

## Genetic Uniqueness for Immune Response Traits in Native Animal Genetic Resources

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Breeding of animals for disease resistance is an important intervention in the era of emerging and re-emerging pathogens in livestock and poultry. Livestock and poultry species of India are considered as an important genetic resource for climate resilience as well as relative less susceptibility to tropical diseases. Indian livestock populations with a broad genetic base, due to availability of diverse breeds and populations, have advantage over the exotic counterpart. Introduction of cross breeding in India, cattle in particular, with exotic germplasm, has further helped in identification of several genes and pathways involved in gaining better immunity against the prevalent diseases. Unique alleles have been identified in the major histocompatibility region and other immune response genes, including toll-like receptors, having important role in disease resistance. Major impediment in associating genetic polymorphism with the disease resistance however, is the non-availability of standardized phenotypic data. Though attention is being diverted towards understanding the genetic basis of disease resistance in native livestock and poultry, there is a need to utilize the latest genome based technologies for fully understanding as well as exploiting the genetic potential of indigenous breeds.

### Introduction

The biotic stress which includes pathogenic insults is the prime selecting factor for the evolution in all vertebrates. The pathogens, invading glands, mucosal surfaces of digestive tract, reproductive tract etc. are sensed first by the physical or chemical barriers of the innate immune system. After the failure of these barriers, the system reacts by initiating a cascade of immune cell-mediated responses. The innate immunity is functionalized by several preexisting defenses such as proteins and peptides secreted by the resident macrophages, mobile neutrophils etc., while the adaptive immune response functions in a more sophisticated way, and enables the living system to create a record of past intruders/foreign particles/antigens. The livestock that have adapted and evolved close to humans have influenced greatly the nutritional status, economic affairs, and social life of their human counterparts. Health status as well as ability of the animals to recover from the infectious diseases determine not only the production/economic aspect, but also poses threats to zoonosis and major outbreaks in the human population as evident from anthropological history. Recent COVID outbreak is a good example of SARS-CoV2 crossing the human-animal barrier and causing havoc.

Livestock sector plays a significant role in India's rural livelihood as it employs a major section of the

labour force and also provides a large share of draft power, being used for the cultivation of crops. India's livestock sector is one of the largest in the world with 535.8 million population, registering growth of 4.6% from the last census of 2012 to 2019. Contribution of livestock sector to the national economy in terms of Gross Domestic Product (GDP) is 4.1% and 25.6% of total agricultural GDP. India is one of the few countries in the world, where most of the varied natural agro-climatic conditions are available within the same region, ranging from the arid/semi-arid Thar Desert, tropical and coastal region, arid cold region of Ladakh. Indian livestock landraces/breeds are well adapted to the diverse geo-climatic conditions of the country. As a result, these breeds can survive the rugged gradient of temperature, humidity, hypoxic and subzero temperature, landscapes etc. without much management interventions, under an extensive system of rearing.

The immune system's function is influenced by multiple factors such as genetic make-up, environmental influences, behavioral patterns, dietary factors, etc. Those animals/livestock, who cannot control the environmental influences are forced to sustain the stresses and eventually shape their genetic makeup and these beneficial traits get fixed in the long run by the virtue of adaptive evolution. Consequently, the local landraces of livestock that have evolved in the native environment, are more resistant to

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pathogen attacks than their exotic counterparts. Several innate and adaptive immune genes have been implicated, when analyzing variations in cohorts of disease-resistant or susceptible production animals. Since the race between the immune system and the ever-evolving pathogenic forces is very dynamic, a thorough understanding of livestock immunology can help in building the strategies to overcome the potential outbreaks. In this aspect, the local animal genetic resources are major germplasm carrying the plethora of beneficiary genetic and physical traits needed to combat the locally prevalent diseases.

