Revue-IRS

Revue Internationale de la Recherche Scientifique (Revue-IRS)

ISSN: 2958-8413

Vol. 2, No. 5, September 2024

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Investigation into the genetic polymorphism of the SLC11A1 gene and its influence on the resistance and susceptibility to bovine tuberculosis in Tunisia

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Abstract: Bovine tuberculosis (bTB), caused by Mycobacterium bovis (M. bovis), is a contagious infectious disease with considerable economic impact on dairy and meat farms worldwide. Host genetics could potentially affect susceptibility or resistance to this disease. The aim of this study was to discover the association between tuberculosis and the polymorphism of a gene playing an important role in resistance/susceptibility to this disease. Blood samples were taken from 200 Holstein cows, including 50 cows classified as cases, tested positive for tuberculosis, and 150 cows as controls, tested negative for tuberculosis. To study polymorphism of the gene coding for SLC11A1, genomic DNA was extracted using the salt method and the analytik jena kit (n=200). DNA was amplified using specific primers. PCR produced 374 pb amplifiates, which were then digested by PCR-RFLP with the PstI restriction enzyme. Two genotypes, CC and CG, with allele frequencies of 0.85 and 0.15 for the C and G alleles respectively, were revealed. Homozygous GG individuals show resistance to the disease, and the observed C> G mutation may play a role in controlling the individual's resistance to the tuberculin reaction, but the results obtained are insufficient to confirm the association between the polymorphisms and susceptibility to tuberculosis. This is the first work to indicate that rs109453173 C>G polymorphisms may contribute to SLC11A1 mediated bTB susceptibility. SLC11A1 may be a useful candidate gene linked to bTB in Holstein cows.

Keywords: *Bovine tuberculosis; Polymorphism; Resistance; SLC11A1; Sensitivity PCR-RFLP.*

Digital Object Identifier (DOI): https://doi.org/10.5281/zenodo.13826228

1 Introduction

Bovine tuberculosis (bTB) is presently among the foremost re-emerging zoonotic illnesses observed across various regions globally [1,2]. It imposes significant economic burdens on livestock and poses substantial challenges for the international trade of animals and their derivatives [3]. Although predominantly affecting cattle, it can spread to other species, including humans [1,4]. Over recent decades, efforts to reduce the risk of human infection have been undertaken, such as milk pasteurization and regular tuberculin testing of cattle, coupled with mandatory culling of animals displaying signs of infection [5]. The livestock sector plays a significant role in financing the livelihoods of farmers, consumers, traders, and workers worldwide. Approximately 70% of our country's population is directly or indirectly involved in agriculture and livestock [6]. The profitability of this sector depends on several factors, including infectious diseases that result in annual losses mainly due to morbidity, mortality, decreased fertility, production losses, and inefficient use of animal feed. Among the major infectious diseases with a significant economic impact on the global and Tunisian agricultural industries is bovine tuberculosis [7]. The annual cost of tuberculosis to the global agriculture is estimated at around three billion US dollars [8]. At the national level, bTB remains a significant threat to public health and causes economic losses in the livestock sector [9]. It also poses a risk to human health, as 10 to 15% of human tuberculosis cases worldwide are attributed to M. bovis infection [10]. Understanding the genetic basis of disease susceptibility has become an increasingly crucial research objective. Today, technological advancements enable the deployment of a growing number of markers in case-control studies aiming to identify genetic factors influencing susceptibility or resistance to infectious diseases [11]. Genome-wide association studies (GWAS) have identified chromosomal regions of the bovine genome associated with susceptibility to bovine tuberculosis, aiming to pinpoint specific candidate genes for further analysis [12-14]. Several candidate genes have been studied and selected in livestock based on their association with resistance or susceptibility to various other diseases and their known role in disease pathogenesis. Among these genes is the Natural Resistance-Associated Macrophage Protein 1 (NRAMP1), also known as Solute Carrier Family 11 A1 (SLC11A1). It is an integral transmembrane protein expressed particularly on the macrophage phagosome [15,16]. The gene (SLC11A1) is located on chromosome 2 in cattle and comprises 16 introns and 15 exons. The role of this gene in innate immunity is crucial, as it prevents bacterial proliferation in macrophages during the early stages of infection [17]. A previous study identified polymorphisms (SNPs) in the bovine SLC11A1 gene associated with susceptibility to bTB [18]. A study on Taiwanese Holstein-Friesian cattle identified SNPs in exon 4 and intron 4 associated with increased susceptibility to bTB [19]. While in a Chinese population of Holstein-Friesian cattle, associations with susceptibility/resistance to bTB were found with SNPs in exon 11 and introns 5 and 9 [20]. The identification of genetic polymorphism is thus a highly effective means to assess this diversity and study the genetic characteristics of cattle in order to select the best individuals that allow us to build disease-free herds. The aim of this study is to investigate the association between polymorphisms in tuberculosis-related genes, specifically the SLC11A1 gene, and the resistance/susceptibility of livestock to this disease, as well as its prevalence in our country. Our objective is to develop a proactive approach for selecting breeding stock, aimed at minimizing the health and economic impact of tuberculosis in Tunisia.

