STUDIES ON ASSOCIATION BETWEEN POLYMORPHISM OF MHC II GENES AND *Haemonchus contortus* RESISTANCE IN THE SHEEP AND GOATS

Thesis

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF VETERINARY SCIENCE

in

VETERINARY PARASITOLOGY

By

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To

DEEMED UNIVERSITY

INDIAN VETERINARY RESEARCH INSTITUTE

Izatnagar – 243 122 (U.P.)

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भारतीय पशु चिकित्सा अनुसंधान संस्थान
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Dated: 12, 6 , 2012

Certificate

Certified that the research work embodied in this thesis entitled **"Studies on association between polymorphism of MHC II genes and *Haemonchus contortus* resistance in the sheep and goats"** *submitted by* **Dr. Vikram Singh Khobra, Roll No. 4965,** *for the award of* **Master of Veterinary Science** *degree in* **Veterinary Parasitology** *at Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate himself under my supervision and guidance.*

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(S.C. GUPTA)
Chairman
Advisory Committee

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Certified that the thesis entitled, **"Studies on association between polymorphism of MHC II genes and *Haemonchus contortus* resistance in the sheep and goats"** submitted by Dr. Vikram Singh Khobra, Roll No. 4965, in partial fulfilment of the requirement of Master of Veterinary Science degree in Veterinary Parasitology, Deemed University, Indian Veterinary Research Institute, Izatnagar, embodies the original work done by the candidate. The candidate has carried out his work sincerely and methodically.

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

It is further certified that the candidate has completed all the prescribed requirements governing the award of Master of Veterinary Science of Indian Veterinary Research Institute.

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(Vikram Singh Khobra)

Abbreviations

A	:	Adenine
Aa	:	Amino acid
A_{260}/A_{280}	:	Absorbance at 260 and 280 nm
AE buffer	:	Elusion buffer
AL buffer	:	Binding buffer
APS	:	Ammonium per-sulphate
APC	:	Antigen presenting cells
AW buffer	:	Washing buffer
bp	:	Base pair
BlastN	:	Basic local alignment search tool
BoLA	:	Bovine leukocyte antigen
C	:	Cytosine
CLA/Cahi	:	Caprine leukocyte antigen
CSWRI	:	Central Sheep and Wool Research Institute
DC	:	Dendritic cells
CD	:	Cluster of differenciation
DLA	:	Dog leukocyte antigen
dNTP	:	Deoxy nucleotide tri-phosphate
DNA	:	De-oxy Ribonucleic Acid
DW	:	Distilled Water
EDTA	:	Ethylene diamine tetra acetic acid
EPG	:	Egg per gram
BPB	:	Bromophenolblue
EtBr	:	Ethidium Bromide
Fig	:	Figure
Frd	:	Forward
G	:	Guanine
gDNA	:	Genomic DNA
GIN	:	Gastrointestinal nematode
Hb	:	Haemoglobin
HLA	:	Human leukocyte antigen
i.e.	:	That is
IVRI	:	Indian Veterinary Research Institute
Kbp	:	Kilo base pair
kDa	:	Kilo Dalton

$MgCl_2$	:	Magnesium Chloride
MHC	:	Major histocompatibility complex
Min	:	Minute
ml	:	Milliliter
No	:	Number
NFW	:	Nuclease Free Water
nt	:	Nucleotide
OD	:	Optical Density
OLA/Ovar	:	Ovine leukocyte antigen
pmol	:	Picomole
PAGE	:	Polyacrylamide Gel Electrophoresis
PBC	:	Peptide binding Cleft
PCR	:	Polymerase Chain Reaction
PCV	:	Packed cell volume
pH	:	Log hydrogen ion concentration
RLA	:	Rabbit leukocyte antigen
RE	:	Restriction Endonuclease
RFLP	:	Restriction fragment length polymorphism
RNA	:	Ribonucleic Acid
rpm	:	Revolutions per minute
SBT	:	Sequence based typing
SE	:	Standard error
SLA	:	Swine leukocyte antigen
SSCP	:	Single Strand conformation polymorphism
SNP	:	Single nucleotide polymorphism
T	:	Thymine
TAE	:	Tris Acetate EDTA
Taq	:	*Thermus aquaticus*
TE	:	Tris EDTA
TEMED	:	N,N,N',N'-Tetramethyl ethylene diamine
Th	:	T helper
Tm	:	Melting Temperature
Tris	:	Tris-hydroxy methyl aminoethane
v/v	:	Volume/volume
UV	:	Ultra Violet
µl	:	Microliter

UNITS OF MEASUREMENT

µ	:	Micron
µg	:	Microgram
µl	:	Microlitre
µM	:	Micromolar
%	:	Percent
°C	:	Degree Celsius
Cm	:	Centimetre
g	:	Gram
h	:	Hours
IU	:	International units
L	:	Litre
M	:	Molar
mg	:	Milligram
min	:	Minutes
ml	:	Milliliter
mM	:	Milli Molar
N	:	Normallity
ng	:	Nano gram
nm	:	Nanometer
rpm	:	Revolutions per minute
sec	:	Second (s)
U	:	Unit(s)
V	:	Volts

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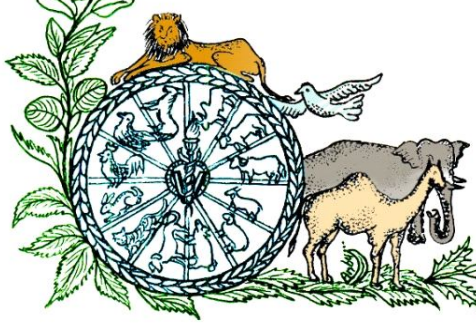


1 Introduction

Increased population and urbanisation in 'developing' countries lead to a strong demand for animal products and world demand for animal protein is expected to rise by 50% by 2020. Efforts to meet these demands in developing countries are focused primarily on increasing numbers of animals rather than increasing productivity per animal. It has led to degradation of rural areas, clearing of forests, rapid increases in the incidence of zoonotic diseases and a build-up of pesticides in the food chain. To avoid further degradation of the environment and meet the increased demand for animal products, increments to production will have to come from higher productivity of meat and milk per unit of land (Steinfeld, 2004). However, animal diseases particularly parasitic diseases are major constraint to obtain optimum productivity. According to the World Organisation for Animal Health, helminthosis (i.e. disease caused by GI parasites) is found to be the top ranking disease, followed by neonatal mortality, foot and mouth disease and ectoparasites.

Among the diseases that constrain the survival and productivity of sheep and goats, gastrointestinal nematodes (GINs) infection ranks highest on a global index, with *Haemonchus contortus* being of overwhelming importance (Perry *et al.,* 2002). Along with *H. contortus*, *Trichostrongylus colubriformis* and *Oesophagostomum columbianum* are common in small ruminants in India. These parasites are highly prevalent in the tropics/subtropics where temperatures and rainfall which generally favours the development and survival. Not only is the environmental conditions favourable, more-or-less continuously, for the free-living stages are endemic in many part of the world. Annual treatment costs due to *H.contortus* alone have been estimated to be $26m, $46m and $103m for Kenya (Anon, 2004), South Africa (Horak,

pers. comm.) and India (McLeod, 2004), respectively. The losses due to GINs are over AUD 400 million annually only in Australian small ruminant industries (Sacket *et al*., 2006).

Besides, other strongylides like *Trichostrongylus* spp. *Teladorsagia* spp., *Cooperia spp*. and *Nematodirus spp*. are also known to affect small ruminants in different pockets of India. Among the GINs, *H. contortus* is prevalent in tropical, subtropical and temperate regions, especially under warm and wet conditions (Jabbar *et al*., 2008; Paraud *et al*., 2010; Khan *et al*., 2010). It is a highly fecund and voracious blood-sucking parasite of the sheep/ goat abomasum and causes significant production losses, especially in growing lambs due to haemorrhages, anorexia, depression, severe chronic anaemia, loss of condition and eventually death of the affected animals (Allonby 1975; Soulsby, 1982; Notter *et al.,* 2003), there considered as most pathogenic parasites of sheep and goats (Kassai, 1999).

The control of nematode parasites traditionally relies on grazing management, anthelmintic treatment, or both are used in developed countries. However, grazing management schemes are often impractical due to expense or to the hardiness of infective larvae on pasture. Further, grazing lands are fragmented and un-organised in developing countries like India. Therefore, the control of GIN parasites mainly relies on chemical treatment (Barger, 1999). Due to continuous/irrational use of anthelminths, there is widespread resistance against anthelmintics and there is serious occupation exposure and environmental impact (Risher *et al*., 1987; Spratt, 1997; Schneider *et al*., 2003). The anthelmintic resistant gastro-intestinal nematode populations constitute a major problem not only in the subtropics and tropics, but also a serious threat to livestock in rest of the world (Conder and Campbell, 1995; Waller, 1997; Sangster, 1999). Anthelmintic resistance in nematode parasites of almost all species of animals is now a firmly established phenomenon througout the world. What is of greatest concern is the fact that multiple resistance (i.e. resistance to all three groups of drugs) can occur and when it does, it can render farm animal production unsustainable. This has already occurred in many parts of South Africa, Australia, North America and particularly South America (Waller *et al*., 1995; Taylor, 1999).

Many alternative strategies are developed and tried to control of nematode infections with varying degree of success. Ethno-veterinary medicines have shown some potential as anthelmintics (Iqbal *et al*., 2001), but they cannot be marketed until appropriate dosage,

toxicity are active ingredients with mode of action are known. In contrast to other methods of control of nematode parasites of livestock, biological control is directed at the free-living stages, rather than parasitic stages within the host. Many micro-organisms have been identified as predators, pathogens or parasites of nematodes (Waller and Faedo, 1996) and the most promising candidates as possible bio-control agents of animal parasitic nematodes are the nematophagous fungi (Gronvold *et al*., 1996; Waller, 1997). Biological control has many obvious attractions and advantages over other non-chemotherapeutic means of parasite control. For example, it will be applicable to the range of nematode parasites not only within, but also between, species of livestock. Finally, because the mechanisms by which fungi kill larvae are complex and multifactorial, it is also difficult to envisage worms developing resistance mechanisms to fungal trapping.

One such approach is the characterization and utilization of host genetic variation for resistance or resilience to endo-parasites. A relatively simple and cheap method of reducing the effects of nematode infestation would be selection and breeding of genetically nematode-resistant animals (Baker, 1999; Bishop and Stear, 1999). Unlike temperate region, one great advantage of a number of indigenous tropical breeds of livestock is their genetic ability to tolerate or resist disease. This has been particularly well demonstrated for resistance to nematode parasites in sheep breeds. The best examples are the East Africa Maasai, Florida Native, Barbados Blackbelly and the St. Croix, but this is likely to apply to many other, as yet untested, breeds (Baker, 1999). As per FAO's Domestic Animal Diversity Information System (DAD-IS), Currently four goat breeds (Carpatina, Cashgora, Jamnapari, Katjang Yei) and 13 sheep breeds (Churra Lebrijana, Criolla Mora, Criollo, Garut, Gulf Coast Native, Kumumawa, Madagascar, Malin (Malaysian Indigenous), Morada Nova, Priangan, Rahmani, Solognot, Tsigai) were reported to DAD-IS as having resistance or tolerance to a certain degree against parasitic diseases in general or against specific parasites.

Selection of animals, which are genetically resistant to infection by worms, is an attractive approach because resistance traits are readily disseminated through the sale of resistant animals, and it can provide long-term solutions. Resistance also reduce pasture contamination which in turn reduces reinfection of animals. Animals are showing resistance to one parasite found to be resistance against other parasite also. Such reduction of the reinfection leads to

improvement in both productivity and animal health. In developing due to lack of intensive selection for production and reproduction, the breed develops some parasitic resistance mechanism, however in developed countries due to intensive selection of animals for production trait leads to increase susceptibility to parasitic infections. Similarly, development and maintenance of resilience/tolerance through nutritional management (Burke and Miller, 2002) on a large scale in many parts of the world is not possible.

A number of studies were performed to identify QTLs (Quantitative Traits Loci) for resistance to gastrointestinal nematodes in ruminants. Divergent sheep selection lines resistant and (or) susceptible to nematode parasites were used to find the respective QTLs (Charon, 2004). The QTL for resistance was localized in chromosome 3, and mapped to about a 5 cM region. The gene located to this region codes for the interferon gamma (IFNG) and is considered a putative candidate gene for resistance to nematode parasites. The ovine major histocompatibility complex (MHC) has been consistently associated with nematode resistance (Schwaiger *et al*., 1995; Dukkipati *et al*., 2006).

Polymorphism in MHC class II antigen is playing important role in resistance to helminths. The widest polymorphism among the MHC genes is found in *locus DRB*. This gene encodes the beta chain of the DR molecule, protein found in high concentrations on the surface of antigen-presenting cells. The most frequently investigated fragment of the *DRB* gene covers exon 2, which codes the binding site for a foreign protein. In a study showed that DRB1 class II antigen was associated with 10-fold reduction in faecal egg count (Schwaiger *et al*., 1995). This result and results of others demonstrate the significant role of the MHC in ruminant resistance to parasites (Outteridge *et al*., 1996; Paterson *et al*., 1998; Van Haeringen *et al*., 1999). The polymorphism of the MHC complex increases the range of parasites recognized by the immune system.

