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A systematic review on genetic resistance to gastrointestinal nematode infection in sheep

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Abstract
Gastrointestinal nematode infection is a major constraint to the sheep farming and cause production losses, increased costs of management and treatment, and mortality in severe cases. The use of conventional disease control methods viz. chemotherapeutic agents, antibiotics, deworming and vaccination protocols, have most frequently had negative consequences like drug-resistant strains, increased production cost, etc., In alternative to that, the breeding programs with the goal of enhancing host resistance to diseases may help to lessen the problems permanently. In this article, current knowledge on the host immune response and markers associated with resistance to gastrointestinal nematodes infection in sheep is reviewed. The genetic variations within and between sheep breeds against the gastrointestinal nematodes have been reported where some individuals/breed are more resistant to the infection. Attempts have been made to identify susceptible and resistant animals based on indicator traits. Candidate genes are genes with known biological function that directly or indirectly regulating the developing processes of the investigated disease. The genes have been shown to be related to disease and more likely to find associations with the target disease traits. The basic idea is to analyze the mutations in susceptible / resistant animals, or different breeds with different susceptibility to a infection. Alleles based on the mutations find in these genes may be useful markers for disease resistance breeding. The identification of the genetic markers will enable the use of marker assisted selection to increase the accuracy of selection in breeding programmes. This knowledge may lead to a better understanding of the mechanisms of susceptibility and resistance of hosts to gastrointestinal nematodes. Understanding the genetic and molecular basis of disease resistance also has many advantages and applications such as the development of novel genetic markers for inclusion in genetic improvement programmes.

Keywords: Gastrointestinal nematode, sheep, host resistance, genetic markers, candidate genes

Introduction
Diseases caused by infectious agents (bacteria, fungi and viruses) and parasites have a harmful effect on livestock, rigorous impact on production and significantly on the overall process of economy of livestock farming. The use of conventional disease control methods viz. chemotherapeutic agents, antibiotics, deworming and vaccination protocols, have most frequently had negative consequences like drug-resistant strains, increased production cost, etc., (Jovanovic *et al*., 2009) [1]. In alternative to that, the breeding programs with the goal of enhancing host resistance to diseases may help to alleviate the problems permanently. Selection of livestock with natural resistance to disease is the best option for substitute to control of disease (Gibson and Bishop, 2005) [2].The genetic or natural or host resistance to disease denotes that some individuals when exposed to disease become ill whereas others do not. Such variation in the susceptibility of species or breeds or individuals is rarely exploited. The genetic markers may help to identify the resistant individual and breeding for resistance may be incorporated in the herd/flock. Hence, it will be more suitable to exploit the genetic variation among livestock to progress resistance against diseases (Douch *et al.*, 1996) [3]. Identification of genes involved in regulating resistance will allow earlier selection of genetically superior animals. The identification of these genetic markers will enable the marker assisted selection to increase the accuracy of selection in breeding programmes. Additionally, this knowledge should lead to a better understanding of the mechanisms of susceptibility and resistance of hosts to diseases (McRae *et al*., 2015) [4].
The development of genetic markers for parasite resistance in domestic sheep has received major attention in the last decade. The identification of genetic markers for parasite resistance in sheep will be useful for establishing breeding systems with the objective of selection for parasite resistant animals.

Identifying genes which contribute to the variation in resistance provides a better understanding of the mechanisms of resistance but more work is needed to determine if such genes, alone or in combination, account for the variation in resistance to allow marker assisted selection (Estrada-Reyes *et al*., 2019) [5]. Though several studies on genetic variation to gastrointestinal nematodes infection in sheep breeds have been made around the world, studies in Indian breeds are limited and mostly related to estimation of genetic parameters. There are not many reports available on the association of genes with regards to parasitic resistance in sheep breeds and efforts are in progress to identify the linkage of gene/markers for parasitic resistance in sheep in India. In this article, current knowledge on the host immune response and markers associated with resistance to gastrointestinal nematodes infection in sheep is reviewed. This information will provide a better knowledge for managing the problem in a sustainable manner.