All the livestock species in India are very well adapted to diverse the agro-climatic conditions and are considered to be better equipped to fight the tropically prevalent infectious diseases in the region, than the exotic breeds and presence of unique genetic attributes could play a major role in that. Genetic selection to increase resistance against infectious diseases, will prove to be a low cost and sustainable practice. Besides, all the above properties, there are some of the common qualities in our pure breeds, cattle in particular. Their skin is having sweat and sebaceous glands, which secrete an oily fluid, acting as repellent to the insects and mosquitoes. It also decreases the rate of most of vector borne infections. In case of chlamydial abortion, Johne's disease, Babesiosis, Tuberculosis, Mycoplasmosis, Leptospirosis, Brucellosis and diarrhea, our native breeds show better resistance. The globulins levels in the blood of pure Indian breeds are high, while creatinine level is less than that of the crossbreds or exotic cattle. Indicus cattle are also considered relatively resistant to mastitis and ticks as well as tick borne protozoan diseases such as Babesiosis and Theileriosis, exotic and crossbred being susceptible to. Studies have shown Holstein and Jersey crossbreds of India having a higher risk (94.54%) of mastitis than the local cattle breeds (31.25%). Similarly local indigenous chicken are reported to be more resistant to *Pasteurella multocida* infection than exotic.

Among the various immune response genes, keeping in mind the disease resistance related traits, major histocompatibility complex (MHC) genes play a major role. The bovine major histocompatibility region, BoLA contains approximately 200 genes, most of which are immune-related. Class II bovine MHC genes polymorphism are considered to be most likely influencing the immune traits, among which the BoLA-DRB3 has shown more influence on ability to evoke the immune response. Selection, genetic drift, and population bottlenecks have significant effect on

the allelic diversity of MHC class I and class II loci in a population. Genetic diversity in MHC genes is also considered as an important marker for the assessment of survival and risk status of animal species. Comparative analysis of indicus and exotic as well as crossbred cattle has shown extensive allelic diversity as well as specific alleles at the DRB3 and DQA loci of MHC region, associated with resistant to sub-clinical and clinical mastitis in indicus cattle breeds of India. A high level of duplication and allelic diversity has been reported for BoLA-DQA in Mewati, Konkani, Tharparkar, Ladakhi, Malnad Gidda, Ongole, Gir, and Sahiwal cattle breeds (Kumari *et al.*, 2021). Similarly, in buffalo extensive duplication and variability have been reported for DQA alleles (Mishra *et al.*, 2020).

Another study conducted on clinical mastitis incidents in indicus and crossbred cattle, clearly indicated that glutamine and glutamic acid at positions 62 and 66 playing an important role in resistance to clinical mastitis. While, arginine at 62 position and deletions of amino acid at positions 58 and 59 make the animals more susceptible to mastitis. Expression of BoLA- DQA, DQB and DRB3 gene of healthy and mastitis affected indicus, exotic and crossbred animals has been studied and there was significant higher expression of the BoLA-DQA gene in healthy indicus cattle compared to mastitis affected indicus cattle and both healthy and mastitis affected exotic and crossbred cattle. Whereas the other studied genes BoLA- DQB and DRB3 presented no significant differences in expression in healthy and mastitis affected indicus, exotic and crossbred cattle. This indicates that duplication of gene and polymorphism probably playing more significant role in differential immune response among indicus and crossbred/exotic cattle at MHC locus. A study on response to vaccination against PPR and MHC locus diversity in Sirohi goat has revealed rich diversity of major histocompatibility complex (MHC) region in Indian goat.

### Pattern Recognition Receptors Diversity

Nucleotide-binding oligomerization domain-containing protein 2 (*NOD2*) and toll-like receptors (*TLR*) are the foremost receptors to identify the pathogen-associated molecular patterns (*PAMPs*). Toll-like receptors are transmembrane proteins with an extra-cellular domain consisting of leucine-rich repeats (LRRs) domain and cytoplasmic (TIR) domain. A total of 13 TLRs have been identified in mammals. In Indian buffalo, all the ten TLR genes reported in bovines, have been