2 Materials and methods

2.1 Animals and tuberculosis diagnosis

The experiment was conducted at the experimental farm located at the north of Tunisia (33°38′00″S 62°25′00″W). 150 Holstein cows were included in the study, with 50 cows classified as cases, testing positive for bTB, and 100 cows as controls, testing negative for bTB. Herds were first examined for bTB, and study animals were selected at random from a list of both infected (cases) and non-infected (control) animals. Since all animals have the same habitat and dietary needs, it was assumed that their exposure to M. bovis in the environment was uniform. The study's animals were all cows, with ages ranging from 4 to 6 years, and artificial insemination was used for reproduction. The animals were housed in a semi-intensive production system consisting of grass supplemented with a concentrated diet, and they were milked twice daily. A Tuberculin skin test was carried out to identify M. bovis infected and non-infected.

2.2 Samples and DNA extraction

The blood samples were collected into 5 ml vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant using a single-use needle to prevent contamination. Subsequently, the samples were kept cold in an insulated container until transportation to the laboratory as soon as possible, where they were stored at a temperature of -20°C before DNA extraction. Genomic DNA was extracted using a commercial extraction kit (Analytik Jena) following the recommended protocol. The protocol of this kit consists of six essential steps: cell lysis, RNA removal, DNA binding, washing, ethanol removal, and DNA elution. DNA separation based on molecular weight (MW) was performed by electrophoresis on a 0.8% agarose gel

2.3 molecular markers used

Primers for the one SNPs (rs109453173) were designed using Oligoanalyser while reported primer for SLC11A1 marker was used for amplification of the loci. The details of primers and restriction enzymes are tabulated in Table 1.

2.4 DNA amplification by PCR

The amplification of the target sequence was carried out using the polymerase chain reaction (PCR) technique, employing a reaction mixture containing specific primers for each gene studied. The optimized PCR conditions were the followings: 10 min of initial denaturation at 95 °C, followed by 30 cycles of 30 s at 95 °C, with the annealing temperature at 55 °C for 30 s, and the extension for 1 min at 72 °C. A final extension step was carried out at 72 °C for 10 min to amplify the used primers. The reaction volume used for each 25 µl sample contained 2.5 µl MgCl2 (25 mM), 0.2 µl dNTP (25 mM), 2.5 µl of each primer (25 pmol/µl), 0.2 µl of Taq polymerase (5 U/µl) , 50 ng DNA and 2.5 µl of buffer (10X). Amplified products were denatured with HiDi TM formamide (8.3 µl). (Table 2)

2.5 PCR-RFLP assay

The amplicons were digested by PstI restriction enzymes (Table 3) using the known mutation detection technique, the restriction fragment length polymorphism. Point mutations within the target sequence were detected using the RFLP technique. The restriction enzyme PstI was utilized to hydrolyze the PCR product in a

reaction mixture consisting of: 2.5 µl of buffer, 0.3 µl of the enzyme, 12 µl of the amplicons (PCR products), and 10.2 µl of dH2O. This mixture was incubated at 37°C for 12 hours. The digestion products were separated on a 2% agarose gel, stained with Gelstain, and visualized under UV light.

