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Impact of Host Genetics on Susceptibility and Resistance to *Mycobacterium avium* Subspecies *Paratuberculosis* Infection in Domestic Ruminants

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Abstract: Johne's disease or Paratuberculosis has emerged as major infectious disease of animals in general and domestic livestock in particular on global basis. There have been major initiatives in developed countries for the control of this incurable malady of animals and human beings alike (inflammatory bowel disease or Crohn's disease). Disease has not received similar attention due to inherent complexities of disease, diagnosis and control, in resource poor countries around the world. However, the rich genetic diversity of the otherwise low productive animal population offers opportunity for the control of Johne's disease and improve per animal productivity. Present review aims to gather and compile information available on genetics or resistance to Johne's disease and its future exploitation by resource poor countries rich in animal diversity. This review will also help to create awareness and share knowledge and experience on prevalence and opportunities for control of Johne's disease in the livestock population to boost per animal productivity among developing and poor countries of the world. Breeding of animals for disease resistance provides good, safe, effective and cheaper way of controlling Johne's disease in animals, with especial reference to domestic livestock of developing and poor countries. Study will help to establish better understanding of the correlation between host cell factors and resistance to MAP infection which may have ultimately help in the control of Johne's disease in future.

Key words: Paratuberculosis, resistance, susceptibility, host, genetics, ruminant

INTRODUCTION

Mycobacteria are either saprophytic or obligatory ancient microbes (Hett and Rubin, 2008; Ventura *et al.*, 2007; Deb and Goswami, 2010) causing infection in even camel (Ghosh *et al.*, 2012). *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the etiological agent of Johne's disease (Verschoor *et al.*, 2010b; Momotani, 2012; Dobson *et al.*, 2013). It is a deadly intestinal ailment leading to chronic enteritis as well as

granulomatous inflammation of lymph nodes in ruminants (Pant *et al.*, 2011; Deb and Goswami, 2011). Disease adversely affects productivity and viability of animal industry across the world by causing significant economic losses by way of reduced productivity, premature culling and mortality (Pant *et al.*, 2010, 2011). Paratuberculosis has been characterized as the most costly infectious disease of dairy cattle. Recent evidences on the potential role of MAP in causation of Inflammatory Bowel's Disease (IBD) or, Crohn's Disease (CD) in human

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beings (Hermon-Taylor, 2009; Greenstein, 2003). Collins (2011) and Sweeney *et al.* (2012) have further underlined the importance of controlling MAP infection both in animal and human population world-wide. Therefore, concerted global efforts are required on priority to restrict the spread of MAP infection both in animals and human beings in order to boost per animal productivity and safeguard human health globally. Control methods so far employed viz., hygienic management practices, test and cull policy and vaccination measures have either failed or did not yield desired results. Therefore, the role of host genetics is an alternative approach which has recently been studied by many leading workers in order to control chronic diseases like Johne's disease (Pant *et al.*, 2010; Kirkpatrick and Shook, 2011). In fact, development of genetically resistant animals through selective breeding for disease resistant traits in the host species is a slow and long term process but its impact are stable and the genetic resistance gained in one generation is likely to remain its next counterpart. Therefore, host genetics and breeding of animals for disease resistant traits may be an alternative and effective approach in reducing prevalence of MAP infection in the domestic ruminants. Selective breeding programme will help in establishment of genetically resistant animals in a population thereby improving the 'herd immunity'. This review focus to establish better understanding of the correlation between host cell factors and natural resistance to MAP infection which may have significant impact in controlling Johne's disease specially in the resource poor countries of the world with enough genetic variability.

BREED RESISTANCE

Breed differences play major role on the genetics of disease resistance (Lindhe and Philipsson, 1998; Raadsma *et al.*, 1998) and can be used as tool for the control of disease (Van Hulzen *et al.*, 2011). Breed effects with respect to MAP infection have also been experienced and studied in the population of domestic ruminants in India (Singh *et al.*, 2012a, b; Singh *et al.*, 2009) reported better adaptation of 'Indian Bison Type' biotype of MAP in different breeds of farm goats of Uttar Pradesh as compared to farm goats in Rajasthan. Local breeds are resistant to these diseases in tropical regions compared to imported breeds imported breeds (Savic *et al.*, 1995) and their crosses. Major role of environment with respect to breeds located in different agro climatic zones cannot be ruled out and it has tremendous impact on evolution or survival of these breeds over the years. Besides physical environment (arid or semi arid, more sunshine, dry

conditions etc.), it is the animal profiles (colour of skin and hairs), livestock husbandry practices, management (stocking density, intensive or extensive or migratory system of management), etc., play important role on the prevalence of disease in particular agro-climatic region.

Channel Island and Shorthorn cattle showed a particularly high incidence of Johne's disease (Withers, 1959) while exotic sheep breeds viz., Scottish Black face, Shetland; cattle viz: Limousin, Channel Island breeds (Jersey and Guemesy) are prone to disease (Clarke, 1997). (Manning and Collins, 2001; Collins *et al.*, 2001). In UK a number of cattle breeds have a reputation for susceptibility to JD. Merino as well as some dairy breeds of sheep may be more susceptible to MAP infection than other breeds, however the genetic resistance to JD has not been identified yet. Variation in genetic susceptibility of breeds have also been observed with respect to different blood lines (Koets *et al.*, 2000), therefore careful selection and description of animals will be important for the eventual disease outcome with respect to disease and reproducibility of the trials.