Gastrointestinal Nematodes Infection in Sheep

Gastrointestinal nematode infection is a major constraint to the sheep industry and cause production losses, increased costs of management and treatment, and mortality in severe cases (Larsen *et al*.,, 1995) [6]. Sheep are popularly known as 'museum of parasites' because of their close grazing habit. Among parasites, gastrointestinal nematode infections such as *Haemonchus contortus*, *Oesophagostomum sp*. and *Bunostomum sp.* impose severe constraints on sheep production. Grazing ruminants are constantly exposed to natural challenge by gastrointestinal nematodes. Infection by such parasites leads to clinical disease and production losses. Gastrointestinal nematodes constitute a major cause of morbidity and mortality in sheep. In India, control of gastrointestinal nematodes is solely dependent on use of anthelmintics. However, control that relies entirely on anthelmintics is at risk due to the widespread occurrence of anthelmintics resistance in addition to the residues in livestock products.There are several reports of parasites becoming resistant to most of the available classes of anthelmintics (Singh *et al*., 2002) [7]. In addition to anthelmintics resistance, residues in livestock products, consumer demands for organic products and adverse effect on environment, have led to the need for new control measures (Bartley *et al*., 2004).

Genetic variation in host resistance to Gastrointestinal Nematodes infection

The genetic variations within and between sheep breeds against the gastrointestinal Nematodes have been reported where some individuals/breed are more resistant to the infection. Attempts have been made to identify susceptible and resistant animals based on indicator traits etc (Larsen *et al.*, 1995) [6]. Several studies reported the genetic basis for resistance to gastrointestinal nematodes in sheep and the differences exist in both between and within breeds. Considerable variation has been reported among sheep breeds on their ability to resist gastrointestinal nematodes. For example, Rhon sheep (Gauly *et al.*, 2001) [8], Red Maasai (Baker *et al.*, 2002) [9], Garole (Nimbkar *et al.*,2003)[10], Barbados Black Belly (Gruner *et al.*, 2003)[11] and Gulf Coast Native (Miller *et al.*,2006) [12] were found to have relatively better resistance against gastrointestinal nematodes. Similarly, within-breed genetic variation has also been demonstrated in diverse sheep populations including Scottish Blackface (Stear *et al.*, 1997) [13], Merino (Woolaston *et al.*, 2001) [14], etc. Mugambi *et al.*, (2005) [15] studied the genetic variation in resistance to parasitic infection and reported that the Red Maasai breed showed higher resistance to *H. contortus* than Black Headed Somali and Dorper sheep and all three breeds were substantially more resistant than the Romney Marsh breed. Woolaston *et al.*, (2001) [14] stated that Merino flocks were successfully selected for high and low immune response to *H. contortus* and *T. colubriformis.* Sayers *et al.*, (2005) [16] carried out molecular genetics study to identify the variation at the ovine MHC-DRB1 locus in Suffolk and Texel sheep. Ovar-DRB1 alleles and faecal egg count were determined for Texel and Suffolk lambs and concluded that the Ovar-DRB1 gene plays an important role in resistance to nematode infection in the Suffolk breed.

In India, a few studies have been carried out on the breed susceptibility to nematode parasitism. Yadav *et al.*, (1993) [17] observed body weight gain, haemoglobin, and packed cell volume after artificial infection with *H. contortus* to be significantly lower in Hisardale lambs (Nali x Corriedale) and peripheral eosinophil count to be significantly higher in Munjal lamb (Nali x Lohi). They concluded that Hisardale lambs have significantly higher susceptibility to experimental *H. contortus* infection than Munjal lamb. Swarnkar *et al.*, (2000) [18] observed susceptibility variation in different sheep breeds in FEC of natural infected *H. contortus* and observed that Malpura lambs had the lowest FEC followed by Avikalin and the highest FEC in Bharat Merino. Singh *et al*.,(2002) [7] evaluated the progenies of Avikalin (Rambouillet × Malpura) breed for FEC at native and exposed stage of natural infection and two divergent lines were created by selecting progenies from sires with low and high mean FEC. The heritability estimate of FEC at native and exposed stage of natural infection was 0.223 ± 0.194 and 0.114 ± 0.112, respectively revealing existence of genetic variation for both innate and acquired resistance to infection. Results reveal that resistance to *H. contortus* is moderately inheritable and selection for resistance is possible and will not adversely affect production parameters in semi-arid environment. Nimbkar *et al.*, (2003) [10] stated that the Garole breed had significantly higher internal nematode resistance than the Deccani and Bannur breeds. Prince *et al.*, (2010) [19] estimated genetic parameters for faecal egg count in Avikalin sheep of India. Direct heritability for the trait was 0.149±0.096. Effect of faecal egg count on the growth characteristics was observed to be significant and further stated that the direct genetic and maternal permanent environmental effects were important for this trait; thus, they need to be considered for improvement in the trait.

