IDENTIFICATION OF STRs POLYMORPHISM AND ITS ASSOCIATION WITH TUBERCULOSIS IN CATTLE

Thesis

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Veterinary Science
(Animal Genetics and Breeding)

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Dedicated to....

My guide

Dr. Amit Kumar

Special Thanks

to

Dr. Deepak Sharma

&

Dr. Arvind Sonvane

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Dated: 31-05- **2013**

Certificate

This is to be certify that the research work embodied in this thesis entitled **"Identification of STRs polymorphism and its association with tuberculosis in cattle"** *submitted by* **Dr. Renjith R., Roll No. 5022,** *for the award of* **Master of Veterinary Science** *Degree in* **Animal Genetics and Breeding** *at Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate himself under my supervision and guidance.*

It is further certified that **Dr. Renjith R., Roll No. 5022,** *has worked for more than 21 months in the Institute and has put in more than 150 days attendance under me from the date of registration for the* **Master of Veterinary Science** *Degree in this Deemed University, as required under the relevant ordinance.*

Amit Kumar

(AMIT KUMAR)
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We the undersigned members of Advisory Committee of **Dr. Renjith R.,** *Roll No. 5022 a candidate for the degree of* **Master of Veterinary Science** *with the major discipline* **Animal Genetics and Breeding,** *agree that the thesis entitled* **"Identification of STRs polymorphism and its association with tuberculosis in cattle"** *may be submitted in partial fulfillment of the requirement for the degree.*

We have gone through the contents of the thesis and are fully satisfied with the work carried out by the candidate, which is being presented for the award of by **Master of Veterinary Science** *Degree of this Institute.*

It is further certified that the candidate has completed all the prescribed requirements governing the award of **Master of Veterinary Science** *Degree of the Deemed University, Indian Veterinary Research Institute, Izatnagar.*

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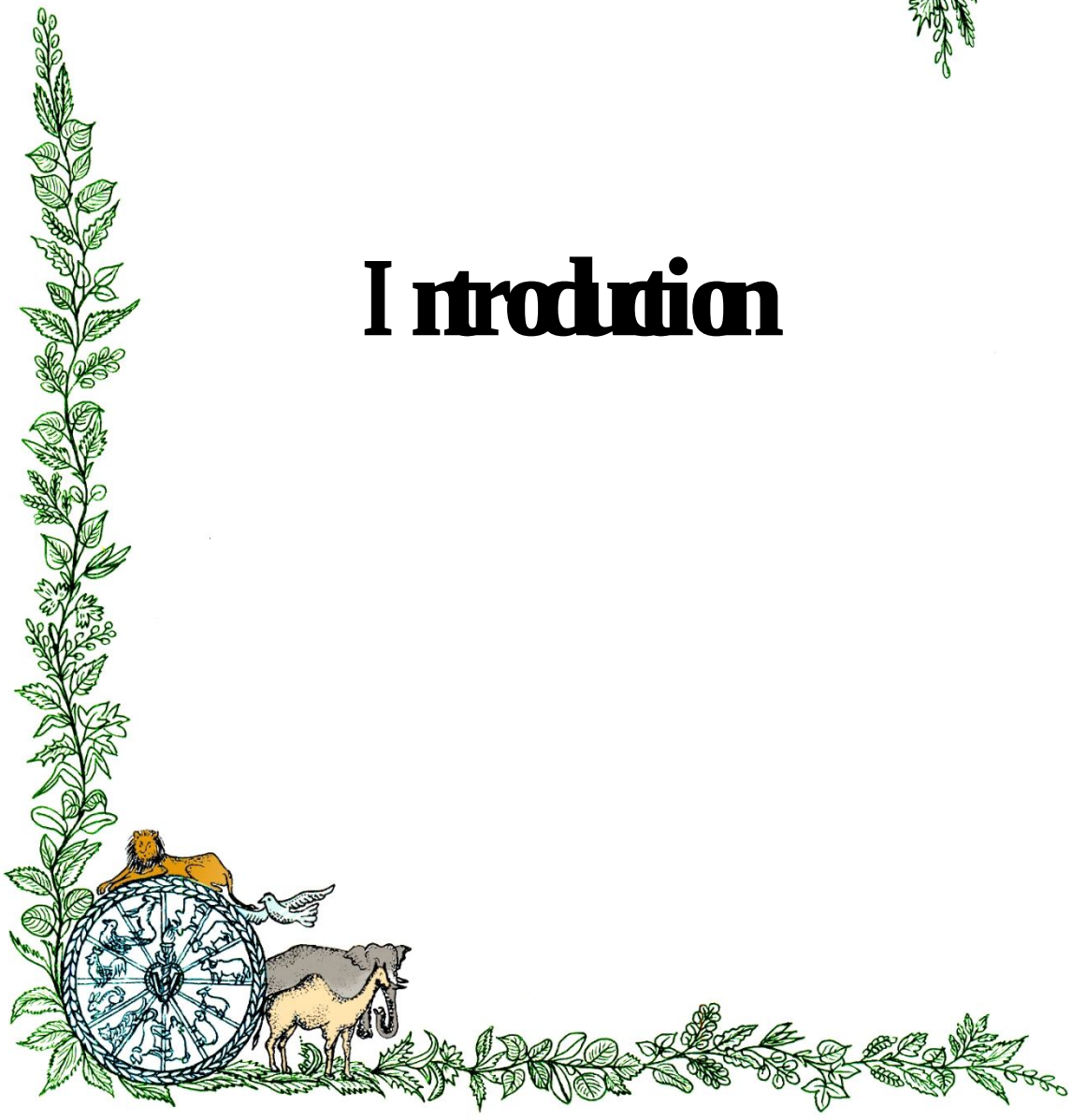


1 INTRODUCTION

Livestock is an important sub-sector of Indian agricultural economy. It plays a versatile role in providing a sustainable livelihood security to the rural population. The contribution of livestock and fisheries sector together at current prices in agriculture and allied sector has significantly increased from 16 % in 1997-71 to 31.6 % in 2007-08 to the GDP of India. As per the 18th Indian Livestock Census Report-2007, India is home to a total of 529.70 million livestock population. India is ranked 1st in buffaloes, 2nd in cattle and goat, 3rd in sheep, 4th in duck, 5th in chicken, and 6th in camel and 7th in mule population in the world. India has also emerged the largest producer of milk in the world producing 121.8 million tonnes of milk/year during the year 2009-10.

Outbreaks of diseases are common in commercial and private farms as well as livestock kept under crop livestock production system. In spite of government's efforts to provide subsidized veterinary services through a network of physical and human infrastructure; infectious diseases continue to hamper production and productivity of livestock. Diseases like rinderpest have been eradicated from the country, but still there are many which pose potential threat to Indian livestock. An estimated 40 million animals die every year in the country due to natural causes, disease and other problems (Singh, 2010). With regard to prevention and control of infectious diseases, the conventional measures include use of antibiotics and antibacterial agents, vaccination and quarantine procedures. These strategies are used with varying degrees of success to prevent and control many livestock diseases. In addition to these measures, the approach should be the utilization of superior genotypes, which are high producing and are simultaneously resistant to diseases.

Tuberculosis is one of the major chronic disease in humans, especially in India at par with AIDS and Cancer. It is caused by many organisms belonging to the genus *Mycobacterium* spp. The common infectious agent causing tuberculosis in human is *Mycobacterium tuberculosis*. An important disease of domesticated cattle that has a major economic and health impact throughout the world (Smith *et al*., 2006). Infection due to *Mycobacterium bovis* typically occurs in cattle but has been reported in other animals including dogs, cats, swine, rabbits, birds and man (Pavlas, 1982; Delahay *et al*., 2002) and it has wild animal reservoirs host. Prevalence data on bTB in cattle are generally scarce, but official data reported by member countries of the OIE (World Organization for Animal Health) suggest that the disease in domestic animals is widely distributed around the world and present on nearly all continents (Cosivi *et al*., 1998). Worldwide, agricultural losses due to bTB are estimated around $3 billion annually (Garnier *et al*., 2003).Studies revealed that among humans, the disease incidence is higher in farmers, abattoir workers and others who worked with cattle. Tuberculosis in humans causes almost two million deaths annually (Mathers *et al*., 2009).In India; however there is little information available on the transmission of bovine tuberculosis and its impact on human health. The organism is susceptible to heat treatment, but drinking of milk without boiling is major cause of zoonotic spread of disease. Generally treatment for bTB is not recommended in animals since there is no cost effective treatment for bTB. The incidence of bTB has been significantly reduced or eradicated from domestic cattle in many developed countries by state compensation of a test and cull policy that removes infected cattle (Ayele *et al*., 2004).The TB-free New Zealand programme is regarded as "world-leading" and has successfully reduced cattle and deer herd infection rates from more than 1700 in 1994 to fewer than 100 herds in July 2011. Even though developed countries have successfully checked the disease; the same is not the case with developing countries. Hence our focus should be to develop cattle which are comparatively resistant to the disease. The traditional animal breeding strategies (i.e. animal performance and pedigree recording for genetic evaluations of animals) for developing resistant strains of live-stock species is difficult to implicate due to organizational, operational, technical and infrastructural difficulties. However, the use of genetic markers for the genetic improvement of resistance of the host is a critical component of effective disease control. Better knowledge on host genetic mechanisms of susceptibility and/or resistance are prerequisites for the development of animal breeding tools which may open ways for possible

effective and sustainable methods of bTB control (Ali *et al*., 2013). Thus the basic principle is the identification of already resistant animals and further use of such superior genotype for multiplication. Identification of genes related to disease resistance has already been carried out in various researches. Now the part left is identification of these genes and their possible polymorphisms related to disease resistance in our population. One approach in this regard could be to exploit the host genetic variation in response to bTB (Mackintosh *et al*., 2000). In a study on a large dataset of tuberculin skin tests from Irish and British dairy cows (Bermingham *et al*., 2009) estimated heritability were ranged from 0.14 to 0.18 (se: ± 0.044). A recent quantitative genetic study, which analyzed TB skin test data from British dairy cattle herds, estimated heritability for TB resistance of 0.18 ± 0.04 on the liability scale (Brotherstone *et al*., 2010) .These results suggest that exploitable genetic variation exists and that selection for resistance to bTB, is feasible. Interestingly, in cattle it has been demonstrated that differences in susceptibility to BTB is at the level of genus, (Vordermeier *et al*., 2012) indicating that *Bos indicus* cattle are more resistant than *Bos taurus*. These encouraging findings indicate a role for genetics in a wider risk management strategy. For example, in dairy cattle, exploitation of genetic variability has already been established and used in selection programmes for mastitis resistance (Rupp *et al*., 2003). Although the heritability of clinical mastitis is low and has an adverse correlation with production traits, selection for mastitis resistance is nevertheless implemented in selection programmes in many countries, notably in Scandinavia (Heringstad *et al*. 2003). In principle, the same could be done for resistance to TB in cattle breeding programmes.

Till date no report about genetic basis of resistance against tuberculosis is available in cattle in India. Mostly people have worked on virulence of organism but host pathogen interaction study is totally lacking. Also, no report is available about development preventive/prophylactic measures against tuberculosis in cattle.

Candidate gene approaches in bTB resistance research have concentrated mainly on the bovine natural resistance-associated macrophage protein (NRAMP1) gene owing to the ubiquity with which it has been identified as a candidate in mouse and human studies. Although there is no particular gene known to be responsible for differences in BTB infection susceptibility in cattle, natural resistant associated macrophage protein 1 (NRAMP1) was a known candidate

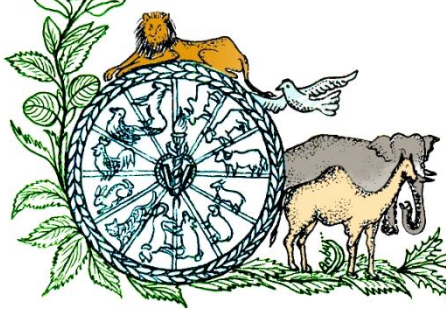
gene; it is associated with natural resistance to infection with Mycobacterium spp, in cattle (Kadarmideen *et al*.,2011). A microsatellite in the 30' untranslated regions (UTR) of the bovine NRAMP gene has been found to be associated with natural resistance to brucellosis infection in cattle (Adams *et al*., 1998) and in macrophages the 'resistant' allele appeared to be associated with the survival of M. bovi BCG (Qureshi *et al*., 1996). In a study, comprising 33 cattle with positive results to the tuberculin test, of which nine were assigned a resistant phenotype and 24 a susceptible phenotype, no association was observed between the bovine 30 UTR microsatellites and resistance to *M. bovis* infection (Barthel *et al*., 2000., Ameni *et al*. 2007 reported disease risks were also estimated, with Holstein cattle 2.32 times more likely to be diseased than zebu cattle. Despite considerable evidence of a genetic component to TB resistance, modest effort has been directed towards identifying bovine genetic susceptibility loci. It is only recently that effort has been directed towards quantifying the host genetic influence (Bermingham *et al*., 2009 and Magee *et al*., 2012) revealed that increased number of down regulated genes is showing that *M. bovis* infection is associated with the repression of host gene expression. These studies provide sub-stantial evidence that polymorphisms in the genome sequence play an essential role in determining resistance/ susceptibility to bTB infection.

However, there is a lack of information on the potential genetic markers for bTB resistance in Zebu cattle of India and Indian Cross breed cattle. Hence, once association between alleles of microsatellites and the immune profiles of tuberculosis tolerance/susceptibility are identified then it could be incorporated into selection and breeding programs. Keeping all these factors in view, the present study is proposed with the following objectives:

i) To find polymorphism of microsatellite markers conferring resistance against Tuberculosis in Indigenous and Cross bred animals

ii) To find association of allelic variants of microsatellite markers with resistance/ susceptibility of bovine tuberculosis and immunological parameters

Review of Literature



2 REVIEW OF LITERATURE

2.1. Bovine tuberculosis

Bovine tuberculosis is caused by an intra cellular, acid-fast bacterium, *Mycobacterium bovis*. *Mycobacterium bovis* typically attacks cattle, but reports on infections diagnosed in other species like dogs, cats, human etc are available (Pavlas *et al*., 1982; Moda *et al*, 1996). The infection will hamper the health of the animal and will result in serious economic losses (Pollock et *al*., 2002). Since the bacterium affects man, it poses serious public health concern. Infected cattle are a potential source of infection to other animals and humans. European B. taurus cattle appear to be more susceptible to *M. bovis* infection than *B. indicus* cattle (Ameni *et al*., 2007).

2.2. Diagonosis of Bovine tuberculosis

It is important to address the area of TB diagnostics in this review as it has major implications for the design of any genetic epidemiology study tasked with determining the heritability of TB susceptibility and the individual loci contributing to this phenotype. The identification of the TB-susceptible (case) or TB-resistant (control) phenotypes will include information derived from the diagnostic test itself, along with other co-variables. *Mycobacterium bovis* is an obligate pathogen and the presence of *M. bovis* in an animal indicates infection. Proper diagnosis is important for the identification and elimination of the infected animals. Diagnosis of chronic diseases like tuberculosis is difficult as compared to that of acute diseases. Different signals of the disease are produced at different stages of the progress of the disease and are very useful in the diagnosis of infection. So a clear understanding of the pathophysiology of the disease will prove beneficial for the diagnosis. Diagnostic tests may be grouped into

surrogate and pathognomonic categories. Surrogate tests are those that detect a signal which indirectly indicates *M. bovis* infection, whereas pathognomonic tests are those that directly detect the *M. bovis* organism itself and thus by definition are 100% specific (Adams, 2001). Confirmatory tests are used when *M. bovis* infection is strongly suspected. Confirmatory tests must have a specificity approaching 100%, but lower sensitivity is acceptable ((Adams, 2001).

Bovine tuberculosis infection in cattle is usually diagnosed in the live animal on the basis of delayed hypersensitivity reactions along with clinical signs such as weakness, anorexia, emaciation, dyspnoea, enlargement of lymph nodes, and cough, particularly with advanced tuberculosis. After death, infection is diagnosed by necropsy and histopathological and bacteriological techniques. Rapid nucleic acid methodologies, such as the polymerase chain reaction (PCR), may also be used although these are demanding techniques and should only be used when appropriately validated.

2.2.1. Cell-mediated immunity-based diagnostics

The cell mediated immune response during *Mycobacterium* infection in cattle is more important than humoral immune response. Tuberculosis induces a spectrum of immune responses dominated by the cell mediated response to intracellular infection (Pollock *et al*., 2002; Welsh *et al*., 2005)

A. Tuberculin intradermal delayed type hypersensitivity

The test is totally based on the proper cell-mediated immunological response of the host developed during the various phases of disease pathogenesis (Ashutosh *et al*., 2012). OIE prescribed delayed hypersensitivity test as a reliable test for the diagnosis (Anon, 2008.) and It is one of the oldest and the most widely used in vivo and surrogate diagnostic tests (Adams, 2001). This intra dermal test is performed by injecting 0.1 ml of bovine tuberculin in the skin of the animal and detecting the change in the thickness of the skin at the site of injection after 72 hours and measurement of the change in skin thickness is done using a vernier callipers (Grasser et *al*., ,1986). The site of injection of PPD is reviewed often and intradermal injection at cervical region is found to be most convenient compared to caudal fold test (Francis *et al*., 1978; Costello *et al*., 1997). . Tuberculins are complex mixtures of soluble antigens (Daniel *et*

al., 1978) produced by mycobacteria (*M. bovis* or *M. avium*). The Tuberculin Skin Test (TST) sometimes results in false-positive reactions due to exposure of some animals to environmental mycobacteria such as *M. avium* and MAP (Domenech *et al.*, 2006). TST can also cause false-negative reactions due to suppression of the immune response of the animals long time since exposure to field strain, , injecting sub potent dose of tuberculin, use of Tuberculins of reduced potency, immunosuppression during the early postpartum, desensitisation following tuberculin tests, and variability among observers (Doherty *et al.*, 1995; Monaghan *et al.*,1994; Domenech *et al.*, 2006)

B. Interferon gamma assay

The alternative IFN-γ assay is an in vitro blood test based on measuring the CMI response of the animals infected with MB (Vordermeier,*et al.*, 1999) . Cytokine release assays, particularly the IFN-γ test, are being increasingly used internationally for the diagnosis of tuberculosis in animals (Schiller *et al.*, 2009) due to the ready availability of commercial reagents (Bovigam test kits, Prionics, Switzerland). Since the introduction of the IFN-γ assay for bovine TB in 1998(Rothel *et al.*, 1990; Wood *et al.*, 1990) a number of studies have demonstrated its value as an ancillary test for the diagnosis of TB in cattle (de la Rua-Domenech *et al.*, 2006; Schiller *et al.*, 2010). The IFN-γ assay is usually performed using PPD as antigen, although recent studies have evaluated ESAT-6 and CFP- 10 (Palmer *et al.*, 2006). A problem with the IFN-γ assay is that it is a costly process that requires well-trained personnel to carry out the test (Dalley *et al.*, 1999).

2.2.2. Invitro method of diagnosis of Bovine TB

The gross and histological examination of six pairs of lymph nodes (meditational, medial retropharyngeal, bronchial, parotid, prescapular and prefemoral) together with the mesenteric nodes and lungs will detect 95% of cattle with gross tuberculous lesions (Barthel et *al.*, 2000; Corner, 1994). The detection of granulomatous lesions of tuberculosis is soon followed by the Ziehl-Neelsen acid-fast staining. Once the bacilli are confirmed to be acid-fast, then Immunohistochemical staining with antibodies specific to M. tuberculosis complex (Cassidy *et al.*, 1999) or polymerase chain reaction (Liebana *et al.*, 1995; Miller *et al.*, 1997), may be performed to specifically identify *M. tuberculosis* complex organisms in tissues or paraffin-

embedded histological sections. Miller *et al*., 1997, reported that the tests based on PCR performed on formalin-fixed paraffin-embedded sections with typical tuberculous lesions and acid-fast bacilli were 93% positive. Similarly, PCR-based tests on fresh tissues were found to be more accurate at 9 1% positive (Wards *et al*., 1995). Immunohistochemical and PCR-based procedures are rapid, specific, confirmatory tests for *M. bovis* infection, if the tuberculin test results and necropsy findings are available. The nucleic acid based molecular

Methods are now used for the diagnosis of bovine tuberculosis. From the early DNA fingerprint analysis by restriction endonuclease procedures by Collins and de Lisle (1984), to the restriction fragment lengthpolymorphism (RFLP) analysis by Thierry *et al.*, (1990),

Followed later by the spoligotyping methods of van Soolingen *et al*., (2001), now the multiplex PCR procedures of Sreevatsan *et al*., (2000), for the detection and differentiation of *Mycobacterium* spp. by nucleic techniques clearly hold great promise for epidemiological investigationsat local, national and international population levels.

2.2.3. Mycobacterial culture

It is the most confirmatory test for the detection of the infection. For the culture of mycobacterium an agar based medium, Stonebrink or Löwenstein-Jensen with pyruvate (Veerman *et al*., 1986). The direct detection of M. bovis using culture methods to isolate the bacterium from post mortem samples is generally regarded as the most sensitive and therefore the 'gold standard' for determining infection status (Corner *et al*., 1990; Crawshaw *et al*., 2008). However the sensitivity of tissue culture is greatly influenced by a number of factors most notably the number of tissues examined, the aseptic methods used to take tissues, the processing of tissues prior to culture and the methods and materials used for culture including the number and type of media used and the length of Incubation time (Sam *et al*., 2011).

2.3. Microsatellite as markers

With the revolution in molecular biology and with the discovery of polymerase chain reaction (Mullis *et al*., 1986), the protein markers are completely superseded by molecular markers (Bostein *et al*., 1980). Microsatellites systems are composed of DNA repeats in tandem at each locus. The origin of such polymorphism is still under debate though it appears

most likely to be due to slippage events during DNA replication (Schltterer *et al*., 1992). Despite the fact that the mechanism of microsatellite evolution is still unclear, SSRs were being widely employed in many fields soon after their first description (Litt & Luty, 1989; Weber *et al*., 1989) because of the high variability which makes them very powerful genetic markers.

At present, microsatellites are the markers of choice for genome mapping, genetic dissection of complex traits and genetic diversity studies because of their highly polymorphic nature, co-dominant mode of inheritance and ease of typing (Barendse *et al*.1994; Crawford *et al*., 1995; Rohrer *et al*., 1996) .Microsatellites have proven to be an extremely valuable tool for genome mapping in many organisms (Schuler *et al*.,1996; Knapik *et al*,. 1998), but their applications span over different areas ranging from ancient and forensic DNA studies, to population genetics and conservation/management of biological resources (Jarne *et al*., 1996). Microsatellites have been found in all organisms so far. In the human genome poly (A)/poly (T) stretches are most common repeat types. However the poly (A) and poly (T) type is not suitable as genetic markers because of instability during PCR reactions. The study shows that other mammalian genome scans seems to have similar repeat compositions as the human genome. Microsatellites are assumed to be evenly distributed throughout the genomes but rare within coding regions (Hancock, 1995). Microsatellites are useful markers for a vast variety of purposes such as genome mapping, parentage determination, disease research, cancer research and determination of genetic variation genetic distance and genetic diversity (Caskey *et al*., 1992). Scientists have characterised hundreds of microsatellite markers from bovine, ovine and porcine genomes and thus led to the production of high density linkage maps (Barendse *et al*., 1994; Kappes *et al*., 1997). The advantage of using microsatellites is that it is convenient and any sample containing the animals DNA can be used (Ozkan *et al*., 2009).

2.4. Microsatellite markers in disease resistance studies

Weller *et al*. (1995) analyzed eleven microsatellite markers and the Dairy Bull DNA Repository (DBDR) and concluded that several markers were associated with significant effects on milk production and health traits like Somatic cell score (SCS), which is related to Mastitis.

Ashwell *et al*., (1996) identified potential QTL for SCS near marker 513 and concluded that in future alleles of this marker can be used in marker assisted selection to select offspring that will have reduced mastitis incidence. Ashwell *et al*., (1997), detected significant marker allele differences for SCS on BTA23 for markers 513, BM1818, BM1443 and BM4505.

McElroy *et al*., (2005) reported significant association of Seventeen markers with Mareks disease survival. Le Flèche *et al*. (2006) proposed set of eight microsatellite loci for Brucella MLVA typing assay found to be extremely discriminate and highly efficient to distinguish strains within a local outbreak, but is unable to correctly predict the biovar or even the species of an isolate. Dukkipati *et al*.(2010) reported association of microsatellite polymorphisms with immune responses to a killed mycobacterium avium subsp. paratuberculosis vaccine in merino sheep. Pinedo *et al*. (2008) did a statistical analysis demonstrating significant differences in allelic frequencies between cases and controls for microsatellites in BoIFNG-SNP12781 and SLC11A1 genes, indicating a significant association between infection and variant Alleles and in the analysis of genotypes, a significant association was also found between infection status and BoIFNG-SNP12781 and SLC11A1-275-279-281 microsatellites.

2.5. Candidate gene approach in disease resistance aspect

Ruiz O *et al*. (2007) established genetic association between bovine NRAMP1 and CARD15 genes with infection caused by Mycobacterium avium subsp. Paratuberculosis. Pinedo *et al*. (2009) reported no correlations between MAP susceptibility and three TLR4 polymorphisms in a cohort of 431 cattle. In another study Pinedo *et al*. (2009) reported, sourced from a previous bovine study, 32 TLR4 SNPs across 20 haplotypes. Pinedo *et al*., 2009 reported that candidate gene polymorphisms in BoIFNG, TLR4, and SLC11A1 are risk factors for paratuberculosis infection in cattle. Verschoor *et al*. (2010) stated that polymorphism in the gene encoding bovine interleukin-10 receptor alpha are associated with Mycobacterium avium ssp. Paratuberculosis infection in cattle. Reddacliff *et al*. (2005) reported an association of MHC polymorphisms with susceptibility to MAP infection. Koets *et al*. (2010) reported 21 different SNP in a study conducted in population of 24 cattle and found out that the TLR2-1903 T/C SNP was significantly associated with resistance to Map and they again extent the study of this and four additional TLR2 SNP in a subsequent observational

field study with 553 cows from farms with paratuberculosis and ascertain the same result. Delgado *et al*. (2009) studied the expression of NRAMP1 and iNOS in Mycobacterium avium subsp. Paratuberculosis in a naturally infected cattle population and showed strong specific immunolabeling against both NRAMP1 and iNOS molecules, throughout granulomatous PTB-compatible lesions in ileum and ileocaecum.