SKIN OR COAT COLOUR

Skin or coat colour has impact on the genetic resistance against various diseases. Different skin and coat colours in different breeds of domestic animals have emerged as part of nature's strategy for survival and mitigate heat stress and diseases in various climatic conditions. Melanin is the main participants in colour based resistance or susceptibility to environment and disease. Melanin has several physiological roles in maintaining health viz. synthesis of vitamin D which works as a modulator of the different processes of the immune system, skin pigmentation and thermo-regulation and protect the body from harmful ultraviolet radiation. Autoimmune diseases in certain breeds is influenced by latitude (Shoenfeld *et al.*, 2009). Genes for melanism in felines may provide resistance to viral infections. In Java and Malaysia, there is high prevalence of black leopards and black servals, the reason might be the high altitude adaptation since black fur absorbs more heat (Seidensticker and Lumpkin, 2006). Later on, the study published in New Scientist magazine in 2003 suggested that recessive-gene melanism is linked to disease resistance instead of altitude. Resistance power is higher in Melanistic cats compared to cats having normal coat colour. Similar advantage of dark colour goat breeds and sheep with wool coat have been experienced by the author in last 29 years of experience in working with different breeds of goats and sheep with respect to

Johne's disease. Disease is endemic in farm herds of goats and sheep at CIRG, Makhdoom, where dark colour breeds (Jakhrana, Marwari and Sirohi) have shown better adaptability to MAP infection (Singh *et al.*, 2009). Though the role of other factors like duration of existence of breeds at the farm, since breeds from Rajasthan were introduced in 1990s as compared to Barbari and Jamunapari breeds of goats which are maintained since 1976, The better performance of the only sheep breed (Muzaffarinagri) located at CIRG, Makhdoom, is due to fast development of rumen, better capacity of sheep to drive nutritional requirement from poor quality and deficient grazing resources. Sheep graze as compared to goat which browses and needs good plantation which is not available as these farm exists on the wastelands of river Yamuna on CIRG campus. Earlier in sheep unit there existed crosses of Suffock and Dorset with native Muzaffarnagri breed and suffered from different kinds of health problems, were therefore removed in favour of pure Muzaffarnagri breed. Lastly the goat adapted MAP strain may be acting as 'vaccine strain' for minority species, i.e., sheep. Similarly better performance of Sirohi goats in the Central Sheep and Wool Research Institute is due to adaptation of MAP strain to sheep and 'vaccine strain' for goats the minority species. In this interplay of events, availability of energy resources is critical and is directly affected by skin and coat colour especially in winter climate.

ENVIRONMENTAL RESISTANCE

Each living creature on earth is also affected by its physical non-living (abiotic) and biotic environment and innate potential viz., reproductive and growth rate, ability to migrate and invade new habitats, ability to cope with adverse conditions, defence mechanisms. Factors responsible for development of environmental resistance are adverse conditions viz., insufficient nutrients and water, unsuitable habitat, adverse weather, predators, disease and competition. Malnutrition generally alters resistance of the host to infection and infectious disease exaggerates existing malnutrition (WHO monographs series 57). There is increasing reports that slow and mild exposure to some environmental pollutants may disturb immune responsiveness and change the susceptibility of animals to pathogens (Bradley and Morahan, 1982). Authors have experienced the role of nutrition in immune response to vaccination against Johne's disease and observed dramatic results in intensively fed goats/sheep/cattle as compared to poorly fed animals where vaccine response was slow in endemically infected herds and flocks.

HOST'S GENOME

Study of host genetics for the identification of host genes involved in susceptibility and resistance to infectious diseases uses diverse designs including animal models, observation of individuals with marked susceptibility or resistance, study of candidate genes for common infections, race or family-based, genome-wide, linkage studies etc. Genome-wide linkage studies enable the identification of regions containing major disease-susceptibility loci (Pant *et al.*, 2010; Deb *et al.*, 2012). This approach is very systematic and comprehensive but has very low power. It will therefore not detect genes which exert a moderate effect on population-wide disease risks. Association-based, candidate gene studies have comparatively much greater power. A case-control study of candidate gene approach, when used alone; it failed to detect the gene exerting the largest population effects on disease susceptibility. Identification of the genes responsible for susceptibility to atypical mycobacterial infections provides valuable insight into the host immune response. The identified molecular marker through candidate gene studies and whole genome association studies for resistance to Johne's disease will help in enhancing the overall 'herd resistance' by encouraging the selective breeding of resistant animals. Similarly, genetic variations that exists in the population of domestic animals in terms of breeds and strains emerged in particular agro-climatic regions contribute to host susceptibility to MAP infection are also very important to uniform animal improvement programmes aimed at reducing susceptibility to infection and for gaining a better understanding of the mechanisms of disease. Overall, the study may aid in designing the more efficacious and safer strategies for the control and eradication of Johne's disease from endemically infected herds/flocks.

HOST RESPONSE AGAINST PARATUBERCULOSIS

Survivality of Mycobacteria is similar to other Mycobacteria. One feature of mycobacteria, including MAP, is their propensity to infect macrophages. Within macrophages MAP interferes with the maturation of the phagosome by unknown mechanisms, thereby evading the host's normal first line of defence against bacterial pathogens. Moreover, MAP has been shown to decrease MHC expression by macrophages (Weiss *et al.*, 2008). Due to impaired innate responses local macrophages will be unable to destroy the phagocytosed MAP and significant bacterial multiplication within macrophages

occurs. The host immune system starts a series of attacks against MAP infected macrophages, including the rapid deployment of gamma delta T cells, CD4+T cells and cytolytic CD8+T cells (Charavaryamath *et al.*, 2013). Macrophages may be lysed (by the direct effects of bacteria or by cytotoxic cells) and release bacteria, or infected macrophages may themselves divide. The acid fast organism invades sheep macrophages (Rajya and Singh, 1961). Alonso-Hearn *et al.* (2008) reported that, MAP3464 gene of *M. avium* subsp. Paratuberculosis which codes for Oxidoreductase are linked with activation Cdc42 in the host cell.