Genetic Variation within Breed

Genetic variation within breed has been exploited to develop sheep resistant to *H. contortus.* It has been demonstrated that Romney sheep could be selected divergently for nematode resistance (Bisset, *et al*., 1996) [20], and the same was shown in Merino flocks that were successfully selected for high and low immune response to *H. contortus* and *T. colubriformis* (Woolaston and Piper, 1996) [21]. The heritability of resistance to infection in sheep, as measured by FEC, varied from 0.22 to 0.43. Woolaston *et al*. (1991) [22] reported estimated heritabilities after artificial challenge with *H. contortus* of FEC in Merino ewes as 0.27, 0.22 and 0.31. Cummins *et al*. (1991) [23] reported estimated heritability after naturally acquired Ostertagia infection of FEC in Merino sheep as 0.42.

In other study, Baker *et al*. (1991) [24] reported estimated heritability after natural acquired mixed species infection of FEC in Romney sheep as 0.34. The heritable variations in FEC were also reported by several workers (Bishop *et al*., 1996 [25]; Stear *et al*., 1997 [13]; Morris *et al*., 2000) [26]. Resistance to natural infections of *H. contortus* was studied in Merino sheep and lambs, which were sampled for FEC and PCV. The results showed that the FEC could be used as a selection criterion for resistance to *H. contortus* infection in Merino sheep (Nieuwoudt *et al*., 2002) [27]. Recently, genetic correlation between resistance to *H. contortus* and *T. colubriformis* has been reported in INRA 410 sheep. The results showed that the heritability of FEC of *H. contortus* ranged from 0.39 to 0.48 and genetic correlation between FEC after the first and second infection with the same or different species was near 1. The similar heritability (0.47) was found with *T. colubriformis* and genetic correlation within and between species was also near to 1. These studies indicated that the faecal egg count might assist in the selection of sheep for resistance to nematode parasite (Gruner *et al*., 2003) [11].

Genetic Variation between Breeds

The genetic variation between sheep breeds in the levels of resistance to internal parasite is of significant interest to animal breeder for economic reasons (Beh and Maddox, 1996) [28]. It has been established that some sheep breeds are more resistant to nematode infection compared to other breeds. For example, Bahirathan *et al*. (1996) [29] and Miller *et al*. (1998) [12] showed that Gulf coast native sheep are more resistant than Suffolk sheep. In Indonesia, Romjali *et al*. (1997) [30] found that introduced St Croix ewes are more resistant than local Sumatra ewes to the parasites. The Indonesian Thin Tail sheep exhibit superior resistance to *H. contortus* (Subandriyo *et al* 1996) [31] and express a very high level of innate and acquired resistance to *Fasciola gigantica* as compared to Indonesian Fat Tail and Merino Sheep. Wanyangu *et al*. (1997) [32] found that the Red Maasai sheep were more resistant to *H. contortus* than Dorper sheep, by producing lower FEC and higher immunological parameters after artificial infection with parasite. Yadav *et al*. (1993) [17] investigated the differences in susceptibility to *H. contortus* infection between seven lambs each of two different crossbreeds: Nali X Lohi (Munjal) and Nali X Corriedale (Hisardale). They found the body weight gain, haemoglobin and packed cell volume after artificial infection with *H. contortus* to be significantly lower in Hisardale lambs and the peripheral eosinophil count to be significantly higher in Munjal lambs. They conclude that Hisardale lambs have significantly greater susceptibility to experimental H contortus infection than Munjal lambs and suggest that these genetic differences in susceptibility should be investigated with an appropriate experimental design. Nimbhkar *et al*. (2003) [10] found the Garole sheep known for its prolificacy to be more resistant to gastrointestinal nematodes.

Mechanism of Genetic Resistance

Genetically resistant sheep are increasingly considered as viable alternatives to increase animal production and to learn novel mechanisms of resistance not described in commercial breeds (Amarante *et al*., 2004) [33]. Importantly, gastrointestinal nematodes parasites may not adapt to these resistance mechanisms in such selected sheep. However, to date the mechanisms underlying the genetic resistance of sheep to gastrointestinal nematodes infections are largely unknown. Fecundity (faecal egg counts and eggs in utero) has been shown to correlate positively with worm burdens and worm length. These significant correlations between parasitological parameters are often reported in studies where the early parasitic larval stages are proposed as the target of immunity and little direct effect is seen on the surviving adult populations. In resistant sheep the latter mechanism against adult worms is more prominent, while susceptible sheep fight the early parasitic larval stages of helminths (Kemper *et al*., 2009) [34].