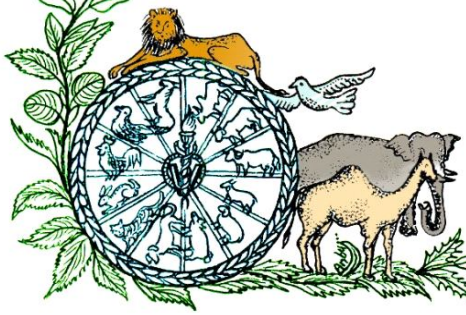
The immune mechanisms responsible for resistance are not fully understood (Andronicos *et al*., 2010; Kemper *et al*., 2009; Meeusen, 1999) however, it is generally agreed that the immune system plays a key role in the manifestation of inherited resistance (Wakelin, 1985) and is polygenic in nature (Kemper *et al*., 2009). GINs induce a Th type 2 (Th2) antibody-dependent responses (Andronicos *et al.,* 2010; Svetic *et al*., 1993), whereas intracellular parasites induce a Th type 1 response. Th2 responses involve the influx of inflammatory cells

into the local mucosa and generation of immunoglobulin (IgE) antibody (Huntley *et al*., 2001; Harrison *et al*., 1999).

Keeping in view the above facts, the present study was proposed with the following objectives:

1. **To investigate the polymorphism in DQA1 and DRB1 gene of sheep and DQA1 and DRB3 gene of goat**
2. **To study the association of polymorphism in *Ovar* and *Cahi* MHC genes with resistance to *Haemonchus contortus***

Review of Literature



2 Review of Literature

Internal parasite infections pose a major health problem to domestic livestock worldwide, particularly in those animals reared in the grazing livestock systems. In sheep and goats, gastrointestinal nematode (GIN) parasites cause huge economic losses. Economic losses are caused by GI nematodes in a variety of ways. Parasitism causes a reduction in food intake and lower weight gains, milk production and mortality can occur in heavily parasitised animals. The direct losses are also because of the control of the infection and veterinary care. Additionally, there are indirect losses due to a reduction in performance, which is in many cases caused by the subclinical effects of the parasitism and also the parasitic infections may predispose to other bacterial and viral infections. Several studies have shown that subclinical diseases can result in a reduction in live weight, wool weight and reproductive performances. Effective control of internal parasites in small ruminants is one of the most difficult challenges encountered by veterinary in practice. The last decade has witnessed a major contraction throughout the world in the number of research centers and staff involved in applied veterinary parasitology research. This coincides with a time when these livestock industries need the most help. Resistance to anthelmintic drugs amongst the major nematode parasites of sheep and goats has now reached alarming proportions throughout the world and threatens the future viability of continued small ruminant production in many countries (Fleming *et al*., 2006; Larson, 2006).

GI parasitism is very common in tropics and sub-tropic due to temperature and humidity. These environmental conditions promote survival, rapid growth of nematodes and extended periods of transmission, which are making the parasites prevalent for most of the year (Kaplan, 2004). Gastrointestinal nematodes causes various gastrointestinal pathology depend upon the

location of parasite, level of infection, immune status of the animal. Important species of nematode affecting ruminants in the tropics include the abomasa worms *Haemonchus* spp., blood-sucking worms that cause anaemia and weight loss, *Teladorsagia circumcincta* causes atrophy/ hypertrophy of gastric glands and *Trichostrongylus axei,* which causes gastritis, diarrhoea and weight loss; worms of the small intestine such as *Trichostrongylus colubriformis* and *Cooperia* spp.*,* heavy infestations of which cause villous atrophy, diarrhoea, loss of condition and loss of appetite; *Bunostomum* and *Gaigeria* hookworms of the small intestine, which can cause anaemia due to blood sucking, diarrhoea and loss of condition; and *Oesophagostomum* spp., worms of the large intestine, which cause diarrhoea and loss of condition (Hunter 1994; Hall, 1985). It has been calculated that internal parasitism, largely by trichostrongylid nematodes, causes losses equivalent to 19 percent of the sheep industry (Barriga, 1997). Stomach/intestinal worms have been and still are the number one concern of small ruminants industries.

The extensive proliferation of epithelial cells in parasitized gastrointestinal tract results in replacement of differentiated functional cells with immature immature non-functional cells with imperfectly formed intercellular junctional complex (Murray *et al*., 1970). This results in leakage of macromolecules through the mucosa into the intestine. Leakage of protein into intestine leads to hypoproteinaemia particularly hypoalbuminaemia.

There are three phases of hypoalbuminaemia: In the initial phase, there is fairly rapid fall (20-30%) in serum albumin level and in the second phase, the host compensates for the protein loss by adjusting endogenous protein catabolism. These alterations in protein metabolism are reflected by an increase in the incorporation of amino acids into liver protein synthesis, associated with increased plasma protein synthesis. The albumin level may be below normal however at the same time there is reduced skeletal muscle protein synthesis. In the third phase, severe hypoalbuminaemia is seen due to exhaustion of body reserve. In the infected animal due to deamination of amino acids there is elevated plasma urea level and increased excretion of urinary nitrogen contribute to inferior nitrogen balance (Roseby and Leng 1974). There is increased production of HCl by the parietal cells and resultant lack of conversion of pepsinogen to pepsin (Murray *et al*., 1970) and interfere with protein digestion in oestertagiosis. Villus atrophy and deficiency of brush border enzymes like alkaline phosphatise, maltase and dipeptidase were observed in trichostrongylosis and cooperiosis. Gastrointestinal parasitism

also induces disorder in mineral metabolism. There is reduced absorption of calcium, phosphorus, magnesium results in reduced bone growth in young animals (Reveron *et al*., 1974).

Epidemiology of gastrointestinal nematodosis in India

The epidemiology of gastrointestinal nematodes is governed by weather conditions particularly precipitation, temperature and humidity and management practices that regulate the development as well as survival of exogenous stages of parasites (Bali, 1973, Mishra *et al*., 1974; Dhar *et al*., 1982) The epidemiological picture of ovine GIN in India can be described under 4 major agroclimatic zones:

Northern Plains:

Predominant parasites: *H. contortus, Oesophagostomum spp., Trichostrongylus spp., Bunostomum spp.*

The usual monsoon period in this region remained from June to October causing high rise of GIN in small ruminants during monsoon and post monsoon period (Ahmed and Ansari, 1987; Mishra *et al*., 1974; Bali, 1973).The incidence of *H. contortus* was maximum during July to October and minimum during March to June. The climatic conditions favourable for development of trichostrongyle larvae on pasture during February to April lead to occurrence of peri-parturient rise (PPR) in faecal egg counts (Gupta *et al.*, 1988). However, hypobiosis was reported to be absent. The occurrence of *H. contortus* and *Trichostrongylus spp*. was throughout the year while prevalence of *Oesophagostomum spp.* was restricted mainly from August to November and *Ostertagia spp*. was recorded in the month of May (Anon, 2008).

Semiarid western Region

Predominant parasites: *H. contortus, Trichostrongylus spp., Oesophagostomum spp.*

Most of precipitation occurs from July to September in this region. Therefore, exogenous stages of parasites level in the pasture is heavy from July to October and peak intensity of infection in host was recorded from July to early September and in young from august to early November (Singh *et al*., 2005; Khan *et al.*, 1999). Maximum survival period of infective

larva on pasture was 9 weeks in September and 2 months resting period was required to sterilize the contaminated pasture. Intensity of infection started rising with onset of monsoon and persisted up to September, followed by decline in succeeding months. Comparatively higher prevalence of *Trichostrongylus spp.* was recorded in arid region of Rajasthan than semiarid region. (Swarnkar *et al.,* 1997). Incidence of gastro-intestinal nematodes was higher during monsoon in all climatic zones of Rajasthan.

Sub-temperate southern Humid Region:

Predominant parasites*: H. contortus, Trichostrongylus spp., Oesophagostomum spp.*

Rainy and autumn seasons (June to November) are best suited for survival and migration of exogenous stages of nematodes (Sanyal, 1989) and a higher incidence of GIN was recorded during south-west and north-east monsoon (Anon, 2004). Gour (1984) observed prevalence of *H. contortus* (70%), *T. colubriformis* (20%), *O. venulosum* (20%) and occasional *Strongyloides* in sheep and goats of this region. The migration of infective larvae on grass blades was more in autumn, while all the pre-parasitic activities were low in winter due to scanty rainfall and moderate to heavy winter and the lowest pasture contamination was observed in the month of December which is attributed to harsh climate as a result of low temperature and sparse rainfall. The survivability of infective larvae was more than 11 weeks in rainy and post rainy season.

Temperate and Sub Temperate Himalayan Region:

Predominant parasites: *H. contortus, Trichostrongylus spp, Bunostomum spp*, *Teladorsagia circumcincta, Oesophagostomum spp*

The monsoon period started by middle of June and exists up to early September. The moderate temperature makes the environment favourable for development and survival of pre parasitic stages leading to availability of larvae of pasture. Peak intensity of infection in host occurred from July to September with maximum incidence from March to November (Dhar *et al.,* 1982).

Chemical control

Broad Spectrum Anthelmintics

Inhibition of microtubule formation, beta-tubulin binding

Benzimidazoles and its prodrugs

Benzimidazoles (BZ) are one of the broad-spectrum antihelminthics, most widely used to control gastrointestinal nematode infections in small domestic ruminants. It has high therapeutic index, absence of drug residue in milk and meat and economically viable (Humbert *et al*., 2001). Prodrugs of benzimidazoles are converted to active benzimidazoles by metabolic processes in the host animal so that it is the active metabolites that are responsible for the anthelmintic action. Friedman (1979) showed that BZs selectively bind to tubulin in parasitic nematodes leading to inhibition of microtubule formation. Alpha- and beta-tubulin molecules are soluble proteins which aggregate to form heterodimers, the building blocks of the insoluble polymeric microtubules. These undergo a constant process of proliferation and dissociation at their opposite ends (Lacey, 1988). Microtubules play a fundamental role in several important cell functions, like cell division, shape and motility or intracellular substrate transport. These actions reduce the absorption of nutrients in the parasite and leads to death of parasite. When alpha-/beta-tubulin with bound BZ is incorporated into the growing end of the microtubule, further heterodimers are prevented from being associated. This process was termed 'capping' (Lacey, 1988). Since dissociation continues at the opposite end, the microtubules become degraded and finally completely disappear. The role of tubulin as the BZ target molecule was further substantiated by Russell and Lacey (1992) who found that BZ-binding was significantly reduced in BZ-resistant as compared with BZ-susceptible *H. contortus* and *T. colubriformis* isolates.

The tertiary benzimidazoles, such as fenbendazole, oxfendazole and albendazole, are most potent anthelmintics. They remain in the host body for longer periods (15 to 24 hours) after dosing as compared to thiabendazole, oxibendazole and parbendazole (4 to 6 hours) (Prichard *et al.*, 1978).

Nicotinic agonists

Nicotinic agonists are the imidazothiazoles (levamisole and butamisole); the tetrahydropyrimidines (pyrantel, morantel and oxantel); the quaternary ammonium salts

(bephenium and thenium) and the pyrimidines (methyridine). These compounds act selectively as agonists at synaptic and extrasynaptic nicotinic acetylcholine receptors on nematode muscle cells and produce contraction and spastic paralysis. Following oral administration, they are absorbed by the host almost immediately and cause rapid paralysis in those parasites that are exposed (Prichard *et al.*, 1978). Once paralysed, the nematodes are swept out of the host along with the ingesta.

Glutamate-gated chloride (Glucl) receptor potentiators

Ivermectin, abamectin, doramectin, milbemycin D, moxidectin

The avermectins are a group of broadspectrum, macrocyclic lactone antibiotic anthelmintics used to control nematode parasites inman and animals (Campbell & Benz, 1984), and assumed to have the same mode of action in both. The macrocyclic lactones, or the avermectins/ milbimycins, are a family of 16 membered macrocyclic lactones, originally isolated from an actinomycete, *Streptomyces avermitilis*. They are all potent nematocides and insecticides, thus commonly referred to as end-ectocides. They are used to control onchocerciasis (river blindness) in humans and gastrointestinal, cardiac and respiratory nematode parasites of domestic animals. The mode of action of the avermectins is to selectively paralyse the parasite by increasing muscle CI- permeability.

Organophosporus cholinesterase inhibitors

The mode of action of these compounds is to block the action of the parasite enzyme, acetylcholinesterase, leading to the excessive build-up of the neurotransmitter, acetylcholine. This mode of action also predisposes towards toxicity in the host animal where acetylcholinesterase enzymes are also present. Because more selective combined anthehnintic+insecticidal agents (avermectins and milbemycin) are available, the organophosphorus compounds are now used less frequently. Although acetylcholineesterase is responsible for the breakdown of acetylcholine and involved in motor action in nematodes, it is also secreted into the external environment in large quantities by parasitic nematodes. The function of the secreted acehtylcholinesterase may be to reduce the effects of host acetylcholine in the intestine, perhaps decreasing mucosal glandular secretion by the host. The identification of the function of secreted cholinesterase by parasitic nematodes, and the ability to antagonize

this enzyme, may lead to the increased use of anticholinesterases in the future to facilitate removal of gut nematodes (Martin, 1997).