2.6. Candidate gene approach in Tuberculosis resistance aspect

Resistance against diseases was studied genetically from the beginning of 20th century. Wright *et al*. (1921), reported resistance against Tuberculosis in Guinea pigs. Lurie,(1941) reported genetic resistance against Tuberculosis in Rabbits. The bovine MHC, or BoLA region, has shown association with several diseases in cattle (Lewin *et al*., 1999) and in particular, BoLA DRB alleles have been associated with variation in T-cell responses to M. bovis antigens studied in vivo in a population of 47 Holstein calves (Casati *et al*., 1995). Similarly, the bovine orthologue of human DC-specific ICAM-3 grabbing non-integrin C-type lectin (DC-SIGN) gene has recently been identified and functionally characterized (Yamakawa *et al*., 2008). Results demonstrate that the DC-SIGN receptor interacts and binds to M. bovis BCG (Yamakawa *et al*., 2008). It is worth speculating that in cattle, as in humans, polymorphic variation in the DC-SIGN gene may be associated with TB resistance. Few HLA class II alleles and association with tuberculosis has been reported in populations from India (Ravikumar *et al*., 1999). Certain mannose-binding–protein alleles have been shown to influence tuberculosis in a population from India (Selvaraj *et al*., 1999). Four polymorphic mutations of natural resistance–associated macrophage protein 1 (NRAMP1) were shown to predispose individuals to tuberculosis in human population in Gambia (Bellamy *et al*., 1998). In the same population, Bellamy *et al*. (1999), again reported an association with polymorphisms in the vitamin D–receptor gene. Polymorphisms in the genes encoding the cytokine interleukin (IL)-1beta and its receptor antagonist IL-1Ra were found to be associated with tuberculosis in patients of Gujarati origin who were living in England. Newport *et al*. (1996) reported that a mutation in the interferon-gamma-receptor gene causes susceptibility to mycobacterial infection and further that interferon-gamma pathway is important in the response to intracellular pathogens such as mycobacteria, in a human study. Means *et al*. (1999), reported the cellular activation by

Mycobacterium tuberculosis of the Human toll-like receptors. Kramnik *et al*.,(2000), identified a mouse gene which influences susceptibility to tuberculosis, linked to NRAMP 1 on the chromosome 1. van Crevel *et al.* (2009) reported Infection with Mycobacterium tuberculosis Beijing Genotype Strains is Associated with polymorphisms in SLC11A1/NRAMP1 in a study on Indonesian Patients affected with Tuberculosis. Songane *et al.* (2012) reported that autophagy is important for intracellular killing of Mycobacterium tuberculosis, and they found out polymorphisms in the autophagy gene IRGM linked with susceptibility to Human tuberculosis. Recently, a genome-wide associa-tion study on Holstein-Friesian herds in Irish cattle that used the bovine 50 k SNP chip has identified three SNPs in a 65 kb genomic region on chromosome 22 associated with bTB susceptibility (Finlay *et al*., 2012).

2.7. Microsatellite markers in Bovine Tuberculosis

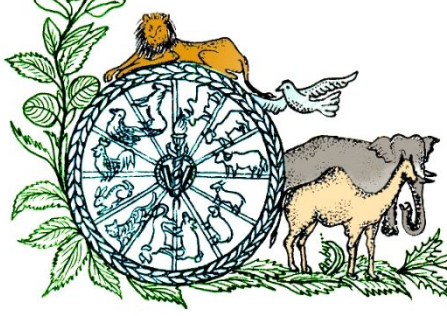
Zanotti *et al*. (2002) studied the effect of four microsatellites, in linkage with NRAMP1 gene, on the evolution of bovine tuberculosis (TB) has been estimated in a sample of 135 cattle bred in five Italian TB infected farms. Only 3 microsatellites were polymorphic (BM64444 (GT) 16, AR028 (GT) and HORIN) with 3, 7 and 2 alleles, respectively. No allele at any locus significantly affected the progression of TB estimated by the release of γ-interferon (γ-IFN) and by peripheral blood mononuclear cells grown in vitro in the presence of reference bovine and avian PPD. On the contrary, the genotype 152/154 of BM644 microsatellite significantly affected the susceptibility to TB and 267/270 of AR028 microsatellite affected resistance. In the latter case the effect was more than one standard deviation ($P<0.001$). Farm and age were not statistically significant and no effect was observed for heterozygous vs. homozygous genotypes. Ali *et al*. (2009) studied association between microsatellite genetic markers and a candidate gene with tuberculosis-related traits in African zebu cattleand in the study a total of 249 of Chadian zebu cattle were genotyped for 23 microsatellites and for a known candidate gene NRAMP1 (natural resistance associated macrophage protein 1). These animals were measured for two tuberculosis-related traits, namely, single intra-dermal comparative cervical tuberculin (SICCT) test on live animals and lung lesion (LL) from the same animals at slaughter. A generalised linear mixed model (GLM) treating both traits as binomially distributed were fitted using probit link function. Eleven out of 21 microsatellite markers tested were significantly associated with presence of LL (P-value < 0.001 to P-value

<0.01). For SICCT trait, only BM2113 marker was significant (P-value = 0.012). NRAMP 1 gene (Chr 1) was significantly associated with LL at P-value = 0.006, but not associated with SICCT test (P-value = 0.488). Reasons for this disagreement were considered. These results showed that these genetic markers and NRAMP1 gene could be potentially used in marker-assisted selection (MAS) strategies in breeding programs to control the spread of Mycobaterium bovis, which is a causative agent of bovine tuberculosis. Driscoll *et al*., (2011) reported that bovine tuberculosis (bTB) impacts greatly the UK cattle industry, yet genetic predispositions have yet to be identified. Hence they used a candidate gene approach to study 384 cattle, of which 160 had reacted positively to an antigenic skin test ('reactors'). Their approach was unusual in that it used microsatellite markers, embraced high breed diversity and focused particularly on detecting genes showing heterozygote advantage, a mode of action often overlooked in SNP-based studies. A panel of neutral markers was used to control for population substructure and using a general linear model-based approach they were also able to control for age. They found that substructure was surprisingly weak and identified two genomic regions that were strongly associated with reactor status, identified by markers INRA111 and BMS2753. In general the strength of association detected tended to vary depending on whether age was included in the model. At INRA111 a single genotype appears strongly protective with an overall odds ratio of 2.2, the effect being consistent across nine diverse breeds. Their results suggest that breeding strategies could be devised that would appreciably increase genetic resistance of cattle to bTB (strictly, reduce the frequency of incidence of re actors) with implications for the current debate concerning badger-culling.

Kadarmideen *et al*. (2011) investigated associations between polymorphism at the solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1 gene (SLC11A1, previously known as natural resistant associated macrophage protein 1, NRAMP1), with BTB phenotypes in Chadian cattle. Phenotypes were (i) single intradermal comparative cervical tuberculin test (SICCT) outcome, (ii) presence of gross visible lung lesions, (iii) a bacteriological culture test outcome and (iv) a predicted true bTB infection status using a Bayesian model. All traits were recorded as binary (presence or absence) traits. A total of 211 cattle were genotyped for a microsatellite within the SLC11A1 candidate gene. Standard linear and threshold-liability models regressing bTB traits on copy number of SLC11A1 alleles

revealed statistically significant effects of SLC11A1 alleles ($P < 0.001$) on most bTB traits. Polymorphisms (alleles 211, 215 and 217) were significantly related to lower incidence of bTB traits in Chadian cattle. This was the first study to report the association of SLC11A1 gene polymorphisms with bTB traits in Chadian or any other African cattle breeds. Amos *et al*. (2013) reported that at marker INRA111 a relatively common '22' genotype occurred significantly more frequently in non-reactor cattle. Here we test the possibility that the putative protective '22' genotype does not confer resistance but instead causes cattle that carry it to react less strongly to the prescribed test, and hence avoid slaughter, potentially even though they are infected. They showed that, after controlling for age and breed, '22' cattle react less strongly to the immunological challenge and may therefore be less likely to be classified as a reactor. Ali *et al.* (2013) studied 20 microsatellite marker in relation to bovine TB and reported that out of 20 markers tested were significantly associated with at least one trait considered; these were ILSTS005, ILSTS006, TGLA227, BM2113 and CSRM66.

Materials and Methods



3 MATERIALS AND METHODS

3.1 Resource panel

The present study was conducted on animals of mixed cattle population (containing Kosi, Sahiwal and crossbred animals), maintained at Shri Mataji Goshala, Barsana, Mathura. The population included male and females of various age groups. The animals were maintained under similar feeding and management conditions. A total of 245 animals were screened for bovine tuberculosis (bTB) by performing single intradermal comparative cervical tuberculin test (SICCT) keeping in view of prevalence rate of tuberculosis. On the basis of the result of SICCT two groups were formed i.e. tuberculin test positive animals affected with tuberculosis and the second group were negative for tuberculin test and supposed to be free of tuberculosis. Subsequently, the data on the selected animals based on SICCT was recorded and 35 tuberculin positive and 41 tuberculin negative animals were included in our investigation for genotyping.

3.2 Tuberculin test procedure (SICCT)

Preliminary screening for TB was carried out using the purified protein derivative (PPD) tuberculin skin test (SICCT). Prior to inoculation with PPD the skin thickness at site of injection was measured. Approximately, 0.1 ml PPD was injected into the mid-neck, and the skin-fold thickness of each injection site was measured after 72 h. The reactions were then categorized as positive (>4 mm thickness) and negative (<2 mm thickness). The thickness between 2mm to 4 mm is assumed as inconclusive animals and were not included in any group.

3.3 Collection and storage of blood

About 6 ml of venous blood was collected from jugular vein of each of the selected animal in a sterile polypropylene tube containing 0.5 ml of 2.7% EDTA as an anticoagulant.

The tubes containing blood was then kept on ice until it was transferred to the laboratory. For the isolation of serum about 10 ml of blood was collected in a sterile vacutainer tube with anti coagulant and was kept in slanting position for 1 hour for the separation of serum. It was then centrifuged at 2000 rpm for 15 minutes for further separation of the serum. The serum thus separated will be transferred to aliquot kept under refrigeration (-20^{o} C).

3.4 Isolation of DNA

DNA from the whole blood collected from the experimental animals was isolated using Promega Wizard® Genomic DNA extraction Kit, using following procedures.

1. Added 900µl of Cell Lysis Solution to a sterile 1.5ml micro centrifuge tube.
2. Gently rocked the tube of blood until thoroughly mixed; then transferred 300 µl of blood to the tube containing the Cell Lysis Solution. Inverted the tubes 5–6 times to mix properly.
3. Incubated the mixture for 10 minutes at room temperature (invert 2–3 times once during the incubation) to lyse the red blood cells. Centrifuge at 13,000–16,000 rpm for 20 seconds at room temperature.
4. Removed and discarded as much supernatant as possible without disturbing the visible white pellet. Approximately 10–20µl of residual liquid would be remained in the tube had been frozen, repeat Steps 1–4 until pellet was white.
5. Vortex the tube vigorously until the white blood cells was resuspended for 10–15 seconds. Completely resuspended the white blood cells to obtain efficient cell lysis.
6. Added Nuclei Lysis Solution at the rate of 300µl for 300µl sample volume the tube containing the resuspended cells. Pipetted the solution 5–6 times to lyse the white blood cells. The solution should become very viscous. If clumps of cells were visible after mixing, incubate the solution at 37°C until the clumps were disrupted. If the clumps were still visible after 1 hour, added 100µl of additional Nuclei Lysis Solution.
7. Added 1.5 µl of RNase Solution to the nuclear lysate, and mixed the sample by inverting the tube 2–5 times. The mixture was incubated at 37°C for 15 minutes, and then cooled to room temperature.

8. Added 100µl of Protein Precipitation Solution to the nuclear lysate, and vortexed vigorously for 10–20 seconds. Small protein clumps might be visible after vortexing.

9. Centrifuged at 13,000 rpm for 3 minutes at room temperature. A dark brown protein pellet should be visible at this stage.

10. Transferred the supernatant to another clean 1.5ml micro centrifuge tube containing 300µl of room-temperature isopropanol. Left this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

11. Gently mixed the solution by inversion until the white thread-like strands of DNA form a visible mass.

12. Centrifuged at 13,000 rpm for 1 minute at room temperature. The DNA would be visible as a small white pellet.

13. Decanted the supernatant, and added 300µl of room temperature 70% ethanol to the DNA. Gently inverted the tube several times to wash the DNA pellet and the sides of the micro centrifuge tube.

14. Centrifuged at 13,000 rpm for 1 minute at room temperature. The DNA would be visible as a small white pellet.

15. Carefully aspirated the ethanol using a sequencing pipette tip. The DNA pellet was very loose at this point and care was taken to avoid aspirating the pellet into the pipette. Inverted the tube on clean absorbent paper and air-dried the pellet for 10–15 minutes.

16. Added 100 µl of DNA Rehydration Solution to the tube and rehydrated the DNA by incubating at 65°C for 1 hour. Periodically mixed the solution by gently tapping the tube.

17. The DNA was then stored at -20^{o} c.

3.4.1 Quantification of DNA

DNA quantification was done using Qubit® 2.0 Fluorometer (Invitrogen,USA). The procedure was one using 1-2 µL of DNA solution. The procedure was carried out as follows

1. Set up two Assay Tubes for the standards and one tube for each user sample.

2. Prepared the Qubit™ Working Solution by diluting the Qubit™ reagent 1:200 in Qubit™ buffer. Prepared 200 µL of Working Solution for each standard and sample.

3. Prepare the Assay Tubes according to the table below.

	Standard assay tubes	Sample assay tubes
Volume of Working Solution	190 µL	198-199 µL
Volume of Standard	10 µL	—
Volume of User Sample	—	1–2 µL
Total Volume in each Assay Tube	200 µL	200 µL

4. Vortexed all tubes for 2–3 seconds.

5. Incubated the tubes for 2 minutes at room temperature

6. Insert the tubes in the Qubit® 2.0 Fluorometer and take readings.

7. Using the Dilution Calculator feature of the Qubit® 2.0 Fluorometer, the stock concentration of the original sample was determined.

Those DNA samples having a minimum concentration of 50 µg/ml were used for further study.

3.4.2 Quality checking of DNA

The Quality of extracted genomic DNA will be assessed through 1 % horizontal submarine agarose gel electrophoresis as below-

1. The gel casting plate was sealed with adhesive tape and placed on a leveled table surface.

2. Agarose (1 % w/v) was boiled in 1X TBE (Refer Annexure for composition) buffer. After boiling it was cooled to 55ºC and then ethidium bromide (0.5µg/ml) was added at the rate of 3 µl per 10 ml of agarose solution. The gel was gently poured into the casting tray avoiding bubble formation and was allowed to solidify at room temperature.

3. After solidification, the comb and adhesive tape were removed.

4. The gel casting tray was submerged in gel tank of electrophoresis unit having 1 X TBE buffer.

5. DNA samples for loading were prepared by mixing 2µl of genomic DNA, 8 µl of 1 X TBE and 2µl of 1X loading dye. Samples were carefully loaded in the wells.

Electrophoresis was performed at 2-5 volts/cm for one hour and then gel was visualized and photographed under UV transilluminator. Uncut Lambda (Bangalore Genei, India) DNA (500ng) will be used as reference DNA. After electrophoresis gel was examined on safe image viewer for any sheared DNA. Photographs of gels were taken using Digital camera and were documented and prints were taken as necessary. The good quality DNA samples having intact DNA bands without any smearing were selected for further analysis.

The genomic DNA from each animal was diluted into the working solution using nuclease free water (NFW) so as to get a final concentration of 50µg/ml.

3.5 Microsatellite analysis

A set of 21 di-nucleotide microsatellite primer pairs were used for the genotyping of the 76 selected animals for the study. The relevant information about the microsatellite loci under the study is given in the **table**

3.5.1 Genotyping on horizontal gel electrophoresis using metaphor agarose

22 microsatellite markers were used for genotyping on metaphor agarose gel electrophoresis.

3.6 PCR Assay

The Polymerase Chain Reaction (PCR) is a powerful technique which results in rapid production of DNA, sometimes referred to as “molecular photocopying” was invented by Kary Mullis in 1980s. It is an in vitro method for enzymatically synthesizing defined sequence of DNA. The reaction used two oligonucleotide primers that hybridize to apposite strands and flank the target DNA sequence to be amplified. A heat stables DNA polymerase such as Taq polymerase catalyses the elongation of the primers. Template denaturation at 94°C, primer

annealing generally between 52-64 °C (depending on primer annealing temperature), and extension at 72°C of the annealed primers by the polymerase results in exponential accumulation of a specific DNA fragment. The primer extension products synthesized in a given cycle can serve as the template in the next cycle. The number of target DNA copies approximately doubles every cycle.

3.6.1 Template DNA

The DNA concentrations of the isolated DNA samples were determined and their working solutions were prepared by diluting up to the final concentration of 50 ng/µl with NFW.

3.6.2 Primers

The success of a PCR experiment is almost wholly dependent upon the oligonucleotide primers. The primers need to be designed such that one recognizes the sense strand of the DNA to be replicated (i.e. is the same sequence as the antisense strand) while the other recognizes the antisense strand of the target DNA (i.e. is the same sequence as the sense strand). Typically, primers will have the following characteristics. They will be between 17 and 30 nucleotides in length – sufficient to allow unique annealing to a single sequence within a genome. They will have a GC content of approximately 50 per cent.

The primers for the current study was taken from already published database from reputed sites (www.ncbi.nlm.nih.gov and www.thearkdb.org) the list of primers is given in the table

3.6.3 PCR reaction setup

Initially varying various parameters like annealing temperature and $Mgcl_2$ concentration, the amplification conditions were standardized. Finally these standardized conditions were used Table

PCR amplification was carried out in a final volume of 25 µl reaction mixture (Ogawa *et al*, 1996), in 0.2 ml thin PCR tubes. Each PCR tubes containing 50 ng(Approx.) genomic DNA, 1.5 mM $MgCl_2$, 1X reaction mixture, 200 µM of each dNTP(dATP,dGTP,dCTP and dTTP), 1 U of *taq* DNA polymerase enzyme and 5 pmol of each forward and reverse primer. In tabular form it is given below.

Sl. No.	Reaction Component	Volume	Final Concentration
1	5X Taq buffer with	5 µl	
2	$MgCl_2$	1.5-3.0 µl	1.5mM
3	dNTP mix (2.5 mM)	0.2 µl	200µM
4	W Forward primer (1µg/µl)	1 µl	1µM
5	W Reverse primer (1µg/µl)	1 µl	1µM
6	Taq DNA polymerase (3U/ µl)	0.2 µl	1U
7	Autoclaved distilled water	Ad to 24 µl	-
8	Template DNA (50 ng/ µl)	1.0 µl	50 ng

3.6.4 PCR amplification conditions

The amplification for all the primers was carried out in a DNA Engine Thermal Cycler Machine (M/s BioRad, USA) in the below mentioned PCR program.

1. Initial denaturation at 94^{0}C for 4 minutes, followed by 25 cycles of PCR, each cycle consists of:

   i. Denaturation at 94^{0}C for 30 seconds.

   ii. Annealing at Tm ^{0}C for 30 seconds.

   iii. Extension at 72^{0}C for 30 seconds.

   2. Final extension at 72^{0}C for 5 minutes.

   3. Finally 4^{0}C forever.

3.6.5 Checking of amplified product

The amplified PCR products was checked by submarine horizontal gel electrophoresis with agarose gel of 1.0 % w/v 1X TBE for the conformation of amplification. The amplified products will be visualized as a single compact band of expected size under UV light and will be documented by gel documentation system. Following this the mass PCR was carried out and the amplified product was checked using 2.4% agarose gel.

3.7 Resolution and Documentation of Microsatellite Alleles

After the completion of the PCR reaction, five micro litres of the 6X loading dye was added and the samples were stored at 4^{o}C till further use.

The amplification products from the microsatellite markers were resolved on 4 % metaphor agarose gel, since the product size was between 90 and 300 bp.The procedure for the preparation and loading was as given below

1. Chose a beaker of 1litre volume (that is 2 - 4 times the volume of the solution).
2. Added chilled 1X TBE buffer and a magnetic stir rod to the beaker.
3. Sprinkled in the pre-measured agarose powder while the solution is rapidly stirred.
4. Removed the stir bar (if not Teflon coated).
5. Weighed the beaker and solution before heating and covered the beaker with a plastic wrap.
6. Pierced a small hole in the plastic wrap for ventilation.
7. Heated the beaker in the microwave oven on power until bubbles appear.
8. Removed the beaker from the microwave oven.
9. Gently swirled the beaker to resuspend any settled powder and gel pieces.
10. Reheated the beaker on high power until the solution comes to a boil.
11. Held at boiling point for 1 minute or until all of the particles were dissolved.
12. Remove the beaker from the microwave oven.
13. Gently swirl the beaker to mix the agarose solution thoroughly.
14. After dissolution, added sufficient hot distilled water to obtain the initial weight and mixed thoroughly.
15. Cooled the solution to 60°C prior to casting.
16. Added 50 µl of ethidium bromide(0.5µg/ml) to this solution and mixed well
17. The gel was poured on to electrophoresis trough and comb was inserted. The gel was allowed to set on a flat surface for about 30 min.
18. The combs were removed after solidification of the gel and the gel along with the casting tray is immersed in 1X TBE running buffer in the tank of the electrophoresis apparatus.
19. The dye premixed PCR products were then loaded in the well of the metaphor gel and parallel to this 20 bp ladder marker was also loaded.
20. The electrophoresis was carried out at 120 V for varying time periods depends on the resolution of the products on the gel.

Molecular sizes of various alleles of microsatellite markers were estimated by using 20 bp DNA ladder (Bangalore Genei) as molecular size marker. The alleles at different microsatellite locus were sized using Alpha DigiDoc 1000 computer software. The results were then entered manually into a MS excel work sheet for statistical analysis.

3.8 ELISA assay

The humoral immune response (HIR) is the aspect of immunity that is mediated by macromolecules (as opposed to cell mediated immunity) found in extracellular fluids such as secreted antibodies, complement proteins and certain antimicrobial peptides. Humoral immunity is so named because it involves substances found in the humours, or body fluids. Enzyme-linked immunosorbent assay (ELISA) is a test that uses antibodies and color change to identify a substance. In this study association of allelic variants of microsatellite markers with the amount of immunoglobulin G1 (IgG1) amount were investigated using Bovine IgG1 ELISA Quantitation Set from Bethyl Laboratories, Inc.sandwitch ELISA was performed for the Igg1 detection. Antibody-sandwich ELISAs is the most useful of the immunosorbent assays for detecting antigen because they are frequently between 2 and 5 times more sensitive than those in which antigen is directly bound to the solid phase. To detect antigen, the wells of microtiter plates are coated with specific (capture) antibody followed by incubation with test solutions containing antigen. Unbound antigen is washed out and an antigen-specific antibody conjugated to enzyme (i.e., developing reagent) is added, followed by incubation. Enzyme labeled antibody can be produced in the same animal that produced passively adsorbed antibody, or from a different species immunized with the same antigen that is captured. Unbound conjugate was washed out and substrate is added. After incubation, the degree of substrate hydrolysis was measured. The amount of substrate hydrolyzed is proportional to the amount of antigen in the test solution.

The assay was performed as per given below

1. Added 100 µl of diluted coating antibody to each well.
2. Incubated at room temperature (20-25^{o}C) for 1 hour.
3. Washed plate FIVE times, using washing buffer.
4. Added 200 µl of Blocking Solution to each well.
5. Incubated at room temperature for 30 minutes.
6. Washed plate FIVE times, using washing buffer.

7. Added 100 µl of standard or sample to well.
8. Incubated at room temperature for 1 hour.
9. Washed plate FIVE times, using washing buffer.
10. Added 100 µl of diluted HRP detection antibody to each well.
11. Incubated at room temperature for 1 hour.
12. Washed plate FIVE times, using washing buffer.
13. Added 100 µl of TMB serum solution to each well.
14. Developed the plate in the dark at room temperature for 15 minutes.
15. Stopped reaction by adding 100 µl of Stop Solution to each well.
16. Measured absorbance on a plate reader at 450 nm.

The OD values obtained for the standards and the samples were compared by plotting a linear graph and using the linear equation thus obtained the amount of IgG1 present in the serum was calculated and manually entered the value in MS excel file sheet for statistical analysis.

3.9 Allele frequencies and their distribution

Gene and genotype frequencies of various identified alleles/genotypes were calculated.

Genotype frequency= Number of individuals of a particular genotype/Total Number of individuals of all genotypes.

Gene frequency = (2D+H)/2N

Where, D=Number of homozygote animals of a particular genotype

H= Number of heterozygote animals having both alleles

N= Total number of individuals

Genotype of every animal was recorded manually from the autoradiograph. Genotyping involved the recording of the homozygous or heterozygous state of the animal, as well as the size of the respective alleles in base pairs. On population basis, the number of alleles, their size and frequencies for different markers was recorded for each breed.

3.10 Heterozygosity

Expected heterozygosity or maximum likelihood estimate (MLE) of heterozygosity will be calculated from allele frequency data as follows with the assumption that the population is in Hardy-Weinberg equilibrium:

$$Hl = 1 - \Sigma\ plu2$$

$$H = 1 \frac{1}{m\ l\ u} \Sigma\ \Sigma\ plu2$$

Where, plu is the frequency of uth allele at lth locus. Hl and are the heterozygosities at lth locus and average over many loci, respectively.

The unbiased estimates (-Hu) of the heterozygosity will be calculated as per;

$$-Hu = -H.\ n / (n-1)$$

Where, n is the number of alleles observed in the sample.

3.11 Polymorphism information content (PIC)

Expected value of PIC for each locus will be calculated as per (Botstein *et al.,* 1980):

$$PIC = 1 - \sum_{i=1}^{n} pi2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2pi2\ pj2$$

3.12 Statistical analysis

The association, if any between various allelic variants with Bovine tolerance/susceptibility was worked out by suitable statistical techniques using different procedures of SAS 9.3. The PROC ALLELE procedure of the SAS 9.3 was used for the estimation of PIC, HWE and heterozygosity. The PROC LOGISTIC procedure of SAS 9.3 was used to find association of allelic and genotypic frequencies with bTB and to found the overall association of the microsatellite loci with the disease status of the animal. The following model was used

$$y_{ij} = \mu + \sum_{i=1}^{S} (a_i w_i + d_i v_j) + \sum_{k=1}^{4} a_k m_k + e_{ij}$$

The ODDs ratio of genotypes was calculated in affected population versus their contemporary genotypes using the PROC FREQ procedure of SAS 9.3. The ELISA results were compared to check the significance of IGg1 level in control and case populations using the PROC GLM of the SAS software.