The immune response of sheep to nematode parasites depends on Th2 cytokines such as IL-4, IL-5 and IL-13 which recruit mast cells and eosinophils into the abomasal and intestinal mucosa. These recruited cells release potent inflammatory mediators, such as histamine, and also arachidonic acid metabolites such as leukotrienes and prostaglandins. These mediators, as well as potent vasodilators such as bradykinin, probably act to remove worm larvae by causing leakage of plasma protein into the abomasum and intestinal lumen, and contracting nonvascular smooth muscle. (Williams *et al*., 2010) [35].

Genetic markers for resistance to gastrointestinal nematodes infection

The first genetic marker used in the selection of resistance was hemoglobin type. Sheep have two alleles (A and B) for hemoglobin and animals with haemoglobin type AA were more resistant than AB, which more resistant than BB with *H. contortus* infection. However, these haemoglobin types could not be effective as markers for resistance to *H. contortus.* The second attempt was the use of candidate gene approach to investigate the major histocompatability complex (MHC) variability. The MHC consists of a group of closely linked genes involved in antigen presentation to the vertebrate immune system and shows extremely high levels of heterogeneity at certain genes contained within the complex (Klein *et al*., 1993) [36]. Compared to other domesticated species, sheep MHC is poorly characterized and have distinct class I and II regions. Sheep MHC class II gene has been shown to be highly polymorphic and believed to play a major role in immune defense against macroparasites. Allelic variations have been reported in different DQA1, DQA2, DQB, DRA and DRB loci of MHC class II region in sheep. Although, majority of the polymorphism in sheep appears to be greatest in exon 2 which, in both A and B genes, encodes the antigen binding groove of the expressed protein (Escayg *et al*., 1996) [37]. Grain *et al*. (1993) [38] reported the restriction fragment length polymorphism (RFLP) of DQB and DRB class II genes of ovine MHC using probes, but no genetic association with parasite resistance could be established. A microsatelllite region next to intron 2 of MHC-DRB gene of sheep was amplified. The SSCP (single strand conformational polymorphism) technique demonstrated that the segregation of alleles was found with both of intronic microsatellites and exon 2 variable regions (Outteridge *et al*., 1996) [40].

Candidate gene analysis

Candidate genes are genes with known biological function that directly or indirectly regulating the developing processes of the investigated disease. The genes have been shown to be related to disease and more likely to find associations with the target disease traits. The basic idea is to analyze the mutations in susceptible/resistant animals, or different breeds with

different susceptibility to a infection. Alleles based on the mutations find in these genes may be useful markers for disease resistance breeding. Molecular markers, revealing polymorphisms at the DNA level, especially Single Nucleotide Polymorphism are being used for this type of polymorphism and subsequent association studies.

Candidate Genes on Disease Resistant Traits

1. Major Histocompatability Complex Gene

Major Histocompatability Complex (MHC) is a gene family found in most vertebrates. It plays an important role in the immune system, autoimmunity and reproductive success. The proteins from this gene encoded by the MHC are expressed on the surface of cells and these proteins display antigen to a type of white blood cell. This white blood cell has the capacity to kill or coordinate the killing of pathogens, infected or malfunctioning cells.

The association between MHC-DRB1 allele and fecal egg count following natural *O. circumcinta* infection in Scottish Blackface sheep was reported. Nineteen DRB1 alleles were identified within the intron between exon 2 and 3 and suggests that the MHC complex plays an important role in the development of resistance to *O. circumcinta* (Schwaiger *et al*., 1995) [41]. Paterson *et al*. (1998) [42] analyzed MHC variation in Soay sheep using five microsatellite markers. Markers OLADRB and OLADRBps are located within MHC class II expressed and non-expressed genes, respectively, while OMHC1 is located within the MHC class I region (Groth and Wetherall, 1994) [43] and BM1815 and BM1818 used as flanking markers. They found that OLA-DRB locus is strongly associated with juvenile survival and alleles significantly associated with parasite resistance in lambs and yearling. Interestingly, the OLADRB 257 allele was significantly associated with both decreased parasite resistance and decrease survival in lambs, while the OLADRB 263 allele is associated both increased parasite resistance and increased survival in yearlings. It has been concluded that the parasites are likely to play a major role in the maintenance of MHC diversity in the population.