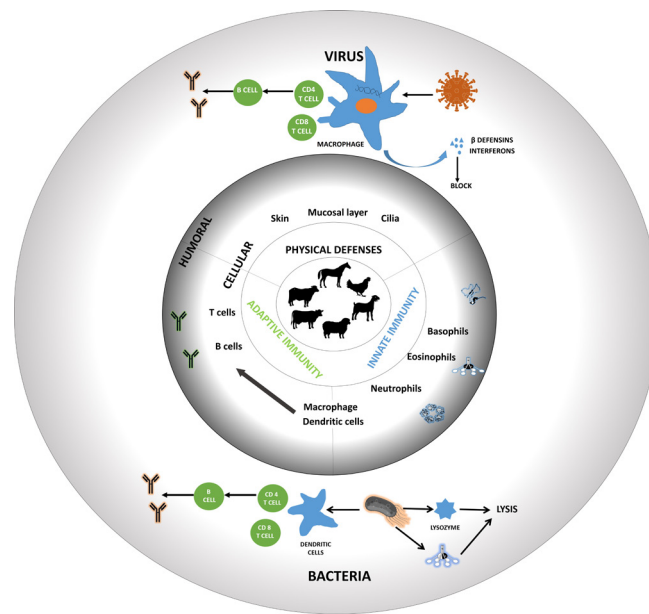


Fig. 1. The immune cell components and host-pathogen interaction in livestock

characterized, documenting the complete gene sequence based structural variability across bovines (Dubey *et al.*, 2013). The studies have shown the differential evolutionary lineages of buffalo TLRs compared to cattle and other ruminant species. The non-synonymous SNPs in other receptor gene, *NOD2* have been associated with incidences of mastitis in buffaloes (Dhiman *et al.*, 2017). The *NOD2* works in synergy with other PRRs, *TLR2* in a dose-dependent manner, and also cross talks of *NOD2* and *TLR2* leads to the activation of *NOD2-TLR2* specific gene panel, otherwise, they both have different signaling cascades.

### Other Immune Response Genes

Several candidate genes have been identified with genetic variability having significant association with disease resistance in Indian livestock breeds. *NRAMP1* (natural resistance-associated macrophage proteins), is a divalent iron transporter, pumping iron out of the phagosome, which is considered as an important defense against mycobacterial infections in particular. The polymorphism within the gene has also been linked to other intra-cellular pathogens like *Brucella* and *Salmonella* infections. The cases of bovine tuberculosis (bTB) are reported higher in cattle than the buffaloes, particularly, the prevalence of bTB is more in cross-bred than the indigenous cattle of India. The beta-defensins have been widely associated with incidences of mastitis. Particularly, the *LAP* and

*BNBD4* expression is reported to be upregulated in PBMCs of mastitis-affected cattle and buffalo (Gurao *et al.*, 2018). Polymorphism and unique alleles within several other candidate genes including cytokines have been identified in indigenous livestock breeds, which need to be further investigated. Targeted sequencing of exonic regions of *CHGA*, *CHGB*, *CHGC*, *NRAMP1*, *NRAMP2*, *DEFB1* and *TAP* genes in Gir cattle and Murrah buffalo revealed 54 and 224 SNPs, respectively. Polymorphism identification in innate immune genes would advance our knowledge about the role of these genes in elucidating the disease resistance/susceptibility in Indian breeds.

### Whole Genome Based Studies

Molecular markers are more important in breeding of animals through selection for traits like disease resistance, with low to moderate heritability and for which measurement of phenotype is difficult. Advanced technologies, including whole genome sequencing, transcriptome and proteome analysis will help in dissecting the genomic variation and developing molecular markers useful for selection of livestock and poultry resistant to diseases. Several breeds/populations still need to be explored genetically to answer the question of being resistant to many tropical diseases. Transcriptome analysis of Foot and Mouth Disease (FMDV) serotype O/IND/R2/75 infected two indigenous

cattle breeds, Malnad Gidda, and Hallikar, and an exotic breed Holstein Frisian, identified MHC class II, *IFN- $\alpha$* , *IFN- $\gamma$* , *IFN- $\lambda$ 3*, and *RSAD2* (viperin) transcripts' to be upregulated in the indigenous cattle than in the exotic cattle breed. This indicates that the indigenous cattle breeds could neutralize the FMDV better than the exotic breeds (Saravanan *et al.*, 2021).

A whole-genome sequence based comparative analysis of Indian cattle breeds, Kangayam, Tharparkar, Sahiwal, Red Sindhi, and Hariana, identified the genetic variants, within the genes and pathways involved in the innate immune response (Vijayakumar *et al.*, 2022). Additionally, the workers suggested non-synonymous polymorphism in antiviral effectors genes such as *MX1*, *OASL*, *IFITM5*, *IFITM3*, *IFIT5*, *TGFBI*, *GBP1*, *GBP7* and *RSAD2* in the same panel of indigenous cattle breeds, should be considered for association between BVDV persistence and antiviral activity. Major variants identified were within toll-like receptors, retinoic acid-inducible gene I-like receptors, NOD-like receptors, Jak-

STAT signaling pathways, and several non-synonymous variants in the candidate immune response genes. These pathways and genes directly or indirectly contribute to the disease resistance of *Bos indicus* cattle breeds. Genome wide analysis in indicus Sahiwal cows could identify important candidate genes for clinical mastitis, which included *DNAJB9*, *ELMO1*, *ARHGAP26*, *NR3C1*, *CACNB2*, *RAB4A*, *GRB2*, *NUP85*, *SUMO2*, *RBPJ*, and *RAB33B* genes. The findings helped in elucidation of the genetic architecture of the disease in *Bos indicus*, and potential regions for the fine mapping and future exploration.