2.6 Statistical analysis

SAS software (SAS Institute Inc. SAS® 9.4. 2014) was used to analyze the data. The chi-square test was used to examine the association between genetic polymorphisms and animal's susceptibility to infection. If the theoretical value for a cell was <5 in the chi-square test, Fisher's exact test was applied. The degree of association between polymorphisms and BTB was statistically assessed using estimated odds ratios (ORs) and 95% confidence intervals (CIs). For every SNP, the Hardy-Weinberg equilibrium (HWE) was examined. At $p < 0.05$, differences were deemed statistically significant.

Figure 1. Electrophoresis profile on 1% agarose gel of genomic DNA samples from the analyzed individuals.

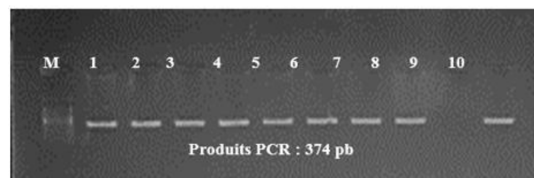

Figure 2. Electrophoretic profile of the PCR reaction after migration on a 2% agarose gel.

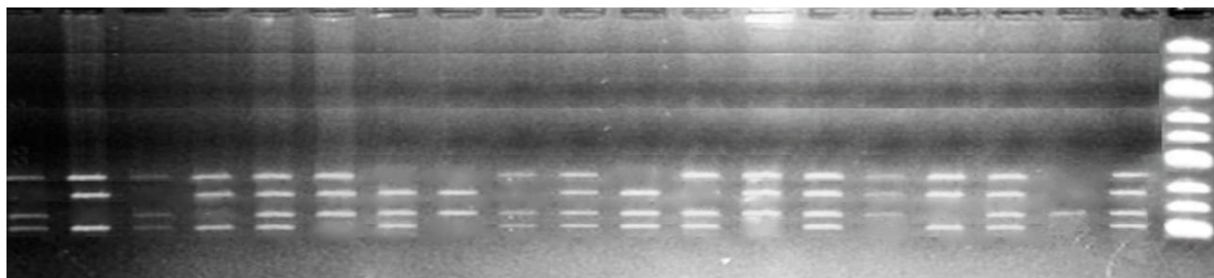

Figure 3. Digestion profile of the SLC11A1 gene amplification products with the PstI enzyme on a 2.5% agarose gel.

3 Results and discussions

3.1 Genomic DNA extraction and evaluation of the PCR-RFLP protocol

A 1% agarose gel analytical electrophoresis was performed for all samples. This step allowed for the evaluation of the quality and quantity of the extracted DNA. Under ultraviolet (UV) light visualization, the treated samples were found to be of good quality as the bands were clear, intense, and free of degraded DNA. Figure 1 illustrates the electrophoretic profile of some treated samples. When visualizing the electrophoretic profile of the amplicons of the gene studied in our present research under UV light, clear and intense bands are seen, demonstrating the proper functioning of the polymerase chain reaction (Figure 2). The successful execution of PCR primarily depends on the optimization of the annealing temperature and the concentration of deoxyribonucleotides (dNTP).

3.2 Genotypic frequencies and allelic frequencies

The amplified products were digested with the restriction enzyme PstI, which has a restriction site of 5'CTGCAG3'. The presence of the restriction site indicates the presence of the mutation. In our case, the enzyme used for genotyping at the SNP C/G locus rs109453173 of the SLC11A1 gene allowed for the distinction of:

- Wild-type homozygous genotypes: These exhibit bands of 374 bp, the same as the PCR product bands, indicating that these individuals with the C//C genotype do not have the mutation responsible for susceptibility/resistance to tuberculosis.
- Heterozygous genotypes: These exhibit two bands of different sizes, one being the 374 bp PCR product band and the other being a 293 bp band, indicating that these individuals with the C//G genotype have the mutation responsible for susceptibility/resistance to the disease.