Table 3.1 : Details of 21 microsatellites markers used in the present study

Sl no	Marker Name	Repeat sequence	ChrT	annel (^{0}C)	Primers	Amplicon length (bp)
1	BM1818	(TG)n	23	56	F: AGCTGGGAATATAACCAAAGG R: AGTGCTTTCAAGGTCCATGC	254-278
2	BM1824	(GT)n	1	56	F: GAGCAAGGTGTTTTTCCAATC R: CATTCTCCAACTGCTTCCTTG	176-188
3	ETH3	(GT)nAC(GT)6	19	63	F: GAACCTGCCTCTCCTGCATTGG R: ACTCTGCCTGTGGCCAAGTAGG	99-127
4	ETH10	(AC)n	5	63	F: GTTCAGGACTGGCCCTGCTAACA R: CCTCCAGCCCACTTTC TCTTCTC	206-222
5	ILSTS006	(GT)n	7	56	F: TGTCTGTATTTCTGCTGTGG R: ACACGGAAGC GATCTAAACG	279-297
6	TGLA126	(TG)n	20	56	F: CTAATTTAGAATGAGAGAGGCTTCT R: TTGGTCCTCTATTCTCTGAATATTCC	111-127
7	Slc11A1	(CA)n	2	61	F: GGA ATG AGT GGG CAC AGT GGC R: CCTTCC AGA ACT CCC TCT CCG	NA
8	BM 2113	(CA)n	2	53	F: GCTGCCTTCTACCAAATAC R: CTTCCTGAGAGAAGCAACACC	122-156
9	ETH185	(GT) n	17	65	F: TGCATGGACAGAGCAGCCTGGC R: GCACCCCAACGAAAGC TCCCAG	222 - 243
10	ETH 152	NA	5	60	F: AGGGAGGGTCACCTCTGC R: CTTGTACTCGTAGGGCAGGC	181-211
11	Slc11A1	(GT) n	2	50	F: GAT TTC TCT AGT GAG TAACA R: TCT TGC CCA GAT GTT CTT AG	NA
12	BMS2753	(GT) n (GA) n	9	56	F: TCAAAAAGTTGGACATGACTGA R: AGGTTTTCAAATGAGAGACTTTTC	104-110
13	BMS499	(CA) n	17	56	F: CAGGCTTAAGTATCAAACTTTCTTC R: TTTAAGGTAGATGGGTAGTTGTACG	102-130
14	BMS468	NA	23	58	F: GTTAAGCAGAGGGTTTCCCC R: TATTCCCAGGTGCTCTGAGG	127-134
15	BMS2213	NA	18	58	F: ATGGGCAGCTTAGGGATTG R: CTTCAAGAGCCTTCAGTGGG	118-146

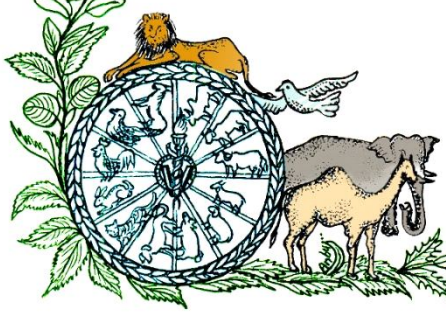
Table 3.1 : Contd...

Sl no	Marker Name	Repeat sequence	ChrT	annel (^{0}C)	Primers	Amplicon length (bp)

Sl no	Marker Name	Repeat sequence	ChrT	annel (^{0}C)	Primers	Amplicon length (bp)

16	INRA131	(CA) n	11	56	F: GGTAAAATCCTGCAAAACACAG R: TGACTGTATAGACTGAAGCAAC	96-126
17	INRA111	(CA) n	11	57	F: TTGTCGGTGTGGAGAAGCACC R: GTTTCCCGGTAACCAATTCC	121-139
18	BM7169	(CA) n	11	57	F: TGGTATGTAGTTACAGCAGCCC R: CCATTGAAACAGACATGAATGC	208-244
19	BMS1724	(GT) n	9	57	F: GACTTGCCCCAATCCTACTG R: ATTTCAGGTTTGTTGGTTCCC	153-165
20	BM7209	(GT) n	9	59	F: TTTTCTGCTCATGCTTCAGTG R: GCAGGCTATAGTCCATGACATC	111-129
21	BMS495	(CA) n	4	56	F: CTTTCACAACCGAAAGACAATC R: TCACACCCCTTCCTCTCTTC	145-161

Results



4 RESULTS

The allele and genotypic frequencies of 21 microsatellite markers were obtained after 4 % horizonta metaphor agarose electrophoresis.

At BM1818 microsatellite locus a total of 7 alleles of '254', '256', '258', '264', '266', '268' and '270' bp length and total 12 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 1** and **Table 2** respectively. At the microsatellite marker locus BM1824 total 4 alleles of '182', '186', '194' and '196' bp length and 5 genotypes were observed as summarized in the **table 3 and table 4**. At the microsatellite marker locus ETH3 total 4 alleles of '99', '109', '111' and '121' bp length and 9 genotypes were observed as summarized in the **table 5** and **table 6** respectively. At the microsatellite marker locus ETH 10, a total of 14 alleles within range of '198' to '228' bp and 22 genotypes were observed as summarized in the **table 7** and **table 8** respectively. At ILSTS006 microsatellite locus a total 6 alleles of '264', '268', '274', '276', '278' and '280' bp length and 9 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 9** and **Table 10** respectively. A total of 10 alleles and 22 genotypes were observed for the locus TGLA126 and the gene and genotypic frequency are tabulated in the **tables 11** and **table 12** respectively. A total of 6 alleles ranged from '227' bp to '249' bp and 6 genotypes were observed in the investigation of the microsatellite locus SLCLLA1-I for which the allelic and genotypic frequencies are summarized in **table 13** and **table 14.** A total of 10 alleles of ranged from '127' bp, to '153' bp length and 22 genotypes were observed in the investigation at microsatellite locus BM2113 for which the allelic and genotypic frequencies are summarized in **table 15** and **table 16**. At ETH185 microsatellite locus total 8 alleles of

'214' bp, '220' bp, '224' bp, '230' bp, '236' bp, '240' bp, '244' bp and '248' bp length and total 18 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 17** and **Table 18** respectively. At ETH185 microsatellite locus total 8 alleles of '166' bp, '168' bp, '174' bp, '176' bp, '178' bp, '180' bp, '182' bp and '184' bp length and total 15 genotypes were observed for which the allelic and genotypic frequencies are tabulated and is shown in the **Table 19** and **Table 20** respectively**.** At **SLC11A1-II** microsatellite locus total 12 alleles of the range varying from '238' bp to '260' bp and total 23 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 21** and **Table 22** respectively. At BMS2753 microsatellite locus total 6 alleles of '108' bp, '110' bp, '112' bp, '114' bp, '116' bp and '118' bp length and total 15 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 23** and **Table 24** respectively. At BMS499 microsatellite locus total 11 alleles of the range varying from '106' bp to '148' bp and total 27 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 25** and **Table 26** respectively. At BMS468 microsatellite locus total 10 alleles of '128', '130', '132', '134', '136' , '138', '140', '142', '144' and '146' bp and 20 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 27** and **Table 28** respectively. At BMS2213 microsatellite locus total 20 alleles of the range varying from '122' to '168' bp length and total 55 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 29** and **Table 30** respectively. At INRA131 microsatellite locus total 16 alleles ranging from '96' bp to '134' bp and total 43 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 31** and **Table 32** respectively. At INRA111 microsatellite locus total 8 alleles of '124' , '128', '130', '132', '138', '140', '142' and '144' bp length and total 15 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 33** and **Table 34** respectively. At BM7169 microsatellite locus total 16 alleles ranging from '208' to '250' bp length and total 33 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 35** and **Table 36** respectively. At BMS1724 microsatellite locus total 10 alleles of the range varying from '110' bp to '170' bp length and total 20 genotypes were observed for which the allelic and genotypic frequencies are tabulated

in **Table 37** and **Table 38** respectively. At BM7209 microsatellite locus total 13 alleles the range between '102' and '148' bp length and total 21 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 39** and **Table 40** respectively. At BMS495 microsatellite locus total 8 alleles of '144', '148', '154', '156', '160', '164', '166' and '168' bp length and total 16 genotypes were observed for which the allelic and genotypic frequencies are tabulated in **Table 41** and **Table 42** respectively.

In the present investigation, an attempt was done to study the association of selected microsatellite markers with the incidence of bTB in Indian Zebu and crossbred cattle. A panel of 21 microsatellite markers was selected as per availability with FAO and other reports. The microsatellites were amplified by the PCR technique using specific primers and the PCR products were resolved in 4 % metaphor agarose gel. Based on the resolution of the agarose allele sizing is done using alpha digi doc software. The results were analyzed and the number of alleles per locus, expected heterozygosity, observed heterozygosity and PIC was calculated for all the 21 microsatellite markers included in the study. In the current investigation all the 21 microsatellite locus were found to be polymorphic in population under investigation. In total, 226 alleles were found at the 21 microsatellite loci. Across the locus, the number of alleles range from 4 for the loci BM1824 and ETH3 to 21 in BMS2213 with an average of 10 alleles per locus.

The expected heterozygosity **(H_E)**, Polymorphism Information Content **(PIC)** and allelic diversity **(Ad)** were given in the **Table 4.43**. The heterozygosity was estimated as measure of the genetic diversity in a population with assumption that the genotypic frequency is accordance with their allelic frequency. The heterozygosity ranged from 0.2237 (BM1824) to 1.00 (ETH10) for microsatellite loci selected for the study and more the heterozygosity implies high polymorphism in the population. The PIC ranges from 0.23(BM1824) to 0.93 (BMS495). Similarly, the allelic diversity ranged from 0.23 (BM1824) to 0.94 (BMS495). All the microsatellite loci except BMS2213 were significantly ($P \leq 0.05$) departing from hardy Weinberg Equilibrium (HWE) which may be ascribed to the migrated cattle were kept in the *Goshala*. The overall analysis using univariate logistic regression revealed that all the 19 out of the 21 microsatellite marker was significantly affecting the incidence of bovine tuberculosis (bTB). It

was found that effect of breed, sex and age had not significant effect on the incidence of bTB.

ELISA for the detection of the IGG1:

SANDWICH ELISA was performed to detect the serum IGG1.Two groups were formed based on the tuberculin test result as positive and negative group. 21 animals from each group were compared in the assay. In the positive animals the serum immunoglobulin G1 value ranged from 0.31 to 5.31 mg/ml and in the negative control the value was between 0.34 and 10.30 mg/ml **(Table 4.44).** The association, if any between the immunoglobulin levels with Bovine tolerance/susceptibility was worked out by The PROC GLM procedures of SAS 9.3software using linear regression model was used. It was found that there was no significant difference (p=0.35) in the immunoglobulin levels in the negative and positive animals. Thus, the result suggested that humoral immunity have no role in developing resistance against *Mycobacterium* infection.

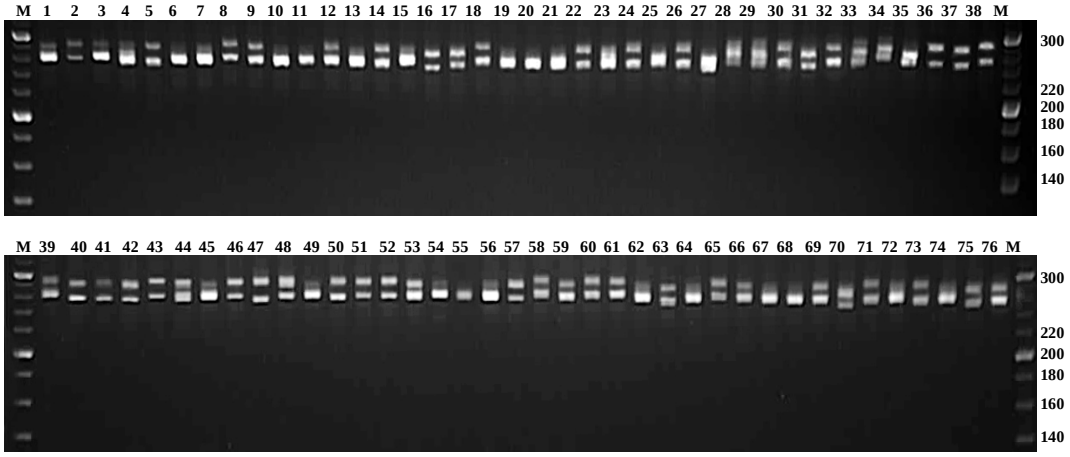


Fig. 4.1 : Microsatellite allele profiling of bTB positive and negative animals generated with marker BM1818 resolved on 4 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

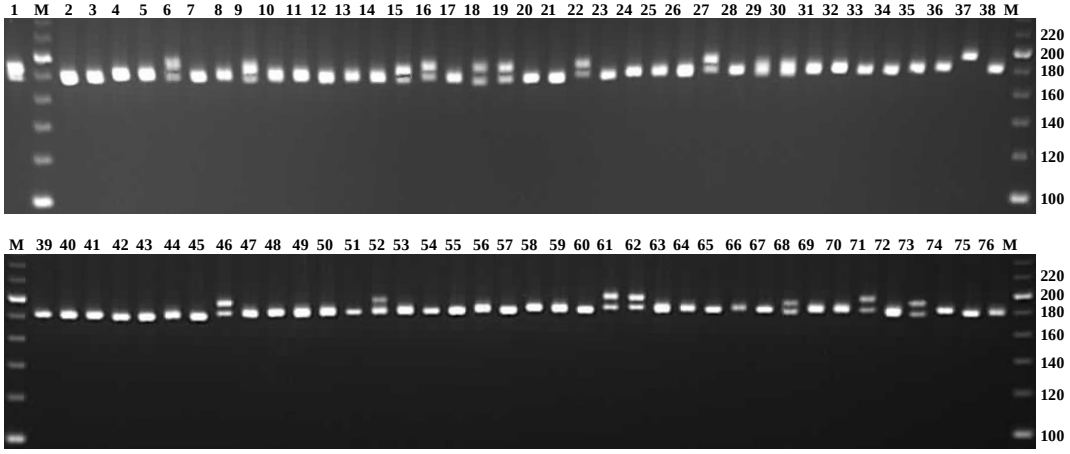


Fig. 4.2 : Microsatellite allele profiling of bTB positive and negative animals generated with marker BM1824 resolved on 5 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

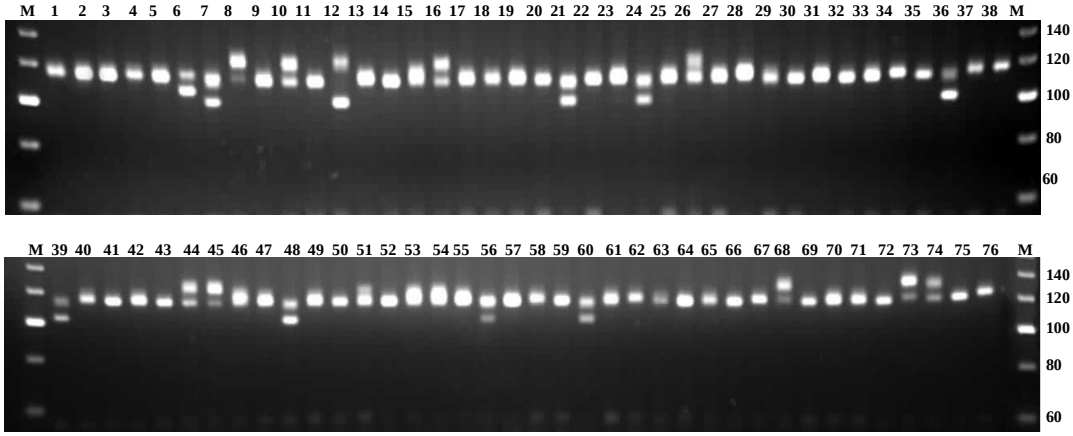


Fig. 4.3 : Microsatellite allele profiling of bTB positive and negative animals generated with marker ETH3 resolved on 5 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

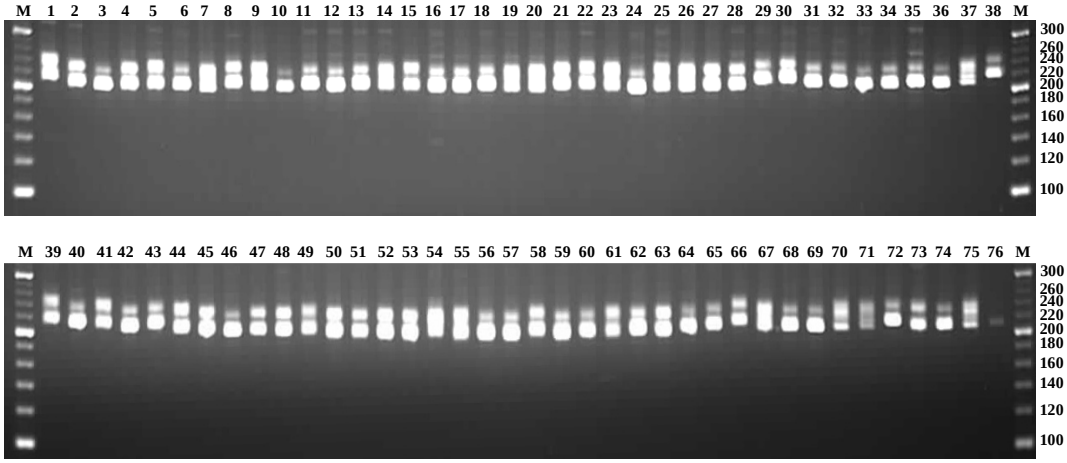


Fig. 4.4 :Microsatellite allele profiling of bTB positive and negative animals generated with marker ETH10 resolved on 5 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

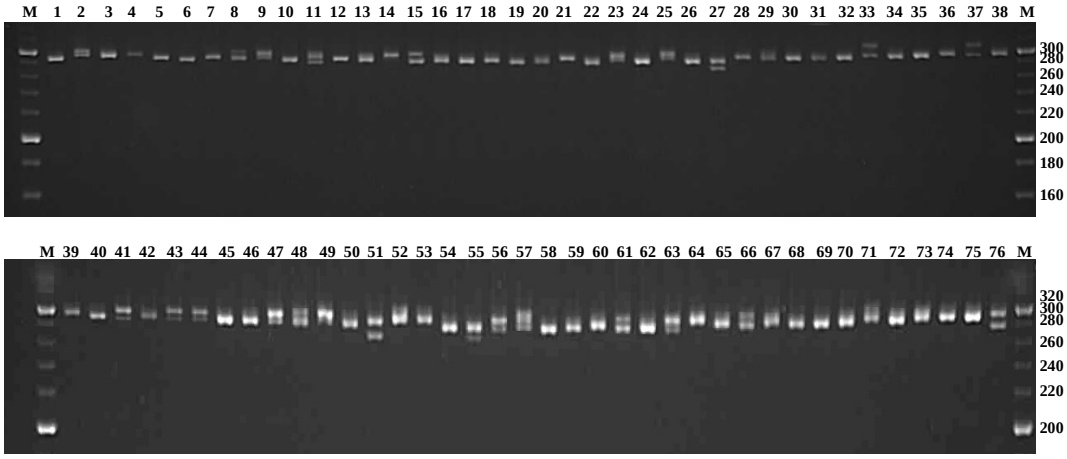


Fig. 4.5 :Microsatellite allele profiling of bTB positive and negative animals generated with marker ILST006 resolved on 5 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

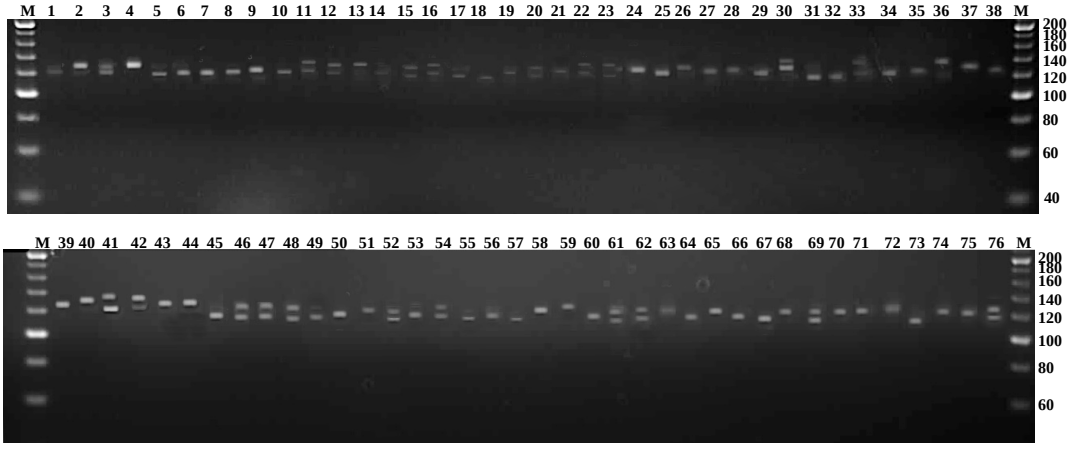


Fig. 4.6 :Microsatellite allele profiling of bTB positive and negative animals generated with marker TGLA126 resolved on 5 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

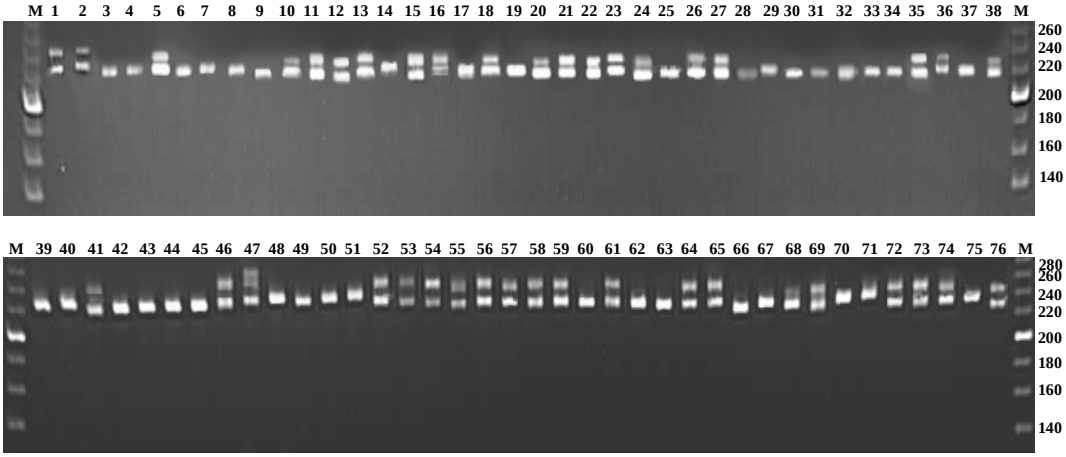


Fig. 4.7 :Microsatellite allele profiling of bTB positive and negative animals generated with marker SLC11A1-1 resolved on 5 % metaphor agarose. Positive: Lane 1-35; Negative: Lane 36-76; 20 bp Marker

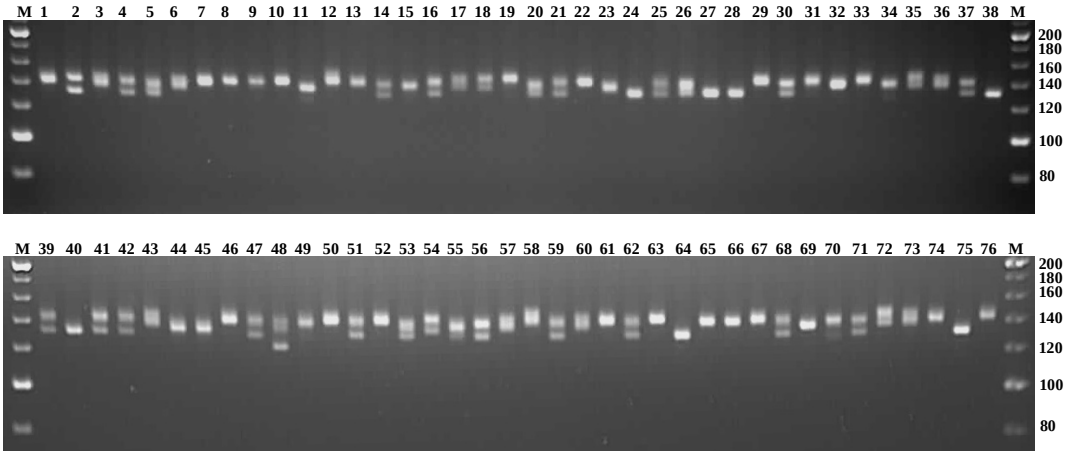


Fig. 4.8 :Microsatellite allele profiling of bTB positive and negative animals generated with marker BM2113 resolved on 4 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

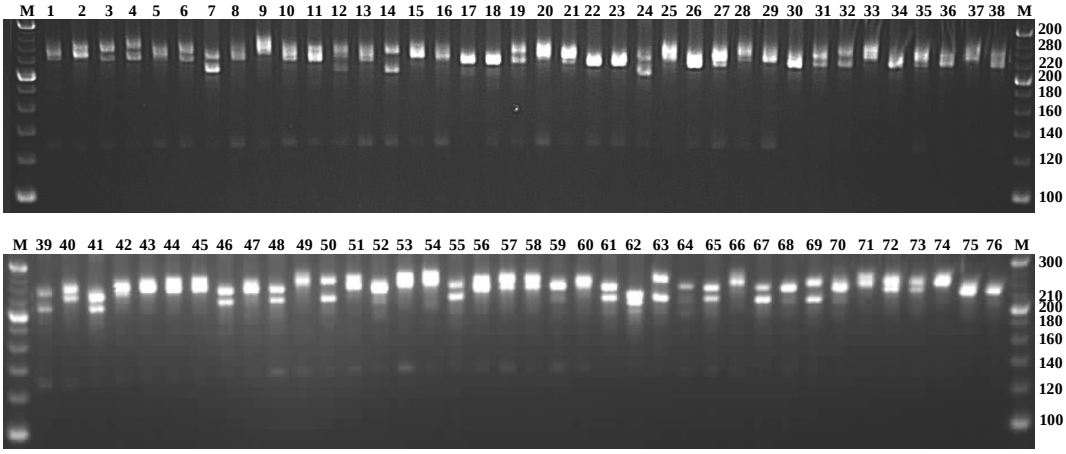


Fig. 4.9 :Microsatellite allele profiling of bTB positive and negative animals generated with marker ETH185 resolved on 4 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