Blood borne monocyte (immature macrophages) are attracted by cytokines released by infected macrophages or by bacterial products and ingest any released bacteria. By this stage sufficient antigens may be present for the initiation of specific immune responses and sufficient organisms may be present for detection of infection by culture of intestinal tissues. It is highly likely that even at this early stage migration of infected macrophages to the regional MLN occurs. The subsequent development of specific immunity might lead to complete elimination or restriction of infection due to strong Th1 responses (with the possibility of later exacerbation), or progression of the disease to the terminal stages due to shift from protective Th1 to non-protective Th2 responses. It is very clear that misdirected immune response due to MAP related host modulation leads to establishment of this debilitating disease. Inhibition of phagosomal maturation, reduction in apoptosis of infected cells, reduced MHC II expression, Th1-Th2 shift and increase of suppressive population of gamma delta ($\gamma\delta$) T cells, inhibition of CD4+ T cell activity, inhibition of TLR9 mediated response, inhibition of gamma interferon induced signalling in monocytes and immune anergy are the seminal events that develops persistency of MAP infection (Sohal *et al.*, 2008; Begg *et al.*, 2011; Dobson *et al.*, 2013; Charavaryamath *et al.*, 2013; Arsenault *et al.*, 2012, 2013).

EVIDENCE THAT GENETIC FACTORS INFLUENCE RESISTANCE TO PARATUBERCULOSIS

It has been hypothesized that during the interaction between host immunity and MAP, a deviation from the proper immune response arises and disrupts the ability of the host to contain the disease. Without doubt, present disease control measures had helped to some extent but are not strong enough to yield desired results and paratuberculosis still continues to be a problem for animal industry world over. Therefore an alternative approach to this problem is genetics of 'disease resistance' which is the inherent capacity of an animal to resist disease when

challenged by the pathogen. Recent studies identified host cell factors MHC, NOD 2/CARD 15, (BoLA) DRB3, IFN γ , TLR, SLC11A1, (solute carrier family 11 member 1) formerly known as NRAMP 1, IL10RA, SP110/Ipr1, PGRP, ANKRA2, CD180 so on., responsible for resistance/susceptibility to MAP infection (Koets *et al.*, 2000; Mortensen *et al.*, 2004; Reddacliff *et al.*, 2005; Gonda *et al.*, 2006; Pinedo *et al.*, 2009a, b; Singh *et al.*, 2009, 2012a, b; Ruiz-Larranaga *et al.*, 2010; Pant *et al.*, 2011; Casas *et al.*, 2011; Rastislav and Mangesh, 2012). Recently, various studies on genome wide profiling of paratuberculosis infection has also emerged (Minozzi *et al.*, 2010).

CANDIDATE GENE STUDIES

Genetic factors have long been suspected in association with susceptibility and resistance to mycobacterial infection including bovine paratuberculosis (Abel and Casanova, 2000; Koets *et al.*, 2000; Purdie *et al.*, 2011; Deb *et al.*, 2012). Several studies reported a breed effect in the variation in susceptibility to paratuberculosis (Cetinkaya *et al.*, 1997; Roussel *et al.*, 2005; Elzo *et al.*, 2006; Singh *et al.*, 2009; Ruiz-Larranaga *et al.*, 2010; Purdie *et al.*, 2011; Rastislav and Mangesh, 2012) and estimations of heritability to MAP infection ranging from 0.041-0.159 have been reported (Koets *et al.*, 2000; Mortensen *et al.*, 2004; Gonda *et al.*, 2006; Van Hulzen *et al.*, 2011).

MHC CLASS OF GENES

In recent years, research on the Major Histocompatibility Complex (MHC) as candidate genes of disease associations has been initiated (Purdie *et al.*, 2011). MHC (genes closely clustered) binds with processed antigen thereby presenting to T cells, thus playing role in immunological functions apart from the non-immunological ones (Deb and Goswami, 2011). MHC class I molecules are present on the membrane of all nucleated cells and present antigens to cytotoxic T cells. MHC class II is mainly located on the cells of the immune system and present antigens to helper T cells. Depending on the antigen or, epitope presented these responses could led to protective immunity to DTH (\circ), to immune suppression (De Vries, 1991). Located between the Class I and Class II gene (in humans at least) are genes for complement factors and TNF all of which are potentially important in immune function.