Despite remarkable achievements in the discovery and development of anthelmintic drugs, nematode parasitic disease remains one of the greatest limiting factors to successful, and sustainable ruminant livestock production worldwide (Perry and Randolph, 2002). Modern anthelmintics are highly effective against the mature and immature stages of virtually all of the important gastrointestinal nematodes as well as many extra-intestinal nematodes. The extensive use and improper dosage of anthelmintics in conjunction with other factors has resulted in drug resistance, causing serious threat to effective control of helminth infections (Sangster, 1999).

Prichard (1978) defined resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and is heritable. Side resistance exists, where the resistance to a compound is the result by selection by another compound with a similar mode of action. Cross-resistance resembles side resistance but involves compounds with different modes of action. Multiple resistances occur when individuals are resistant to two or more different anthelmintic groups either as a result of selection by each group independently or as a result of cross-resistance. Reversion is a decrease in the frequency if resistant individuals in a population following removal of the selecting agent.

Development of anthelmintic resistance

Drug resistance can arise in a limited number of ways: (i) a change in the molecular target, so that the drug no longer recognizes the target and is thus ineffective (ii) a change in metabolism that inactivates or remove the drug, or that prevents its inactivation (iii) a change in the distribution of the drug in the target organism that prevents the drug from accessing its site of action or (iv) amplification of target genes to overcome drug action.

But benzimidazole resistance in nematodes is thought to be preadaptive phenomenon. This implies that resistant populations are often considered to be present in the normal population as a rare allele prior to use of any drug. When the resistant alleles are present as a rare allele in a population, the spread of resistance is more difficult to prevent. The anthelmintic treatments will inevitably constitute a selection pressure in favour of these rare alleles (Humbert *et al*., 2001).

Prichard (1990) summarized the intricate phases of the selected process such as susceptibility phase, intermediate phase and resistant phase. In susceptibility phase, the frequency of resistant individuals within the population is low. The intermediate phase develops when continued exposure to a drug, in which the frequency of heterozygous resistant individuals within the population increases. Finally sustained selection pressure results in resistant phase, where the homozygous resistant individuals predominate within the population. The selection of resistant is most rapid when both heterozygous and homozygous resistant individual survive treatments.

During last 10 -15 years there is increase resistance against anthelmintic used in domesticated animals. It threatens the survivability of small ruminant farming as well as the helminthologists. The problem is most severe in the countries of southern hemisphere like South Africa (Van Wyk *et al*., 1997), Australia (Waller *et al*., 1995), New Zealand (Kettle *et al*., 1983) and many other Latin American countries (Waller *et al.,* 1995). India is slowly and steadily emerging as the resistance epicenter of South Asia (Sanyal, 1998).

Biological control

Biological control is defined as the action of natural enemies, which maintain a host population at levels lower than would occur in the absence of enemies. This not only includes classical un-exploited organisms but also those that are genetically modified to enhance these properties (Waller and Faedo, 1996). The intention of using biological control methods is to lower the density of parasite population below the clinical level and perhaps below the economic threshold above which production losses are obvious owing to a high parasitic population density. All gastro-intestinal nematode parasites of livestock have a life-cycle which involves not only the parasitic stage within the host, but also a free-living or pre-parasitic stage on pasture. The pre-parasitic stages on the pasture are potentially vulnerable to attack by biological control agents. A number of organisms have been identified to exploit the free-living stages of parasites as food source. These organisms include micro-arthropods, protozoa, predacious nematodes, virus, bacteria and fungi. Among these entire biological agent fungi are most suitable for destruction of parasite.

Fungi that exhibit anti-nematode properties have been known for a long time. They consist of a great variety of species characterized by their ability to capture and exploit

nematodes. Nematophagous fungi are soil inhabitants and are found in most soil types throughout the world. Nematophagous fungi form a vegetative hyphal system that produces trapping organs such as sticky nets, knobs or rings (Hertzberg *et al*., 2002).These trapping organs penetrate the nematode cuticle and then grow out and fill the body of the nematode to finally digest it (Gronvold *et al*., 1996). They are divided into three major groups based on their morphology and types of nematode-destroying apparatus (Nordbring-Hertz, 1988; Barron, 1997). These are predacious, endo-parasitic and egg-parasitic fungi. Danish scientists first demonstrated through laboratory and field trials against pre-parasitic stages of nematodes by feeding chlamydospores of this fungus to cattle (Gronvold *et al*., 1996), horses (Fernandez *et al*., 1997), pigs (Nansen *et al.,* 1996) and sheep (Githigia *et al*., 1997).

India initiated the work on biological control of animal nematode parasites using mycological means in 1998 and two species of nematode-trapping fungi, *viz*., *Arthrobotrys oligospora* and *D. flagrans* and two species of egg parasitic fungi, *viz., Paecilomyces lilacinus* and *Verticillium chlamydosporium* were isolated from organic environment of Gujarat and Chhattisgarh (Sanyal, 1989). They were subjected to stringent screening for their suitability as bio-control agents against nematode parasites of ruminants using growth assay, predatory activity, germination potential and ability to survive ruminant gut passage. It is indicated that the isolates of *D. flagrans* and *V. chlamydosporium* fulfilled all the possible criteria. However, *D. flagrans* has been the species under special focus because its spores are able to survive the passage through the digestive tracts of several hosts and to maintain their viability and ability to colonize the host faeces and to exert predatory activity against the L3 larvae. *D. flagrans* produces trapping nets which lasts for approximately 2-3 weeks (Gronvold *et al*., 1996). The optimum temperature for the development of trapping nets is 30°C; a rise in temperature to 35°C and fungus activity when temperature fall below 10°C (Gronvold *et al*., 1996). Unlike the developed world, feeding grains to animals is not a regular husbandry practice in India. Therefore, incorporation of *D. flagrans* chlamydospores in concentrate feed pellets would be the ideal choice. The desiccated chlamydospores having long shelf life and can also use as top dressing of concentrate feed. The application of $1x10^6$ chlamydospores kg-1 body weight or more, virtually eliminated pre-parasitic larvae in both in vitro faecal cultures and on pasture, and there was a substantial reduction in larval numbers at doses of $1x10^5$ or $5x10^5$

chlamydospores kg-1 body weight. Hence, animals receiving as little as $1x10^5$ chlamydospores kg-1 body weight would excrete sufficient chlamydospores in their faeces to reduce larval numbers on pasture sufficiently to prevent substantial larval challenge to the grazing livestock. As the nematode parasites are mostly prevalent during monsoon months and such hot and humid conditions are also favourable for the germination of chlamydospores, feeding dried chalmydospores to ruminants as a top dressing component to concentrate feed at the onset of monsoon might be expected to result in a substantial reduction in the seasonal peaks of larval availability on pasture.

Pasture management

Management of animal farm and pastures is keys to reducing the amount of internal parasite problems in livestock. Internal parasitism in animals may be checked by using effective management strategies because major part of the parasite life cycle is occurring outside of the animal. Pasture rotations have always been a feature of small ruminants production systems and grazing management has been used to minimising the threat of nematode infestations for over 40 years. Although management of the grazing environment is an important weapon in the armoury for worm control, its successful implementationon farm requires detailed understanding of the farmand of the parasites on it. This complexity together with demands that grazing management makes upon types of stock and use of pasture, acts as a powerful deterrent, undoubtedly being among the reasons why grazing management is generally less well exploited.

Grazing management systems that operate without anthelmintics, such as the clean grazing system (Mitchell *et al*., 1984), which involve annual sheep, cattle and conservation rotations can be very effective, but only function wellwithin very tightly defined circumstances. Whenever grazing management is used in conjunction with anthelmintics, then some care has to be taken to ensure that there is sufficient refugia, since if animals are dosed when there are few unexposed worms on pasture or in the host, there can be a strong selection for drug resistance. The rapid rotational grazing strategy is an evasive strategy for application in the humid tropics. It relies firstly on the removal of existing nematode infections in the animals by anthelmintic treatment, secondly the movement of the treated animals to a safe pasture and thirdly (and most critical) to move animals before they re-infect (auto-infect) themselves.

Non -conventional anthelmintic treatment

Copper oxide wire particles (COWP)

The forms of copper most commonly used in animal feed are copper sulphate, copper oxide, copper carbonate, and tribasic copper chloride. Copper is a necessary trace element in the diet. Maximum immune response is dependent on copper as indicted by depressed antibody titers in deficient animals. Because of the effect that copper has on the body (man and animal) COWP have been used for many years to treat copper deficiency (Suttle, 1981; Judson *et al*., 1982, 1984; Langlands *et al.,* 1983; Dewey, 1997). But COWP are not only an efficient and effective means of treating copper deficiency in grazing livestock, it can also be potentially useful as an anthelmintic (Dewey, 1977). After dosing, COWP flow with ingesta from the rumen and lodge in the folds of the sheep's abomasum where the low pH induces the release of high concentrations of soluble copper, which have an adverse effect against abomasal species of nematodes (Knox *et al.*, 2001).

Botanical anthelmintics

In many developing countries around the world, farmers, herders, pastoralists and occasionally veterinary surgeons use plant or plant products to treat cases of parasitism. In traditional societies there seems to be a number of plant remedies deemed suitable for each parasitic disease. Many parts of the plants like, seeds or the foliage of plants such as garlic, onion, mint, walnuts, dill, or parsley have been used to treat animals that suffer from gastrointestinal parasitism, while cucumber and pumpkin seeds have been associated with the expulsion of tapeworms from the gastrointestinal tract (Guarrera, 1999). The use of ethno veterinary plant preparations has been documented from various parts of the world (Anon., 2004, 2008; Bizimana, 1994; Wanyama, 1997; Waller *et al.,* 1995). A number of plants with denoted anthelmintic properties are included in the British pharmacopoeia (British Veterinary Codex, 1953, 1965). For example, oil of chenopodium that derives from *Chenopodium ambrosioides*, was used for many years in the UK to treat nematode parasite. Leaves and dried flowers have been used as an anthelmintic since the early 1900s (Guarrera, 1999). Male fern *Dryopteris filixmas* and *Artemisia* spp. plants have been used against cestodes such as *Moniezia spp*. and nematodes, such as *Ascaridia spp*. in ruminants and poultry, respectively (British Veterinary Codex, 1965). Medicinal plants are the good alternative

for modern synthetic anthelmintics in the developing countries particularly in small farms where cost of chemotherapy will is high. Recent surveys in developing countries have identified many plants that are intended and have the potential to be used as anthelmintics. However, the majority of evidence reported in ethnoveterinary sources is in the form of observations, rather than from controlled studies (Hammond *et al*., 1997). Controlled studies, both *in vitro* and *in vivo* have in some cases supported (Idris *et al*., 1982; Hordegen *et al*., 2003) but in other cases rejected (Ketzis *et al*., 2002; Githiori *et al*., 2002, 2003) previously reported anthelmintic evidence of certain plants.

Despite ample evidence of anti-parasitic properties of several plants (or plant products), there is still a need to provide validated experimental data of biological meaningful reductions in infection levels to support the view that plants may play a direct role in the sustainable control of helminth infections under farming situations. This is the case with herbal remedies that show an effect after single or few administrations, and it is the case with the substantial amount of research available on forages rich in condensed tannins used for longer term grazing of small ruminants. As relatively few plants have shown high activity against nematode parasites within sheep and goats there is still a need to look further for plants with higher levels of anti-parasitic activity. Plant products that have showed high activity against nematode parasites *in vitro* need to be evaluated and tested in ruminant hosts. However, plants with moderate anthelmintic activity should still be considered; maybe not as a sole alternative to anthelmintic drugs, but as part of an integrated approach specifically designed to achieve sustainable parasite control in ruminant production systems.

Host/Nematode Interaction

To complete their life cycle, nematode parasites have to develop and lay eggs in the host. The host becomes infected by consuming infective third-stage larvae during grazing. After ingestion, the larvae lose their protective sheath and invade the mucosa of the abomasum, small intestine, or large intestine depending on which nematode is involved. While in the mucosa, larvae develop to the fourth larval stage and then return to the surface of the gut mucosa where they become adult worms. The major host defense mechanism is immunity. When infectious agents enter the body, the immune system reacts through a series of activities that mobilize various components (e.g., antibodies, lymphocytes, mast cells, eosinophils), which then attack

and eliminate the invaders. These components act on the larval stages in the mucosa and adults in the lumen. The immune system matures with age; therefore, young animals are most susceptible to infection and become more resistant with age. Young animals usually harbour the heaviest infection levels and suffer the most severe consequences. Adult animals have stronger immunity and usually harbour lower infection levels. The prepatent period (time from ingestion of infective larvae to mature egg-laying adults) of most nematodes is about 3 wk, but this period can be extended for those that have the capability to enter a period of delayed development called hypobiosis, where fourth-stage larvae stop development and remain in the mucosa for an extended period of 3 to 4 month. There is no apparent host response to these hypobiotic larvae. Hypobiosis usually occurs when there is insufficient moisture in the environment or temperatures are too cold for pasture larval development and survival. This can be in summer or winter depending on the nematode and geographical location. Two well-recognized (and thought to be immune-mediated) host responses to infection are immune exclusion and the self-cure phenomenon.