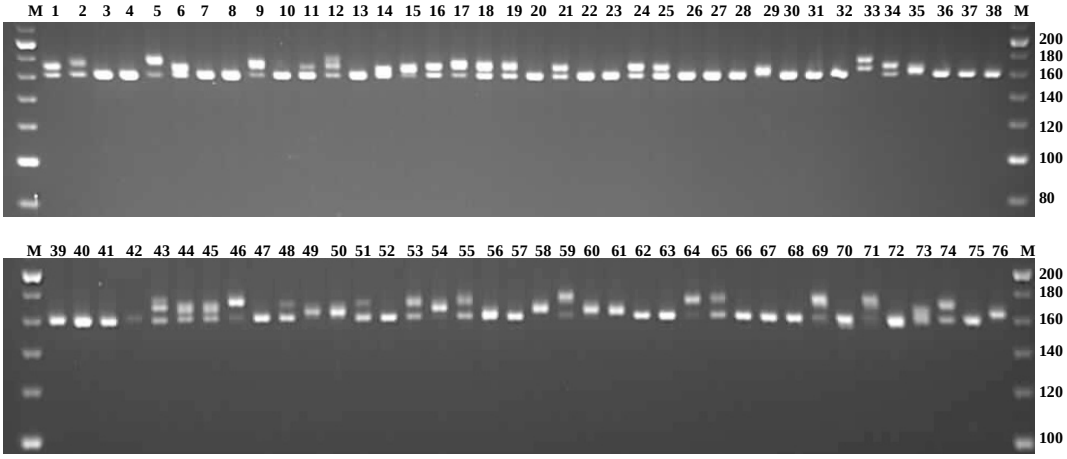


Fig. 4.10 :Microsatellite allele profiling of bTB positive and negative animals generated with marker ETH152 resolved on 4 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

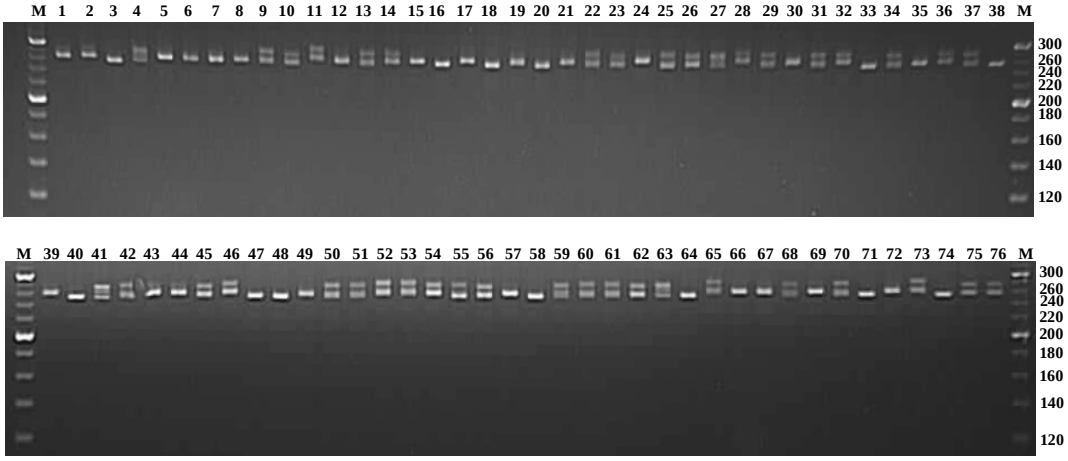


Fig. 4.11 :Microsatellite allele profiling of bTB positive and negative animals generated with marker SLC11A1-II resolved on 5 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

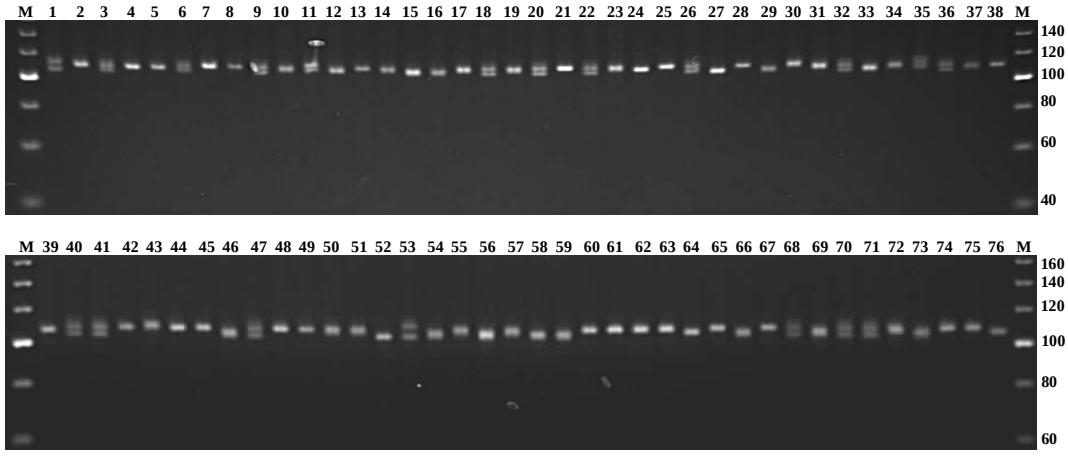


Fig. 4.12 :Microsatellite allele profiling of bTB positive and negative animals generated with marker BM2753 resolved on 5 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

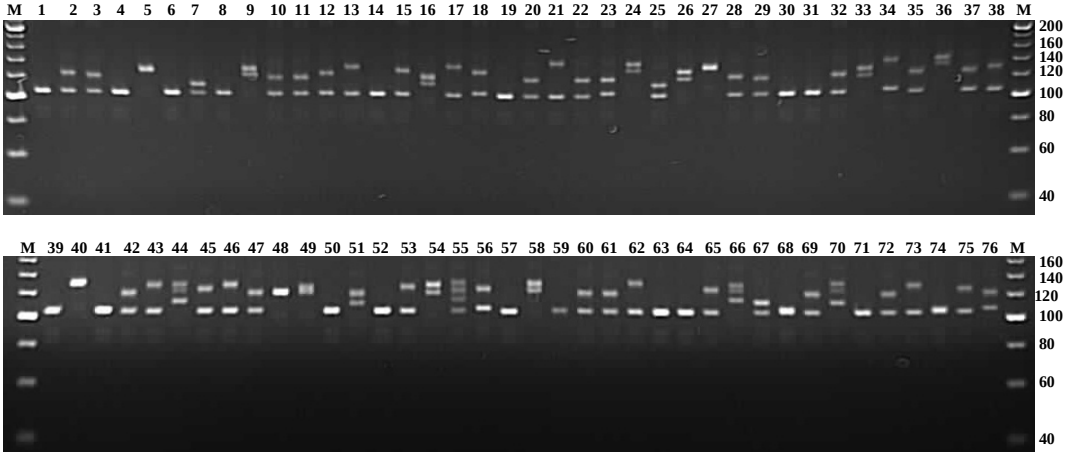


Fig. 4.13 :Microsatellite allele profiling of bTB positive and negative animals generated with marker BMS499 resolved on 4 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

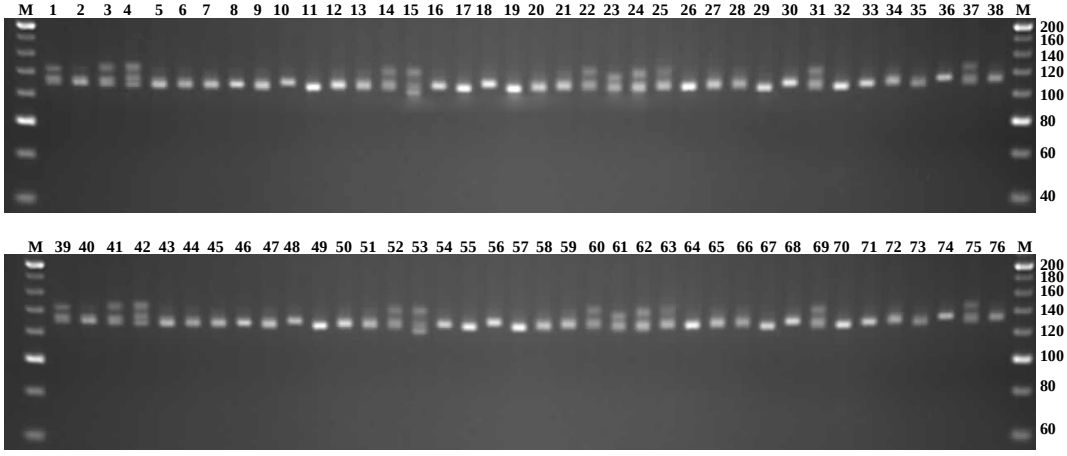


Fig. 4.14 :Microsatellite allele profiling of bTB positive and negative animals generated with marker BMS468 resolved on 5 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

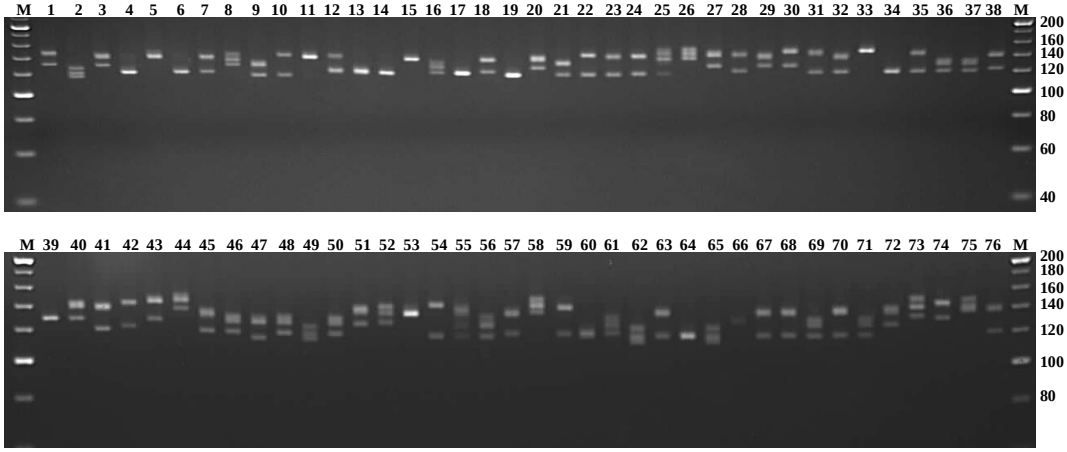


Fig. 4.15 :Microsatellite allele profiling of bTB positive and negative animals generated with marker BMS2213 resolved on 4 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

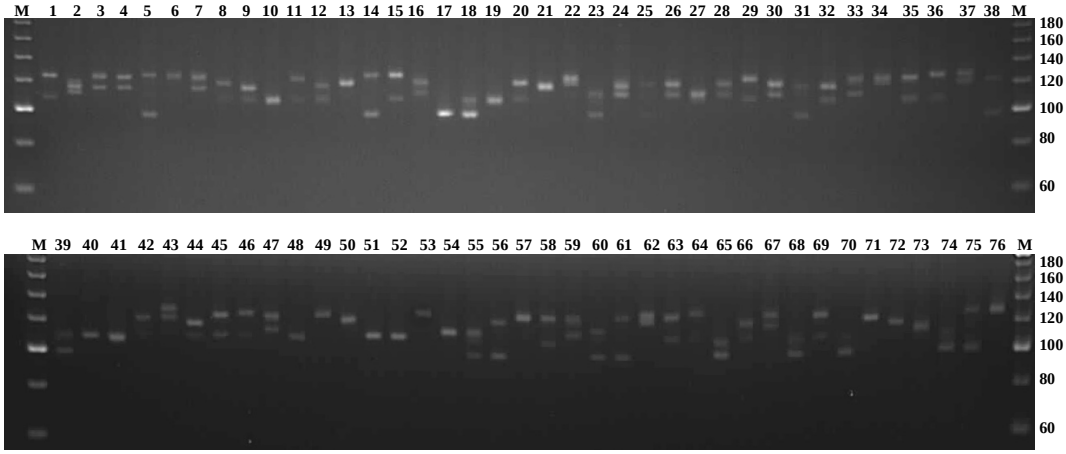


Fig. 4.16 :Microsatellite allele profiling of bTB positive and negative animals generated with marker INRA131 resolved on 5 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

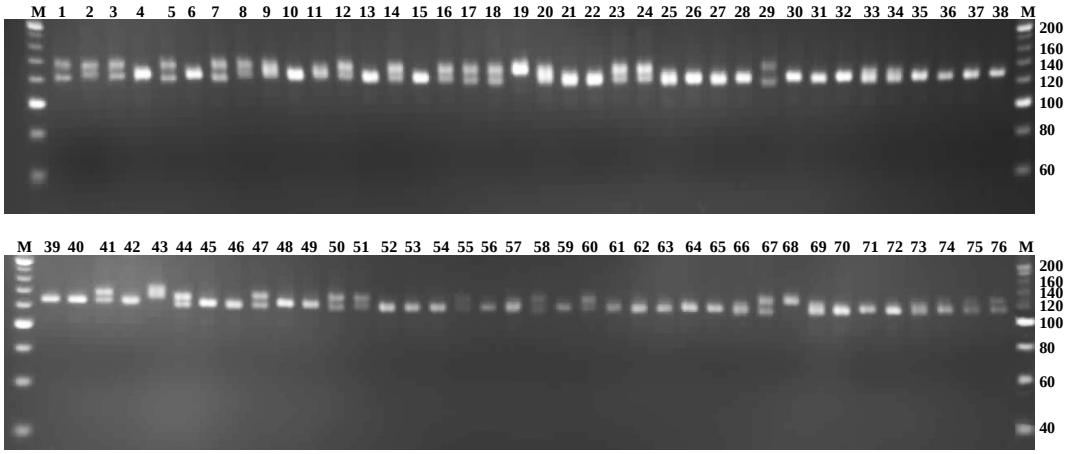


Fig. 4.17 :Microsatellite allele profiling of bTB positive and negative animals generated with marker INRA111 resolved on 4 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

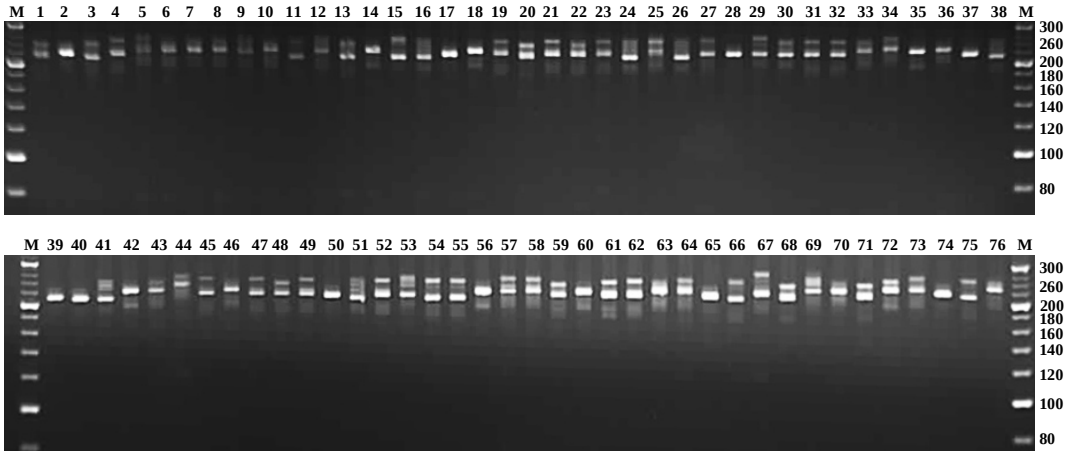


Fig. 4.18 :Microsatellite allele profiling of bTB positive and negative animals generated with marker BM7169 resolved on 4 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

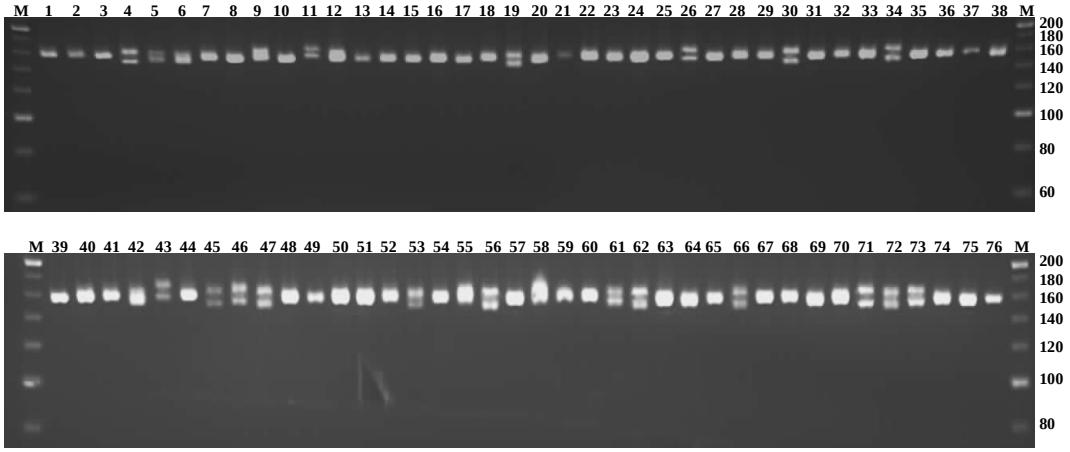


Fig. 4.19 :Microsatellite allele profiling of bTB positive and negative animals generated with marker BMS1724 resolved on 5 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

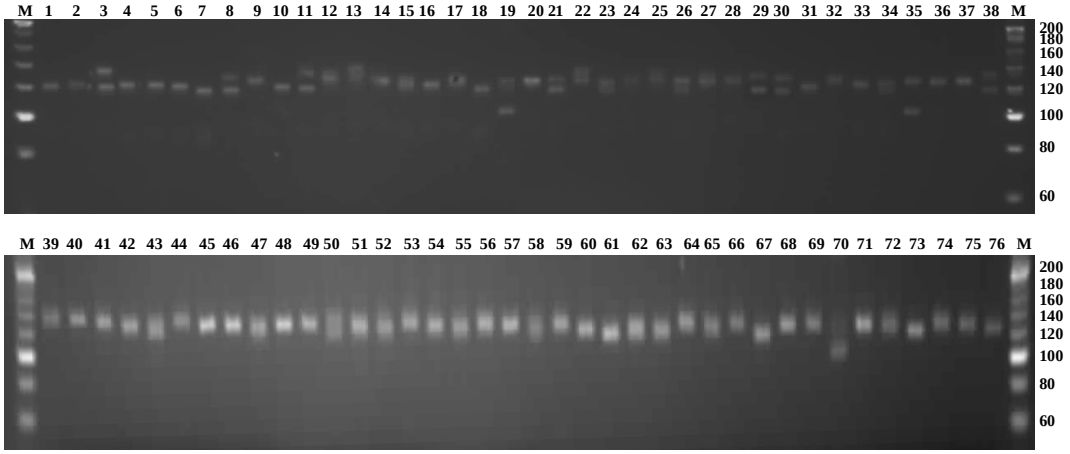


Fig. 4.20 :Microsatellite allele profiling of bTB positive and negative animals generated with marker BM7209 resolved on 5 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

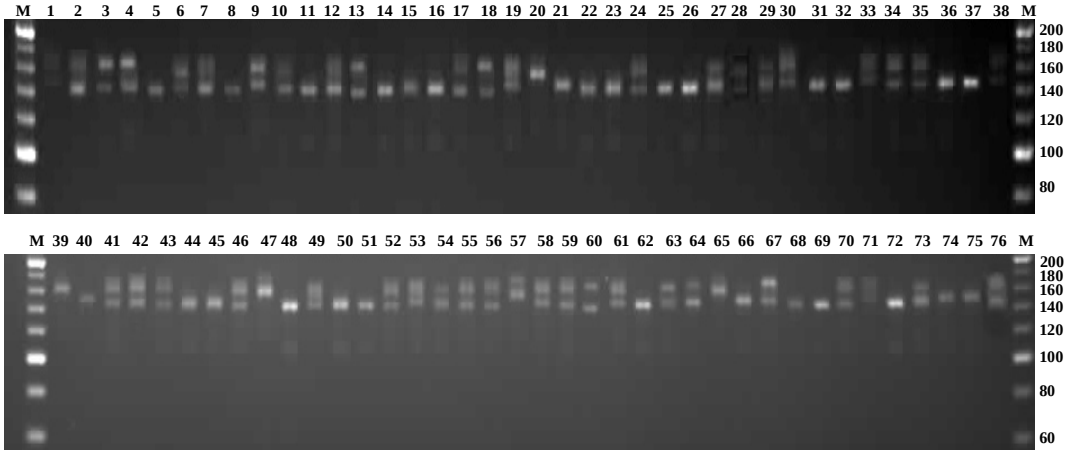


Fig. 4.21 :Microsatellite allele profiling of bTB positive and negative animals generated with marker BMS495 resolved on 4 % metaphor agarose. Positive: Lane 1-35; **Negative**: Lane 36-76; 20 bp Marker

Table 4.1 : Allelic frequencies with their χ2 probabilities at BM1818 locus in case versus control animals

BM1818	Negative		Positive		χ2	Odds ratio
	Frequency	Percent	Frequency	Percent		(95 % CI)
254	38	46.34	41	58.57	0.13	1.64(0.86-3.12)
256	14	17.07	9	12.86	0.47	0.72(0.29-1.78)
258	3	3.66	1	1.43	0.39	0.38(0.04-3.75)
264	5	6.10	2	2.86	0.45	0.45(0.0851-2.4108)
266	**1**	**1.22**	**7**	**10.00**	**0.02**	**9.00(1.08-75.06)**
268	12	14.63	10	14.29	0.95	0.97(0.39-2.41)
270	**9**	**10.98**	**0**	**0.00**	**<0.01**	**~0**

Table 4.2 : Genotypic frequencies with their χ2 probabilities at BM1818 locus in case versus control animals

BM1818	Negative		Positive		χ2	Odds ratio
	Frequency	Percent	Frequency	Percent		(95 % CI)
254/254	9	21.95	14	40.00	0.09	2.37(0.87-6.4568)
254/258	1	2.44	0	0.00	1.00	~0
254/264	5	12.20	2	5.71	0.44	0.44(0.079-2.40)
254/266	1	2.44	5	14.29	0.09	6.67(0.74-60.08)
254/268	9	21.95	6	17.14	0.60	0.74(0.23-2.32)
254/270	4	9.76	0	0.00	0.12	~0
256/256	2	4.88	3	8.57	0.66	1.83(0.29 -11.62)
256/258	2	4.88	1	2.86	1.00	0.57(0.05 -6.61)
256/268	3	7.32	2	5.71	1.00	0.77(0.12 -4.88)
256/270	5	12.20	0	0.00	0.06	~0
266/266	0	0.00	1	2.86	0.46	∞
268/268	0	0.00	1	2.86	0.46	∞

Table 4.3 : Allelic frequencies with their χ2 probabilities at BM1824 locus in case versus control animal

BM1824	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
182	**0**	**0.00**	1	1.43	0.46	∞
186	73	89.02	59	84.29	0.39	0.66 (0.257 -1.70)
194	**7**	**8.54**	**0**	**0.00**	**0.02**	**~0**
196	**2**	**2.44**	**10**	**14.29**	**<0.01**	**6.66 (1.41-31.56)**

Table 4.4 : Genotypic frequencies with their χ2 probabilities at BM1824 locus in case versus control animals

BM1824	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
182/196	0	0.00	1	2.86	0.46	Infty
186/186	33	80.49	25	71.43	0.36	0.61(0.21-1.76)
186/196	**0**	**0.00**	**9**	**25.71**	**<0.01**	∞
186/194	**7**	**17.07**	**0**	**0.00**	**0.04**	**~0**
196/196	1	2.44	0	0.00	1.00	~0

Table 4.5 : Allelic frequencies with their χ2 probabilities at ETH 3 locus in case versus control animals

	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
99	5	6.10	5	7.14	1.00	1.18(0.33-4.27)
109	**26**	**31.71**	**9**	**12.86**	**<0.01**	**0.32(0.14-0.74)**
111	**46**	**56.10**	**50**	**71.43**	**0.05**	**1.96(0.99-3.85)**
121	5	6.10	6	8.57	0.56	1.44(0.42-4.95)

Table 4.6 : Genotypic frequencies with their χ2 probabilities at BM1824 locus in case versus control animals

ETH3	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
99/109	4	9.76	1	2.86	0.37	0.27(0.03-2.56)
99/111	1	2.44	3	8.57	1.00	1.19(0.22-6.29)
99/121	1	2.86	0	0.00	1.00	~0
109/109	9	21.95	3	8.57	0.11	0.33(0.08-1.35)
109/111	2	4.88	0	0.00	0.50	~0
109/121	2	4.88	2	5.71	1.00	1.18(0.16-8.86)
111/111	20	48.78	23	65.71	0.14	2.01(0.80-5.10)
111/121	3	7.32	1	2.86	0.62	0.37(0.04-3.75)
121/121	0	0.00	1	2.86	0.46	∞

Table 4.7 : Allelic frequencies with their χ2 probabilities at ETH 10 locus in case versus control animals

ETH10	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
198	6	7.32	9	12.86	0.25	1.87(0.63-5.54)
200	13	15.85	11	15.71	0.98	0.99(0.41-2.37)
202	15	18.29	14	20.00	0.79	1.12(0.50-2.51)
204	5	6.10	1	1.43	0.22	0.22(0.03-1.96)
206	1	1.22	0	0.00	1.00	~0
208	1	1.22	0	0.00	1.00	~0
210	2	2.44	1	1.43	1.00	0.58(0.05-6.53)
214	1	1.22	2	2.86	0.59	2.38(0.21-26.85)
216	3	3.66	4	5.71	0.70	1.60(0.34-7.39)
218	11	13.41	6	8.57	0.35	0.605(0.21-1.73)
220	11	13.41	17	24.29	0.09	2.07(0.90-4.78)
222	10	12.20	4	5.71	0.17	0.44(0.13-1.46)
224	1	1.22	1	1.43	1.00	1.17(0.07-19.12)
228	2	2.44	0	0.00	0.50	~0

Table 4.8 : Genotypic frequencies with their χ2 probabilities at ETH 10 locus in case versus control animals

ETH10	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
198/214	1	2.44	2	5.71	0.59	2.42(0.21- 27.93)
198/216	3	7.32	3	8.57	1.00	1.19(0.22- 6.30)
198/218	2	4.88	3	8.57	0.66	1.83(0.28-11.68)
198/220	0	0.00	1	2.86	0.46	∞
200/210	0	0.00	1	2.86	0.46	∞
200/216	0	0.00	1	2.86	0.46	∞
200/218	7	17.07	3	8.57	0.33	0.46(0.10-1.91)
200/220	**0**	**0.00**	**6**	**17.14**	**<0.01**	∞
200/220	5	12.20	10	28.57	0.07	2.89(0.87-9.45)
200/222	1	2.44	0	0.00	1.00	~0
202/210	1	2.44	0	0.00	1.00	~0
202/218	2	4.88	0	0.00	0.50	~0
202/220	4	9.76	0	0.00	0.12	~0
202/222	7	17.07	4	11.43	0.49	0.63(0.16-2.35)
202/224	1	2.44	0	0.00	1.00	~0
204/210	1	2.44	0	0.00	1.00	~0
204/220	1	2.44	0	0.00	1.00	~0
204224	0	0.00	1	2.86	0.46	∞
204/222	2	4.88	0	0.00	0.49	~0
204/228	1	2.44	0	0.00	1.00	~0
206/220	1	2.44	0	0.00	1.00	~0
208/228	1	2.44	0	0.00	1.00	~0