Genetic associations between the MHC and susceptibility to certain infectious diseases like tuberculosis, leprosy etc., have been identified in human

beings (Goldfeld *et al.*, 1998; Ravikumar *et al.*, 1999). Studies have demonstrated that caprine MHC I and II genes are highly polymorphic (Cameron *et al.*, 1990) in which DRB regions are most polymorphic (Andersson and Rask, 1988) as well as play major role in disease resistance (Longenecker and Gallatin, 1978; Schierman and Collins, 1987; Kaufman and Venugopal, 1998; Reddacliff *et al.*, 2005; Sayers *et al.*, 2005; Li *et al.*, 2010). Two Single Nucleotide Polymorphisms (SNPs) in the DRB region (detected by PCR-RFLP method using PstI and TaqI restriction endonuclease) were described by Amills *et al.* (1995), leading to amino acid substitutions in the antigen binding site of the caprine MHC molecules. Bovine Leucocyte Antigen (BoLA) is a part of the Major Histocompatibility Complex (MHC) of cattle. The BoLA-DRB3 gene, one of the MHC II groups of genes, plays a key role in the immune response by presenting peptides derived from extracellular proteins. A single nucleotide polymorphism at the antigen recognition site of the Bovine Leucocyte Antigen (BoLA) DRB3 gene was studied in healthy and MAP infected cattle. Four mutations, Val53Glu (OR 453.7), VAL53Leu (OR 453.7), Asp57His (OR 1.944) and Arg84Gly (OR 0.453) were linked with high resistance indicating important mutations in the protein-binding site of DRB3, responsible for activation of immune response against MAP (Rastislav and Mangesh, 2012).

In a recent study on 203 Jamunapari goats (Indian vulnerable breed extremely liable to paratuberculosis), polymorphism was analyzed within the exon-2 of the caprine MHC Class II DRB region and its association with status (resistance or susceptibility) to paratuberculosis (Singh *et al.*, 2012a). On the basis of clinical signs and laboratory examination viz., microscopic, faecal culture, ELISA and PCR, sixty and 143 goats were classified as resistant and liable to paratuberculosis, respectively. PCR-based restriction fragment length polymorphism (PCR-RFLP) with 2 enzymes (PstI and TaqI) was conjointly performed to assess variations within the DRB gene(s). The frequency of p and t alleles of individual pp and tt and of composed pptt genotypes were considerably higher ($P_{corr} < 0.001$) within the 'resistant' group as compared to the 'susceptible' group, whereas the P and T alleles were related with susceptibility ($P_{corr} < 0.001$) In heterozygous genotypes, susceptibility was dominant over the resistance (Singh *et al.*, 2012b).

Therefore, investigations of possible relationships between these critical SNPs with resistance or susceptibility to JD are of potential importance. Despite of few evidences indicating the important role of MHC Class II gene in the susceptibility to paratuberculosis, there is a lack of more comprehensive work to further explore the role of this gene in JD occurrence.

NOD2 (CARD15) GENES

The Nucleotide-binding Oligomerization Domain-containing 2 protein gene (NOD2), previously referred to as the Caspase Recruitment Domain 15 protein gene (CARD15), is well characterized gene that contributes to predisposition to Crohn's disease in human beings (Hugot, 2006; Purdie *et al.*, 2011). The product of CARD15 gene is an intracellular element responsible for the indirect recognition of bacterial peptidoglycan by monocytes, macrophages, dendritic cells and intestinal epithelial cells (Ogura *et al.*, 2001a). NOD2 having 5' and 3' flanking and partial intronic regions is considered as a candidate gene in a wide variety of cattle based on similarities between Crohn's disease and JD (Taylor *et al.*, 2006). Most of the animals belonging to several different breeds have shown polymorphism but association of infection with polymorphisms could not be tested due to study being conducted in small number of animals. Subsequently, Pinedo *et al.*, 2009a) tested association of three of the NOD2 polymorphisms identified by Taylor *et al.* (2006) in a case-control study in dairy cattle and significant association has been found between two non-synonymous NOD2 SNPs based haplotypes and infection status that are independent of the breed factor. Future comprehensive investigations are required to have exhaustive information on the role of this genetic element in paratuberculosis infection. Based on genetic association, NOD2, has also found to be a candidate gene for MAP infection in a Bos taurus × Bos indicus crossbreds. Study on Holstein Friesian reported that the C allele of SNP c.*1908C>T, located in the 3'-UTR region of the gene occurred more frequently in infected animals, indicating the role of bovine NOD2 gene in MAP susceptibility (Ruiz-Larranaga *et al.*, 2010).

Mutation in a gene ATG16L, NOD2/CARD15, IBD5, CTLA4, TNFSF15 and IL23R genes are associated with Crohn's disease (Ogura *et al.*, 2001b; Fielding, 1986; Grant *et al.*, 2008; Gazouli *et al.*, 2010; Naser *et al.*, 2012), susceptibility being observed in certain phenotypes mapped to chromosome 16 (Cuthbert *et al.*, 2002; Hugot *et al.*, 2001). Three independent studies reported that mutation within the NOD2/CARD15 gene were strongly linked to Crohn's disease in Europeans (Ogura *et al.*, 2001b; Hugot *et al.*, 2001; Hampe *et al.*, 2001). Crohn's disease associated gene without confirmation of association has been reported (Greenstein, 2003), suggesting the tendency of development of the disease in genetically identifiable sub-populations (Inoue *et al.*, 2002).

Crohn's disease in Sardinian population was carried out on several specimens (few showing no association)

based on NOD2/CARD15 gene (insC3020, G908R and R702W alleles) analysis, indicating 70% susceptibility (Sechi *et al.*, 2005).

These NOD2 variants Change in the structure of the leucine-rich repeat domain of the protein occurs due to frame shift variant and two mis-sense variants of NOD2 encoding a member of the Apaf-1/ Ced-4 superfamily of apoptosis regulators expressed in monocytes. This NOD2 may also activate another nuclear factor NF- κ B which is regulated by the carboxy-terminal leucine-rich repeat domain. Therefore, NOD2 gene product is responsible for susceptibility of individuals to Crohn's disease by changing the recognition of these components and/or by over-activating NF- κ B in monocytes (Ogura *et al.*, 2001b).

