. A. and Rossetti C. A. 2014 Polymorphisms at the 3' untranslated region of SLC11A1 gene are associated with protection to Brucella infection in goats. *Vet. Immunol. Immunopathol*. **160**, 230-234.

Halldorsdottir S., Englund S., Nilsen S. F. and Olsaker I. 2002 Detection of Mycobacterium avium subsp. paratuberculosis by buoyant density centrifugation, sequence capture PCR and dot blot hybridization. *Vet. Microbiol.* **87**, 327–340.

Kadarmideen H. N., Ali A. A., Thomson P. C., Muller B. and Zinsstag J. 2011 Polymorphisms of the SLC11A1 gene and resistance to bovine tuberculosis in African Zebu cattle. *Anim. Genet.* **42**, 656-658.

Korou L. M., Liandris E., Gazouli M. and Ikonomopoulos J. 2010 Investigation of the association of the SLC11A1 gene with resistance/sensitivity of goats (Capra hircus) to paratuberculosis. *Vet. Microbiol*. **144**, 353-358.

Liandris E., Gazouli M. and Ikonomopoulos, J. 2009 Characterization of the caprine (Capra hircus) SLC11A1 gene: innate resistance to paratuberculosis. *Online J. Vet. Res.* **13**, 41-52.

Martínez R., Dunner S., Barrera G.and Cañon J. 2008. Novel variants within the coding regions of the Slc11a1 gene identified in Bos taurus and Bos indcus breeds. *J. Anim. Breed. Genet.* **125**, 57–62.

Pinedo P. J., Buergelt C. D., Donovan G. A., Melendez P., Morel L., Wu R., Langaee T. Y. and Rae D. O. 2009 Candidate gene polymorphisms (BoIFNG, TLR4, SLC11A1) as risk factors for paratuberculosis infection in cattle. *Prev. Vet. Med.* **91**, 189-196.

Piras G., Pazzola M., Balia F., Pira E., Dettori M. L., Carcangiu V. and Vacca G. M. 2011 Polymorphism of caprine SLC11A1 gene and relationships with hygienic characteristics of milk. *Agric. Conspec. Sci.* **76**, 175-178.

Reddacliff L. A., Beh K., McGregor H. and Whittington R. J. 2005 A preliminary study of possible genetic influences on the susceptibility of sheep to Johne's disease. *Aust. Vet. J.* **83**, 435–441.

Singh S. V., Singh A. V., Singh P. K. and Sohal J. S. 2008 Prevalence of juvenile capri tuberculosis and genotyping of Mycobacterium avium subsp. paratuberculosis recovered from postnatal kids. *Indian J. Anim. Sci.* **79**,235-238.

Singh A. V., Singh S. V., Makharia G. K., Singh P. K., Sohal J. S. 2008 Presence and characterization of Mycobacterium avium subspecies paratuberculosis from clinical and suspected cases of Crohn's disease and in the healthy human population in India. *Int. J. Infect. Dis*. **12(2):**190-197.

Taka S., Liandris E., Gazouli M., Sotirakoglou K., Theodoropoulos G., Bountouri M., Andreadou M. and Ikonomopoulos J. 2013 In vitro expression of the SLC11A1 gene in goat monocyte-derived macrophages challenged with Mycobacterium avium subsp paratuberculosis. *Infect. Genet. Evol.* **17**, 8–15.

Taka S., Gazouli M., Sotirakoglou K., Liandis E., Andreadou M., Triantaphyllopoulos K. and Ikonomopoulos J. 2015 Functional analysis of 3'UTR polymorphisms in the caprine SLC11A1 gene and its association with the Mycobacterium avium subsp. paratuberculosis infection. *Vet. Immunol. Immunopathol.* **167**, 75-79.

Thomas N. and Joseph S. 2012 Role of SLC11A1 gene in disease resistance. *Biotechnol. Anim. Husbandry* **28(1),** 99-106.

Vacca G. M., Pazzola M., Pisano C., Carcangiu V., Diaz M. L., Nieddu M., Robledo R., Mezzanotte R. and Dettori, M. L. 2011 Chromosomal localization and genetic variation of the SLC11A1 gene in goats (Capra hircus). *Vet. J.* **190**, 60-65.