Table 4.9 : Allelic frequencies with their χ2 probabilities at ILST006 locus in case versus control animals

ILST006	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
264	1	1.22	0	0.00	1.00	~0
268	0	0.00	1	1.43	0.46	∞
274	**56**	**68.29**	**34**	**48.57**	**0.01**	**0.44(0.23-0.85)**
276	0	0.00	1	1.43	0.46	∞
278	**10**	**12.20**	**31**	**44.29**	**<0.01**	**6.52(2.90-14.63)**
280	**15**	**18.29**	**3**	**4.29**	**<0.01**	**0.20(0.06-0.72)**

Table4.10: Genotypic frequencies with their χ2 probabilities at ILST006 locus in case versus control animals

ILST006	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
264/274	1	2.44	0	0.00	1.00	~0
268/274	0	0.00	1	2.86	0.46	∞
274/274	21	51.22	14	40.00	0.33	0.63(0.25-1.58)
274/278	1	2.44	5	13.20	0.09	6.67(0.74-60.08)
274/280	**12**	**29.27**	**0**	**0.00**	**<0.01**	**~0**
278/276	0	0.00	1	2.86	0.46	∞
278/278	**4**	**9.76**	**11**	**31.43**	**<0.01**	**5.09(1.43-18.06)**
278/280	1	2.44	3	8.57	0.33	3.75(0.37-37.79)
280/280	1	2.44	0	0.00	1.00	~0

Table 4.11: Allelic frequencies with their χ2 probabilities at TGLA126 locus in case versus control animals

ILSTS006	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
118	11	13.41	5	7.14	0.21	0.50(0.16-1.51)
120	**21**	**25.61**	**8**	**11.43**	**0.03**	**0.37(0.15-0.91)**
122	**0**	**0.00**	**4**	**5.71**	**0.04**	∞
124	**18**	**21.95**	**3**	**4.29**	**<0.01**	**0.16(0.04-0.57)**
126	23	28.05	15	21.43	0.35	0.70(0.33-1.48)
128	**7**	**8.54**	**15**	**21.43**	**0.02**	**2.92(1.12-7.65)**
130	**0**	**0.00**	**7**	**10.00**	**<0.01**	∞
132	2	2.44	6	8.57	0.14	3.75(0.73-19.21)
134	**0**	**0.00**	**4**	**5.71**	**0.04**	∞
136	0	0.00	3	4.29	0.10	∞

Table 4.12: Genotypic frequencies with their χ2 probabilities at TGLA126 locus in case versus control animals

TGLA126	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
118/118	3	7.32	2	5.71	1.00	0.77(0.12-4.88)
118/124	3	7.32	0	0.00	0.24	~0
118/126	1	2.44	0	0.00	1.00	~0
118/128	1	2.44	0	0.00	1.00	~0
118/130	0	0.00	1	2.86	0.46	∞
120/120	7	17.07	2	5.71	0.17	0.29(0.06-1.52)
120/126	6	14.63	1	2.86	0.12	0.17(0.02-1.50)
120/128	1	2.44	2	5.71	0.59	2.42(0.21-27.93)
120/132	0	0.00	1	2.86	0.46	∞
122/132	0	0.00	2	5.71	0.50	∞
122/122	0	0.00	1	2.86	0.46	∞
124/124	**7**	**17.07**	**0**	**0.00**	**0.01**	**~0**
124/128	1	2.44	0	0.00	1.00	~0
124/130	0	0.00	2	5.71	0.50	∞
124/132	0	0.00	1	2.86	0.46	∞
126/126	8	19.51	6	17.14	0.79	0.85(0.26-2.75)
126/132	0	0.00	1	2.86	0.46	∞
128/132	0	0.00	1	2.86	0.46	∞
130/130	2	5.71	6	17.14	0.13	4.03(0.76-21.45)
134/134	0	0.00	1	2.86	0.46	∞
134/136	0	0.00	1	2.86	0.46	∞
136/136	0	0.00	1	2.86	0.46	∞

Table 4.13: Allelic frequencies with their χ2 probabilities at SLCLLA1-I locus in case versus control animals

SLCLLA1-I	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
227	27	32.93	28	40.00	0.37	1.36(0.70-2.64)
229	28	34.15	26	37.14	0.70	1.14(0.59-2.21)
231	1	1.22	0	0.00	1.00	~0
233	**4**	**4.88**	**0**	**0.00**	**0.04**	**~0**
245	21	25.61	16	22.86	0.70	0.86(0.41-1.82)
249	1	1.22	0	0.00	1.00	∞

Table 4.14: Genotypic frequencies with their χ2 probabilities at SLCLLA1 locus in case versus control animals

SLCLLA1-I	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
227/227	12	29.27	12	34.29	0.64	1.26(0.48-3.32)
227/245	3	7.32	4	11.43	0.70	1.63(0.34-7.86)
229/229	5	12.20	7	20.00	0.35	1.80(0.52-6.28)
229/245	18	43.90	12	34.29	0.39	0.67(0.26-1.70)
231/249	1	2.44	0	0.00	1.00	~0
233/233	2	4.88	0	0.00	0.50	~0

Table 4.15: Allelic frequencies with their χ2 probabilities at BM2113 locus in case versus control animals

BM2113	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
127	1	1.22	**0**	**0.00**	1.00	~0
137	**11**	**13.41**	**2**	**2.86**	**0.02**	**0.19(0.04-0.89)**
139	11	13.41	13	18.57	0.38	1.47(0.61-3.532)
141	4	4.88	1	1.43	0.37	0.28(0.03-2.59)
143	2	2.44	2	2.86	1.00	1.18(0.16-8.58)
145	11	13.41	9	12.86	0.91	0.95(0.37-2.45)
147	**16**	**19.51**	**4**	**5.71**	**0.01**	**0.25(0.08-0.78)**
149	22	26.83	28	40.00	0.08	1.82(0.92-3.60)
151	**0**	**0.00**	1	1.43	0.46	∞
153	**4**	**4.88**	**10**	**14.29**	**0.05**	**3.25(0.98-10.87)**

Table 4.16: Genotypic frequencies with their χ2 probabilities at BM2113 locus in case versus control animals

BM2113	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
127/147	1	2.44	0	0.00	1.00	~0
137/137	1	2.44	0	0.00	1.00	~0
137/143	2	4.88	0	0.00	0.50	~0
137/145	2	4.88	0	0.00	0.50	~0
137/147	4	9.76	1	2.86	0.37	0.27(0.03-2.55)
137/149	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
139/139	3	7.32	3	8.57	1.00	1.19(0.22-6.30)
139//145	0	0.00	2	5.71	0.21	∞
139/147	0	0.00	1	2.86	0.46	∞
139/149	3	7.32	3	8.57	1.00	1.19(0.22-6.30)
139/151	0	0.00	1	2.86	0.46	∞
139/153	2	4.88	0	0.00	0.50	~0
141/141	2	4.88	0	0.00	0.50	~0
141/153	0	0.00	1	2.86	0.46	∞
143/143	0	0.00	1	2.86	0.46	∞
145/145	4	9.76	2	5.71	0.68	0.56(0.10-3.26)
145/153	1	2.44	3	8.57	0.33	3.75(0.37-37.79)
147/147	5	12.20	0	0.00	0.06	~0
147/149	0	0.00	2	5.71	0.21	∞
147/153	1	2.44	0	0.00	1.00	~0
149/149	9	21.95	11	31.43	0.35	1.63(0.58-4.55)
153/153	0	0.00	3	8.57	0.09	∞

Table 4.17: Allele frequencies with their χ2 probabilities at ETH185 locus in case versus control animals

ETH185	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
214	3	3.66	4	5.71	0.70	1.60(0.34-7.39)
220	**18**	**21.95**	**2**	**2.86**	**<0.01**	**0.10(0.02-0.47)**
224	**0**	**0.00**	**4**	**5.71**	**0.04**	∞
230	**10**	**12.20**	**23**	**32.86**	**<0.01**	**3.52(1.54-8.06)**
236	21	25.61	18	25.71	0.99	1.00(0.48-2.09)
240	15	18.29	16	22.86	0.49	1.32(0.60-2.92)
244	3	3.66	2	2.86	0.78	0.77(0.13-4.77)
248	12	14.63	1	1.43	0.06	0.34(0.10-1.09)

Table 4.18: Genotypic frequencies with their χ2 probabilities at ETH185 locus in case versus control animals

ETH185	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
214/240	0	0.00	2	5.71	0.21	∞
214/236	3	7.32	2	5.71	1.00	0.77(0.12-4.88)
220/220	4	9.76	0	0.00	0.12	~0
220/230	3	7.32	1	2.86	0.62	0.37(0.04-3.75)
220/236	5	12.20	1	2.86	0.21	0.21(0.02-1.91)
220/244	1	2.44	0	0.00	1.00	~0
220/248	1	2.44	0	0.00	1.00	~0
224/224	0	0.00	2	5.71	0.21	∞
230/230	2	4.88	6	17.14	0.13	4.03(0.76-21.45)
230/236	2	4.88	2	5.71	1.0	1.18(0.16-8.86)
230/240	**1**	**2.44**	**7**	**20.00**	**0.02**	**10.00(1.16-85.87)**
230/248	0	0.00	1	2.86	0.46	∞
236/236	4	9.76	6	17.14	0.50	1.91(0.49-7.42)
236/240	0	0.00	1	2.86	0.46	∞
236/248	3	7.32	0	0.00	0.24	~0
240/240	7	17.07	3	8.57	0.33	0.46(0.11-1.91)
244/244	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
248/248	4	9.76	0	0.00	0.12	∞

Table 4.19: Gene frequencies with their χ2 probabilities at ETH152 locus in case versus control animals

ETH152	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
166	**6**	**7.32**	**24**	**34.29**	**<0.01**	**6.60(2.51-17.37)**
168	**41**	**50.00**	**23**	**32.86**	**0.03**	**0.49(0.25-0.95)**
174	18	21.95	11	15.71	0.33	0.66(0.29-1.52)
176	**2**	**2.44**	**8**	**11.43**	**0.04**	**5.16(1.06-25.18)**
178	**9**	**10.98**	**2**	**2.86**	**0.05**	**0.24(0.05-1.14)**
180	5	6.10	0	0.00	0.06	~0
182	**0**	**0.00**	2	2.86	0.21	∞
184	1	1.22	**0**	**0.00**	1.00	~0

Table 4.20: Gene frequencies with their χ2 probabilities at ETH152 locus in case versus control animals

ETH152	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
166/166	3	7.32	9	25.71	0.03	4.38(1.08-17.76)
166/174	0	0.00	3	8.57	0.09	∞
166/176	0	0.00	3	8.57	0.09	∞
168/168	16	39.02	6	17.14	0.04	0.32(0.11- 0.95)
168/174	0	0.00	3	8.57	0.09	∞
168/176	2	4.88	5	14.29	0.24	3.25(0.59-17.92)
168/178	6	14.63	2	5.71	0.28	0.35(0.07-1.88)
168/180	1	2.44	0	0.00	1.00	~0
168/182	0	0.00	1	2.86	0.46	∞
174/174	8	19.51	2	5.71	0.10	0.25(0.05-1.27)
174/178	1	2.44	0	0.00	1.00	~0
174/182	0	0.00	1	2.86	0.46	∞
174/184	1	2.44	0	0.00	1.00	~0
178/178	1	2.44	0	0.00	1.00	~0
180/180	2	4.88	0	0.00	0.50	~0

Table 4.21: Gene frequencies with their $\chi 2$ probabilities at SLC11A1-II locus in case versus control animals

SLC11A1-II	Negative		Positive		$\chi 2$	Odds ratio
	Frequency	Percent	Frequency	Percent		(95 % CI)
238	**0**	**0.00**	**7**	**10.00**	**<0.01**	∞
240	**3**	**3.66**	**9**	**12.86**	**0.04**	**3.89(1.01-14.97)**
242	**1**	**1.22**	**13**	**18.57**	**<0.01**	**18.47(2.35-145.23)**
244	8	9.76	11	15.71	0.27	1.72(0.65-4.56)
246	**14**	**17.07**	**4**	**5.71**	**0.03**	**0.29(0.09-0.94)**
248	10	12.20	4	5.71	0.17	0.44(0.13-1.46)
250	24	29.27	14	20.00	0.19	0.60 (0.28-1.28)
252	1	1.22	5	7.14	0.1	6.23(0.71-54.66)
254	1	1.22	0	0.00	1.00	~0
256	7	8.54	2	2.86	0.17	0.32(0.06-1.57)
258	**10**	**12.20**	**1**	**1.43**	**0.01**	**0.10(0.01-0.87)**
260	3	3.66	0	0.00	0.25	~0

Table 4.22: Gene frequencies with their χ2 probabilities at Slc11A1-II locus in case versus control animals

SLC11A1-II	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
238/238	0	0.00	3	8.57	0.09	∞
238/250	0	0.00	1	2.86	0.46	∞
240/240	1	2.44	2	5.71	0.59	2.42(0.21- 27.9)
240/250	1	2.44	5	14.29	0.08	6.67(0.74-60.08)
242/242	0	0.00	4	11.43	0.04	∞
242/250	0	0.00	2	5.71	0.20	∞
242/252	1	2.44	3	8.57	0.32	3.75(0.37-37.79)
244/244	3	7.32	3	8.57	1.00	1.19(0.22-6.29)
244/248	0	0.00	1	2.86	0.46	∞
244/250	1	2.44	0	0.00	1.00	~0
244/252	0	0.00	2	5.71	0.20	∞
244/254	1	2.44	0	0.00	1.00	~0
244/256	0	0.00	2	5.71	0.20	∞
246/246	4	9.76	2	5.71	0.68	0.56(0.10-3.26)
246/256	4	9.76	0	0.00	0.11	~0
246/258	2	4.88	0	0.00	0.49	~0
248/248	2	4.88	1	2.86	1.00	0.57(0.05-6.61)
248/256	2	4.88	0	0.00	0.49	~0
248/258	4	9.76	1	2.86	0.36	0.27(0.03-2.56)
250/250	7	17.07	3	8.57	0.32	0.46(0.11-1.91)
250/256	1	2.44	0	0.00	1.00	~0
250/258	4	9.76	0	0.00	0.11	~0
250/260	3	7.32	0	0.00	0.24	~0

Table 4.23: Gene frequencies with their χ2 probabilities at BMS2753 locus in case versus control animals

BMS2753	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
108	19	23.17	8	11.43	0.06	0.43(0.17-1.05)
110	21	25.61	20	28.57	0.68	1.16(0.57-2.39)
112	**31**	**37.80**	**11**	**15.71**	**<0.01**	**0.31(0.14-0.67)**
114	**7**	**8.54**	**20**	**28.57**	**<0.01**	**4.29(1.69-10.89)**
116	4	4.88	7	10.00	0.22	2.17(0.61-7.73)
118	0	0.00	4	5.71	0.04	∞

Table 4.24: Genotype frequencies with their χ2 probabilities at BMS2753 locus in case versus control animals

BMS2753	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
108/108	4	9.76	2	5.71	0.68	0.56(0.10-3.26)
108/110	5	12.20	1	2.86	0.20	0.21(0.02-1.90)
108/112	2	4.88	2	5.71	1.00	1.18(0.16 -8.85)
108/114	3	7.32	1	2.86	0.62	0.37(0.04-3.75)
108/116	1	2.44	0	0.00	1.00	~0
110/110	7	17.07	6	17.14	0.99	1.00(0.30-3.34)
110/112	2	4.88	1	2.86	1.00	0.57(0.05-6.60)
110/114	0	0.00	3	8.57	0.09	∞
110/116	0	0.00	1	2.86	0.46	∞
110/118	0	0.00	2	5.71	0.21	∞
112/112	**13**	**31.71**	**4**	**11.43**	**0.04**	**0.28(0.08-0.95)**
112/116	1	2.44	0	0.00	1.00	~0
114/114	**2**	**4.88**	**8**	**22.86**	**0.04**	**5.78(1.14-29.35)**
116/116	1	2.44	3	8.57	0.33	3.76(0.37-37.80)
118/118	0	0.00	1	2.86	0.46	∞

Table 4.25: Genotype frequencies with their χ2 probabilities at BMS499 locus in case versus control animals

BMS499	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
106	**0**	**0.00**	**21**	**30.00**	**<.0.01**	∞
110	**39**	**47.56**	**16**	**22.86**	**<.0.01**	**0.33(0.16-0.66)**
112	3	3.66	1	1.43	0.62	0.38(0.04-3.75)
118	5	6.10	5	7.14	1.00	1.18 (0.32-4.2726)
128	14	17.07	13	18.57	0.81	1.11(0.48-2.54)
130	4	4.88	6	8.57	0.51	1.83(0.49-6.75)
134	3	3.66	2	2.86	1.00	0.77(0.12-4.77)
138	**12**	**14.63**	**3**	**4.29**	**0.03**	**0.25(0.06-0.93)**
140	1	1.22	0	0.00	1.00	~0
142	0	0.00	2	2.86	0.21	∞
148	1	1.22	1	1.43	1.00	1.17(0.07-19.12)

Table 4.26: Genotype frequencies with their χ2 probabilities at BMS499 locus in case versus control animals

BMS499	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
106/106	0	0.00	3	8.57	0.09	∞
106/110	0	0.00	1	2.86	0.46	∞
106/118	0	0.00	3	8.57	0.09	∞
106/128	**0**	**0.00**	**6**	**17.14**	**<0.01**	∞
106/130	0	0.00	3	8.57	0.09	∞
106/138	0	0.00	1	2.86	0.46	∞
106/142	0	0.00	1	2.86	0.46	∞
110/110	11	26.83	5	14.29	0.18	0.45 (0.14-1.47)
110/118	1	2.44	1	2.86	1.00	1.18(0.07-19.52)
110/128	8	19.51	3	8.57	0.18	0.39 (0.09-1.59)
110/130	2	4.88	1	2.86	1.00	0.57 (0.05-6.60)
110/134	1	2.44	0	0.00	1.00	~0
110/138	5	12.20	0	0.00	0.06	~0
112/130	1	2.44	0	0.00	1.00	~0
112/134	1	2.44	0	0.00	1.00	~0
112/138	1	2.44	0	0.00	1.00	~0
112/148	0	0.00	1	2.86	0.46	∞
118/128	1	2.44	1	2.86	1.00	1.18 (0.07-19.53
118/134	1	2.44	0	0.00	1.00	~0
118/138	2	4.88	0	0.00	0.50	~0
128/128	1	2.44	0	0.00	1.00	~0
128/130	1	2.44	1	2.86	1.00	1.18 (0.07-19.53)
128/138	2	4.88	2	5.71	1.00	1.18 (0.15-8.85)
130/142	0	0.00	1	2.86	0.46	∞
134/134	0	0.00	1	2.86	0.46	∞
138/138	1	2.44	0	0.00	1.00	~0
140/148	1	2.44	0	0.00	1.00	~0

Table 4.27 : Genotype frequencies with their $\chi 2$ probabilities at BMS468 locus in case versus control animals

BMS468	Negative		Positive		$\chi 2$	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
128	1	1.22	2	2.86	0.61	2.09(0.18-23.56)
130	**5**	**6.10**	**16**	**22.86**	**<0.01**	**4.56(1.57-13.20)**
132	27	32.93	18	25.71	0.33	0.71(0.34-1.43)
134	22	26.83	17	24.29	0.72	0.87(0.42-1.82)
136	12	14.63	8	11.43	0.56	0.75(0.28-1.96)
138	3	3.66	1	1.43	0.62	0.38(0.03-3.75)
140	1	1.22	0	0.00	1.00	~0
142	0	0.00	1	1.43	0.46	∞
144	5	6.10	6	8.57	0.56	1.44(0.42-4.95)
146	6	7.32	1	1.43	0.12	0.18(0.02-1.56)

Table 4.28 : Genotype frequencies with their χ2 probabilities at BMS468 locus in case versus control animals

BMS468	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
128/128	0	0.00	1	2.86	0.46	∞
128/144	1	2.44	0	0.00	1.00	~0
130/130	2	4.88	6	17.14	0.13	4.03(0.75-21.45)
130/138	0	0.00	1	2.86	0.46	∞
130/142	0	0.00	1	2.86	0.46	∞
130/144	0	0.00	1	2.86	0.46	∞
130/146	0	0.00	1	2.86	0.46	∞
132/132	11	26.83	8	22.86	0.69	0.81(0.28-2.30)
132/140	1	2.44	0	0.00	1.00	~0
132/144	2	4.88	2	5.71	1.00	1.18(0.16-8.85)
132/146	2	4.88	0	0.00	0.50	~0
134/130	1	2.44	0	0.00	1.00	~0
134/134	**9**	**21.95**	**0**	**0.00**	**<0.01**	**~0**
134/136	1	2.44	1	2.86	1.00	1.18(0.07-19.52)
134/144	**2**	**4.88**	**8**	**22.86**	**0.03**	**5.78(1.14-29.35)**
136/136	4	9.76	2	5.71	0.68	0.56(0.10-3.26)
136/144	0	0.00	3	8.57	0.09	∞
136/146	3	7.32	0	0.00	0.24	~0
138/138	1	2.44	0	0.00	1.00	~0
138/146	1	2.44	0	0.00	1.00	~0

Table 4.29 : Gene frequencies with their χ2 probabilities at BMS468 locus in case versus control animals

BMS2213	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
122	1	1.22	0	0.00	1.00	~0
124	10	12.20	8	11.4	0.88	0.92(0.34-2.49)
126	9	10.98	5	7.14	0.41	0.62(0.19-1.95)
128	6	7.32	10	14.2	0.16	2.11(0.72-6.13)
130	4	4.88	6	8.57	0.51	1.82(0.49-6.75)
132	1	1.22	1	1.43	1.00	1.17(0.07-19.11)
134	4	4.88	2	2.86	0.68	0.57(0.10-3.22)
136	5	6.10	3	4.29	0.72	0.68(0.15-2.99)
138	**6**	**7.32**	**0**	**0.00**	**0.03**	**~0**
140	7	8.54	5	7.14	0.75	0.82(0.24-2.72)
142	0	0.00	1	1.43	0.46	∞
144	4	4.88	2	2.86	0.68	0.57(0.10-3.22)
146	8	9.76	4	5.71	0.35	0.56(0.16-1.94)
148	3	3.66	3	4.29	1.00	1.17(0.23-6.03)
150	6	7.32	5	7.14	0.96	0.97(0.28-3.34)
152	**2**	**2.44**	**9**	**12.86**	**0.01**	**5.90(1.23-28.30)**
154	1	1.22	2	2.86	0.59	2.38(0.21-26.84)
156	1	1.22	3	4.29	0.33	3.62(0.36-35.67)
158	3	3.66	1	1.43	0.62	0.38(0.03-3.75)
168	1	1.22	0	0.00	1.00	∞

Table 4.30 : Genotype frequencies with their $\chi 2$ probabilities at BMS2213 locus in case versus control animals

BMS2213	Negative		Positive		$\chi 2$	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
122/130	1	2.44	0	0.00	1.00	~0
124/124	1	2.44	0	0.00	1.00	~0
124/132	1	2.44	0	0.00	1.00	~0
124/134	0	0.00	1	2.86	0.46	∞
124/136	1	2.44	1	2.86	1.00	1.17 (0.07-19.53)
124/138	1	2.44	0	0.00	1.00	~0
124/140	0	0.00	1	2.86	0.46	∞
124/144	1	2.44	1	2.86	1.00	1.17(0.07-19.53)
124/146	2	4.88	1	2.86	1.00	0.57(0.05-6.60)
124/148	1	2.44	1	2.86	1.00	1.17(0.07-19.53)
124/150	1	2.44	2	5.72	0.59	2.42(0.21-27.93)
126/128	1	2.44	0	0.00	1.00	~0
126/130	1	2.44	0	0.00	1.00	~0
126/136	2	4.88	0	0.00	0.50	~0
126/138	1	2.44	0	0.00	1.00	~0
126/140	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
126/144	0	0.00	1	2.86	0.46	∞
126/146	2	4.88	0	0.00	0.50	~0
126/150	0	0.00	1	2.86	0.46	∞
126/152	0	0.00	1	2.86	0.46	∞
126/154	0	0.00	1	2.86	0.46	∞
126/168	1	2.44	0	0.00	1.00	~0
128/128	0	0.00	2	5.71	0.21	∞
128/130	0	0.00	1	2.86	0.46	∞
128/138	1	2.44	0	0.00	1.00	~0
128/140	2	4.88	1	2.86	1.00	0.57(0.05-6.60)
128/144	1	2.44	0	0.00	1.00	~0
128/146	0	0.00	1	2.86	0.46	∞
128/148	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
128/152	0	0.00	1	2.86	0.46	∞
130/130	0	0.00	1	2.86	0.46	∞
130/152	0	0.00	2	5.71	0.21	∞
130/150	2	4.88	0	0.00	0.50	~0
132/150	0	0.00	1	2.86	0.46	∞
134/146	3	7.32	0	0.00	0.24	~0
134/152	0	0.00	1	2.86	0.46	∞

Table 4.30: Contd...