In immune exclusion, ingested larvae fail to establish in heavily infected animals (Miller *et al*., 1983; Newlands *et al*., 1990). If the infection is removed by deworming, then ingested larvae will become established. The self-cure phenomenon occurs when established adult nematodes are spontaneously expelled when there is a massive larval invasion over a very short exposure period (Stewart, 1955; Adams, 1983; Hong *et al*., 1989). This is usually observed after heavy rain that liberates large numbers of sequestered infective larvae from faeces. This larval invasion results in an increase in abomasal pH and thus nematode expulsion. ImmunoglobulinE (IgE)- mediated hypersensitivity are also involved.

Helminth immunity

The mechanisms by which nematodes are eliminated are unclear. Despite the significant efforts over the last three decades, it is still not possible to define exactly the immune response(s) remove parasites from the host owing to the high degree of complexity and redundancy among various immunological responses. Worms might be damaged directly by the effector cells and molecules of the immune system (Viney, 2002). Alternatively, they might be damaged by the physiological stress of their efforts to resist attack. Thus, the interaction between worms and the host immune response can be considered as the interaction of opposing forces in that

nematodes actively attempt to persist in the face of attack by the host immune response. At extremes, the outcome is either that the infection persists obviously or that the worms are killed or expelled (Amarante *et al.*, 2004). An intermediate outcome is that infection persists, but features of nematode survival and fecundity are reduced below some maximum (Viney, 2002).

The protective immune response against many helminth parasites has been variously referred to as the type 2 response, TH2 (T helper 2) response or TH2-type response. During helminth infection, parasite antigens are presented to CD4+ T cells in mesenteric lymph nodes and other gut-associated lymphoid tissues. Upon activation of CD4+ T cells, these cells produce various cytokines such as interleukin-4 (IL-4), IL−13, IL−9 and IL−5. Interleukin -5 acts on the eosinophils and triggers eosinophilia in blood and these eosinophils rapidly migrate to the site of infection, where they degranulate, releasing major basic protein-1 (MBP-1), eosinophil peroxidase (EPO), eosinophil derived neurotoxin (EDN) and eosinophil cationic protein (ECP). EPO and ECP are potent helminth toxins, MBP can induce histamine release from mast cells, while both EDN and ECP can act as ribonucleases. A major effector function of eosinophils and their eosinophil secondary granule proteins is tissue remodelling and debris clearance following tissue injury, a general activity that might help to mediate the wound healing response following parasite tissue invasion.

Eosinophils also have a regulatory role through the production of cytokines, including IL−4 and IL-13. Interleukin-5 in conjunction with IL−4, IL−9, and IL−13, and the crosslinking of FcεRIs (high-affinity Fc receptors for IgE) result in baspohilia in the blood and tissue. Mucosal mast cells activation leads to degranulation and release of histamine, leucotrienes and prostaglandins. Degranulation stimulates increased smooth muscle-cell contractility, increased intestinal permeability and elevated goblet-cell mucous secretion which lead to expulsion of the parasite from the intestine (weep and sweep). The major function of mucosal mast cell protease-1 (mMCP−1) is to impair epithelial-cell barriers by degrading the tight junction protein occludin and thereby increasing luminal fluid flow. IL-4 and IL-13 signalling through the IL-4R can increase the sensitivity of target cells to basophil and mast cell derived mediators. Therefore, IgE effects are amplified in the presence of these TH2-type cytokines and these results in enhanced responses including increased vascular permeability, smooth muscle contractility, and also the recruitment of TH2-type effector cells, including eosinophils and TH2 cell.

Interleukin-4, IL?13 and IL−21, drive development of alternatively activated macrophages (AAMs).

AAMs have three major functions, regulation of immune response, wound healing and resistance to parasite invasion. AAMs cause down regulation of Th 1 and Th 17 cell response through IL-10 independent mechanism which is partially mediated by tranforming growth factor ((TGFβ). AAMs might contribute to wound healing by clearing matrix and cell debris and by releasing cytokines, growth factors and angiogenic factors that promote fibroplasia and angiogenesis even at sterile sites. Activation of AAMs leads to upregulation of genes involved in tissue remodeling including: matrix metalloproteinases, extracellular matrix proteins, fibronectin, tenascin-C, tissue inhibitors of matrix metalloproteinases, and several types of collagens. AAMs are upregulating arginase-1 and ChaFFs (chitinase and FIZZ (found in inflammatory zones) family member proteins) which also contribute to healing.

ChaFFs are secreted by AAMs are prime candidates for mediating host resistance (Nair *et al*., 2005). ChaFFs are recently described secretory proteins that are induced during T helper 2 (TH2)-type responses and contribute to allergic asthma, fibrosis or helminth immunity (Nair *et al*., 2005). Some of these molecules are related to enzymes that degrade chitin, a molecule that is common to some pathogens, including some helminth larvae and fungi. Resistin-like molecules (RELMs) have sequence homology to resistin (also known as FIZZ3), a hormone released by adipocytes that mediates insulin resistance (Steppan, 2004).

Breeding for improved resistance to gastrointestinal nematodes

Of the novel approaches to parasite control, breeding small ruminants for resistance seems to be the most encouraging application that can be used to complement the strategic use of dewormers and improved pasture management. Resistance to infection refers to the animal's ability to suppress the establishment and/or development of parasites. From an economic point of view, resistance allows sheep to maintain existing levels of production even if they are infected. The idea is to use only those animals for breeding that shows either an inherently occurring resistance or resilience to nematode challenges (Bishop *et al*., 1996). This idea has, as other alternative strategies, come into the fore since the extent of the spreading anthelmintic resistance of parasites has become more and more obvious.

Many studies showed that it is possible to exploit genetic variation in resistance to nematode parasites of sheep by selection and the selection for resistant animals is achievable within different animals as well as within different breeds (SAC, Stapledon Report, 2000). Various studies have been done on this issue, some focused on the varying degrees of resistance amongst differing breeds (Baker *et al*., 2003; Amarante *et al.,* 2004), and others focused on the occurrence of resistance and its heritability within one breed (Bisset *et al*., 1996; Bouix *et al.,* 1998; Gauly *et al.*, 2002). The outcome of these studies confirmed that within and amongst populations of animals that are challenged by internal parasites there are always animals that perform better than others, they are either resistant, resilient or tolerant (Bishop and Stear, 1999). Breeding in recent years has concentrated on resistance last but not least because resilience is far less heritable then resistance (Eady *et al.,* 2006). Tolerance on the other hand is not a desirable trait at all because tolerant perform well indeed but contribute to pasture contamination. There is a substantial body of evidence that supports the fact that some breeds are naturally more resistant to internal parasites in sheep than other breeds (Table.1).

The immune mechanisms responsible for resistance are not completely understood, but it has a significant effect in inherited resistance. Relatively resistant animals show better local and generalised immune response as compared to susceptible. Immune response against GINs is influenced by many physiological and genetic factors. Resistant animals respond/ eliminate parasitic load earlier than susceptible through AAMs, eosinophils and basophils mediated mechanisms. Determination of specific genes linked with host resistance will provide a valuable approach to find out the molecular mechanism of host resistance to GINs. Resistance has been reported to reduce pasture contamination, which in turn reduces re-infection and thus the requirement of the frequent anthelmintic treatments. A plethora of information is available for the variation (within and between breeds) in sheep/goat resistance to GINs. Resistance and resilience are the two terms generally used in the breed selection strategies. Resistance is the ability to suppress the establishment and/or subsequent development of infection (Albers *et al.*, 1987), whereasresilience (or tolerance) is the ability of the host to survive and be productive in the face of parasite challenge (Clunies-Ross 1932; Woolaston and Baker 1996).

Successful selection of animals for genetic resistance is related to the markers, which are used and depends on the correlation with the trait, heritabilities and cost of testing. A

Table.1 Breeds showing resistance to gastrointestinal parasites

Resistant Breed	Comparison Breed	Parasite	Marker used	Refrence
Taghee	Rambouillet	*Ostertagia spp*, *Nematodirus spp*	FEC, PCV	Scrivner *et al*.,1984
Florida Native	Rambouille, Barbados Black Belly, Suffolk	*H.* contortus	FEC, PCV	Cortney *et al*., 1985
Florida Native	Dorset x Rambouillet	*H. contortus*	FEC, PCV	Zajac *et al.,* 1988
Scottish Black Face	Finn Dorset	*H. contortus*	FEC, PCV	Altaif and Dargie, 1978
Red Masai	Dorper	*H. contortus*	FEC, PCV	Baker *et al.,* 2003
St. Croix	Suffolk, Katahdin, Rambouillet	*H. contortus*	FEC, PCV	Suba *et al*., 2002
Texel	Suffolk	*H. contortus*	FEC, PCV	Good *et al*. 2006
Indonesian Thin Tailed	Merino	*H. contortus*	FEC, PCV	Subandriyo *et al*., 2002
Santa Ines	Suffolk, IIe de France	*H. contortus*	FEC, PCV, Mean Plasma protein	Rocha *et al*., 2004; Amarante *et al*., 2004
Gulf Coast native	Suffolk	*H. contortus*	FEC, PCV	Shaqkya *et al*., 2009
Small East African	Galla	*H. contortus*	FEC, PCV	Baker *et al.,* 2003
Scottish Black Face		Romney		
	Ostertagia circumcincta		FEC, PCV	Stear *et a*l., 1995
Garole	Deccani	*H. contortus*	FEC, PCV	Ghalsasi *et al.,* 1994
Garole x Deccani	Bannur x Deccani	*H. contortus*	FEC, PCV	Nimbkar *et al*., 2003
Nali x Lohi	Nali x Corriedale	*H. contortus*	FEC, PCV	Yadav *et al*. 1993

number of phenotypic traits such as FEC, worm burden, serum antibodies, peripheral eosinophilia, pepsinogen, fructosamine and plasma albumin concentration have been used to identify animals with increased resistance to infection (Beh and Maddox 1996; Dominik, 2005). Of these traits, the principal and most practicable measurement used to evaluate resistance in small ruminants undergoing similar parasite challenge is FEC. FEC has been proposed as the only proven way of selecting sheep for parasite resistance in many breeds (Woolaston 1992; Bisset and Morris 1996). Both FEC and PCV are traits of value, as FEC is an indirect measure

of resistance and PCV is an indicator of resilience. In ideal circumstances, it would be desirable to select for both decreased FEC and increased PCV. Determination of specific genes associated with resistance to GIN infections is important for a better understanding of genetic resistance, biological pathways as well as the biology of the host response to GINs (Beh and Maddox, 1996; Andronicos *et al.,* 2010). A successful search for resistance/susceptibility genes is dependent upon having a number of resources available. When the genes for resistance and their functions are identified, this will provide valuable insights into the molecular basis of the host resistance to GINs.

MHC class II molecule

MHC complex is group of genes on a single chromosome that codes the MHC antigens. Histocompatibility molecules of one individual act as antigens when introduced into a different individual. George Snell, Jean Dausset and Baruj Benacerraf received the Nobel Prize in 1980 for their contributions to the discovery and understanding of the MHC in mice and humans. The physiologic function of MHC molecules is the presentation of peptide antigen to T lymphocytes. These antigens and their genes can be divided into three major classes: class I, class II and class III (Fig 1).

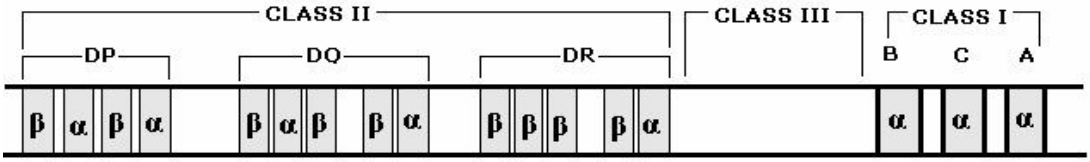


Fig 1. Division of MHC molecule in major classes

Expression

All nucleated cells express MHC class I molecule, however only antigen presenting cells express MHC class II molecules. CD8+ T lymphocytes recognizes peptide antigen only when it is presented by the antigen presenting cell in the peptide binding groove of MHC I molecules. Class I molecules present peptide fragments in the cytosol (endogenous antigen, which could be fragments of viral or tumor proteins) to the CD8+ lymphocytes. MHC class II molecules comprise two non-identical and non-covalently associated polypeptide chains (α and β). These two chains have amino ends on the surface, a short transmembrane stretch and intracytoplasmic carboxyl ends. Both α chain and β chain are MHC-encoded and polymorphic.

A peptide binding groove is formed in between α1 and β1 domains with a beta pleated floor. As in the case for class I MHC, the greatest polymorphic variability in the amino acids is in those facing the groove. This in turn determines the chemical structure of the groove and influences the specificity and affinity of peptide binding. Peptides associated with class II MHC are 13-25 amino acids long. The ends of peptide binding clefts are open so that peptides of 30 residues or more also can fit. As with class I MHC, anchor sites for one or more amino acids also exist in the groove of the class II MHC molecule. α2 and β2 are largely non-polymorphic. During antigen presentation, CD4 molecule of Helper T lymphocyte binds to β2 domain of the class II MHC molecules. B cells, dendritic cells and macrophage are principal cell types that present antigen to $CD4^+$ T cells. Macrophages that have engulfed microbe but unable to clear it presents microbial peptide antigen with MHC II proteins to antigen-specific $CD4^+$ T cells. The T cell in turn activates the macrophage and helps to eliminate the microbe. Similarly, B cells present endocytosed antigen with MHC II proteins to CD4+ T cells. The CD4+ T cells then stimulate the B cells finally resulting in production of antibodies against the foreign antigen.