(BOLA) DRB3 GENE

A single nucleotide polymorphism at the antigen recognition site of the Bovine Leucocyte Antigen (BoLA) DRB3 gene may also be important for activation of proper immune response against MAP in cattle (Rastislav and Mangesh, 2012). Mutations like Val53Glu (OR 453.7), Val53Leu (OR 453.7), Asp57His (OR 1.944) and Arg84Gly (OR 1.458), are associated with increased susceptibility to infection while, Asp57Asn (OR 0) and Phe60Tyr (OR 0.453) are linked with increased resistance to MAP infection in cattle.

Interferons: Interferons are inducible cytokines of multigene family. Interferon- γ (IFN- γ) plays a crucial role in the innate host response to intracellular bacteria, including mycobacteria (Huang *et al.*, 1993; Shtrichman and Samuel, 2001; Mackintosh *et al.*, 2011; Deb and Goswami, 2011). Release of IFN- γ (protective Th1 response) after the initial MAP entry into the host is considered as key factor in the control of infection and manifestation of the disease (Coussens *et al.*, 2002; Coussens, 2004; Arsenault *et al.*, 2012; Dobson *et al.*, 2013). MAP infection is influenced by Interferon- γ gene (Pinedo *et al.*, 2009a). Increased gamma interferon (IFN- γ) expression locally at the site of infection are reported in sub-clinical stage (Sweeney *et al.*, 1998) and higher IFN- γ production in culture supernatants after stimulation of Peripheral Blood Mononuclear Cells (PBMC) with MAP antigens (Stabel, 2000). With the shifting of MAP-infected animals towards the clinical state, there is decrease in the production of local and peripheral IFN- γ (Stabel, 2000, Sweeney *et al.*, 1998). Exogenous IFN- γ stimulates monocyte for destroying the intracellular pathogen (Zhao *et al.*, 1997). A similar upregulation of IFN- γ production during the 'contained stage' of the tuberculosis has also been reported (Dlugovitzky *et al.*,

1997; Orme, 1993). Growth inhibition of *Mycobacterium tuberculosis* and *Mycobacterium bovis* by macrophages is observed due to addition of recombinant human IFN- γ to monocyte cell cultures (Dlugovitzky *et al.*, 1997; Orme, 1993). Conversely, infection of IFN- γ knockout mice with a sub-lethal dose of *Mycobacterium bovis* or *Mycobacterium tuberculosis* resulted in increased mortality and high bacteria counts from the organs viz., spleen, liver and lung of recovered mice (Dalton *et al.*, 1993; Cooper *et al.*, 1993).

Maintaining of mycobacteriosis within persistently infected macrophage and activating newly required macrophages at sites of MAP infection require locally high concentration of IFN- γ . High IFN γ responses are protective and can play stimulatory effect on B lymphocyte and antibody production (Abbas *et al.*, 1996); sheep with pathological lesions and multibacillary condition in intestine have been found to be IFN- γ negative (Perez *et al.*, 1999); also clinically infected cows had low IFN γ concentrations compared to sub-clinically infected cows (Stabel, 2000). It has been reported that IFN γ results found to be higher in cattle with clinical paratuberculosis than sub-clinical (Billman-Jacobe *et al.*, 1992). Sub-clinical phase of MAP infection is characterized by increasing IFN γ response while clinical paratuberculosis is characterized by high antibody titre in blood, low IFN γ response and high bacterial shedding in feces (Sohal *et al.*, 2008).

TOLL-LIKE RECEPTORS GENES

Toll- Like Receptors (TLR) are a family of trans membrane structures capable of recognizing several class of pathogens and are responsible for co-ordination with appropriate innate and adaptive immune responses (Wang *et al.*, 2002; Quesniaux *et al.*, 2004; Ruiz-Larranaga *et al.*, 2011; Purdie *et al.*, 2011) TLR4 mediates cytokine production and stimulates host defense and is implicated in the recognition of mycobacterial antigens (Quesniaux *et al.*, 2004; Yadav and Schorey, 2006; Ferwerda *et al.*, 2007; Weiss *et al.*, 2008; Byun *et al.*, 2012). Importance of TLRs in mycobacterial recognition has been reviewed by Jo *et al.* (2007). TLRs signaling occurs through MyD88 protein (Common adaptor protein) and studies using MyD88-deficient mice revealed that TLRs are vital for launch of innate response as mice were highly sensitive to infection with *M. tuberculosis*, but MyD88 deficiency allowed emergence of adaptive responses (Ryffel *et al.*, 2006). Examination of TLR 1, 2 and 4 genes for the evidence of polymorphism in test and control groups of three Slovakian cattle herds were observed for all 3 genes, showing association with

increased incidence of infection in one case (TLR1) of polymorphism (Mucha *et al.*, 2009). Pinedo *et al.* (2009b). But there is no association of TLR4 with infection (White *et al.*, 2003).