BMS2213	Negative		Positive		$\chi 2$	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
134/154	1	2.44	0	0.00	1.00	~0
136/136	1	2.44	0	0.00	1.00	~0
136/148	0	0.00	1	2.86	0.46	∞
136/154	0	0.00	1	2.86	0.46	∞
138/156	1	2.44	0	0.00	1.00	~0
138/158	1	2.44	0	0.00	1.00	~0
140/140	1	2.44	0	0.00	1.00	~0
140/152	2	4.88	2	5.71	1.000	1.18(0.158-8.85)
142/158	0	0.00	1	2.86	0.46	∞
144/144	1	2.44	0	0.00	1.00	~0
146/128	0	0.00	1	2.86	0.46	∞
146/138	1	2.44	0	0.00	1.00	~0
146/156	0	0.00	1	2.86	0.46	∞
148/158	1	2.44	0	0.00	1.00	~0
150/150	1	2.44	0	0.00	1.00	~0
150/152	0	0.00	1	2.86	0.46	∞
150/158	1	2.44	0	0.00	1.00	~0
152/130	0	0.00	1	2.86	0.46	∞
156/156	0	0.00	1	2.86	0.46	∞

Table 4.31: Genotype frequencies with their χ2 probabilities at INRA131 locus in case versus control animals

BMS131	Negative		Positive		χ2	Odds ratio
	Frequency	Percent	Frequency	Percent		(95 % CI)
96	3	3.66	1	1.43	0.62	0.38(0.03-3.75)
100	8	9.76	5	7.14	0.56	0.71(0.22-2.28)
104	1	1.22	0	0.00	1.00	~0
108	3	3.66	4	5.71	0.70	1.59(0.34-7.38)
110	20	24.39	10	14.29	0.12	0.51(0.22-1.19)
112	0	0.00	2	2.86	0.21	∞
114	5	6.10	9	12.86	0.15	2.27(0.72-7.13)
116	1	1.22	0	0.00	1.00	~0
118	1	1.22	4	5.71	0.18	4.90(0.53-44.98)
120	**2**	**2.44**	**11**	**15.71**	**<0.01**	**7.45(1.59-34.91)**
122	3	3.66	6	8.57	0.20	2.46(0.59- 10.26)
124	6	7.32	1	1.43	0.12	0.18(0.02-1.56)
126	13	15.85	6	8.57	0.17	0.49(0.17-1.38)
128	8	9.76	3	4.29	0.19	0.41(0.10-1.62)
130	4	4.88	8	11.43	0.13	2.51(0.72-8.74)
134	4	4.88	0	0.00	0.12	~0

Table 4.32: Genotype frequencies with their χ2 probabilities at INRA131 locus in case versus control animals

BMS131	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
96/108	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
96/114	1	2.44	0	0.00	1.00	~0
96/124	1	2.44	0	0.00	1.00	~0
100/100	0	0.00	1	2.86	0.46	∞
100/110	3	7.32	0	0.00	0.24	~0
100/114	2	4.88	1	2.86	1.00	0.57(0.05-6.61)
100/122	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
100/128	1	2.44	0	0.00	1.00	~0
100/130	0	0.00	1	2.86	0.46	∞
100/134	1	2.44	0	0.00	1.00	~0
104/126	1	2.44	0	0.00	1.00	~0
108/108	0	0.00	1	2.86	0.46	∞
108/120	0	0.00	1	2.86	0.46	∞
108/126	1	2.44	0	0.00	1.00	~0
108/130	1	2.44	0	0.00	1.00	~0
110/110	5	12.20	1	2.86	0.21	0.21(0.02-1.91)
110/118	0	0.00	1	2.86	0.46	∞
110/120	1	2.44	2	5.71	0.59	2.42(0.21-27.93)
110/122	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
110/124	1	2.44	0	0.00	1.00	~0
110/128	2	4.88	2	5.71	1.00	1.18(0.16-8.85)
110/130	2	4.88	2	5.71	1.00	1.18(0.16-8.85)
112/114	0	0.00	1	2.86	0.46	∞
112/118	0	0.00	1	2.86	0.46	∞
114/114	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
114/120	0	0.00	3	8.57	0.09	∞
114/124	0	0.00	1	2.86	0.46	∞
114/126	0	0.00	1	2.86	0.46	∞
116/126	1	2.44	0	0.00	1.00	~0
118/126	1	2.44	0	0.00	1.00	~0
118/128	0	0.00	1	2.86	0.46	∞
118/130	0	0.00	1	2.86	0.46	∞
120/120	0	0.00	2	5.71	0.21	∞
122/128	1	2.44	0	0.00	1.00	~0
124/124	2	4.88	0	0.00	0.49	~0
120/126	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
122122	0	0.00	2	5.71	0.21	∞

Table 4. 32: Contd...

BMS131	Negative		Positive		$\chi 2$	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
126/126	3	7.32	2	5.71	1.00	0.78(0.12-4.88)
126/130	1	2.44	0	0.00	1.00	~0
126/134	1	2.44	0	0.00	1.00	~0
128/128	2	4.88	0	0.00	0.47	~0
130/130	0	0.00	2	5.71	0.21	∞
134/134	1	2.44	0	0.00	1.00	~0

Table 4.33: Gene frequencies with their $\chi 2$ probabilities at INRA111 locus in case versus control animals

INRA111	Negative		Positive		$\chi 2$	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
124	2	2.44	**1**	**1.43**	1.00	0.57(0.05-6.53)
128	**17**	**20.73**	**6**	**8.57**	**0.03**	**0.35(0.13-0.967)**
130	**35**	**42.68**	**19**	**27.14**	**0.04**	**0.50(0.25-0.99)**
132	**11**	**13.41**	**27**	**38.57**	**<0.01**	**4.05(1.82-8.99)**
138	2	2.44	**0**	**0.00**	0.49	~0
140	**1**	**1.22**	**6**	**8.57**	**0.04**	**7.59(0.89-64.68)**
142	5	6.10	**1**	**1.43**	0.21	0.22(0.02-1.95)
144	9	10.98	**10**	**14.29**	0.53	1.35(0.51-3.54)

Table 4.34: Genotype frequencies with their χ2 probabilities at INRA111 locus in case versus control animals

INRA111	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
124/138	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
124/144	1	2.44	0	0.00	1.00	~0
128/128	6	14.63	1	2.86	0.12	0.17(0.02-1.50)
128/140	0	0.00	3	8.57	0.09	∞
128/142	1	2.44	0	0.00	1.00	~0
128/144	4	9.76	1	2.86	0.37	0.27(0.03-2.56)
130/130	15	36.59	7	20.00	0.11	0.43(0.15-1.23)
130/140	0	0.00	1	2.86	0.46	∞
130/142	3	7.32	0	0.00	0.24	~0
130/144	2	4.88	4	11.43	0.41	2.52 (0.43-14.65)
132/132	**5**	**12.20**	**11**	**31.43**	**0.04**	**3.30(1.01-10.70)**
132/142	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
132/144	**0**	**0.00**	**4**	**11.43**	**0.04**	∞
140/144	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
144/138	1	2.44	0	0.00	1.00	~0

Table 4.35: Genotype frequencies with their χ2 probabilities at BM7169 locus in case versus control animals

BM7169	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
208	3	3.66	5	7.14	0.47	2.02(0.46-8.79)
210	5	6.10	2	2.86	0.45	0.45(0.08-2.41)
212	1	1.22	1	1.43	1.00	1.17(0.07-19.11)
214	**8**	**9.76**	**18**	**25.71**	**<0.01**	**3.20(1.29- 7.916)**
218	13	15.85	17	24.29	0.19	1.70(0.76-3.81)
220	**17**	**20.73**	**2**	**2.86**	**<0.01**	**0.11(0.02-0.50)**
224	2	2.44	0	0.00	0.49	~0
226	5	6.10	2	2.86	0.24	3.07(0.57-16.38)
228	5	6.10	4	5.71	1.00	0.93(0.24-3.61)
230	2	2.44	0	0.00	0.49	~0
232	**1**	**1.22**	**10**	**14.29**	**<0.01**	**13.50(1.68-108.33)**
234	4	4.88	7	10.00	0.22	2.16(0.60-7.73)
238	**13**	**15.85**	**1**	**1.43**	**<0.01**	**0.07(0.001- 0.60)**
240	2	2.44	0	0.00	0.49	~0
244	1	1.22	0	0.00	1.00	~0
250	0	0.00	1	1.43	0.46	∞

Table 4.36: Genotype frequencies with their χ2 probabilities at BM7169 locus in case versus control animals

BM7169	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
208/208	1	2.44	0	0.00	1.00	~0
208/228	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
208/232	0	0.00	2	5.71	0.21	∞
208/234	0	0.00	2	5.71	0.21	∞
210/210	2	4.88	0	0.00	0.50	~0
210/218	0	0.00	1	2.86	0.46	∞
210/226	0	0.00	1	2.86	0.46	∞
210/234	1	2.44	0	0.00	1.00	~0
212/228	0	0.00	1	2.86	0.46	∞
212/238	1	2.44	0	0.00	1.00	~0
214/214	2	4.88	3	8.57	0.66	1.82(0.29-11.68)
214/228	1	2.44	1	2.86	1.00	1.18(0.07-19.57)
214/230	**1**	**2.44**	**7**	**20.00**	**0.02**	**10.00(1.16-85.87)**
214/234	**0**	**0.00**	**4**	**11.43**	**0.04**	∞
214/232	1	2.44	0	0.00	1.00	~0
214/238	1	2.44	0	0.00	1.00	~0
218/218	3	7.32	6	17.14	0.28	2.62(0.60-11.37)
218/224	1	2.44	0	0.00	1.00	~0
218/228	0	0.00	1	2.86	0.46	∞
218/232	0	0.00	1	2.86	0.46	∞
218/234	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
218/238	3	7.32	0	0.00	0.24	~0
218/240	2	4.88	0	0.00	0.50	~0
218/250	0	0.00	1	2.86	0.46	∞
220/220	4	9.76	1	2.86	0.37	0.27(0.03-2.55)
220/228	1	2.44	0	0.00	1.00	~0
220/234	2	4.88	0	0.00	0.50	~0
220/238	**6**	**14.63**	**0**	**0.00**	**0.03**	~0
224/238	1	2.44	0	0.00	1.00	~0
226/226	2	4.88	0	0.00	0.50	~0
226/238	1	2.44	**1**	**2.86**	1.00	1.18(0.07-19.57)
228/228	1	2.44	0	0.00	1.00	~0
230/244	1	2.44	0	0.00	1.00	~0

Table 4.37: Genotype frequencies with their χ2 probabilities at BMS1724 locus in case versus control animals

BMS1724	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
100	0	0.00	1	1.43	0.46	∞
116	0	0.00	1	1.43	0.46	∞
152	**12**	**14.63**	**2**	**2.86**	**0.01**	**0.17(0.03-0.79)**
154	6	7.32	2	2.86	0.28	0.37(0.07-1.90)
156	23	28.05	29	41.43	0.08	1.81(0.92-3.57)
160	**3**	**3.66**	**11**	**15.71**	**0.01**	**4.91(1.31-18.38)**
162	**0**	**0.00**	**15**	**21.43**	**<.0.01**	∞
164	**15**	**18.29**	**4**	**5.71**	**0.02**	**0.27 (0.08-0.85)**
166	**19**	**23.17**	**4**	**5.71**	**<.0.01**	**0.20(0.06-0.62)**
170	4	4.88	1	1.43	0.37	0.28(0.03-2.58)

Table 4.38: Genotype frequencies with their χ2 probabilities at BMS1724 locus in case versus control animals

BMS1724	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
152/160	1	2.44	0	0.00	1.00	~0
152/162	0	0.00	1	2.86	0.46	∞
152/164	2	4.88	0	0.00	0.49	~0
152/166	**8**	**19.51**	**1**	**2.86**	**0.03**	**0.12(0.01-1.02)**
152/170	1	2.44	0	0.00	1.00	∞
154/160	0	0.00	1	2.86	0.46	~0
154/164	2	4.88	0	0.00	0.49	∞
154/166	3	7.32	1	2.86	0.61	0.37(0.0-3.75)
154/170	1	2.44	0	0.00	1.00	~0
156/156	7	17.07	8	22.87	0.52	1.44(0.46-4.47)
156/160	**0**	**0.00**	**6**	**17.14**	**0.01**	∞
156/162	**0**	**0.00**	**7**	**20.00**	**<0.01**	∞
156/164	**0**	**0.00**	**4**	**11.43**	**0.04**	∞
156/166	8	19.51	2	5.71	0.09	0.25 (0.05-1.27)
156/170	1	2.44	0	0.00	1.00	~0
160/160	1	2.44	2	5.71	0.53	2.42 (0.21-27.93)
162162	0	0.00	3	8.57	0.09	∞
164/164	5	12.20	0	0.00	0.06	~0
162/170	0	0.00	1	2.86	0.46	∞
164/170	1	2.44	0	0.00	1.00	~0

Table 4.39: Gene frequencies with their χ2 probabilities at BM7209 locus in case versus control animals

BMS7209	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
102	0	0.00	3	4.29	0.25	∞
124	5	6.10	9	12.86	0.15	2.27(0.72-7.13)
126	7	8.54	12	17.15	0.39	0.64(0.24-1.74)
128	14	17.07	10	14.29	0.64	0.80(0.33-1.95)
130	**17**	**20.73**	**6**	**8.57**	**0.04**	**0.35(0.13-0.96)**
132	6	7.32	5	7.14	0.9	0.97(0.28-3.34)
134	17	20.73	13	18.57	0.73	0.87(0.38-1.95)
136	4	4.88	5	7.14	0.73	1.50(0.38-5.81)
138	5	6.10	3	4.29	0.72	0.68(0.15-2.99)
140	0	0.00	1	1.43	0.46	∞
142	0	0.00	1	1.43	0.46	∞
144	7	8.54	1	1.43	0.70	0.35(0.13-0.96)
148	0	0.00	1	1.43	0.46	∞

Table 40: Genotype frequencies with their χ2 probabilities at BM7209 locus in case versus control animals

BMS7209	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
102/102	0	0.00	1	2.86	0.46	∞
102/134	0	0.00	1	2.86	0.46	∞
124/124	2	4.88	3	8.57	0.6	1.83(0.29-11.61)
124/138	1	2.44	0	0.00	1.00	~0
124/128	0	0.00	1	2.86	0.46	∞
124/136	0	0.00	1	2.86	0.46	∞
124/138	0	0.00	1	2.86	0.46	∞
126/126	1	2.44	3	8.57	0.39	3.75(0.37-37.79)
126/130	3	7.32	0	0.00	0.24	~0
126/134	0	0.00	1	2.86	0.46	∞
126/138	2	4.88	1	2.86	1.00	0.57(0.05-6.60)
126/140	0	0.00	1	2.86	0.46	∞
126/142	0	0.00	1	2.86	0.46	∞
128/128	6	14.63	5	14.29	0.97	0.97(0.27- 3.51)
128/138	2	4.88	1	2.86	1.00	0.57(0.05- 6.61)
130/130	7	17.07	3	8.57	0.33	0.46(0.11- 1.91)
132/132	3	7.32	2	5.71	1.00	0.77(0.12-4.88)
132/148	0	0.00	1	2.86	0.46	∞
134/134	5	12.20	5	14.29	1.00	1.20(0.32- 4.54)
134/144	7	17.07	1	2.86	0.06	0.14(0.02- 1.22)
136/136	2	4.88	2	5.71	1.00	1.18(0.16-8.85)

Table 4.41: Genotype frequencies with their χ2 probabilities at BMS495 locus in case versus control animals

BMS495	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
144	**10**	**12.20**	**41**	**58.57**	**<0.01**	**10.18(4.50-22.99)**
148	**39**	**47.56**	**7**	**10.00**	**<0.01**	**0.123(0.05-0.29)**
154	3	3.66	0	0.00	0.28	~0
156	1	1.22	0	0.00	1.00	~0
160	5	6.10	7	10.00	0.38	1.71(0.52-5.65)
164	8	9.76	5	7.14	0.57	0.72(0.22-2.29)
166	5	6.10	0	0.00	0.06	~0
168	11	13.41	10	14.29	0.84	1.10(0.43-2.75)

Table 4.42: Genotype frequencies with their χ2 probabilities at BMS495 locus in case versus control animals

BMS495	Negative		Positive		χ2	Odds ratio (95 % CI)
	Frequency	Percent	Frequency	Percent		
144/144	**2**	**4.88**	**13**	**37.14**	**<0.01**	**11.52 (2.38-55.82)**
144/148	1	2.44	0	0.00	1.00	~0
144/160	2	4.88	4	11.43	0.41	2.51 (0.43-14.65)
144/164	**0**	**0.00**	**4**	**11.43**	**0.04**	∞
144/166	2	4.88	0	0.00	0.50	~0
144/168	**1**	**2.44**	**7**	**20.00**	**0.02**	**10.00(1.16- 85.87)**
148/148	**11**	**26.83**	**1**	**2.86**	**<0.01**	**0.08(0.009-0.66)**
148/160	1	2.44	1	2.86	1.00	1.18(0.07-19.57)
148/164	6	14.63	1	2.86	0.16	0.17(0.02-1.50)
148/168	9	21.95	3	8.57	0.11	0.33(0.08-1.35)
154/154	1	2.44	0	0.00	1.00	~0
154/168	1	2.44	0	0.00	1.00	~0
156/166	1	2.44	0	0.00	1.00	~0
160/160	1	2.44	1	2.86	1.00	1.18(0.07-19.53)
164/164	1	2.44	0	0.00	1.00	~0
166/166	1	2.44	0	0.00	1.00	~0

Table 4.43 : The overall H_E, PIC and test of association

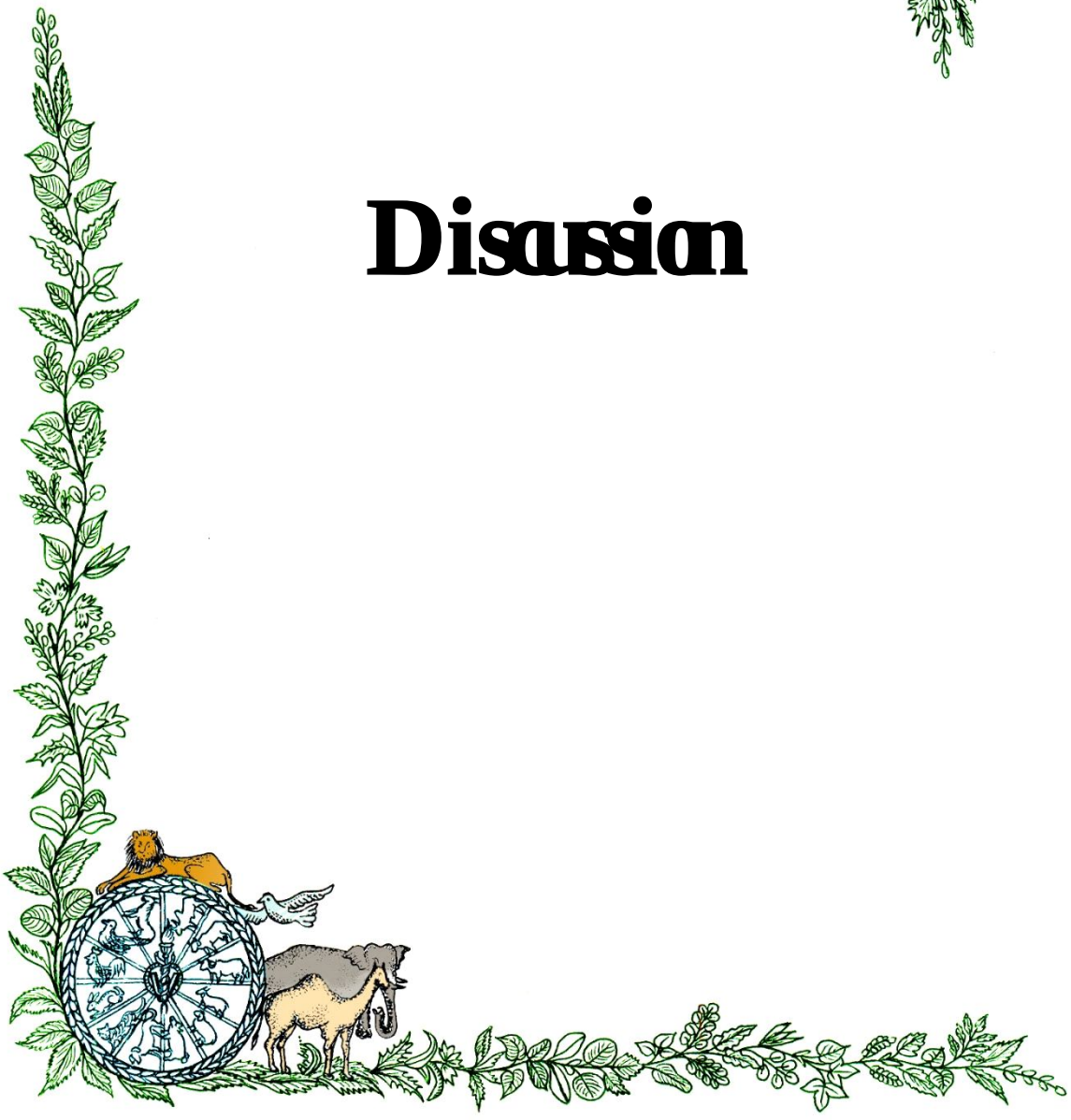
Locus	N	Alleles	PIC	Heterozygosity	Allelic Diversity	Test for HWE			Test for Association with BTB
						Chi-Square	DF	Pr > ChiSq	
BM1818	76	7	0.64	0.60	0.67	55.95	21	<0.01	0.02
BM1824	76	4	0.22	0.22	0.23	13.30	6	0.04	<0.01
ETH3	76	4	0.48	0.26	0.53	46.81	6	<0.01	<0.01
ETH10	76	14	0.85	1.00	0.86	300.81	91	<0.01	0.12
ILSTS006	76	6	0.49	0.32	0.56	36.97	15	<0.01	<0.01
TGLA126	76	10	0.82	0.35	0.84	254.61	45	<0.01	<0.01
Slc11A1	76	6	0.61	0.50	0.68	297.02	15	<0.01	0.37
BM 2113	76	10	0.79	0.42	0.81	184.87	45	<0.01	<0.01
ETH185	76	8	0.79	0.47	0.81	203.17	28	<0.01	<0.01
ETH 152	76	8	0.70	0.38	0.73	138.18	28	<0.01	<0.01
Slc11A1	76	12	0.85	0.53	0.87	248.94	66	<0.01	<0.01
BMS2753	76	6	0.74	0.32	0.78	137.61	15	<0.01	0.03
BMS499	76	11	0.77	0.71	0.79	152.04	55	<0.01	<0.01
BMS468	76	10	0.77	0.31	0.80	209.57	45	<0.01	0.03
BMS2213	76	21	0.92	0.88	0.92	238.86	210	0.08	0.04
INRA131	76	16	0.89	0.65	0.90	193.04	120	<0.01	0.03
INRA111	76	8	0.73	0.39	0.76	138.14	28	<0.01	<0.01
BM7169	76	16	0.87	0.67	0.88	268.04	120	<0.01	<0.01
BMS1724	76	10	0.79	0.71	0.81	93.63	45	<0.01	<0.01
BM7209	76	15	0.86	0.34	0.88	532.95	105	<0.01	0.14
BMS495	76	8	0.72	0.57	0.76	120.33	28	<0.01	<0.01
BMS499	76	16	0.82	0.68	0.83	244.65	120	<0.01	0.06

Table 4.44 : Comparison of IgG1 values in positive and negative animals

Sl No	Positive		Negative	
	Animal	IgG1 value (mg/ml)	Animal	IgG1 value (mg/ml)
1	1	4.68948	36	0.89668
2	2	5.31012	37	3.68956
3	3	5.75836	38	0.27604
4	4	5.27564	39	0.345
5	5	3.24132	40	2.37932
6	6	5.3446	41	8.03404
7	7	2.20692	42	1.79316
8	8	0.31052	43	0.27604
9	9	1.86212	44	10.30972
10	10	1.27596	45	6.65484
11	11	1.06908	46	2.55172
12	12	0.55188	47	3.31028
13	13	0.72428	48	3.93092
14	14	2.44828	49	3.06892
15	15	2.89652	50	0.82772
16	16	2.17244	51	1.68972
17	17	0.79324	52	2.86204
18	18	0.72428	53	9.03396
19	19	1.5518	54	1.34492
20	20	0.89668	55	0.58636
21	21	0.37948	56	0.96564

Table 4.45 : Mean of IgG1 in positive and negative animals

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	5.6053824	5.6053824	0.91	0.3461
Error	40	246.6381845	6.1659546		
Corrected Total	41	252.2435669			



Discussion

5 DISCUSSION

The association each microsatellite makers with bTB were investigated and being presented for discussion at individual locus.

5.1 BM1818 microsatellite locus: The univariate logistic regression analysis revealed that at BM1818 microsatellite marker only one allele of '266' bp was differing significantly in case and control population and ODD ratio (OR) of '266' bp allele verses other genotype was 9.00 (1.08- 75.06; 95% CI) in tuberculin positive cases as compared to control population. It suggested that the allele of '266' bp was responsible for making animal susceptible for bovine tuberculosis. Similarly the '270' bp allele was significantly ($P<0.01$) and exclusively present in tuberculosis affected animals and hence its ODDs was towards infinity. The overall effect of BM1818 microsatellite locus was found to be significant ($P=0.02$) on incidences of bovine tuberculosis. Hence, individual genotypic frequencies at BM1818 microsatellite locus was compared and it was found that none of the genotype was differing significantly ($P<0.05$) with each other in case verses control group (Table 2). But there was an increasing trend of '254/254' and '254/266' genotype at BM1818 microsatellite locus in tuberculin negative animals. At same locus '256/270' genotype was present in five negative animals and was nil in positive population. So these genotypes might have made the overall impact on BM1818 microsatellite locus for making it significant in present study and chances of getting more reliable result is warranted only in larger population. Similar to present study, Ali *et al*. (2009), reported that the microsatellite locus BM1818 was significantly ($p<0.01$) with positive skin tuberculin test. The heterozygosity (H_E) and PIC were 0.60 and 0.60 respectively in the populations.

5.2 BM1824 microsatellite locus: The univariate logistic regression analysis revealed that at this microsatellite locus two alleles, '194' bp and '196' bp were significantly differing in case and control populations. The allele '194' was exclusively present in the control population and hence its odds ratio approaches infinity. The allele '194' was a private allele for tuberculin positive animals. The OR of '196' bp allele was 6.66 (1.41-31.56; 95% CI) which indicated that proportion of 196 bp allele was significantly higher in cases of tuberculin positive animals. The H_E and PIC of the population was 0.22 and 0.22 in the population.

The overall effect of the marker on tuberculin positive reaction was found to be highly significant ($p<0.001$) and further the individual genotypes were compared and two genotypes '186/196' and '186/194' was differing significantly ($P<0.05$) in case verses control populations with an odds ratio of infinity. The '186/196' genotype was exclusively present in positive population whereas 186/194 was exclusively present in tuberculin negative animals.. The one genotype of '196/196' was observed only in control population. Ali *et al*. (2009) reported significant ($p<0.01$) association with respect to positive tuberculin test. They also reported association with lung lesion in Arab and Morocco breeds of cattle. Thus the two studies were almost having the same results.

5.3 ETH 3 microsatellite locus: The univariate logistic regression analysis revealed that at ETH 3 microsatellite marker, two alleles of '109' and '111' bp were differing significantly in case and control population and ODD ratio (OR) of '109' bp and '111' bp allele verses other genotype were 0.32 (0.14-0.74; 95% CI) and 1.96 (0.99-3.85; 95% CI), respectively in tuberculosis affected animals as compared to control population. It suggested that the allele of '109' bp was responsible for making the animal resistant to tuberculosis and the higher proportion of '111' bp in the affected animals revealed its role in increasing susceptibility of animals to bovine tuberculosis. The other two alleles did not reveal any significant results with respect to the bTB status of the animals.