Polymorphism studies on small ruminants MHC

At least 160 OLA-DRB1 alleles are reported from various sheep breeds (Konnai *et al*., 2003). Among MHC class II genes, the *DRB1* locus are found to be highly polymorphic (Ballingall *et al*., 1992; Schwaiger *et al*., 1995; Kostia *et al*., 1998; Paterson *et al.*, 1998; Jugo *et al* ., 2000; Konnai *et al*., 2003). A high polymorphism level is present in exon 2, which encodes the antigen-binding site (Outteridge *et al*., 1996; Escayg *et al*., 1997; Konnai *et al*., 2003a). Variation in these genes may impact immune responses to pathogens, which may lead to variation in disease susceptibility (Tizard, 2003). Most of the alleles identified in the polymorphism studies are associated with susceptibility of several infectious diseases including *T. circumcincta* infection (Hassan *et al.,* 2011). Other reports also revealed association of OLA-DRB 1 and resistance to development of bovine leukemia virus induced – ovine lymphoma (Nagaoka *et al*., 1999). Several studies (Schwaiger *et al.*, 1995; Buitkamp *et al*., 1996; Charon *et al.,* 2002) revealed the association of OLA-DRB1 alleles with reduced faecal egg counts in parasitic infestations. Antibody productions are also influenced by MHC microsatellite alleles in the nematode infestation (Outteridge *et al.,* 1996).

Nineteen alleles are identified OLA- DRB A , B, C, D, E, F, G1, G2, H1, H2, I, K, L, M, N, O, P, Q, R from Scottish black face sheep and allele G was found to be resistance against *T. circumcincta* infection in lambs (Schwaiger *et al*., 1995). In another study carried on Russian Romanoff and Karelian sheep by Kostia *et al* (1998) showed 19 alleles and out of these alleles OLA-*DRB1*01* found to be specific for Romanoff sheep and all Grey sheep found to possess only *DRB1*0201* allele.

The caprine lymphocyte antigen (CLA) systems, the major histocompatibility complex of goat, have two expressed class II antigens, DQ and DR (Takada et al., 1998). Till today two DRB loci have been characterized (Schwaiger *et al*., 1993; Amills *et al*., 1995). The CLA-DRB3 exon 2 (CLA-DRB3*02) encodes the first domain of the DR molecule, which is in close contact with the foreign antigen and displays a very high degree of polymorphism with more than 25 different sequences identified to date. The extensive polymorphism of CLA-DRB3*02 is considered to be responsible for the differences among individuals in the immune response to infectious agents. Most of alleles identified are associated with resistance to disease (e.g. Cowdriosis and nematode infection) and production traits (e.g. meat and milk) of goat. In one study carried out on Chinese indigenous goats by Hua Li *et al*. (2006) it showed that , Six alleles and 18 restriction digestion profiles are found by digestion of PCR amplification product of CLA-DRB3*02 with *Hae* III. A new allele CLA-DRB3*0206 are identified only in three goat populations from the three-gorge reservoir and the breed-specific allele CLA-DRB3*0205 was merely detected in the three populations of Tibetan goat.

Three genotypes were revealed in DRB3.2 gene of Changthangi goat (Sheikh *et al*., 2006) Raeini Cashmere goat (Baghizadeh *et al.,* 2009) by RFLP. Frequencies of these three (TT, Tt, tt) genotypes are 0.46, 0.44 and 0.10, respectively and T and t alleles frequencies are calculated as 0.68 and 0.32 respectively. *Taq*I RFLP shows that in TT genotype, there was only one recognition site in homologous pair of chromosomes, in Tt genotype there was presence of one recognition site in one chromosome and absence of recognition site on its homologous pair, whereas in genotype tt, absence of recognition site for *Taq*I enzyme was examined. However, *Pst*I and *Hae*III enzymes showed 13 restriction patterns and eight alleles (A, B, C, D, E, F, G and H) in CLA-DRB3*02 gene of domestic goat breeds of Southwest China (Zhao *et al*., 2011).

In OLA-DQA 2, 24 allelic sequences have been identified till today (Hickford *et al.,* 2004; Zhou and Hickford, 2004). Hickford *et al.* (2004) reported 21 DQA2 alleles in New Zealand sheep using PCRM-SSCP technique namely: *0101–*1401, 19.8% ; *1201, 16.8% ; *0602, 10.46% ; *1101, 7.8% ; *0501, 6.6%; *0103, 6.3% ; *0901, 6.1% ; *0401–*1501, 4.2% ; *0601, 3.5%; *08011, 3.3%; *0102–*1401, 2.5% ; *08012–*0201, 2.3% ; *0402–*1701, 2.2% ; *0301, 1.8% ; *0701–*1401, 1.6% ; *0702–*1401, 1.6% ; *1001, 1.5% ; *0401–*1401, 1.0% ; *0102–*1601, 0.6% ; *0701–*1301, <0.1% and *0101–*1601, <0.1%. One hundred and seventy one different genotypes were observed ranging in frequency up to 5.5%. Among these alleles, *1001 and *1201 alleles were associated with strongyle spp. count at weaning, while alleles *1001, *1201 and *0601 were found to be associated with weaning *Nematodirus* spp. counts. Alleles *1001 and *1201 were found to be associated with total FECs at weaning. The presence of allele *1201 tended to be associated with increased strongyle spp. count, while the presence of haplotype *0402–*1701 tended to be associated with reduced counts. Allele *0103 was not found to have a significant effect on strongyle spp. counts. Alleles *1201 and *1001 were found to have significant independent effects on *Nematodirus* spp. counts, the presence of these alleles being associated with increased counts. In contrast, the presence of allele *0601 was found to be significantly associated with reduced *Nematodirus* spp. counts. Alleles *0702–*1401 and *0301 were not found to have a significant effect on *Nematodirus* spp. counts. Alleles *1201 and *1001 were found to have significant independent effects on total FECs; both alleles being associated with increased counts were not found to have significant effects on total FECs at weaning.

Breed resistance to GI parasites and Cytokine expression

Immune response against gastrointestinal nematodes characterised by activation and development of a Th2 profile and the accompanied production of elevated levels of IgE and IgG1 antibodies, eosinophilia, mucosal mastocytosis and goblet cell hyperplasia (Meeusen, 1999). During the development of immune response there is primary detection of the infectious agent, mediated by the Toll-like receptor (TLR) family and associated signalling pathways (McGuinness *et al*., 2003). Activation of this pathway in turn leads to highly coordinated expression of a number of cytokines and chemokines that control the Th2 nature of the response (Dissanayake, 2004). Ultimately, cells are recruited and activated to effect removal of the invading nematode through expression of antimicrobial peptides, antibody,

complement and free radicals, as well as manipulation of gut barrier integrity, pH, contractility or viscosity (Mulcahy *et al.,* 2004). Ingham *et al.* (2008) studied gene expression changes in resistant sheep against *H. contortus* and *T. Colubriformis* (Table.2).

Table 2: Candidate genes quantified in the mucosa of the ovine gastrointestinal tract following challenge with either *Trichostrongylus colubriformis* or *Haemonchus contortus*

Functional grouping	Genes
Detection through Toll-like receptor signalling	TLR1-10, IRF3, M YD 88, NFKBIA, IKBKB
Cytokine receptor signalling	IL1B, IL2,IL2RA-CD25, IL4-6,IL8, IL-10, IL-12B, IL13, IL-18, INFγ, TNF, TNFSF-13B, TGFA, TGFβ1-3, MIFDLL4, JAG1, GATA3, TBX21
Generation of reactive oxygen or nitrogen	ARG2, CYBA, CYBB, DUOX1, DUOX2, NCFI, NOS2 A, XDH
Extracellular matrix	MUC2, MUC3, MUC5AC, TFF2, TFF3
Epithelial integrity	ASAM, CLDNI-4, CMA1, CTSB, CTSF, CTSL2, CTSS, LGMN, CDH2, OCLN, BCL2, CASP3, SPRR2A, SLC11A1, MYLK, ERBB2, NRG1
T cell development	DLL4, JAG1, GATA3, TBX21

The reactive oxygen producer dual oxidase 1 (DUOX1), xanthine dehydrogenase (XDH), transforming growth factor (TGFb2) and toll-like receptor 2 (TLR2) expression levels were found increased following *T. colubriformis* infection. The increase in the DUOX1 transcript was particularly marked in both the *H. contortus* flock and *T. colubriformis* flock at the second infection (909 and 200 fold, respectively). In susceptible sheep TLR signalling pathway members (NFKBIA and IKBKB), cathepsin proteases (CTSF, CTSL) and mast cell protease (CMA1) are increased. No expression found in DUOX2, SPRR2A, SLC11A1, IL-5, IL-13 and MUC3A during *T. colubriformis* infection. Following *H. contortus* infection in resistant animals, there is increase expression of MS4A2, TLR10, TLR7, CDH2, IL-2RA, IFNc, IL-10, TGFβ-2 and MUC5AC. *Haemonchus* resistant flock produced significantly more of the high affinity IgE receptor transcript (MS4A2) than susceptible flock but increased expression of XDH gene in *Haemonchus* susceptible sheep. *Haemonchus* resistant sheep produced significantly more of the IFNγ at the "innate" time and four of the Toll-like receptors (TLR2, 4, 8 and 9) at the "acquired" stage of the response.

In animals that mount an effective anti-nematode response, the early inflammatory response is followed by induction of a regulatory environment characterised by IL10, TGFβ and TReg (CD4+CD25+) cells (Maizels and Yazdanbakhsh, 2003). The increased expression of CD25 (IL RA) was observed in *T. colubriformis* and *H. contortus* resistant sheep. This study also revealed increased level of regulatory cytokine gene, IL10 and TGFβ in resistant sheep following primary challenge. Nitric oxide (NO) has been reported as being cytotoxic to a range of parasites (Cloasanti *et al*., 2002). NO and reactive oxygen causes modification of mucus form or production (Shao and Nadel, 2005). In resistant animals inducible NOS2A, which functions in the production of reactive nitrogen, as well as reactive oxygen producers DUOX1 and neutrophil cytosolic factor 1 (NCF1) are increased. DUOX1 and NCF1 genes were expressed above control levels in resistant animals whereas NOS2A was drop below control levels in susceptible sheep.

Viscosity of luminal contents has great impact on parasite colonisation. The Mucin MUC5AC transcript level was higher in *T. colubriformis* resistant sheep at the "acquired" time point but more abundant in *H. contortus* susceptible sheep at both "innate" and "acquired" time points in response to challenge.

Epithelium membrane integrity plays important role in nematode infection. In the case of infection a temporary disruption of membrane integrity may be beneficial in allowing increased flow of immune cells and related mediators into the lumen. Tight junction and protease gene (OCLN, CTSF, and CTSL2) were found to be more abundant in susceptible animals and at levels higher than a naive control, following *T. colubriformis* challenge. The cadherin gene CDH2 was more abundant in *T. colubriformis* and *H.contortus* resistant animals following challenge.

Gene expression profiling of naive resistant and susceptible sheep was done by Keane *et al.* (2006) using microarray. Forty one unique genes were differentially expressed between the resistant and susceptible animals using northern blotting.

Intron 1 of Interferon γ gene

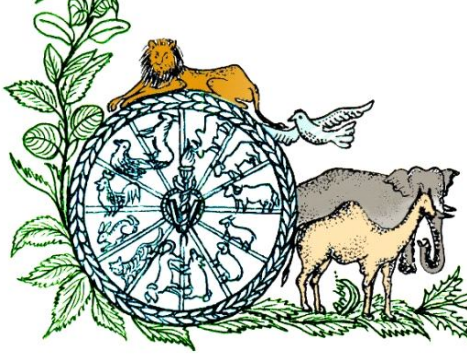
Genes for parasitic resistance are located on q arm of chromosome 3 in sheep. In this region most likely known candidate is interferon gamma, which has been mapped to 3q23 by

in situ hybridization (Goldammer *et al*. 1996). The IFN-gamma gene is associated with nematode resistance in both domestic and free-living sheep breeds (Coltman *et al*., 2001). There were four markers on ovine chromosome 3, which included one microsatellite marker located in intron 1 of the IFN-gamma gene. This microsatellite has been characterised as a bi-allelic (GTTT) repeat, allele A has six repeats and allele B, five repeats (Schmidt *et al*., 1996). In addition, allele A has a G 49 bp downstream of the microsatellite (refered to as haplotype A) while allele B has an A at the corresponding position (refered to as haplotype B). Frequency of haplotype A in Texels and Suffolks breed are 79% and 20% and haplotype C and D are 30% and 20% in Suffolk breed. Suffolk breed found to be more susceptible to nematode infection than Texel breed. Haplotype B was associated with nematode resistance in in Texel breed, however it is not found in Suffolk breed of sheep. A conclusion was drawn that the INF- γ genes are not directly associated with nematode resistance, however a locus closely related with INF-γ gene is responsible. Also, suggested that this locus and INF-γ are in linkage disequilibrium in Texel breed however it is not associated with Suffolk breed.