For the SLC11A1 gene, we observed two alleles, C and G, with the dominance of the C allele being very notable, with an allele frequency of 0.85.

Our results are in agreement with those found by [21], within an Indian herd, but they do not match those found by [22] in China, where the genetic polymorphism of the SLC11A1 gene showed two fragments containing 633 bp and 303 bp for the GG and TT genotypes; two fragments containing 709 bp and 227 bp for the CC and AA genotypes; four fragments containing 709 bp, 406 bp, 303 bp, and 227 bp for the CG and AT genotypes; and four fragments containing 709 bp, 633 bp, 303 bp, and 227 bp for the CG and AA genotypes.

According to the digestion results, we obtained homozygous individuals with the C//C genotype who are considered healthy. This genotype indicates individuals resistant to the tuberculin reaction, which contradicts the findings of [23]. However, our results agree with those obtained by [24], who found that the C//C genotype might confer significant resistance to tuberculosis and thus identify a healthy phenotype.

Our hypotheses can be confirmed by increasing the sample size and obtaining an intradermal test confirming that individuals predicted to be phenotypically healthy are affected. Thus, we can consider that the C > G mutation plays a role in susceptibility to tuberculosis, and the heterozygous genotype marks individuals sensitive to the tuberculin reaction.

This result will contradict the one found [24], who acknowledge that the C//G genotype can also be resistant to tuberculosis. Therefore, significant associations between SLC11A1 polymorphisms and susceptibility to tuberculosis have been reported, as documented by [25].

Indeed, in a study in humans, the G>C mutation in intron 4 of the NRAMP1 gene has been reported as a factor predisposing to paratuberculosis [26]. However, studies in Russians have revealed that the G/C (INT4) polymorphism is not associated with tuberculosis [27]. Therefore, not all studies support an association between SLC11A1 polymorphisms and susceptibility to M. bovis infection, and it is important to note that the sensitive phenotype is due to a nucleotide substitution resulting in an amino acid change rather than a polymorphism in microsatellite sequences [28].

For the SLC11A1 gene, we observed two alleles, C and G, with the dominance of the C allele being very notable, with an allele frequency of 0.85. However, in India, a dominant frequency for the G allele was found by [29]. whereas in China, dominance was for the C allele, as reported by [24]. From these results, we can conclude that there is an association between the C allele and disease resistance, which aligns with the findings of [19].

In this regard, we can confirm the existence of a mutation in the SLC11A1 gene, which is a substitution of the C allele with the G allele at the rs109453173 locus, resulting in two genotypes: the first being the wild-type homozygous C//C and the second being the heterozygous C//G. However, this mutation may play a role in the association of SLC11A1 with resistance/susceptibility to the tuberculin reaction. However, these results require further validation through markers in a larger population, including additional confirmatory diagnostic tests.

4 Conclusion

For the SLC11A1 gene, two alleles, C and G, were observed, with the dominance of the C allele being very notable, with an allele frequency of 0.85. It can be considered that the C > G mutation plays a role in susceptibility to tuberculosis.

Table 1. Primers for the target sequence of the SLC11A1 gene.

Gene	Primers	PCR size
SLC11A1	AF: 5'- ATCTCCTTCCTACTGCCCG-3' AR: 5'-CACAAACTGTCCCGCGTAC-3'	374 pb
F : bait direction ; R : anti-sense. (Baqir et al.,2015)		

Table 2. PCR reaction components

Components	Initial concentration	Vi (µl) / one sample
Tampon (tampon)	10X	2.5
$MgCl_2$	50mM	1.25
dNTP	100mM	2.5
Primer sense	25mM	2.5
Anti-sense primer	25mM	2.5
Taq polymerase	5U/µl	0.25

Autoclaved water volume	-	11.5
Volume mix	-	23
Genomic DNA	-	2
Total volume	-	25

Table 3. Restriction enzyme and hybridization temperature.