The overall effect of the marker on tuberculin positive reaction was found to be highly significant ($p<0.01$) and further the individual genotypes were compared and none of the genotypes were differing significantly ($P<0.05$) in case verses control populations. The genotypes '99/121' bp and '109/111'bp were present only in the control animal population with their frequency 1 and 2 respectively out of 41 animals tested tuberculin negative. Similarly a single

genotype of '121/121' bp was observed in the tuberculin positive animals (1 out of 35 positive animals) and the exclusive presence of these 3 genotypes cannot be concluded for resistance/ susceptibility unless a larger population is investigated. Ali *et al*. (2009) reported a significance value of p=0.002 for this microsatellite loci. The H_E and PIC values were 0.26 and 0.48 respectively within the population.

5.4 ETH 10 microsatellite locus: The univariate logistic regression analysis revealed that at ETH 10 microsatellite marker none of the alleles were differing significantly (P<0.05) in case and control population. The alleles of '206', '208' and '228' bp were present exclusively in the negative control population and in frequencies 1, 1 and 2 respectively out of 41 animals tested as tuberculin negative. So study on a larger population is required to confirm this observation. The univariate logistic regression analysis revealed a non-significant (p=0.12) association of ETH 10 locus on incidences of bTB. This result is in accordance with the result obtained in allele wise and genotype wise analysis where no significant analysis is obtained for the alleles separately. The allele '200/220' bp is present exclusively in tuberculin positive populations and the genotype is found to be highly significant in case versus control population (p=<0.01) and with this genotype was restricted to tuberculin positive population with the OR almost approaching infinity. The H_E and PIC values for the microsatellite in the population was 1.00 and 0.85 respectively. The significant effect of this genotype might be neutralized by the other genotypes making the marker non-significant as such with respect to tuberculin positive status. On contrary, Ali *et al*. (2009) reported that the ETH10 microsatellite marker was highly significant (p<0.001).

5.5 ILST006 Microsatellite locus: Three alleles, '280' bp, '274' bp and'278' bp were found to be significantly differing in case and control populations (p<0.05 respectively). A higher proportion of '274' and '280' bp alleles verses other genotype was observed in the negative control population than the tuberculosis positive animal.

On over all analysis the microsatellite locus ILSTS006 was found to be significant (p<.0.01) on the incidence of bovine tuberculosis. The frequencies of the genotypes were further analyzed for association and the '274/280' genotype was found to be significantly (P<0.01) and exclusively in the negative control population**.** The significance of the allele '278/ 278' was expected considering the high proportion of the allele '278' in tuberculin positive

animals. The proportion of '278/278' genotype was significantly higher in tuberculin positive animal with its OR 5.09 (1.43-18.06; 95 % CI) verses other genotypes of positive animals. A single observation of '264/274' bp and '280/280' bp was there in the negative population and similarly one each of '268/274' bp and '278/276' bp were present in the positive population. The H_E and PIC were 0.32 and 0.49 respectively within the population. In Ali *et al*. (2009) research the allele was found to be significant at a p-value of 0.003 in Morocco and Arab cattle breeds.

5.6 Microsatellite loci TGLA126: The univariate logistic regression analysis revealed a significant association ($p<0.01$) for the locus with the incidence of bTB in the study population. The individual allele frequencies and genotype frequencies were further analyzed using for their association with bTB. It is found that the alleles '120', '122', '124', '128', '130' and '134' bp were significantly differing (respectively<0.05) in case versus control population. The allele of '130' bp was present in exclusively (7 out of 70) in the case population which suggested the presence of the allele '130' bp as a private allele for the locus TGLA126. Similarly alleles of '122' bp and '136' bp were present exclusively in the case population with frequencies of 4 and 3 out of 70 respectively. Only one genotype '124/124' bp was found to be significantly affecting the tuberculin test and was exclusively found in the negative control population. The genotypes '118/124' bp, '118/126' bp, '118/128' bp, '124/124' bp and '124/128' bp were present only in the control population but with too low frequency to draw a valuable conclusion. Similarly the alleles '118/130' bp, '120/132' bp, '122/132' bp, '122/122' bp, '124/130' bp, '124/132' bp, '126/132' bp, '128/132' bp, '134/134' bp, '134/136' bp and '136/136' bp were exclusively present in the tuberculin positive population. The HE and PIC of the marker in the population was 0.35 and 0.82 respectively. Similarly Ali *et al*. (2009) reported a significant ($p<0.003$) effect for this locus, on bTB in Morocco and African Zebu cattle.

5.7 SLCLLA1-I microsatellite loci: The univariate logistic regression analysis revealed that at SLCLLA1-I microsatellite marker only one allele of, proportion of '233' bp was differing significantly ($p=0.04$) case-control animals with the odds ratio infinity. A non-significant association ($p=0.37$) with the incidence of bovine TB was obtained for this microsatellite locus by the univariate logistic regression analysis. Further it was revealed that all genotypes differed non-significantly in case versus control populations. However the alleles '231' bp, '233' bp

and '249' bp were present exclusively in the control population with their frequencies 1, 4 and 1 respectively out of total 82 alleles observed. The presence of the genotypes '231/249' bp and '233/233' bp exclusively on the negative populations but may not be valuable information considering the small size of the population under investigation. The HE value and PIC value of the markers were 0.50 and 0.61 respectively. On contrary to our findings, Felicitas *et al*. (2006) reported an overall significant association of SLCLLA1-I microsatellite marker with bTB.

5.8 BM2113 Microsatellite locus: The univariate logistic regression analysis revealed that at this microsatellite marker locus three alleles, '137' bp, '147' bp and '153' bp were differing significantly ($P \leq 0.05$) in tuberculosis positive cases as compared to control population with the ORs of 3.25 (0.98- 10.87; 95 % CI), 0.25 (0.08- 0.78; 95 % CI) and 0.19 (0.04- 0.89; 95 % CI), respectively. The alleles '127' bp and '151' bp were present exclusively in control and case populations respectively (only 1 in each population) and required study on a large population would be required to validate this finding. The allele '149' though was non-significant was present in higher proportion in the tuberculin positive population (40.00 %) as compared to the negative population (26.83 %) and might have made contribution towards making the microsatellite locus significant to tuberculosis. The rest 6 alleles were not having any significant difference in case and control populations.

The microsatellite marker BM2113 had significant ($P<0.001$) effect on incidences of bovine tuberculosis as revealed from univariate logistic regression analysis. Hence, individual genotypic frequencies at BM2113 microsatellite locus was compared, but it was found that none of the genotype was differing significantly ($P<0.05$) with each other in case verses control group (Table 16). But an increased proportion of '147/147' bp genotype was present in the control population than control population. The H_E and PIC values of the marker BM2113 in the population were 0.42 and 0.79 respectively. Similar to our report of Ali *et al*. (2009) reported significant association of BM2113 microsatellite locus with positive tuberculosis positive animals.

5.9 ETH185 Microsatellite locus: The univariate logistic regression analysis of this microsatellite locus revealed that proportion of three alleles, '220' bp, '224' bp and '230' bp were differing significantly ($P<0.05$) in case verses control population. The ODD ratio (OR)

of 220 bp allele verses other genotype was 0.10 (0.02-0.47; 95 % CI); '230' bp allele verses other genotype was 3.52 (1.54-8.06; 95 % CI) in tuberculosis positive cases as compared to control population. The allele '224' bp was limited only to the tuberculosis positive animals and hence its odds ratio was found to be approaching infinity. So the allele '224' bp was a private allele for studying tuberculin reactor status. Thus it was concluded that the higher proportion of the '224' bp allele was present in tuberculosis positive animals.

The overall effect of ETH185 microsatellite locus was found to be significant ($P<0.01$) on incidences of bovine tuberculosis. Further, the individual genotypic frequencies at this microsatellite locus were compared and interestingly only one genotype of '130/240' was differing significantly ($P=0.02$) in case verses control animals (Table 2). The genotype '248/ 248' bp, '220/220' bp and '236/248' bp were observed only in negative animals for tuberculosis and hence a possible association of these genotypes with tuberculosis negative status cannot be inferred. The H_E and PIC values were 0.47 and 0.79 respectively in the population. Similarly Ali *et al*. (2009) reported significant ($p<0.001$) association for this marker with respect to bTB.

5.10 ETH152 Microsatellite locus: The alleles of '166' bp, '168' bp, '176' bp, and '178' bp were found to be differing significantly ($P<0.05$) in the case-control animals with their odds ratios 6.60 (2.51-17.37; 95 % CI), 0.49 (0.25-0.95; 95 % CI), 5.16 (1.06-25.18; 95 % CI) and 0.24 (0.05-1.14; 95 % CI), respectively. The allele '180' bp was found exclusively in the control population (1 out of 41 animals in control) and its frequency was not large enough to draw any valuable conclusion.

The microsatellite locus ETH152 was found to be highly significant ($P<0.01$) in for incidences of bTB in cattle. To investigate further individual genotypic frequencies at BM1818 microsatellite locus were compared and interestingly it was found that only 2 out of 15 genotypes were found to be differing significantly ($P<0.05$) with each other in case verses control group (Table 20). The genotype '166/166' was in higher proportion among tuberculin positive animals than in the control animals and hence it could be a candidate potential marker for marking animals susceptible for tuberculosis. At same time at this locus the genotypes '168/178' and '174/174' were observed in larger proportion in the control population than the case population (6 and 8 in the negative control against 2 in positive animals). So all these genotypes might

have made an overall impact on ETH152 microsatellite locus for making it significant in present study and its validation on a larger population will warrant these conclusions. The HE and PIC values of the population were 0.38 and 0.70 respectively. This result was in agreement with Ali *et al*. (2009) who reported ETH152 microsatellite locus was significant (p=0.02) with positive skin test in African Zebu animals.

5.11 SlC11A1-II Microsatellite locus: The univariate logistic regression analysis revealed that at Slc11A-II microsatellite marker three alleles of '238' bp, '240' bp, '242' bp, '246' bp and '258' bp were differing significantly (P<0.05) respectively) in the tuberculosis positive animals verses negative animals. The allele of '238' bp was present exclusively on the tuberculin positive population and hence its ODD ratio was tending to infinity. The ODD ratio (OR) of the alleles '240' bp, '242' bp and '246' bp verses other genotype were 3.89 (1.01-14.97; 95 % CI), 18.47 (2.35-145.23; 95 % CI), and 0.29(0.09-0.94; 95 % CI) in tuberculin positive cases as compared to control population. The alleles of '240' and '246' bp were in higher proportion in the control population and hence it may be responsible for the tuberculin test negative status of these animals. The 258 bp allele was present in significantly higher proportion in the negative population and with its ODD ratio of 0.10 (0.01-0.87; 95 % CI). The alleles '254' bp and '260' bp were observed restricted to the negative control, but no significant conclusion should be drawn considering the small frequency and hence study on a larger population only reveal the trend of these two alleles. The H_E and PIC values were 0.53 and 0.85 respectively for the population.

The overall effect of Slc11A1 microsatellite locus was found to be significant (P<0.01) on incidences of bovine tuberculosis. Hence, individual genotypic frequencies at Slc11A1 microsatellite locus was compared and it was found that only one out of the 22 genotypes was differing significantly (P<0.05) with each other in case verses control group (Table 22). The genotype, '242/242' was found to be significant (p=0.04) and was found exclusively on the animals tested positive for tuberculosis. Similar to our findings, Felicitas *et al*. (2006) reported that SlC11A1-II microsatellite locus is present in the ARO28 region of the Slc11A1 (NRAMP) gene significantly (p<0.001) associated with bTB. Felicitas *et al*. (2006) reported 12 alleles for this microsatellite locus of 246 to 288 bp lengths.

5.12 BMS2753 microsatellite locus: Three out of the 6 alleles, namely '112' bp, '114' bp and '118' bp were found to differ significantly with the case and control animals as revealed by the univariate regression analysis. The alleles of '112' bp and '114' bp were present in significantly ($p < 0.01$ for both the alleles) higher proportions in the tuberculosis negative animals and the ODD ratio (OR) of these alleles verses other genotype were 0.31(0.14-0.67) and 4.29(1.69-10.89) respectively in tuberculosis positive cases as compared to control population. The third significant allele was of '118' bp and was observed exclusively in the bTB positive animals (4 out of 35 animals).

The overall effect of BMS2753 microsatellite locus was found to be significant (P=0.03) on incidences of bovine tuberculosis. To investigate further the individual genotypic frequencies at the microsatellite locus were compared and it was found that only two out of the 15 observed genotypes was differing significantly (P=0.04) with each other in case verses control group (Table 24). The genotypes '108/116', '110/108' and '112/116' were present only in the negative control whereas, genotypes '118/118' bp, '110/118' bp, '110/116' bp and '110/114' bp were present only in the positive animal population (table 24). The H_E and PIC values were 0.32 and 0.74 respectively. Similar to our findings, Driscoll *et al.* (2011) in a study on a European cattle population found significant (p=0.018) association for the microsatellite locus BMS2753 with bTB.

5.13 BMS499 microsatellite locus: The univariate logistic regression analysis revealed that at BMS499 microsatellite marker only three alleles of '106' bp, '110' bp and '138' bp were differing significantly ($P<0.05$) in the case and control populations. It was noticed that the '106' bp allele was present only in the animals positive for tuberculin test (21 out of total 70 alleles) and it could be a private allele for incidence of bovine tuberculosis. The '130' bp allele for significantly presenting in positive animals. The '140' bp allele was present in the tuberculin negative population and on contrary to this the allele '142' bp was present in the tuberculin positive animals, but their numbers were too low to draw some valuable conclusions and hence a study on larger population is required to be done.

The overall effect of BMS499 microsatellite locus was found to be highly significant ($P<0.01$) on incidences of bovine tuberculosis. Hence, individual genotypic frequencies at BMS499 microsatellite locus was compared and it was found that only one out of the 27

genotypes was differing significantly (P<0.05) with each other in case verses control group (Table 2). The genotype, '106/128' was found to be significant (p<0.01) and was found exclusively in tuberculosis positive animals. Hence, this genotype was associated with the tuberculin test positive status and will be of useful in identifying the animals susceptible to tuberculosis. Also there was a higher proportion of the genotype '110/110' bp at the microsatellite locus with a frequency of 26.83 % in the negative population (table 26). Most of the other genotypes observed were either present in positive animals or in negative animals, that too in smaller proportions and hence it would be very difficult to draw some valuable conclusions considering the smaller size of the population under study. The H_E and PIC values were 0.71 and 0.77 respectively for the population. On contrary to our finding Driscoll *et al*. (2011) reported a non-significant (p>0.05) association for the locus in the European cattle population.

5.14 BMS468 microsatellite locus: The univariate logistic regression analysis revealed that at BMS468 microsatellite marker only one allele of '130' bp was differing significantly (p<0.01) with its ODD ratio (OR) of 4.56 (1.57- 13.20; 95 % CI) verses other genotype in tuberculosis positive cases as compared to control animals. It suggested that the allele of '130' bp was responsible for making animal bovine tuberculosis resistance. The other alleles were not showing any significant difference in the study.

The overall effect of BMS468 microsatellite locus was found to be significant (P=0.03) on incidences of bovine tuberculosis. Further, individual genotypic frequencies at BMS468 microsatellite locus was compared and two out of the 20 genotypes, '134/134' and '134/ 144' were found differing significantly (P<0.01 and P=0.03, respectively) with each other in case verses control group (Table 28). The genotype '134/134' was present exclusively in control animals and hence can be associated with the tuberculosis resistence status. The genotype '130/130' bp was present in higher proportion in the positive animals as compared to the negative control. The H_E and PIC values were 0.31 and 0.77 respectively. In contrary Driscoll *et al*. (2011) reported a non-significant association of BMS468 microsatellite locus with bTB in European cattle breeds.

5.15 BMS2213 microsatellite locus: Out of this 20 alleles, at BMS2213 microsatellite marker only two allele of '138' bp and '152' bp were differing significantly

(P=0.03 and P=0.01, respectively) in case and control population. The allele of 138 bp size was found restricted to the tuberculosis negative population and hence the ODD ratio was calculated to be infinity and whereas OR for allele of '152' bp verses other genotype was 5.90 (1.23-28.30) in tuberculin positive cases as compared to control population. Hence the allele 138 bp was a private allele with respect to the incidence of bovine tuberculosis. It suggested that the alleles of '138' bp was responsible for making animal bovine tuberculosis resistant since its proportion was more in the negative population and allele of '152' bp length which is found in higher proportions among the tuberculin positive group of animals. The alleles '122' bp and '168' bp were found exclusively in the negative control only, but in smaller proportions and hence no valuable conclusion was drawn from it.

The overall effect of BMS2213 microsatellite locus was found to be significant (P=0.04) on incidences of bovine tuberculosis. Hence, individual genotypic frequencies at BMS2213 microsatellite locus were compared and it was found that none of the genotype was differing significantly (P<0.05) with each other in case verses control group (Table30). In the investigation most of the genotypes were present in either case or control populations. So all of these genotypes might have made the overall impact on BMS2213 microsatellite locus for making it significant in present study and chances of getting more reliable result is warranted only in larger population. The H_E and PIC value were observed to be highest for this microsatellite with the values 0.88 and 0.92 respectively. On the contrary to Driscoll *et al*. (2011) reported a non-significant association for the microsatellite locus BM2213 with bTB in European cattle population.

5.16 INRA131 microsatellite locus: The univariate logistic regression analysis revealed that at INRA131 microsatellite marker only one allele of '120' bp was differing significantly (P<0.01) in case and control animals and its ODDs verses other genotype was 7.45 (1.59-34.91; 95 % CI) in tuberculosis positive cases as compared to control animals.

An overall analysis of the INRA131 microsatellite locus revealed that its effect was significant (P=0.03) on incidences of bovine tuberculosis. Hence, individual genotypic frequencies at INRA131 microsatellite locus was compared and it was found that none of the genotype was differing significantly (P<0.05) with each other in case verses control group (Table 32). The most of the genotypes were found either in positive or negative populations with a lower

frequency. The HE and PIC values were 0.65 and 0.89 respectively for the microsatellite locus in the investigated population. On the contrary to Driscoll *et al*. (2011) reported a non-significant association for the microsatellite locus INRA131 with bTB in European cattle population.

5.17 INRA111 microsatellite locus:The univariate logistic regression analysis revealed that at INRA111 microsatellite marker four alleles of '128' bp, '130' bp, '132' bp and '140' bp ware differing significantly ($p<0.05$) in case and control population and ODD ratios (ORs) of 103, 132 and 140 bp alleles verses other genotype were 0.50 (0.25-0.99; 95 % CI), 4.05 (1.82-8.99; 95 % CI) and 7.59 (0.89-64.68; (% % CI), respectively in tuberculosis positive cases as compared to control population. It suggested that these alleles were responsible for making the microsatellite marker locus INRA 111 significant with respect to the occurrence of bovine TB. Similarly the 138 bp allele was exclusively present in tuberculosis affected animals but its role in the disease resistance cannot ascertain unless a study on larger population is carried out.

The overall effect of INRA111 microsatellite locus was found to be significant ($P=<0.01$) on incidences of bovine tuberculosis. To investigate further the individual genotypic frequencies at INRA111 microsatellite locus were compared and it was found that two genotypes '132/ 132' and '132/134' were significantly ($P<0.05$) affecting the bovine tuberculosis. The genotype '132/134' bp was present only in the tuberculin positive with a frequency of 11.43 % and it may be significantly associated with the tuberculin negative status of the animals. But there was an increasing trend of '128/128' and '130/130' among tuberculin negative animals at INRA111 microsatellite locus. These two genotypes also might have made the overall impact on INRA111 microsatellite locus for making it significant in present study and chances of getting more reliable result is warranted only in larger population. Single cases of the genotypes '124/144', '128/ 142', '130/142' and '144/138' were in the negative population. Since their frequencies are low, hence valuable conclusions cannot be drawn from the present study and investigation on a larger population may give actual trend of these genotypes. The H_E and PIC were 0.39 and 0.73 respectively. Similar to our findings Driscoll *et al.* (2011) reported a significant ($P<0.01$) association of INRA111microsatellite marker on tuberculosis incidences in European cattle population.

5.18 BM7169 microsatellite locus: Out of these 16 alleles four were significantly (P<0.05) associated with bovine tuberculosis as evident from the univariate logistic regression analysis. The alleles 214, 220, 232 and '238' bp size were found to be significantly (P <0.01) differing in case versus control populations investigated under this study. The ORs of '214', '220', '232' and '238' bp alleles verses other genotypes were 3.20 (1.29- 7.916; 95 % CI), 0.11 (0.02-0.50; 95 % CI), 13.50 (1.68-108.33; 95 % CI) and 0.07(0.001- 0.60; 95 % CI), respectively in tuberculosis affected animals. It suggested that the proportion of '214' and '232' bp allele was significantly (P<0.05) higher in affected animals whereas, the frequency of '220' and '238' bp allele was significantly (P<0.05) lower in control animals**.** The single case of '224' bp, '230' bp, '240' bp and '244' bp alleles were found exclusively in the control animals. The allele of '218' bp was found in higher proportion in the tuberculin positive population (25.71 %) than in the negative population (9.76 %) and it might have contributed much for overall significance of the microsatellite marker locus BM1769. Similarly the allele '218' bp was also found in higher proportion in bTB affected animals.

The overall effect of BM1769 microsatellite locus was found to be significant (P=0.04) on incidences of bovine tuberculosis. To study the effect of the individual locus, frequencies of each genotypes at BM1769 microsatellite locus were compared and it was found that three genotypes of '214/230' , '214/234' and '220/238' were differing significantly (P<0.05) in case verses control group (Table 36). The OR of '214/230' genotypes verses other genotype was 10.00 (1.16-85.87; 95 % CI) whereas ORs of '214/234' and '220/238' were towards infinity. But, there was an increasing trend of '218/218' genotype at BM1769 microsatellite locus with a frequency of 17.14 % in the positive population versus 7.32 % in the negative population and hence these genotype genotypes might have made the overall impact on BM1769 microsatellite locus for making it significant in present study. The HE and PIC values were 0.67 and 0.87 respectively for the population under investigation. Driscoll *et al*. (2011) reported that BM7169 microsatellite locus was not associated significantly with bTB, based on their studies in European cattle (*Bos taurus*).

5.19 BMS1724 microsatellite locus: The univariate logistic regression analysis revealed that at BMS1724 microsatellite marker 5 out of 10 alleles, namely '152' bp, '160' bp, '162' bp, '164' bp and '166' bp were differing significantly in case and control population.

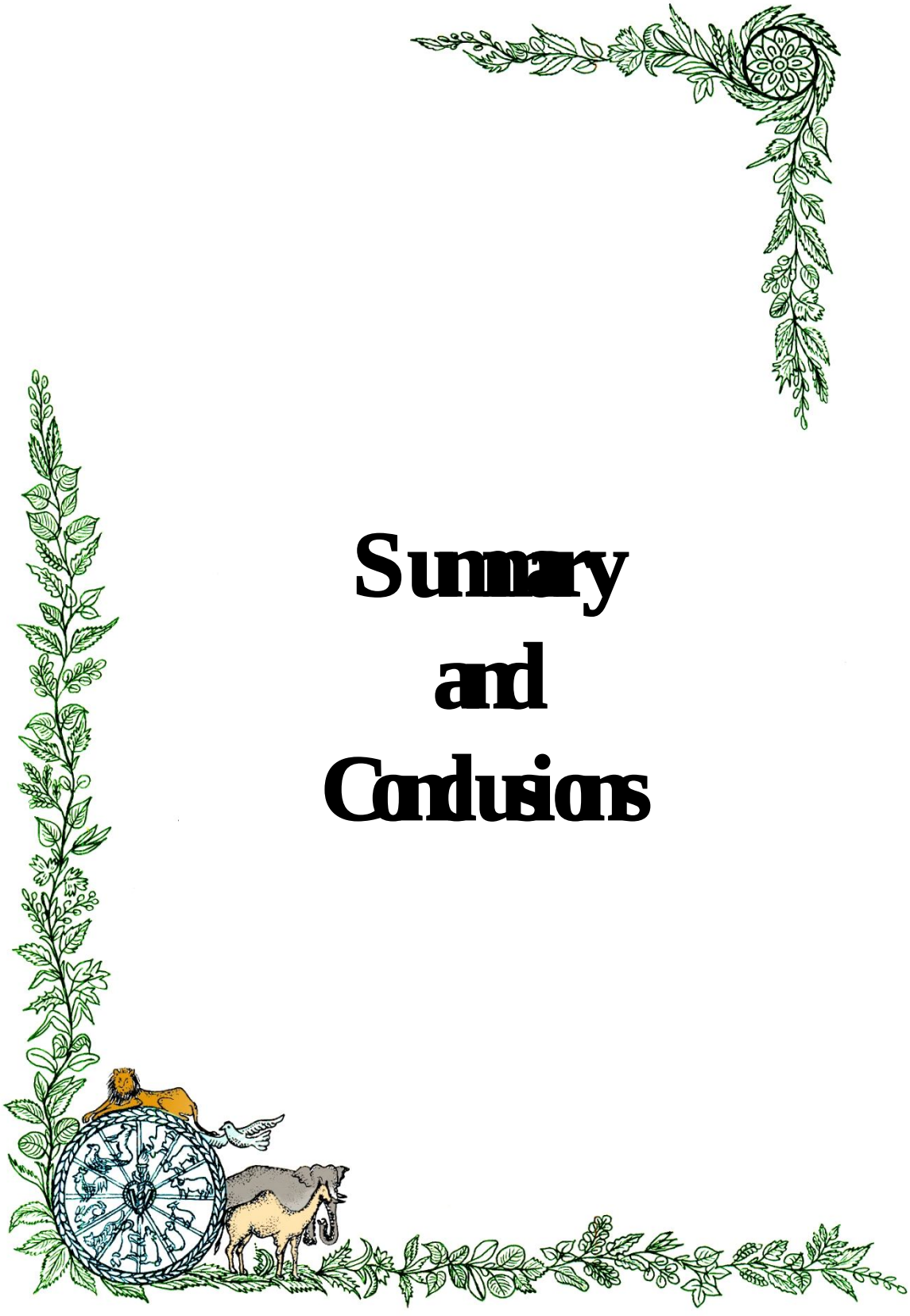
The allele '162' is found exclusively in the animals negative for tuberculosis and it can be designated as a private allele for bovine tuberculosis incidence. The allele '162' bp were a private allele with respect to the bovine tuberculosis. The ORs of '152' bp, '160' bp, '164' bp and '166' bp alleles with respect to other alleles were 0.17 (0.03-0.79; 95 % CI), 4.91(1.31-18.38; 95 % CI), 0.27 (0.08-0.85; 95 % CI) and 0.20(0.06-0.62; 95 % CI), respectively. Thus it was revealed that frequency of '160' bp allele was significantly higher in tuberculosis affected animals whereas the frequency of '152' and '164' and '166' bp alleles were significantly lower in control animals.