IgA heavy alpha chain gene

IgA response varies with GI parasites and an increased in IgA associated with parasite resistance as measured by reduced faecal egg count (Strain *et al*., 2002; Stear *et al*., 1990). The allelic variation occurred in the constant region of the ovine IgA heavy alpha chain gene (IGHA) which encodes the hinge region of the mature protein (Zhou and Hickford, 2004). An elongated hinge region may result in increased flexibility allowing the IgA monomer to bind antigens with wide range of epitope separations. The variation in IGHA gene leads to variation in IgA response against parasite. IgA bind to *Teladorsagia circumcincta* and regulate length of the larva and their fecundity (Stear *et al*., 1995). The presence of IGHA allele *01 was associated with higher EPG in 4 month old lamb challenged with *Nematodirus* sp. and IGHA allele *02 was associate with a higher mean total EPG at 9 months of age lamb challenged with *Trichostrongylus* sp. The presence of IGHA allele *03 found to be associated with higher mean strongyle EPG in 4-month-old lambs under a mixed challenge with no predominant parasite (Lin *et al.,* 2009).

Materials and Methods



3 Materials and Methods

3.1 Materials

i) Experimental Animals

The experimental goat and sheep used in the present study belonged to Indian Veterinary Research Institute (IVRI) and Central Sheep and Wool Research Institute (CSWRI) flocks maintained at Mukteshwar and Avikanagar.

IVRI Mukteshwar (Nainital, Uttarakhand) flock is located in the temperate Himalayan region of India at 29^{0}28'20"N, 79^{0}38'52"E, and has an average elevation of 2,171 metres (7,123 feet) above the mean sea level (msl). The institute goat flock constituted Jamunapari, Jhakhrana and Local hill breeds. Animals were maintained at different stations according to their age group.

CSWRI, Avikanagar (Jaipur, Rajasthan) flock is located in the semi-arid Aravalli region of India at 26^{0}26'N, 75^{0}28'E, and has an average elevation of 320 metres (1,049 feet) above the mean sea level (msl). The institute sheep flock constituted Malpura and Avikalin breeds Animals were maintained at different stations according to their age group.

3.1.1 Blood samples

Blood samples were collected aseptically in plastic containers with anticoagulant (0.1% Ethylene diamine tetraacetic acid (EDTA) from the jugular vein of sheep and goat.

i) IVRI Mukteshwar

Blood samples (5 ml) were collected from the goat breeds of Jamunapari, Jhakhrana and local hills at the goat farm, Surmane, IVRI, Mukteshwar (Table.1).

ii) CSWRI Avikanagar

Blood samples (5 ml) were collected from Avikalin and Malpura breeds at the sheep farm, CSWRI, Avikanagar (Table.1).

Table 1. Sample collection from sheep and goats

Goat Farm, IVRI Mukeshwar	Jamunapari	Jakhrana	Local Hills
No. of animals	15	15	15
Sheep sectors, CSWRI, Avikanagar	Malpura	Avikalin	
No. of animals	15	15	

3.1.2 Reagents for DNA related studies

Taq DNA polymerase, dNTP mix, DNA 100 bp ladder plus ruler, 6X gel loading dye, *Hae*III (*BsuR*I), *Hinf*I, *Rsa*I (Fermentas), Green Taq polymerase (Promega), EDTA (SRL, India), Ethidium bromide, Nuclease free water, Tris Acetate EDTA buffer (TAE) 10x (Amresco), Agarose-LE (Invitrogen), Ethanol (Merck), PCR primers (Metabion international AG, Eurofins MWG/operon) were purchased and used.

3.1.3 Kits

DNA isolation kit (DNeasy Blood and Tissue Kit), QIAquick PCR purification kit (Qiagen) were used in the study.

3.1.4 General chemicals

Glacial acetic acid, Glycerol, Sodium chloride, Sodium bicarbonate, Acrylamide, N,N Methylene Bisacrylamide, Ammonium persulphate (SRL, India), Sodium hydroxide pellets, Hydrochloric acid (Qualigens), Silver nitrate, Tris, Ethanol, Formaldehyde (Amresco), TEMED (Himedia), Boric acid (SDS, India) were procured and used.

3.1.5 Equipments

Refrigerators and freezers: +4^{0}C (Whirlpool), -20^{0}C (Vestfrost). Centrifuge machines: Sigma 3K30. Balances: Single pan analytical balance, for small quantities (Citizen). Laminar flows: Ultra clean systems, ESC. Thermal Cyclers: Matercycler gradient thermocycler

(Eppendorf). Gel Documentation system: BioImaging System GENE (Syngene). NanoDrop 1000: Thermo Scientific Spectrophotometer, USA. Microscope (Olympus CH 20i). Page chamber: AE-6200 (Bioinstruments, ATTO Japan). Hot Water Bath (India). Shaker Incubator: Innova 4230 New Brunswick scientific (USA). Vortex shaker: Spinix, India. Horizontal Electrophoresis Unit: PS9009TX (Bioinstruments, ATTO Japan). Pipettes (µl: 0.5-10, 1-20, 20-200, 10-100, 100-1000) – Eppendorf Research. Microwave oven: Samsung.

3.1.6 Glasswares and Plasticwares

The glasswares and plasticwares used for the present study were purchased from Borosil, Schott Duran, Genaxy, Tarson, Prolab, Nalgene, Nunc, Greiner and Eppendorff.

3.1.7 Primers

Primers for *DRB3.2*, *DQA1.2*, *DRB1.2* were synthesized from commercial manufacturer Metabion international AG, Deutschland, eorophins/mwg/operon, India.

3.2 Methods

3.2.1 Molecular studies on DRB and DQA genes

3.2.1.1 Genomic DNA isolation

Genomic DNA (gDNA) was isolated using the QIAGEN DNeasy Blood and Tissue Kit following manufacturer's instructions using 200 µl. The protocol for isolation of genomic DNA was as follows:-

1. In a 2 ml microcentrifuge tube, 20 µl of proteinase-K was taken to which 200 µl of anti-coagulated blood was added.
2. AL buffer (200 µl) was added to proteinase-K digested blood and the contents were mixed thoroughly by vortexing. Kept for incubation for 10 min at 56^{0}C.
3. After incubation, 200 µl of ethanol (100%) was added and mixed thoroughly by vortexing.
4. Pour mixed sample into DNeasy mini spin column placed in 2 ml collection tube and centrifuged at 8000 rpm for 1 min. The flow through was discarded along with the collection tube and proceeded next step.

5. Place DNeasy mini-spin column in new 2 ml collection tube, add 500 µl of buffer AW1, centrifuge for 1min at 8000 rpm. Discard flow through and collection tube.

6. Place column in new 2 ml collection tube, add 500 µl buffer AW2, centrifuge for 3 min at 14000 rpm.

7. Place column in a 2 ml microcentrifuge tube, pipette 100 µl buffer AE directly onto DNeasy membrane. Incubate at room temperature for 1 min. Centrifuge for 1 min at 8000 rpm to elute. The eluted DNA was labeled properly and stored at -20^{0}C until use.

3.2.1.2 Purity and concentration of DNA

The concentration and purity of the isolated gDNA was determined by UV/VIS spectrometer (NanoDrop 1000- Thermo Scietific Spectrophotometer, USA). Two µl of purified gDNA sample was directly placed on the loading knop. Elution buffer was used as blank. The optical density (OD) of diluted DNA sample was read at A_{260} and $A_{280,}$ keeping triple distilled water as blank. The DNA purity was estimated by determining the ratio of A_{260}/ A_{280} and the quantity measured by using the standard reading of 1 OD at A_{260} for 50 µg DNA per ml.

3.2.1.2.1 Agarose gel electrophoresis

The resolution and purity of the DNA was further analysed by agarose gel electrophoresis in a submarine horizontal electrophoresis unit (PS9009TX ,Bioinstruments, ATTO Japan). Agarose (0.8%) gel was prepared by boiling analytical grade agarose in 1x TAE buffer to dissolve it completely. After cooling to about 50ºC, ethidium bromide was added to the agarose solution to obtain final concentration of 0.5µgml^{-1}. The gel-casting platform was placed on a leveled surface and the open sides were sealed with adhesive tape. The gel comb was then placed across the gel-casting platform and the molten agarose was poured on to the gel-casting platform and it was kept undisturbed to solidify the gel. After the gel gets solidified, the comb was taken out and adhesive tape was removed. The gel-casting platform with the set gel was submerged in the electrophoresis tank with sufficient quantity (about 1mm level) of electrophoresis buffer (TAE 1x) above the surface of the gel. The test DNA sample (4 µl) was mixed with1 µl of 6x-bromophenol blue, loading dye and loaded into the well with

micropipette. Electrophoresis was performed at 5 volts cm^{-1} and progress of mobility was monitored by the migration of dye. The DNA migration and resolution pattern was examined by UV transillumination technique and the picture was documented by photography.

3.2.1.3 PCR amplification of *DRB* and *DQA* genes

3.2.1.3.1 Primers

Primers specific for *DRB 3* exon 2, *DQA1* exon 2 and *DRB1* exon 2 as given by Baxter *et al.* (2008), Takeshima *et al.,* (2007), Konnai *et al*. (2003) were custom synthesized from commercial source Metabion international AG. Details of the primers were as given below in Table 2, 3 and 4. Lyophilisized primers were reconstituted as per the manufacturer's instructions to make them 100 $pmol\mu l^{-1}$ concentration stock solutions. For working stock, 20 µl of original stock solution was mixed with 80 µl of nuclease free water (NFW) to make the 100 µl of working solution of 20 $pmol\mu l^{-1}$ concentrations.

Table 2: Primers for the PCR amplification and sequencing of *DRB3.2* Gene

Orientation	Primer	Sequence (5' - 3')	Length (bases)
Sense	DRB3FRW	CGC TCC TGT GAY CAG ATC TAT CC	23
Antisense	DRB3REV	CAC CCC CGC GCT CAC C	16

Primers are as described by Baxter *et al.* (2008)

Table 3: Primers for the PCR amplification and sequencing of *DQA1.2* Gene

Orientation	Primer	Sequence (5' - 3')	Length (bases)
Sense	DQA intL2	CAC CAA ATG AAG CCC ACA AAT G	20
Antisense	DQA1-677R	CCC TAG GGA AAA AGG GAG TGA	20
Sense	DQA Int L3	GCC CAC AAT GTT TGA TAG TC	20
Antisense	DQA1 ex2 REV ver2.1	GGG RAC CAC ATA CTG TTG GTA G	21

Primers are as described by Takeshima *et al.,* (2007)

Table 4: Primers for the PCR amplification and sequencing of *DRB1.2* Gene

Orientation	Primer	Sequence (5' - 3')	Length (bases)
Sense	DRB1 FRW	AGG AGT CCG CTC CTG TGA CTA	21
Antisense	DRB1 REV	ACT CAC AGT CGT ACA CAC TCG	21
Sense	DRB1 FRW	ATC CTC TCT CTG CAG CAC ATT TCC	24
Antisense	DRB1 REV	TTT AAA TTC GCG CTC ACC CTG CCG CT	26

Primer as described by Konnai *et al*. (2003)

3.2.1.3.2 PCR amplification protocol

Optimization of the annealing temperature and PCR protocol were done for amplifying the *DRB3.2*, *DQA1.2* and *DRB1.2*. The reaction of 50 µl was prepared as follows:

Reagent/chemical	Vol (µl)
10X Taq buffer	10
25mM $MgCl_2$	03
10mM dNTP	01
Primer FRW (20 pmol)	0.75
Primer REV (20 pmol)	0.75
Taq DNA Polymerase (5 $U\mu l^{-1}$)	0.25
Template	01
Nuclease free water up to	50

3.2.1.3.3 Thermal Profile for PCR

Thermal profile optimized for amplification of the *DRB3.2*, *DQA1.2* and *DRB1.2* was as follows:

Table.5 PCR conditions for DRB3.2, DQA1.2 and DRB1.2 genes

Primers	Initial denaturation	Denaturation	Annealing	Extension	Final extension
DRB3	94°C for 5 min	94°C for 45 sec	**63**°C for 30 sec	72°C for 45 sec	72°C for 5 min
		Repeat for 35 cycles			
DQA1 (First reaction)	94°C for 2 min	94°C for 20 sec	**54**°C for 20 sec	72°C for 40 sec	72°C for 5 min
		Repeat for 15 cycles			
DQA1 (Nested reaction)	94°C for 2 min	94°C for 20 sec	**54**°C for 20 sec	72°C for 40 sec	72°C for 5 min
		Repeat for 35 cycles			
DRB1 (First reaction)	94°C for 4 min	94°C for 30 sec	**55**°C for 30 sec	72°C for 40 sec	72°C for 5 min
		Repeat for 15 cycles			
DRB1 (Nested reaction)	94°C for 4 min	94°C for 30 sec	**62**°C for 30 sec	72°C for 40 sec	72 °C for 5 min
		Repeat for 35 cycles			

3.2.1.4 Fractanation of PCR product

The amplicons were resolved by 1.5% agarose gel electrophoresis as per the method described earlier. The DNA migration and resolution pattern were examined by UV transillumination technique and the picture was documented by photography.

3.2.1.5 Purification of amplified product

The purification of amplified product was done by QIAquick PCR purification kit following the manufacturer's protocol:

1. The amplicons (1X) were mixed with 5 volumes of buffer PB (Binding buffer).
2. Put the mixed sample into the QIAquick column and centrifuge at 13000 rpm for 30-60 sec.
3. Discard the flow-through and place the QIAquick column back into the same tube.
4. Add 0.75 ml buffer PE (Wash buffer) into QIA column and centrifuge at 13000 rpm and 30-60 sec.
5. Discard the flow-through and place the QIA column back into the same tube.
6. Centrifuge the QIA column once more in the provided 2 ml collection tube for 1 min to remove the residual wash buffer.