### Future Prospectives

Although the work carried out so far has clearly indicated that the indigenous livestock species carry significant genetic variation, making them resistant to many tropical diseases, exotic germplasm is susceptible to. Recently developed next generation sequencing tools, have been helping in delineating the molecular basis

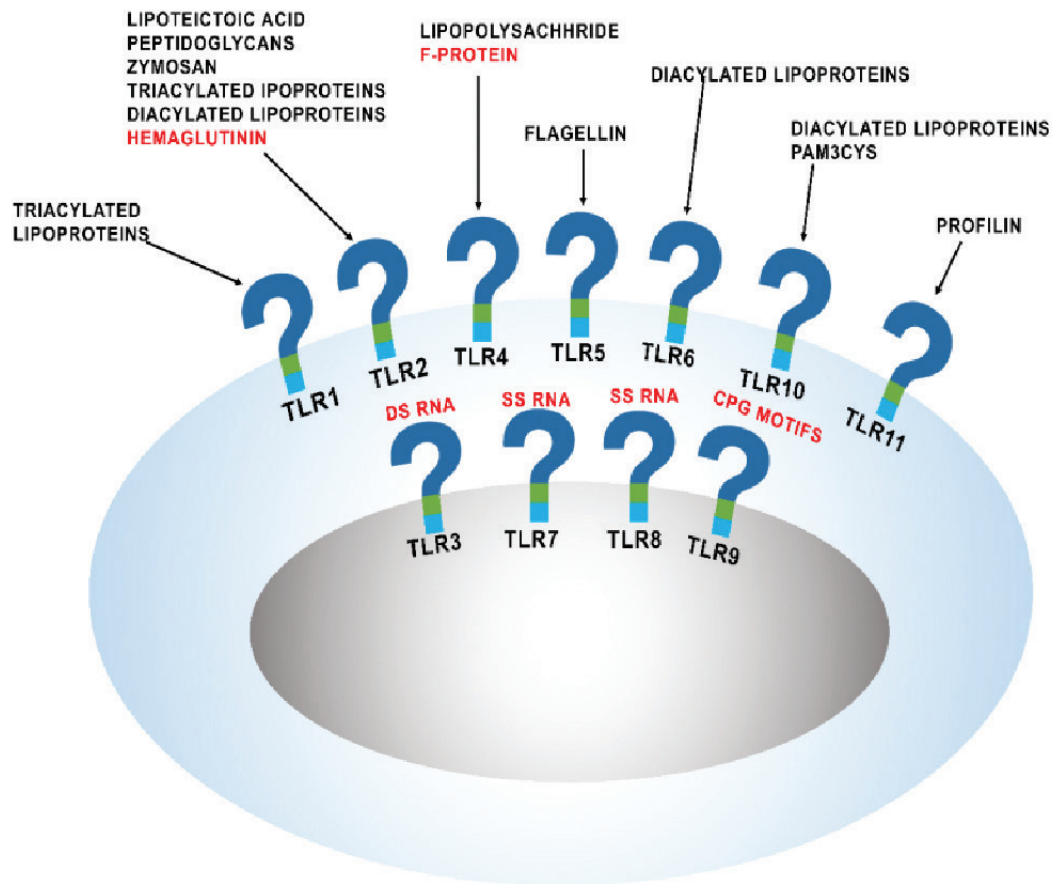


Fig. 2. Cellular localization and the ligand for various Toll-like receptors

of such privilege, original native livestock species are endowed with. Genome sequencing supplemented with transcriptome, proteome and epigenome exploration will pave the new ways for deep understanding as well as exploitation of native germplasm for breeding animals more resistant to diseases. Prediction of genomic breeding value through identification of SNPs in native germplasm will fasten the genetic gains against disease resistance traits. However the availability of quality phenotypic data on the disease resistance traits is the major issue, which needs to be resolved for faster implementation of genetic selection of animals more resilient to tropical diseases.

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