The TLR2-1903 T/C and some other TLR2 SNPs were significantly associated with resistance to MAP and could be useful in marker-assisted breeding strategies for the control of Johne's disease (Koets *et al.*, 2010). Additionally, the functional studies reported that genetic polymorphisms in bovine TLR2 which result in higher macrophage activity may continue to enhance T cell activation and a lower susceptibility to paratuberculosis. Another study showed that chicken resistance to enteric bacteria like Salmonella infection is coupled to TLR4. The magnitude of the TLR4 impact in the differential resistance or susceptibility of chicken lines C and W1 is comparable thereto determined with NRAMP1. Chickens carrying a minimum of one W1 (resistant) allele at NRAMP1 and TLR4 showed the best degree of resistance to infection (93% of the offspring survived infection) compared to chickens bearing C alleles at NRAMP1 and TLR4 (58% survived the initial 1st week of infection). In this study, the odds ratio for survival till day 7 is 0.62. In conjunction with NRAMP1, TLR4 explains 35 percent of the phenotypic variance and suggests that extra enteric bacteria (Salmonella) resistance genes involved in innate or acquired immunity have yet to be known within the chicken. A genome scan performed on the same backcross panel verified the numerous linkage of these 2 loci with resistance to enteric bacteria (Salmonella) infection within chickens confirming the approach of comparative genomics to identify host resistance genes. The importance of TLR4 within the host response of birds to infection with serovar Typhimurium must be additionally explored; but the role of TLRs in the control of innate and adaptive immunity makes them good targets for genetic intervention (Leveque *et al.*, 2003).

SLC11A1 GENES

The SLC11A1 (Solute Carrier Family 11 member 1) gene (coding for Natural Resistance-Associated Macrophage protein 1, NRAMP1) is the Bcg gene consists of 15 exons spanning 11.5 kb and encoding a 90-100 kDa membrane-bound protein containing 12 hydrophobic transmembrane domains. It is associated with natural resistance against intracellular bacteria viz., *Mycobacterium* spp., *Salmonella* spp. and protozoan viz., *Leishmania* spp., playing an important role in innate defense mechanism, preventing the bacterial growth in macrophages during the initial phase of infection (Paixao *et al.*, 2007). Profound differences specifically due

to susceptibility to SLC11A1 alleles was examined recently in mouse as is the case with other pathogens (Roupie *et al.*, 2008; Korou *et al.*, 2010). SLC11A1 functions being the part of the innate defense mechanism help in blocking of bacterial replication during the early stages of infection but the associations between SLC11A1 gene and MHC region was not reported in ovine JD (Reddacliff *et al.*, 2005).

Relationship between Polymorphisms in SLC11A1 has been linked to several autoimmune diseases apart from mycobacterial infections. It has associated with leprosy (Abel *et al.*, 1998), tuberculosis (Bellamy *et al.*, 1998), rheumatoid arthritis (Ates *et al.*, 2009), visceral leishmaniasis (Mohamed *et al.*, 2003), multiple sclerosis (Kotze *et al.*, 2001), type 1 diabetes mellitus (Paccagnini *et al.*, 2009) and Inflammatory Bowel Disease (IBD) (Hofmeister *et al.*, 1997; Sechi *et al.*, 2006; Kotlowski *et al.*, 2008; Gazouli *et al.*, 2008). Though SLC11A1 contains a number of single nucleotide polymorphisms (SNPs) viz. SLC11A1 1730G>A (rs17235409; D543N) and SLC11A1 469+14G>C (rs3731865; INT4G>C), most of these disease associations have been with a promoter dinucleotide microsatellite (GTn) that is known to affect SLC11A1 expression levels (Searle and Blackwell, 1999).

SLC11A1 is a biologically plausible candidate risk gene for the handling and elimination of intracellular pathogens due to its association with mycobacterial diseases. Various studies counsel defects in genes concerned in microorganism detection, handling and elimination are central to CD pathogenesis. Moreover the assertion, albeit controversial that MAP is an initial trigger for CD provides a further explanation to analyze SLC11A1 as a candidate risk gene for IBD. As a result, this study had two aims. The first aim was to attempt the first of the association of SLC11A1 1730G>A and SLC11A1 469+14G>C with IBD. The second aim was to use previously collected MAP IS900 data (Bentley *et al.*, 2008) to test for association of SLC11A1 genotypes with occurrence of MAP DNA in peripheral blood. Genotyping for SLC11A1 1730A>G and 469+14G>C was done in 1468 (94.7%) and 1432 (92.4%) of study participants, correspondingly. No deviations from HWE were found in cases or controls for either SNP ($p > 0.05$). Minor allele frequency (MAF) percentage of SLC11A1 1730G>A and SLC11A1 469+14G>C was 2 and 30%, respectively. SLC11A1 SNP is not associated with overall CD, UC or IBD susceptibility. Similarly, the minor allele and genotype frequencies of SLC11A1 1730G>A and 469+14G>C is not associated with age at time of onset of disease, behaviour of disease, its location, or any requirement of surgical intervention. A significantly higher frequency of the SLC11A1 1730A allele was seen in IBD patients who did

not require immune-modulator therapy, compared to those who did require this treatment approach (PIBD = 0.002, OR: 0.29, 95% CI: 0.13-0.66, PCD = 0.03, OR: 0.38, 95% CI: 0.15-0.95, PUC = 0.01, OR: 0.75, 95% CI: 0.71-0.79). There was no significant association of SLC11A1 1730G>A with MAP status, whereas the SLC11A1 469+14C allele was associated with increased incidence of MAP DNA in peripheral blood ($p = 0.02$, OR: 1.56, 95% CI: 1.06-2.23) (Stewart *et al.*, 2010).

IL10R α GENE

IL-10 is a cytokine that primarily acts as a negative feedback mechanism for T lymphocytes and is an essential immune-regulator in bacterial infection. From the perspective of MAP infection, IL-10 prevents excessive Th1 and CD8+T lymphocyte responses that may lead to immunopathology associated with infection (Subharat *et al.*, 2012; Coussens *et al.*, 2012). The cytokine also prevents overproduction of interleukins 4, 5 and 13. The IL-10 receptor alpha (IL-10R α) gene encodes a ligand-binding subunit of the IL-10R and therefore is a determinant of IL-10 responsiveness.