7. Place the QIA column in a clean 1.5 ml microcentrifuge tube and add 50 µl buffer EB (Elusion buffer) to the centre of QIA quick membrane and centrifuge the column for 1 min. The eluted products were labeled properly and stored at -20°C until use.

3.2.1.6 RFLP (Restricted Fragment Length Polymorphism)

RFLP is a technique that exploits variation in homologous DNA sequences. It refers to a difference between samples of homologous DNA molecule that come from different location of restriction enzyme sites, and to a related laboratory technique by which these segments can be illustrated. PCR-RFLP is a simple and reliable technique for studying nucleotide polymorphism in nucleotide sequences in genes. It involves PCR amplification of segment of DNA, followed by restriction enzyme digestion of the PCR product and visualization of restriction fragments in gel.

The enzyme was selected by restriction map analysis of *DRB3.2* and *DQA1.2* sequence of goat and *DRB1.2* sequences of sheep. The PCR-RFLP reaction was performed as 10 µl volume. Following reagents were used for preparing reaction:

Reagents/Chemicals	**Vol (µl)**
Buffer 10X (*Hinf*I/*Hae*III/*Ras*I)	1.0
*Hinf*I/*Hae*III/*Ras*I Enzyme	0.2
PCR product	5
Nuclease free water up to	10

Reaction mixture was kept in incubator at 37°C for 2- 3 h for digestion and digested products patterns were resolved and analysed in polyacrylamide gel.

3.2.1.6.1 Preparation of polyacrylamide gel for RFLP analysis

3.2.1.6.1.1 Composition of gel (8%)

Reagents/Chemicals	**Vol**
29% Acrylamide and 1% Methylene bisacrylamid	2.66 ml
Nuclease free water	5.27 ml
5x TBE	2.00 ml
10 % Ammonium persulphate	0.07 ml
TEMED	3.5 µl

3.2.1.6.1.2 Assembly of the apparatus

1. Glassware, Plates, comb and spacer were washed with detergent, rinsed initially under running tap water till no remains of detergent were left and finally in double distilled water before drying. Before use, glassware, spacers, comb and plates were cleaned with sprit and air dried.

2. Glassware was fitted by putting 1 mm spacer between the two plates at its position and clamp is applied. Then freshly prepared gel mix was poured into space between plates and comb was inserted immediately.

3. The above gel was allowed to polymerize at room temperature at 30- 45 min.

4. After polymerization, gel comb was removed and gel was put in the electrophoresis tank with notch plate facing towards buffer reservoir.

5. The products were loaded on the gel carefully and the electrophoresis was performed at room temperature for 4-5 h at 90-100 volt constant current. The current, voltage and running time was standardized as per the size and composition of PCR product.

6. After running, gel was subjected for the silver staining to visualize the enzyme digestion banding pattern.

3.2.1.7 Silver staining

Silver staining was carried out by the following protocol:

1. Preparation of gel for staining:

Plate holding the gel was placed on the clean surface, facing the notch plate upward. The notch plate was separated gently by taking care to avoid breaking of gel. First row loaded was marked by the cutting the upper portion of gel from that side. The gel along with the lower plate was placed in a suitable tray. The gel was removed carefully into the tray and the gel was fixed.

2. Fixing the gel:

Fixation solution was made using 10 ml of ethanol to which, 500 µl of glacial acetic acid added and distilled water was added to make volume 100 ml. Fixation solution was added in the tray containing gel, tray was gently agitated with fixation solution for 10 – 15 min.

3. Staining the gel:

0.020 - 0.040 g silver nitrate was taken in 100 ml distilled water. Staining solution was added in the tray containing the gel; agitated gently for 15- 20 min. After staining, washing was done for three times with distilled water.

4. Developing the gel:

The developing solution was made using 3 g sodium hydroxide, 750 µl formaldehyde and distilled water up to 100 ml. Solution was added to tray containing gel, agitated gently for 5 to 10 min. When band of DNA was developing properly then stop solution was added.

5. Stop solution:

The stop solution was made using 5 ml of acetic acid with distilled water up to 100 ml. Once bands were visibly clear, stop solution was added.

3.2.2 Fecal egg count

Faecal samples was collected directly from rectum of the animals and put into the faecal bags.

1. Take 1 gm of faecal sample and add 14 ml of saturated salt solution.
2. Triturated well in pestle and mortar and sieved the mixture.
3. Took small amount of mixture in a pipette and charged the Mac-Master chamber.
4. Left the slide for 3 min.
5. Counted the eggs of the marked area under low power of microscope.
6. Counted eggs were multiplied by 100 to obtained Egg per gram (EPG).

3.2.3. Haematology

3.2.3.1 Haemoglobin estimation

Haemoglobin estimation was done by Sahli's acid haematin method

1. Took 200 µl of blood and 0.1 HCl was added to this.
2. Mixed well the mixture and kept for 3 min.
3. The colour was adjusted with the standard colour of haemoglobin meter by adding 0.1 HCl.
4. Took the reading of the column.

3.2.3.2 Haematocrit estimation (PCV)

Haemocrit estimation was dome by Wintrobe method.

1. Filled the Wintrobe tube slowly with blood upto the 10 mark.
2. Centrifuged the Wintrobe tube in centrifuge at 3000 rpm for 10 min.
3. Took the reading at the top of packed erythrocytes immediately adjacent to the buffy coat.

3.2.2 Statistical analysis for goat and sheep

3.2.2.1 Allelic frequency of DRB3.2 gene in goat by PCR-RFLP

PCR-RFLP patterns/alleles obtained after restriction digestion of amplified PCR product with *HaeIII* were used for obtaining the allelic frequency in three goat populations. Allelic frequency was calculated manually, using frequency distribution of alleles in Jamunapari, Jhakhrana and local hill.

3.2.2.2 Descriptive statistics for the parameters

Mean ± Standard error of blood parameters (Haemoglobin and Packed cell volume) and Egg per gram (EPG) were calculated in Jamunapari, Jhakhrana and Local hill using SPSS (16.0).

3.2.2.3. Correlation between Blood parameters and EPG

Pearson's correlation (r) were obtained for blood parameters (Haemoglobin and Packed cell volume) with EPG from Jamunapari, Jhakhrana and Local hill breed using following statistically formula:

$$\rho x, Y \frac{\mathrm{cov}(X,Y)}{\sigma_X \sigma_Y} = \frac{E[(X-\mu_x)(Y-\mu_Y)]}{\sigma_x \sigma_Y}$$

Where, $\rho_{X,Y}$ = Pearson correlation

X = Haemoglobin or PCV

Y = EPG

E = Expected value

Estimates were tested for significance at 5% level.

3.2.2.4. Effect of RFLP-Alleles and breed on EPG

The effect of PCR-RFLP alleles and breed on EPG was studied using two way ANOVA. The statistical model used is described as below.

$$Y_{ijk} = \mu + A_i + B_j + e_{ijk}$$

Where $Y_{ijk=}$ EPG of K^{th} animal that belong to i^{th} allelic group and j^{th} breed

μ = Constant

A_i = i^{th} allele (i = A,B,C)

B_j = j^{th} breed (j = Jamunapari, Jhakhrana, Local hill)

e_{ijk} = Random error with respect to Y_{ijk}

3.2.2.5. Distribution of RFLP-Alleles in different EPG classes

Frequency distribution of PCR-RFLP alleles was studied in each EPG class within each breed. Distribution of PCR-RFLP alleles in Jamunapari, Jhakhrana, Local hill were tested using test. Differences were tested at 5% level of significance

$$X^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

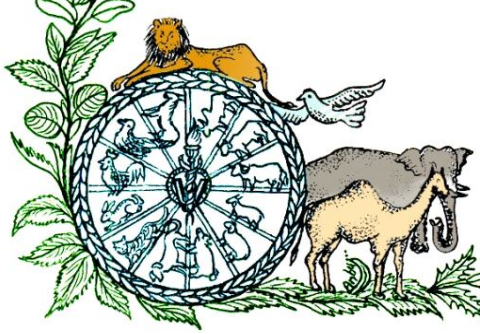
X^2 = Pearson's cumulative test statistic, which asymptotically approaches a distribution

O_i = an observed frequency

E_i = an expected (theoretical) frequency, asserted by the null hypothesis

n = the number of cells in the table

Results



4 Results

4.1. Blood sample collection

A total of 110 blood samples from different breeds of sheep and goats were collected aseptically from the juglar vein into plastic containers containing 0.1% EDTA as anticoagulant. The blood samples were transported to laboratory under ice for further processing.

4.2. gDNA isolation

The blood samples transported to the laboratory was in good condition avoiding coagulation and contamination. DNA was isolated using DNA isolation kit (DNeasy Blood and Tissue Kit). The isolated DNA was resolved by 0.8% Agarose gel and checked for concentration and quality . Quality (ratio of A_{260}/A_{280}) and quantity ($ng\mu l^{-1}$) of the gDNA was estimated using NanoDrop (NanoDrop 1000- Thermo Scientific Spectrophotometer, USA). The DNA was intact without smearing in gel electrophoresis and also the ratio obtained was between 1.76- 1.82 in NanoDrop. The concentration of gDNA obtained was between 160 and 264 $\mu g\mu l^{-1}$ and the concentration used in the amplification reaction was optimized to be around 7 ng per reaction.

4.3. PCR–RFLP of *DRB3.2* and *DQA1.2* gene

Amplification of Cahi – *DRB*3 exon 2 and *DQA1* exon 2 genes from three goat population using PCR generated a single 319 bp (*DRB*3 exon 2) and 349 bp (*DQA*1 exon 2) clear band (Fig 4.1 and Fig 4.2). Restriction enzyme *Hae*III was used for *DRB*3 exon 2 and *Hinf*I was used for *DQA*1 exon 2 gene, respectively. *Hinf*I and *Hae*III PCR-RFLP pattern among the Jamunapari, Jhakhrana and local hills goats are shown in Figure 4.3 and 4.4,

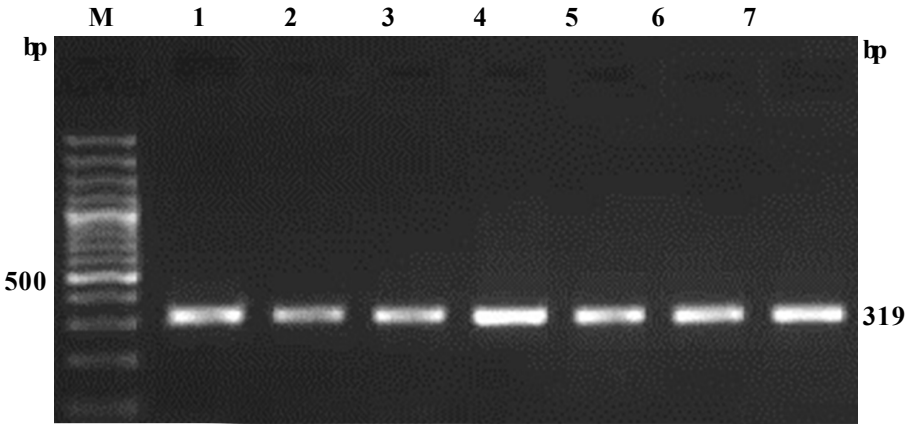


Fig. 4.1 : Amplification of Cahi – DRB3 exon 2 showing a band at 319 bp in three breeds of goat
Lane M : 100 bp plus DNA ladder
Lane 1-7 : 319 bp PCR product of DRB3 exon 2

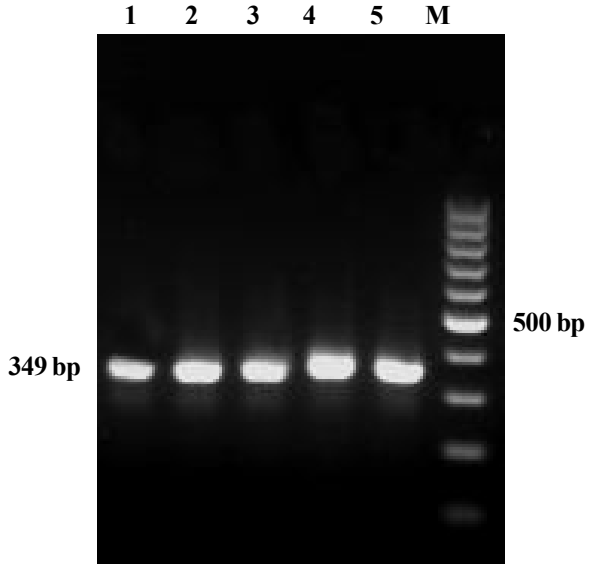


Fig. 4.2 : Amplification of Cahi – DQA1 exon 2 showing a band at 349 bp in three breeds of goat
Lane M : 100 bp plus DNA ladder
Lane 1-5 : 349 bp PCR product of DQA exon 2