Gene	Restriction enzyme	Digestion temperature
SLC11A1	PstI	37°C

REFERENCES

[1] Grange JM (2001) *Mycobacterium bovis* infection in human beings *Tuberculosis*, **81**, 71–77.

[2] Smith RM, Drobniewski F, Gibson A *et al.* (2004) *Mycobacterium bovis* infection, United Kingdom. *Emerging Infectious Disease*, **10**, 539 –541.

[3] Milian Suazo F, Anaya Escalera AM, Gallegos Torres RM (2003) A review of *M. bovis* BCG protection against TB in cattle and other animal species. *Preventive Veterinary Medicine*, **58**, 1–13.

[4] O'Reilly LM, Daborn CJ (1995) The epidemiology of *Mycobacterium bovis* infections in animals and man: a review. *Tubercule and Lung Diseases*, **76**, 1–46.

[5] Minor R (2003) Control of bovine tuberculosis. *Veterinary Record*, **152**, 603–604.

[6] Chebbi, H.E., Amrani, M. et Bouallegui, F. 2023. Le système agricole et agroalimentaire tunisien face aux crises : focus sur la chaîne de valeur céréalière - Constats et réflexions pour la proposition d'un plan de relance à court et à moyen termes. Tunis, FAO. https://doi.org/10.4060/cc6217fr

[7] Prajapati BM, Gupta JP, Pandey DP, Parmar GA, Chaudhari JD. 2017.Molecular markers for resistance against infectious diseases of economic importance, Veterinary World, 10(1): 112-120.

[8] Allen, A. R., Minozzi, G., Glass, E. J., Skuce, R. A., McDowell, S. W., Woolliams, J. A., et al. (2010). Bovine tuberculosis: the genetic basis of host susceptibility. Proc. Biol. Sci. 277, 2737–2745. doi: 10.1098/rspb.2010.0830.

[9] Abid Amira, Kalthoum Sana, Ben Salem Ameni, GuesmiKaouther, Lazgheb Asma, Mansouri Riadh, Benzarti Mhamed, 2019 bulletin zoosanitaire. N°18 Etude rétrospective de la tuberculose bovine et humaine en Tunisie (2006-2016)

[10] Michel, A. L., Muller, B., and van Helden, P. D. (2010). Mycobacterium bovis at the animal-human interface: a problem, or not? Vet. Microbiol. 140, 371–381. doi: 10.1016/j.vetmic.2009.08.029

[11] Freitas, T.M.S.; Dias, J.M.; Guimarães, L.K.P.; Peixoto, S.V.; Silva, R.H.S.d.; Badr, K.R.; Moura, M.I.; Carmo, A.S.d.; Landi, V.; Fioravanti, M.C.S. Genomic Association between SNP Markers and Diseases in the "Curraleiro Pé-Duro" Cattle. Genes 2021, 12, 806. https://doi.org/ 10.3390/genes12060806.

[12] Finlay, E. K., Berry, D. P., Wickham, B., Gormley, E. P., and Bradley, D. G. (2012). A genome wide association scan of bovine tuberculosis susceptibility in Holstein-Friesian dairy cattle. PLoS One 7:e30545. doi: 10.1371/journal.pone. 0030545

[13] Bermingham, M. L., Bishop, S. C., Woolliams, J. A., Pong-Wong, R., Allen, A. R., McBride, S. H., et al. (2014). Genome-wide association study identifies novel loci associated with resistance to bovine tuberculosis. Heredity (Edinb) 112, 543–551. doi: 10.1038/hdy.2013.137

[14] Ring, S. C., Purfield, D. C., Good, M., Breslin, P., Ryan, E., Blom, A., et al. (2019). Variance components for bovine tuberculosis infection and multi-breed genome-wide association analysis using imputed whole genome sequence data. PLoS One 14: e0212067. doi: 10.1371/journal.pone.0212067

[15] Blackwell, J. M., Searle, S., Goswami, T., and Miller, E. N. (2000). Understanding the multiple functions of Nramp1. Microbes Infect. 2, 317–321.