Interleukin-10 receptor alpha (IL10R α) was considered as a candidate gene for susceptibility of bovines to MAP infection, along with other Interleukin-10 (IL-10), based on associations of IL-10 promoter polymorphisms with inflammatory bowel disease in humans (Verschoor *et al.*, 2010a). A total of six anonymous SNPs were identified in IL10R α coding regions and one of these was found to have a significant association with MAP infection after correction for multiple testing (synonymous SNPs are alternative nucleotide triplets that result in the coding of the same amino acid; they produce no change in amino acid sequence of the protein and so are not functionally relevant). The significant SNP was in high linkage disequilibrium with 3 other SNPs, meaning the specific alleles of these 4 SNPs are inherited together as a group in most cases and inheritance at 1 SNP provides the same information as any other.

IL-10R polymorphisms have been associated with bovine MAP infection status (Verschoor *et al.*, 2010a). Verchoor had conducted a candidate gene-based study of MAP susceptibility sourcing Holstein cattle from six commercial farms in Ontario with a history of high MAP prevalence. The infection status was determined by ELISA, with 204 MAP positive and 242 healthy negative cattle included in the study. SNP discovery was performed for IL-10 and its receptor subunits (IL-10R α and IL-10R β), transforming growth factor beta (TGF β 1) and its two receptors (TGFBR1 and 2) and SLC11A1. SNP

genotyping revealed tightly linked groups within the two sets of IL-10R related SNP. Further haplotype analysis was carried out on IL-10R related SNP only. Although a number of SNP were revealed for each gene only four tightly linked SNP related to IL-10R (984G>A, 1098C>T, 1269T>C and 1302A>G) showed statistically significant association with MAP infection, with a strong additive and dominance relationship at the GCTA allele. Cattle with these polymorphisms had a higher probability of MAP infection. None of the SNP from the other genes tested demonstrated an association with MAP susceptibility in this study. Although previous studies have correlated the action of IL-10 to the pathways of other susceptibility related candidate genes (such as SLA11IA), this is the first evidence of a susceptibility correlation with the IL-10 gene itself (Verschoor *et al.*, 2010a).

SP110/IPR1 GENE

Intracellular pathogen resistance 1 (Ipr1) gene of murine model and human ortholog, SP110 nuclear body protein has been reported to play an important role for inducing innate immunity against Mycobacterium tuberculosis infection (Pan *et al.*, 2005; Liang *et al.*, 2011; Lei *et al.*, 2012). Ruiz-Larranaga *et al.* (2010) reported that SNP of SP110 gene are associated with MAP infection in Holstein-Friesian cattle.

PEPTIDOGLYCAN RECOGNITION PROTEIN 1

Peptidoglycan recognition protein (PGRP) are a member of the mammalian innate immune modules, consist of four molecules A, B, C and D with ligand binding clefts situated at A-B and C-D contacts. PGRP binds to lipopolysaccharide (LPS), peptidoglycan (PGN) and lipoteichoic acid (LTA) at their C-D contacts whereas A-B contacts having binding site of fatty acids including mycolic acid of Mycobacterium tuberculosis (Sharma *et al.*, 2013). Holsteins in South western and Eastern Ontario breeds were subjected for analysis for the presence of any association between peptidoglycan recognition protein (PGRP) and occurrence of MAP infection and it was observed that SNP c.480G>A of PGRP are significantly associated with the occurrence of MAP (Pant *et al.*, 2011).

ANKRA2 AND CD180 GENES

Casas *et al.* (2011) also reported that SNP in the ANKRA2 and CD180 genes were significantly associated with the presence or absence of MAP in Brahman \times Angus cattle.

WHOLE GENOME ASSOCIATION STUDIES

Whole Genome Association Studies (WGAS) take a global approach to comprehensively survey the genome of the species of interest for genetic markers associated with a disease or, infection phenotype. Results from WGAS provide information that can be used directly in predicting infection susceptibility genetics as well as providing the preliminary information on which positional candidate gene studies can be based (identification of narrow genomic location in which a gene responsible for susceptibility to infection resides). In Whole genome association studies enables definitive identification of allelic variations between and within diseased individuals or animals, their siblings and other members of family or associated ones.

There are one genome wide linkage analysis and four WGAS for MAP infection or related phenotypes in cattle using commercial dairy herd of Holstein Friesian cattle. Linkage analysis examine the association of alternative alleles inherited from parents in a defined family structure (e.g., often paternal half sib families in cattle), whereas WGAS consider association of alternative alleles at a given genetic marker using animals sampled broadly from the population.

The first genome-wide analysis for MAP infection done in cattle (Gonda *et al.*, 2007) was a linkage analysis considering the contribution of alternative sire alleles. This study used 3 of the largest half sib families (a total of 1263 daughters) from a larger Holstein resource population composed of 4586 cows sired by 12 different bulls. In this study one chromosomal region on bovine chromosome 20 was found significant at a chromosome-wise $p < 0.05$.