The overall effect of BMS1724 microsatellite locus was found to be significant ($P<0.01$) on incidences of bovine tuberculosis. Hence, individual genotypic frequencies at BMS1724 microsatellite locus was compared and it was found that four genotypes, namely '152/166', '156/160','156/162', '156/164' was differing significantly in case verses control group (Table 38). The genotypes '156/160', '156/162' and '156/164' were found exclusively in the tuberculosis positive animals and hence might have contributed for susceptibility for bTB. However the relative frequency of '152/166' genotype was significantly higher in control animals. The HE and PIC values were 0.71and 0.79 respectively in the population studied. On the contrary Driscoll *et al*. (2011) reported that the locus was non-significant in relation to the incidences of bTB.

5.20 BM7209 microsatellite locus: The univariate logistic regression analysis revealed that at BM7209 microsatellite marker only one alleles of '130' bp length was differing significantly ($p=0.04$) in case-control animals and its ODD ratios (ORs) of verses other genotype was 0.35(0.13-0.96; 95 % CI)) in tuberculosis affected cases as compared to control population. It suggested that these alleles were responsible for conferring bTB resistance. The overall effect of BM7209 microsatellite locus was found to be non-significant ($P=0.13$) on incidences of bovine tuberculosis. Also, individual genotypic frequencies at BM7209 microsatellite locus was compared and it was found that none of the genotype was differing significantly ($P<0.05$) with each other in case verses control group (Table 40).Similar to our findings, Driscoll *et al.* (2011) reported non-significant association for BM7209 microsatellite marker with bTB.

5.21 BMS495 microsatellite locus: The univariate logistic regression analysis revealed that at BMS495 microsatellite marker two alleles of '144' bp size and '148' bp size were differing significantly (P <0.01) in case and control population and ODD ratio (OR) of '144' bp allele verses other genotypes was 10.18 (4.50-22.99 ;95 % CI) whereas, the ODD ratio of the allele '148' bp allele verses other genotypes was 0.123 (0.05-0.29 ;95 % CI) in tuberculin positive cases as compared to control population. It suggested that the allele '144' bp was responsible for making animal bovine tuberculosis susceptible but allele of '148' bp was conferring for tuberculosis resistance. Interestingly, the '166' bp allele was found (6.10 %) exclusively in the tuberculin negative population.

The overall effect of BMS495 microsatellite locus was found to be significant (P<0.01) on incidences of bovine tuberculosis. Hence, individual genotypic frequencies at BMS495 microsatellite locus was compared and it was found that 4 genotypes out of the 16 observed genotypes were differing significantly (P<0.05) with each other in case verses control group (Table 42). The relative frequencies of '144/144' and '144/168' genotypes were significantly (P<0.05) higher in the positive animals with their ORs 11.52 (2.38-55.82; 95 % CI) and 10.00 (1.16-85.87; 95 % CI), respectively. The four cases of genotype '144/164' were found exclusively in the tuberculosis positive and hence it could a marker genotype associated with the susceptibility to tuberculosis. The '148/148' was found differing significantly in the case and control populations and its proportion was higher in the negative animals (26.83% in control population against 2.86 % in the positive population) and hence it could be associated with the bovine tuberculosis resistance in cattle. Also there was an increasing trend of '148/ 168' genotype at BMS495 microsatellite locus in control animals. But on the contrary, Driscoll *et al*. (2011) reported non-significant association of BMS495 marker with bTB in European Taurus cattle.



Summary and Conclusions

5 SUMMARY AND CONCLUSIONS

A panel of 21 microsatellite marker (BM1818, BM1824, ETH3, ETH10, ILSTS006, TGLA126,SLC11A1-I,BM2113, ETH185, ETH152, MS2753, BMS468, SLC11A1-II, BMS2213, INRA131, INRA111, BM7169, BMS1724, BM7209, BMS495 and BMS499) were used for study its association with bovine tuberculosis in cattle. The study was conducted on 35 positive and 41 negative animals selected from Sri. Mataji Goshala on the basis of tuberculin skin test. Across the locus, the number of alleles ranged from 4 (BM1824 and ETH3 loci) to 21 (BMS2213 locus) with an average of 10 alleles per locus. The heterozygosity was estimated as measure of the genetic diversity in a population with assumption that whether the genotypic frequency in accordance with their allelic frequency. The heterozygosity ranged from 0.22 for BM1824 to 1.00 for ETH10 microsatellite locus and higher heterozygosity implies for higher polymorphism in the population. The PIC ranges from 0.23 (BM1824) to 0.92 (BMS2213). Similarly, the allelic diversity ranged from 0.23 (BM1824) to 0.92 (BMS495). The all microsatellite loci except BMS2213 were significantly ($P<0.05$) departing from hardy Weinberg Equilibrium (HWE) which may be ascribed due to migrated cattle kept in the *Goshala*. The overall analysis using univariate logistic regression revealed that all the 17 out of the 21 microsatellite marker were significantly affecting the incidence of bovine tuberculosis.

Effect of genotypes at each microsatellite loci were studied. When individual genotypes of these microsatellite markers were compared it was observed that at 17 microsatellite loci genotype/s were differing significantly ($P<0.05$) in case-control animals. Also univariate regression analysis revealed that effect of breed, sex and age had no significant ($P<0.05$) effect on the incidence of bTB.

At BM1818 microsatellite locus a total of 7 alleles ranging from 254 to 270 bp and 12 genotypes were observed where overall effect genotypes was found to be significant ($P \leq 0.02$) on incidences of bTB. Hence, individual genotypic frequencies at BM1818 microsatellite locus was compared and it was found that none of the genotype was differing significantly ($P<0.05$) with each other in case versus control group. At the microsatellite locus BM1824 total 4 alleles of 182, 186, 194 and 196 bp and 5 genotypes were observed. The overall effect of BM1824 locus was highly significant ($p<0.001$) on bTB and two genotypes '186/196' and '186/194' were differing significantly ($P<0.05$) in case versus control populations with their ORs approaching towards infinity. The '186/196' genotype of BM1824 locus was exclusively present in positive population whereas '186/194' was exclusively present in tuberculin negative animals. At ETH3 microsatellite locus total 4 alleles of '99', '109', '111' and '121' bp length and 9 genotypes were observed. Overall effect of genotypes at ETH3 microsatellite locus was significant ($P<0.01$) on incidences of bTB and further the individual genotypes were compared but none of the genotype was differing significantly ($P<0.05$) in case versus control populations. Total of 14 alleles ranged from 198 to 228 bp and 22 genotypes were observed at the microsatellite marker locus ETH10. The univariate logistic regression analysis revealed a non-significant ($P=0.12$) association of ETH10 locus on incidences of bTB but the '200/220' genotype was present exclusively in tuberculosis positive animals and was found to be highly significant in case versus control population ($p=<0.01$). At ILST006 microsatellite locus a total 6 alleles ranged from 264 to 280 bp and 9 genotypes were observed. The ILST006 locus was found to be significant ($P<0.01$) on the incidence of bovine tuberculosis and individual frequencies of genotypes were compared in case-control animals. The proportion of '278/278' genotype was significantly higher in tuberculosis positive animal with its OR 5.09 (1.43-18.06; 95 % CI) versus other genotypes of positive animals at ILST006 locus. At same locus (ILST006) the '274/280' genotype was found to be significantly ($P<0.01$) and exclusively present in the negative control population**.** At TGLA126 microsatellite locus total of 10 alleles (118 to 136 bp) and 22 genotypes were observed. Only one genotype '124/124' was found to be significantly affecting the tuberculin test and was exclusively found in the negative control animals at TGLA126 locus.

BM2113 locus: A total of 10 alleles of ranged from 127 to 153 bp length with 22 genotypes were observed at BM2113 microsatellite locus and frequencies of none of the

genotypes at this locus were differing significantly in case-control animals. At ETH185 microsatellite locus total 8 alleles ranged from '214' to '248' bp length were observed with total 18 genotypes and overall effect of locus was highly significant (P<0.01) on bTB. The individual genotypic frequencies at ETH185 microsatellite locus were compared and interestingly one genotype of '230/240' was differing significantly (P=0.02) with its OR 10.00 (1.16-85.87; 95% CI) in case versus control animals. Hence, selection against, '230/240' genotype would be valuable for reducing incidences of bTB. At ETH152 microsatellite locus total 8 alleles ranging from '166' to '184' bp length and total 15 genotypes were observed. It was found that frequency of 2 out of 15 genotypes were found to be differing significantly (P<0.05) in case versus control group at ETH152 microsatellite locus. The proportion of '166/166' genotype at ETH152 locus was significantly (P=0.03) higher in bovine tuberculosis positive animals with its OR 4.38(1.08-17.76) in affected animals versus control. At same locus (ETH152) the OR of '168/168' genotype versus other genotypes was 0.32 (0.11- 0.95; 95% CI) which indicated that the proportion of '168/168' genotype was significantly (P=0.04) lower in affected animals and will be valuable for selecting disease resistance animals. At BMS2753 microsatellite locus, total 6 alleles ranged from 108 to 118 bp length with total 15 genotypes were observed. The individual genotypic frequencies at BMS2753 locus were compared and it was found that only two ('112/112' and '114/114') out of the 15 observed genotypes were differing significantly (P=0.04) with each other in case versus control group. The proportion of '112/112' genotype versus other genotypes was significantly lower whereas proportion of '114/114' genotype versus other genotypes was significantly higher in bTB affected animals with their ORs 0.28(0.08-0.95; 95 % CI) and 5.78(1.14-29.35; 95 % CI) respectively at BMS2753 microsatellite locus. At BMS468 microsatellite locus total 10 alleles ranging from '128' to '146' bp with 20 genotypes were observed. Further, individual genotypic frequencies at BMS468 microsatellite locus was compared and proportion of '134/134' and '134/144' genotypes (2 out of 20 genotypes) were differing significantly (P<0.01 and P=0.03, respectively) in case versus control animals. The genotype '134/134' was present exclusively in control animals and hence could be associated with the tuberculosis resistance status. At BMS468 microsatellite locus the OR of '134/144' genotype was 5.78 (1.14-29.35; 95% CI) indicated that proportion of this genotype was significantly higher in positive animals than control animals. At BMS2213 microsatellite locus total 20 alleles ranged from 122 to 168 bp

and total 55 genotypes were observed. Although, microsatellite BM2113 locus had significant (P<0.01) effect on bovine tuberculosis as revealed from univariate logistic regression analysis. But, individual genotypic frequencies at BM2113 microsatellite locus were compared and it was found that none of the genotype was differing significantly (Pd"0.05) with each other in case versus control group. At INRA131 microsatellite locus total 16 alleles ranging from 96 to 134 bp with total 43 genotypes were observed and had an overall significant (P=0.03) effect of bTB. But none of the individual genotype at INRA131 microsatellite locus was differing significantly in case-control animals. The overall effect of INRA111 microsatellite locus was found to be significant (P=<0.01) on incidences of bovine tuberculosis. To investigate further the individual genotypic frequencies at INRA111 microsatellite locus were compared and it was found that two genotypes '132/132' and '132/134' were significantly (P<0.05) affecting the bovine tuberculosis. The genotype '132/134' was present only in the tuberculosis positive animals and it was significantly (P=0.04) associated with bTB. The proportion of '132/132' genotype at INRA111 locus was significantly higher in bTB positive cases with it OR 3.30 (1.01-10.70; 95% CI). At BM7169 microsatellite locus total 16 alleles ranging from '208' to '250' bp with total 33 genotypes were observed and overall effect of BM1769 microsatellite locus was found to be significant (P=0.04) on incidences of bovine tuberculosis. To study the effect of the individual locus, frequencies of each genotypes at BM1769 microsatellite locus were compared and it was found that three genotypes of '214/230' , '214/234' and '220/238' were differing significantly (P<0.05) in case versus control group. The OR of '214/230' genotypes versus other genotype was 10.00 (1.16-85.87; 95% CI) whereas ORs of '214/234' and '220/238' were towards infinity at BM1769 locus. At BMS1724 microsatellite locus total 10 alleles ranged from 110 to 170 bp with total 20 genotypes were observed and overall effect of BMS1724 microsatellite locus was found to be significant (P<0.01) on incidences of bovine tuberculosis. Hence, individual genotypic frequencies at BMS1724 locus was compared and it was found that four genotypes, namely '152/166', '156/160','156/162', '156/164' were differing significantly in case versus control group. The genotypes '156/160', '156/162' and '156/164' were found exclusively in the tuberculosis positive animals and hence might have contributed for susceptibility for bTB. However, the relative frequency of '152/166' genotype was significantly higher in control animals. At BM7209 microsatellite locus total 13 alleles the ranged between 102 and 148 bp with total 21 genotypes were

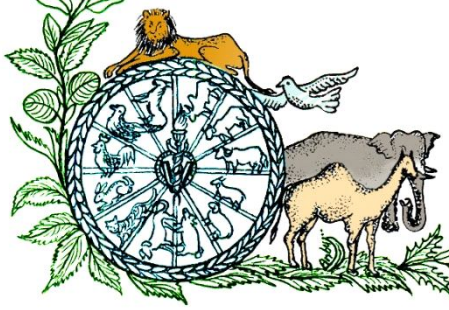
observed and its overall effect was found to be non-significant (P=0.13) on incidences of bovine tuberculosis. Also, individual genotypic frequencies at BM7209 microsatellite locus was compared and it was found that none of the genotype was differing significantly (P<0.05) with each other in case versus control group. At BMS495 microsatellite locus total 8 alleles ranged 144 to 168 bp length and total 16 genotypes were observed and its overall effect was significant (P<0.01) on incidences of bovine tuberculosis. Hence, individual genotypic frequencies at BMS495 microsatellite locus was compared and it was found that 4 genotypes out of the 16 observed genotypes were differing significantly (P<0.05) with each other in case versus control group. The relative frequencies of '144/144' and '144/168' genotypes were significantly (P<0.05) higher in the bTB positive animals with their ORs 11.52 (2.38-55.82; 95 % CI) and 10.00 (1.16-85.87; 95 % CI), respectively. The four cases of '144/164' genotype were found exclusively in the tuberculosis positive and hence it could a marker genotype associated with the susceptibility to tuberculosis. The '148/148' genotype was differing significantly (P<0.01) in the case and control animals and its proportion was higher in the negative animals (26.83% in control animals against 2.86 % in the positive animals) and hence it could be associated with the bovine tuberculosis resistance in cattle. At BMS499 microsatellite locus total 11 alleles ranged from 106 to 148 bp and total 27 genotypes were observed. The overall effect of BMS499 microsatellite locus was found to be highly significant (P<0.01) on incidences of bovine tuberculosis. Hence, individual genotypic frequencies at BMS499 microsatellite locus was compared and it was found that only one out of the 27 genotypes was differing significantly (P<0.05) with each other in case versus control group. The genotype, '106/128' was exclusively present in tuberculosis positive animals (6 out of 35 animals) at '106/128'.

Only a few reports are available on association of microsatellite markers with incidences of bTB which were compared with our findings. Our findings were in agreement with Ali *et al.* (2009) who reported that the microsatellite loci BM1818, BM1824, ETH3, ETH10, ILSTS006, TGLA126, BM2113, ETH185, ETH152, were significantly associated with bTB whereas, the SLC11A1 microsatellite was not associated significantly with bTB in Morocco and African Zebu cattle. On the contrary, Driscoll *et al.*, 2011, reported that microsatellite loci BMS468, BM2213, INRA131, BM7169, BMS1724, BMS495 and BMS499 were not associated significantly with incidences of bTB in in European Taurus cattle. However, similar to our

findings, Driscoll et al. (2011) reported that microsatellite locus INRA111 was significantly associated with bTB and BM7209 microsatellite locus was not associated significantly ($P<0.05$) with bTB. Recently, microsatellite markers ILSTS005, ILSTS006, TGLA227, BM2113 and CSRM66 were found to be associated to bovine tuberculosis (Ali *et al.*, 2013).

Taken altogether, these results indicated that some locus specific microsatellite genotypes were identified as marker for susceptibility or resistance for bTB but these findings needs further validation of pointed markers on larger population or across study evaluation.

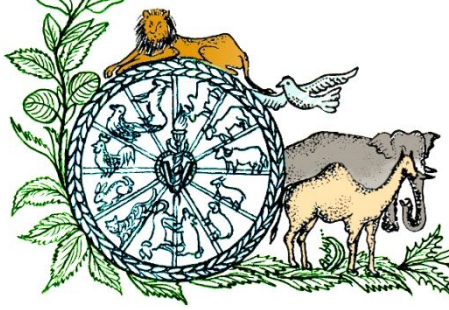
Mini Abstract



6 MINI ABSTRACT

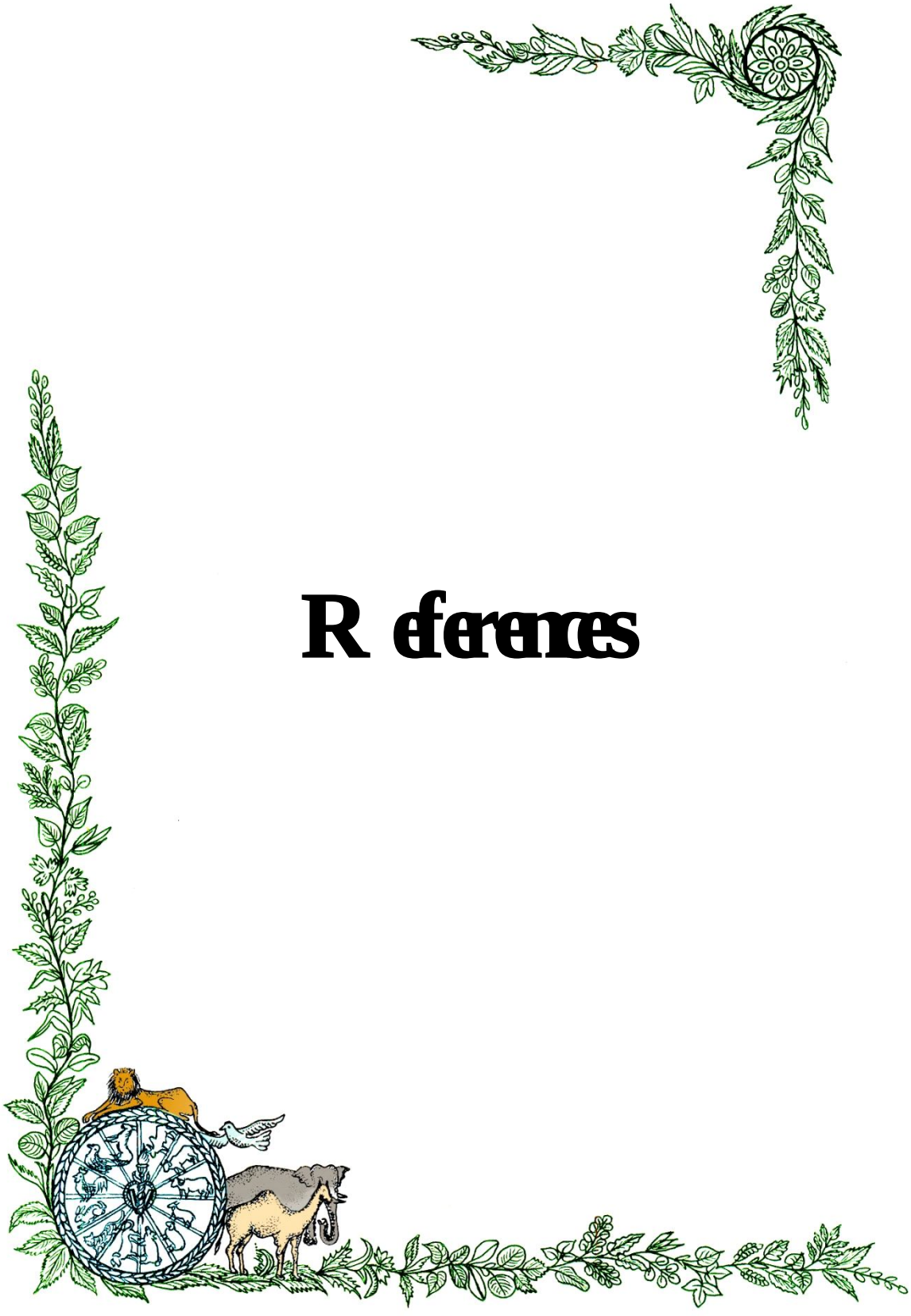
In present study polymorphisms at 21 microsatellite loci were investigated for finding their association with bovine tuberculosis in cattle. Total 245 animals were tested with single intradermal comparative cervical tuberculin test (SICCT) for finding positive animals for tuberculosis. At 17 microsatellite loci genotype/s were differing significantly in case-control animals. . Across the loci, the number of alleles ranged from 4 (BM1824 and ETH3 loci) to 21 (BMS2213 locus) with an average of 10 alleles per locus. The heterozygosity at investigated loci ranged from 0.22 for BM1824 to 1.00 for ETH10 microsatellite locus whereas, PIC ranged from 0.23 (BM1824) to 0.92 (BMS2213). The genotypes '186/196'at BM1824; '200/220' at ETH10; '132/144' at INRA111; '214/234' at BM7169; '156/160', '156/ 162', '156/164' and 144/164 at BMS1724 and '106/128' at BMS499 loci were significantly (Pd"0.05) associated with bTB and were exclusively present in bTB positive animals. Similarly the genotypes '186/194' at BM1824; '274/280' at ILSTS006; '124/124' at TGLA126; '134/134' at BMS468 and '220/238' at BM7169 loci were exclusively present in tuberculosis negative animals. It was concluded that in agreement with earlier reports the microsatellite markers may be valuable for selecting the animals for tuberculosis resistance. The IgG1 levels of the bTB positive and negative animals were compared and found that no significant difference exits in the IgG1 levels in both the animals groups which suggests that humoral immunity have no role in the resistance against tuberculosis.

Yk?k Ikjk"k



7

oreku v/;;u ij 21 ekbØklVykbV ykdh cg:irkvk e i'kvk e xktkrh; {k;jkx d lkFk viu lg;kx dk [kktu d fy, tkp dh xb] dy 245 i'kvk e rifnd d fy, ldkjkRed tkuojk dk [ktu d fy, ,d Ropk d vnj ryukRed xhok Vcjdyhu ijh{k.k ¼,lvkblhlhVh½ d lkFk ijh{k.k fd;k x;kA 17 ekbØklVykbV fonk"k e thukVkbi e fu;=.k i'kvk e dkQh erHkn FkA fcUniFk d ml ikj ,fyy dh l[;k fBdkuk ifr 10 ,fyy dh ,d vklr d lkFk 4 ¼ch,e1824 vkj bVh,p3 LFky½ l 21 ¼ch,e,l2213 fBdkuk½ dk crk;k x;kA 0-23 ¼ch,e1824½ l 0-92 ¼ch,e,l2213½ dk ydj ihvkblh tcfd tkp e fonk"k e fgVklkbxkflVh bVh,p10 ekbØklVykbV fcUniFk d fy, ch,e1824 d fy, 0-22 l 1-00 rd crk;k x;kA thukVkbi d 186@196 vkj ch,e1824] 200@200 bVh,p10 l de] 132@144 vkb,uvkj,11 l de] 214@234 ch,e7169 l de] 156@160] 156@160] 156@162] 156@164 ch,e,l499 fonk"k e vkj ch,e,l1724 l de 144@164 vkj 106@128 ¼ih≤0-05½ e dkQh Fk xktkrh; rifnd d lkFk tMk gvk vkj xktkrh; {k;jkx ldkjkRed tkuojk e vUu; :i l mifLFkr Fk] mlh thukVkbi 186@194 ch,e1824 ij\ 274@280 vkb,y,lVh,l006 ij 124@124 Vhth,y,126 ij 134@134 ch,e,l468 vkj 220@ch,e7169 fonk"k e 238 e rifnd udkjkRed i'kvk e fo'k"k :i l mifLFkr FkA ;g igy dh fijkVk d lkFk le>kr e ekbØklVykbV ekdjk rifnd d ifrjk/k d fy, i'kvk dk p;u dju d fy, eY;oku gk ldrk g ;g fu"d"k fudyk x;k FkkA fd xktkrh; {k;jkx ldkjkRed vkj udkjkRed tkuojk dh vkbthth1 d Lrj dh ryuk fonk"k jkx{kerk irk pyrk g vkj ;g fd tk tkuojk d nkuk legk e vkbthth1 d Lrj e dkb egRoi.k vrj ckgj fudyrk g rifnd d f[kykQ ifrjk/k e dkb Hkfedk ugh ik, x, gA



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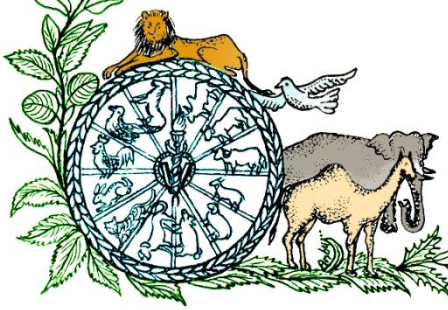
Appendix



APPENDIX

1. Blood collection

0.4M EDTA (Ph 8.0)

EDTA	148.8gm

Dissolved in 500 ml of autoclaved distilled water with the help of magnetic stirrer for 1-2 hours and adjust the pH to 8.0 with NaOH pellets. Make the final volume up to 1000 ml with autoclaved distilled water. Sterilize by autoclaving and store at 4c.

70% ethanol

Ethanol	70ml
Autoclaved distilled water	30ml

Mix thoroughly and store in amber colored bottle at 4 ºC

2. Gel electrophoresis (AGE)

10x Tris borate EDTA (TBE)

Tris base	54.0 g
Boric acid	27.5g
0.5M EDTA (pH 8.0)	3.75 g

Add autoclaved distilled water up to 500 ml.sterilize by autoclaving. Store at room temperature.

3. Setting up of PCR

10X Taq buffer

Tris-HCl (pH 8.8)	100 mM
KCl	500 mM
$MgCl_2$	15 mM
Triton X-100	1%

Store at –20°C.

Taq DNA polymerase enzyme

Taq DNA polymerase	5 units/µl

Store at –20°C.

dNTP solution (pH 7.0)

dATP	10 mM
dCTP	10 mM
dGTP	10 mM
dTTP	10 mM

Store at –20°C.

6X Gel Loading Due

Bromophenol blue	0.25 %
Sucrose	40% (W/V)

Store at 4°C.

20,000X Ethidium Bromide (10 mg/ml)

Ethidium bromide	10 mg
Auto DW	1 ml

Wrap in an aluminum foil (Photosensitive). Store in a dark place at room temperature.

Molecular Weight Marker

100 bp – 10 fragments. 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp.

20 bp- 15 fragments 300,280,260,240,220,200,180,160,140,120,100,80,60,40 and 20

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