2. Toll-Like Receptors

Toll-Like Receptors (TLR) are important components of innate immune system. TLRs form an ancient gene group which is found in invertebrates and vertebrates with related genes in plants (Werling and Jungi, 2003) [44]. It is now well established that in addition to their role in defense against pathogens, the dysregulation of TLRs result in increase of uncontrolled inflammation and metabolic syndromes, which contributes to the development of chronic diseases like, atherosclerosis, rheumatoid arthritis and cancer. TLR gene family has been reported as a promising molecular marker for correlation between host immune responses and bacterial pathogens in mastitis. The term "Toll-like receptors" was proposed in 1997 for mammalian proteins structurally related to the "TOLL" cell surface receptor seen in Drosophila larvae I TolI' means amazing or mad in German. Drosophila Toll (dToll) was the first member of the TLR family to be identified. The Drosophila toll protein was shown to be involved in dorso-ventral pattern formation in fly embryos and implicated as a key component of host immunity against fungal infection (Hashimoto *et al*., 1998) [45]. A year after the discovery of the Drosophila Toll, a mammalian homologue was identified, which together with CD14 molecule, forms the lipopolysaccharide receptor complex. Up to 14 TLRs have been identified in different species, either enabling the host to recognize bacterial components (TLRs 1, 2, 4, 5, 6 and 11), RNAIDNA components (TLRs 3, 7, 8 and 9) or with no known function (TLRs 10, 12, 13 and 14) (Werling and Coffey, 2007) [46]. TLR1 and TLR9 are conserved in both humans and mice. TLRI0 is expressed in human, while TLR11 to TL.R13 are present in mice. In cattle sequences, 10 TLRs have been described, and each TLR is capable of recognizing a distinct PAMP (Werling *et al*., 2006) [47].

Mitra *et al*. (2012) [48] studied TLR4 in Murrah buffalo population in an attempt to sequence nucleotide and detect SNP by PCR-RFLP. Analysis of sequence data by multiple alignments revealed a total of 12 SNPs out of which six were non-synonymous resulting in aminoacid change. Basic Local Alignment Search Tool (BLAST) revealed 97, 97, 99, 98 and 80 per cent sequence homology with Bostaurus, Bosindicus, Ovisaries, Capra hircus and Homo sapiens respectively. Homology identity between TLR4 mRNA of cattle and other species was performed by Wang *et al*. (2008) [49]. They found 97, 84, 81, and 73 per cent identity with sheep, pig, human and mouse respectively. The homology identity of TLR4 protein between cattle and other animal species from sheep, porcine, human and murine were 96, 81, 75 and 66 per cent respectively. The polymorphisms within TLRs were studied by Mariotti *et al*. (2009) [50] using nine primers, which resulted in eight SNPs (three in TLR2, three in TLR4 and two in TLR6). White *et al*. (2003) [51] studied the haplotype variation and predicted positively selected ligand-binding domains in TLR4 gene in cattle belonging to various breeds. Thirty two SNPs were found, out of which 28 were in coding region.

Toll-like receptors are vital for the detection of invading pathogens and are commonly expressed in antigen presenting cells and other immune cells. In resistant sheep infected with *H. contortus* and *T. colubriformis*, upregulation of several TLR genes, including TLR4, was observed in the abomasum. In the same study, susceptible individuals presented lower expression of this gene. Contrary to sheep, susceptible Angus yearlings infected with *Ostertagia*, *Cooperia and Nematodirus spp*., TLR4 showed higher expression in the mesenteric lymph nodes. Resistance to gastrointestinal parasites such as *H. contortus* is likely to be controlled by many loci. Different immune response mechanisms in sheep are used to control *H. Contortus* (Estrada-Reyes *et al*., 2019) [5].

Conclusion

Developing disease resistance population would be more practical if the resistant individuals could be identified with the help of some indicators traits. A number of advantages of incorporating genetic elements in disease management strategies includes the genetic change once it is established it will be permanent, the resistance is consistent, increasing resistance to more than one disease and adding to the diversity of disease management strategies. The information about the genetic constitution of domestic animals and genes controlling mechanisms involved in natural and adaptive resistance and disease pathology will be very helpful in applying genetic selection of livestock for disease resistance. The identification of the genetic markers will enable the use of marker assisted selection to increase the accuracy of selection in breeding programmes. This knowledge may lead to a better understanding of the mechanisms of susceptibility and resistance of hosts to gastrointestinal nematodes. This

will have positive effect of reduced use of anthelmintic drugs, reduced contamination of the pasture and slow down of spread of anthelmintic resistance. Understanding the genetic and molecular basis of disease resistance also has many advantages and applications such as the development of novel genetic markers for inclusion in genetic improvement programmes.

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