Loss of information in estimating allele frequencies from pooled samples along with use resource population partly; sole analysis of paternal genetic contribution (within-family linkage analysis) instead of combined effects of linkage and linkage disequilibrium and sparse marker density have restricted this study. In the WGAS report of first MAP infection (Settles *et al.*, 2009), cows from 3 herds, in New York, Pennsylvania and Vermont and were used in a case-control design. Phenotypic assessment of infection was based on culture of MAP from tissue or lymph nodes of the small intestine or from feces obtained at necropsy. A total of 218 animals were used in the study; 90 animals were classified as tissue positive and 41 out of them were classified as fecal positive. In this study 16 SNPs showed associations exceeding a nominal $p < 5 \times 10^{-5}$ for the various case definition, though given the proximity of some of the significant SNPs this represents 11 unique loci. For 3 of

these 11, the association was observed for 2 case definitions. Subsequent re-analysis of the same data considering tolerance as the phenotype where tolerance was considered as the degree (quantitative) or presence or, absence (case control analysis) of fecal shedding among animals were found to be tissue positive (Zanella *et al.*, 2011). SNP association with the tolerance phenotype that exceeded a nominal $p < 5 \times 10^{-5}$ were observed for 4 genomic locations. A subsequent reanalysis of the same data set suggests an approach for identifying potential candidate genes using data generated from WGAS (Neibergs *et al.*, 2010). In this study, SNP proximity to known genes was evaluated, now considering the relative significance of groups of genes that are part of specific mechanistic pathways or cascades. The advantage of this approach is the multiple genes with modest effects that are part of a common pathway may be discernible as a more significant group, whereas individually their effects might be considered of insufficient significance for further analysis.

A second WGAS for susceptibility to MAP infection used cows ($n = 232$) from 6 dairy herds in Ontario with a prior history of a high prevalence of infection (Pant *et al.*, 2010), cases and controls in this study were defined as animals positive ($n = 90$) or, negative ($n = 142$) to an ELISA for serum antibodies. SNP studies were examined in a 2-stage logistic regression analysis that first considered the effects of the SNPs nominally significant in the preliminary analysis in the context of a chromosome through a principal components approach. A total of 22 SNPs were significantly associated with infection status, representing 13 unique chromosomal region, after accounting for SNPs in close proximity that likely account for the same locus.

A third WGAS of susceptibility of MAP infection used cows from 119 herds in the province of Lodi, Italy. Matching case (ELISA positive, $n = 483$) and control (ELISA negative, $n = 483$) animals were sampled from the same herd on the same day (Minozzi *et al.*, 2010). Whole genome genotype data were used to account for animal relationship in a mixed-model analysis of SNP associations. Ten SNPs were significantly ($p < 5 \times 10^{-5}$) associated with the infection status representing 5 or, 6 unique chromosomal regions. Six of these 10 SNPs were subsequently evaluated on a second group of case and control animals from the same population ($n = 277$) and 5 of the 6 were significant at a nominal $p < 0.01$.

A fourth WGAS of susceptibility to MAP infection used 2 resource populations of approximately 5000 each, the first including daughters of 12 specific Holstein sires sampled from 300 cooperating herds across the United States and the second including all cows from

6 cooperating herds in Wisconsin (Kirkpatrick *et al.*, 2010). The study used a unique approach of a case-reference rather than the typical case control design. Given the extensive availability of 50K SNP genotype data from artificial insemination sires and the availability of pedigree information on the sampled animals, allele frequencies for cases (positive for either blood ELISA or, fecal culture) were compared with allele frequencies for AI sires representative of the herd or, population in question. Use of this approach enabled genotyping of maximum number of case samples (n = 521). Data from the 2 resource population were analyzed both separately and jointly. The latter using a logistic regression approach. Multiple SNP model included seven SNPs in unique chromosomal locations significant at $p < 5 \times 10^{-5}$. The cross validation analysis indicated that the models developed were only fair predictors (correct prediction of sample rank 73% of the time), with the caveat that the alternative grouping of samples was cases (ELISA and/or fecal positive) versus reference (AI sires reflecting the general population). A comparison of case and control (both ELISA and fecal negative) would likely yield an improved predictive ability.

CONCLUSIONS AND FUTURE PERSPECTIVES

The development of sub-clinical or clinical paratuberculosis or other chronic mycobacterial diseases is a result of a complex interaction between the host and pathogen. There are numerous host genes likely to be involved in the progression of disease caused by *Mycobacterium avium* subspecies *paratuberculosis*. Using the variety of study methods, substantial progress has already been made in advancing our understanding of genetic susceptibility or resistance to MAP infection. The identified molecular marker for resistance to John's disease will help in enhancing the overall 'herd resistance and immunity' by encouraging the resistant animals for breeding (selective breeding for disease resistant traits). Similarly, genetic variations that contributes to host susceptibility to MAP infection is also very important both on reducing susceptibility to infection and for gaining a better understanding on the mechanisms of disease. Overall, the review may aid in designing the more efficacious, cost-effective and safer strategies for eradication of John's disease, especially in India, where there is rich diversity of genetic resources in terms of breeds and strain of domestic livestock in different agro-climatic zones. So, for the development of resistant breed of animals and control as well as elimination of paratuberculosis, much effort need to be implemented as there are likely to be many more novel genes to be identified. This review has more relevance in resource

poor or resource-less developing and poor countries, where enough genetic variability exists in the animal population which may help to improve the productivity as well resistant against chronic and incurable infection like MAP and will be helpful in implementation of marker-assisted selective breeding programmes to control paratuberculosis. A delicate balance between breeding of domestic animals for production and for disease resistance to chronic infections like MAP may help to optimize productivity and reduce the levels MAP infection in domestic livestock as well as human population. If paratuberculosis control measures are not initiated, the endemicity of MAP and severity of disease will soon lead to shortage of food for burgeoning human population. Moreover, MAP infection is passes through generations via semen, milk and colostrum, will continue to increase the burden of disease in livestock population and non-activation during pasteurization will pose serious threat to human population of the country.

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