Vidal S. M., Malo D., Vogan K., Skamene E. and Gros P. 1993 Natural resistance to infection with intracellular parasites: Isolation of a candidate for Bcg. *Cell,* **73**, 469-485.

Received 21 November 2016, in final revised form 11 January 2017; accepted 12 January 2017
Unedited version published online: 16 January 2017

Table 1
Direct count heterozygosity, unbiased heterozygosity and PIC value of microsatellite A and B regions at the 3' UTR of *SLC11A1* gene in Indian goats

Microsatellite Region	**Region A**	**Region B**
No. of alleles	8	2
Direct count heterozygosity	0.6833	0.4985
Unbiased heterozygosity	0.6995	0.5036
Polymorphic information content	0.5474	0.2485

Table 2
Genotypes in region A at the 3' UTR of *SLC11A1* gene and paratuberculosis incidence in goats (Based on genetic group)

No	**Genotypes**	**GT Repeats**	**No. of**	**ELISA**	***IS900*PCR**

			Animals	Positive	Positive
MALABARI GOATS					
1	233/233	GT16/GT16	22	3	9
2	221/233	GT10/GT16	11	1	1
3	233/237	GT16/GT18	8	0	1
5	231/233	GT15/GT16	3	0	1
6	233/235	GT16/GT17	2	1	1
7	233/239	GT16/GT19	3	0	0
8	221/221	GT10/GT10	1	1	1
ATTAPADY BLACK GOATS					
1	233/233	GT16/GT16	16	1	1
2	221/233	GT10/GT16	5	0	0
3	233/237	GT16/GT18	4	1	1
4	231/235	GT15/GT17	8	2	2
5	231/233	GT15/GT16	1	0	0
6	233/235	GT16/GT17	2	0	0
7	221/237	GT10/GT18	5	1	1
8	225/237	GT12/GT18	2	0	0
9	233/239	GT16/GT19	1	0	0
10	229/231	GT14/GT15	2	1	1
11	225/225	GT12/GT12	1	0	0
CROSSBRED GOATS					
1	233/233	GT16/GT16	32	5	11
2	221/233	GT10/GT16	7	1	2
3	233/237	GT16/GT18	9	0	2
4	231/233	GT15/GT16	1	0	0
5	225/237	GT12/GT18	3	1	2
6	233/239	GT16/GT19	1	0	0

Table 3

Genotypes in region B at the 3' UTR of *SLC11A1* gene and paratuberculosis incidence in goats (Based on genetic group)

Sl. No.	Genotypes	GT repeats	No. of Animals	ELISA positive	*IS900* PCR positive
MALABARI GOATS					
1	117/117	GT7/GT7	12	0	2
2	117/119	GT7/GT8	26	3	5
3	119/119	GT8/GT8	12	2	8
ATTAPADY BLACK GOATS					
1	117/117	GT7/GT7	6	0	0
2	117/119	GT7/GT8	38	1	2
3	119/119	GT8/GT8	3	4	4
CROSSBRED GOATS					
1	117/117	GT7/GT7	8	0	0

2	117/119	GT7/GT8	42	11	14
3	119/119	GT8/GT8	3	2	4

Table 4

Association of the diagnostic test results with the genotypes of the region A and region B at the 3'UTR of *SLC11A1* gene. Statistical significance was considered for $P < 0.01$ based on Chi square Test.

Sl No	Microsatellite region	Test	*P*-value
1.	Region A	ELISA	0.836^{NS}
2.	Region A	*IS900* PCR	0.508^{NS}
3.	Region B	ELISA	$<0.01^{*}$
4.	Region B	*IS900* PCR	$<0.01^{*}$

Level of Significance P<0.01, * Significant at P < 0.01

Table 5

Association of the results for ELISA and *IS900* PCR with the presence of the B8 allele in homozygous and heterozygous condition, based on the Chi square Test

Sl. No.	Genotype	ELISA		*IS900* PCR	
		+	-	+	-
1	B_7/B_7	0	26	2	24
2	B_7/B_8 and B_8/B_8	23	101	37	87
		P <0.01		*P* <0.01	