[16] Prakash O, Kumar A, Sonwane A, Rathore R, Singh RV, Chauhan A, Kumar P, Renjith R, Yadav R, Bhaladhare A, Baqir M, Sharma D.2014. Polymorphism of cytokine and innate immunity genes associated with bovine brucellosis in cattle. Mol Biol Rep. 41:2815–2825.

[17] Pinedo PJ, Buergelt CD, Donovan GA, Melendez P, Morel L, Wu R, et al,2009. Candidate gene polymorphisms (BoIFNG, TLR4, SLC11A1) as risk factors for paratuberculosis infection in cattle. Prev Vet Med2009;91(2e4):189e96

[18] Cheng Y, Huang C, Tsai HJ, 2015. Relationship of Bovine SLC11A1 (Formerly NRAMP1) Polymorphisms to the Risk of Bovine Tuberculosis in Holstein Cattle. J Veterinar Sci Technol 6:247. doi:10.4172/2157-7579.1000247

[19] Cheng, Y., Huang, C., and Tsai, H. J. (2015). Relationship of Bovine SLC11A1 (Formerly NRAMP1) Polymorphisms to the risk of Bovine tuberculosis in Holstein cattle. J. Veterinar. Sci. Technol. 6:5. doi: 10.4172/2157-7579.100 0247.

[20] Liu, K., Zhang, B., Teng, Z., Wang, Y., Dong, G., Xu, C., et al. (2017). Association between SLC11A1 (NRAMP1) polymorphisms and susceptibility to tuberculosis in Chinese Holstein cattle. Tuberculosis (Edinb) 103, 10–15. doi: 10.1016/j.tube.2016.11.003

[21] Baqira Mohd, Saket Bhushanb, Amit Kumara, Arvind Sonawanea, Ranvir Singha, Anuj Chauhana, Ramji Yadava et al. 2015.Association of polymorphisms in SLC11A1 gene with bovine tuberculosis trait among Indian cattle

[22] liu Kaihua a, Bin Zhang a, Zhaochun Teng a, Youtao Wang a, Guodong Dong b, Cong Xu c , Bo Qin a , Chunlian Song a , et al., 2016.Association between SLC11A1 (NRAMP1) polymorphisms and Q4 susceptibility to tuberculosis in Chinese Holstein cattle.

[23] Baqir, Ashish Bhaladhare, Deepak Sharma, Amit Kumar, Arvind Sonwane, Anuj Chauhan, Ranvir Singh,Pushpendra Kumaret al., 2016 .Single nucleotide polymorphisms in toll-like receptor genes and case-control association studies with bovine tuberculosis

[24] **Pinedo PJ, Buergelt CD, Donovan GA, Melendez P, Morel L, Wu R, et al,2009**. Candidate gene polymorphisms (BoIFNG, TLR4, SLC11A1) as risk factors for paratuberculosis infection in cattle. Prev Vet Med2009;91(2e4):189e96

[25] **Qin B, Liu T, Xu C, Dong G, Wang S, Tu W, Shi X, Zhang Y. 2015.** Correlation study of CARD15 gene style polymorphism and susceptibility of tuberculosis in dairy cows. *China Anim. Husb. Vet. Med.*;42(6):1553–1558

[26] Puzyrev VP, Freĭdin MB, Rudko AA, Strelis AK, Kolokolova OV, 2002.Polymorphisms of the candidate genes for genetic susceptibility totuberculosis in the Slavic population of Siberia: a pilot study. Mol Biol, 36:788–791.

[27] Tatiane A. Paixão, Cintia Ferreira, Álan M. Borges, Denise A.A. Oliveira, Andrey P. Lage, Renato L. Santos (2006). Frequency of bovine *Nramp1* (Slc11a1) alleles in Holstein and Zebu breeds. Veterinary Immunology and Immunopathology Volume 109, Issues 1–2, 15 January 2006, Pages 37-42

[28] Baqira Mohd, Saket Bhushanb, Amit Kumara, Arvind Sonawanea, Ranvir Singha, Anuj Chauhana, Ramji Yadava et al.,2015. Association of polymorphisms in SLC11A1 gene with bovine tuberculosis trait among Indian cattle