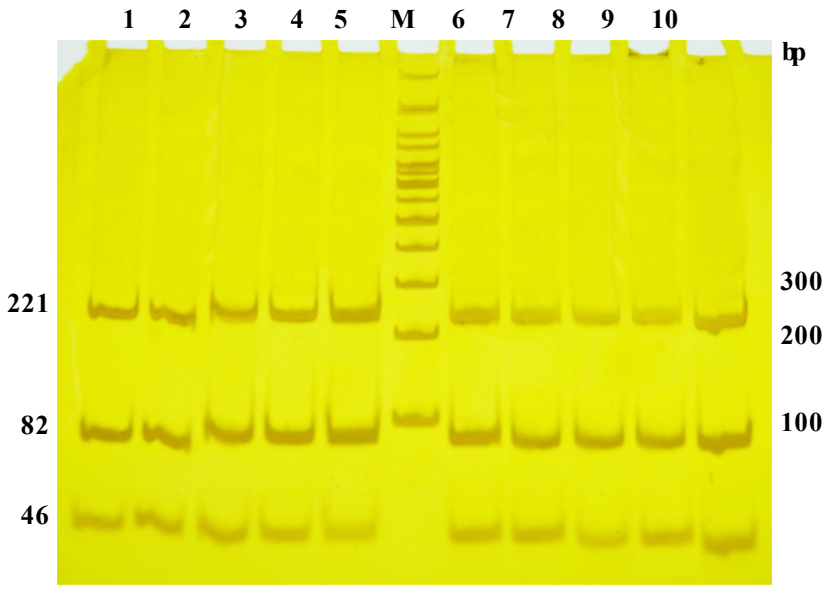


Fig. 4.3 : Restriction digestion of PCR product of DQA1 exon2 gene with Hinf I in three breeds of goat

Lane M : 100 bp plus DNA ladder
Lane 1-10 : Pattern “a” (221, 82 & 46 bp)

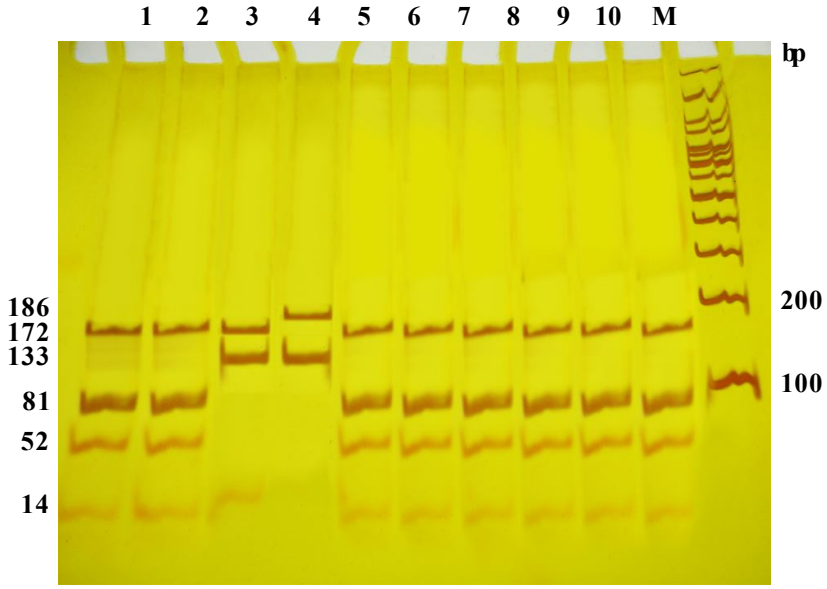


Fig. 4.4 : Restriction digestion of PCR product of DRB3 exon2 gene with HaeIII in three breeds of goat

Lane M : 100 bp plus DNA ladder
Lane 1,2 & 5-10 : Pattern “C” (172, 81, 52 & 14 bp)
Lane 3 : Pattern “B” (172, 133 & 14 bp)
Lane 4 : Pattern “A” (186 & 133 bp)

respectively. *Hae*III PCR-RFLP patterns/alleles were 186/133 (A), 172/133/14 (B) and 172/81/52/14 (C) in all the three goat population observed in the present study. *HinfI* PCR-RFLP yielded a single pattern/allele , 221/82/46 (a).

Allelic frequencies for *Hae*III PCR-RFLP pattern/allel of *DRB3.2* gene among three populations are presented in Table 4.1. The frequency of A type allele was 13.33% and found only in Jamunapari but not in other two breeds of goat. The frequency for B type allele was 13.33, 40 and 46.67 in Jamunapari, Jakhrana and local hill goat, respectively. The frequency of C type allele was 73.33, 60 and 53.33, in Jamunapari, Jakhrana and local hill goat, respectively. In the Jhakhrana and Local hill breed, allele A was absent; while, allele B and C were present in all the three breeds.

Table 4.1: Allelic frequency of *DRB3.2* in goat population

Allelic frequency (%)	Jamunapari (n = 30)	Jhakhrana (n= 30)	Local hills (n= 30)
A	13.30	0	0
B	13.33	40	46.67
C	73.33	60	53.33

4.3.1. Phenotypic parameters

Haemoglobin (Hb), PCV and EPG were taken as phenotypic parameters in the study (Table 4.2). The mean Hb was 6.5, 6.8 and 7.2 in Jamunapari, Jhakhrana and Local hill goats, respectively. The mean PCV of goats was 28, 31 and 34 in Jamunapari, Jhakhrana and Local hill, respectively. The mean EPG was higher in Jamunapari (2326), followed by Jhakhrana (1940) and lowest in Local hill (1497).

Table 4.2: Mean ± Standard error (n=15) for the phenotypic parameters

Parameter	Jamunapari	Jhakhrana	Local hill
Hb	6.5±0.27	6.8±0.20	7.2±0.25
PCV	28.4±1.4	31.4±1.3	34.6±1.1
EPG	2326±345	1940±375	1356±386

4.3.2 Correlation between EPG vis-á-vis Hb and PCV

In the present study, EPG showed a negative correlation with both Hb and PCV that was highly significant (P<0.01). Therefore, it was concluded that if EPG would increase, the Hb and PCV value would decrease, or, otherwise.

Table 4.3: Correlation estimates between the parameters

Pearson Correlation (r)	Jamunapari		Jhakhrana		Local	
	Hb	PCV	Hb	PCV	Hb	PCV
EPG	-0.884	-0.876	-0.600	-0.513	-0.846	-0.821
Sig. (2-tailed)	**	**	*	*	**	*

** r significant at 0.01 level (P<0.01) * r significant at 0.05 level (P<0.05)

4.3.3 Effect of breed and PCR –RFLP allele on EPG

The effect of breed and PCR-RFLP allele on EPG was statistically non-significant (P>0.05); however, it was observed that Jamunapari and Jhakhrana showed higher EPG than that of local hill goats. PCR-RFLP allele A and C were showing higher EPG while it was lower in PCR-RFLP allele B (Table 4.4).

Table 4.4: Effect of breed and PCR-RFLP allele type on EPG count

Classes	Mean±SE¥
Breed (n=15 for each breed)NS	
Jamunapari	2161±436
Jhakhrana	2000±510
Local hill	1473±511
RFLP alleleNS	
A (n=2)	2167±1049
B (15)	1312±377
C (28)	2155±268

NS: non- significant (P>0.05) ¥ Least square mean

4.3.3.1. Frequency distribution of PCR- RFLP alleles in Jamunapari with respect to EPG classes

Egg count per gram (EPG) was categorized into three frequency intervals or classes representing low (<500), moderate (500-2000) and high (>2000) degree of gastrointestinal parasitism and was tabulated against PCR-RFLP allele type. In Jamunapari breed of goat, the RFLP-allele C was found in high EPG class (>2000). The RFLP-allele A and B showed comparable EPG counts (Table 4.5).

Table 4.5: Frequency distribution of PCR-RFLP alleles in Jamunapari with respect to EPG class

Type of PCR-RFLP allele	EPG classes		
	Low (<500)	Moderate (500-2000)	High (>2000)
A	1	0	1
B	0	2	0
C	1	2	8

Chi square test significance P=0.06

4.3.3.2 Frequency distribution of PCR-RFLP alleles in Jhakhrana with respect to EPG classes

In Jhakhrana breed of goat, PCR-RFLP allele A was absent and PCR-RFLP-allele B and C was distributed in a comparable way (P>0.05) among low (<500), medium (500-2000) and high (>2000) EPG classes (Table 4.6).

Table 4.6: Frequency distribution of PCR-RFLP alleles in Jhakrana with respect to EPG classes

Type of PCR-RFLP allele	EPG classes		
	Low (<500)	Moderate (500-2000)	High (>2000)
A	0	0	0
B	2	2	2
C	1	3	5

Chi square test significance was non-significant (P>0.05)

4.3.3.3 Frequency distribution of PCR-RFLP alleles in local hill goats with respect to EPG classes

In local hill breed of goat, PCR-RFLP allele A was absent whereas PCR-RFLP allele B and C showed comparable distribution (P>0.05) among low (<500), medium (500-2000) and high (>2000) EPG classes (Table 4.7).

Table 4.7: Frequency distribution of PCR-RFLP alleles in local hill goat with respect to EPG classes

Type of PCR-RFLP allele	EPG classes		
	Low (<500)	Moderate (500-2000)	High (>2000)
A	0	0	0
B	2	4	1
C	3	3	2

Chi square test significance was non-significant (P>0.05)

4.4 PCR–RFLP of *DQA1.2* and *DRB1.2* gene in the sheep

Amplification of Ovar- *DQA1* exon 2 genes and Ovar – *DRB1* exon 2 from two sheep breeds by PCR generated a single band of 349 bp and 308 bp, respectively (Fig 4.5 and 4.6). Restriction enzyme *HinfI* was used for *DQA1*.2 and *RsaI* was used for *DRB1.2* gene, respectively. *HinfI* and *RsaI* PCR-RFLP pattern among the Avikalin and Malpura sheep are shown in Figure 4.7 and 4.8, respectively. *HinfI* PCR-RFLP revealed three patterns such as 304/45 (*a*), 221/82/46 (*b*) and 267/82 (*c*) while *RsaI* PCR-RFLP yielded two patterns such as 117/68/54/39/30 (*i*) and 232/76 (*j)* in Avikalin and Malpura sheep (Table 4.8).

Table 4.8 : Allelic patterns of *DQA1.2* and *DRB1.2* in Avikalin and Malpura sheep

PCR-RFLP allele type	*Hinf I* (DQA1)	pattern	*RsaI* (DRB1)	Pattern
ai	304, 45	*a*	117,68,54,39,30	*i*
bi	221, 82, 46	*b*	117,68,54,39,30	*i*
ci	267, 82	*c*	117,68,54,39,30	*i*
bj	221, 82, 46	*b*	232,76	*j*

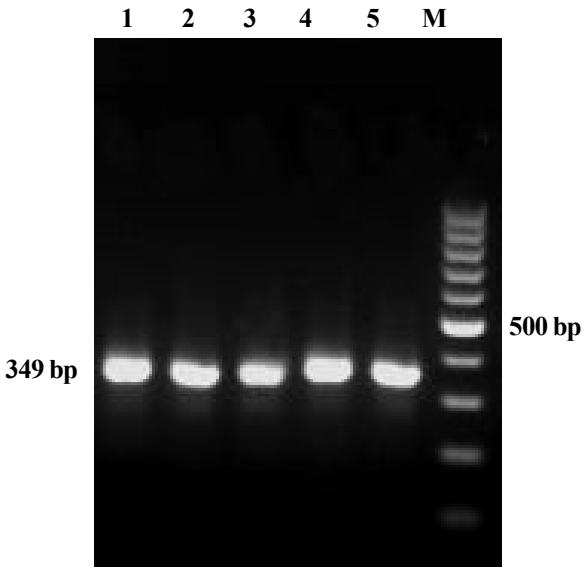


Fig. 4.5 : Amplification of Ovar – DQA1 exon 2 showing a band at 349 bp in Avikalin and Malpura breeds of sheep
Lane M : 100 bp plus DNA ladder
Lane 1-5 : 349 bp PCR product of DQA exon 2

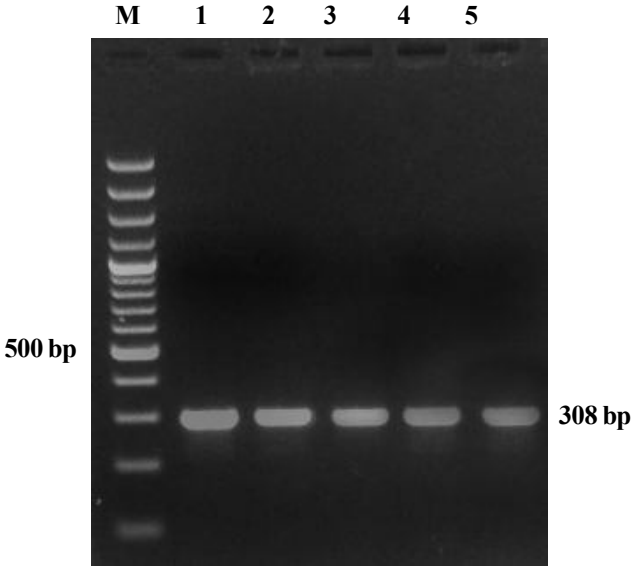


Fig. 4.6 : Amplification of Ovar – DRB1 exon 2 showing a band at 308 bp in Avikalin and Mulpura breed of sheep
Lane M : 100 bp plus DNA ladder
Lane 1-5 : 308 bp PCR product of DRB1 exon 2

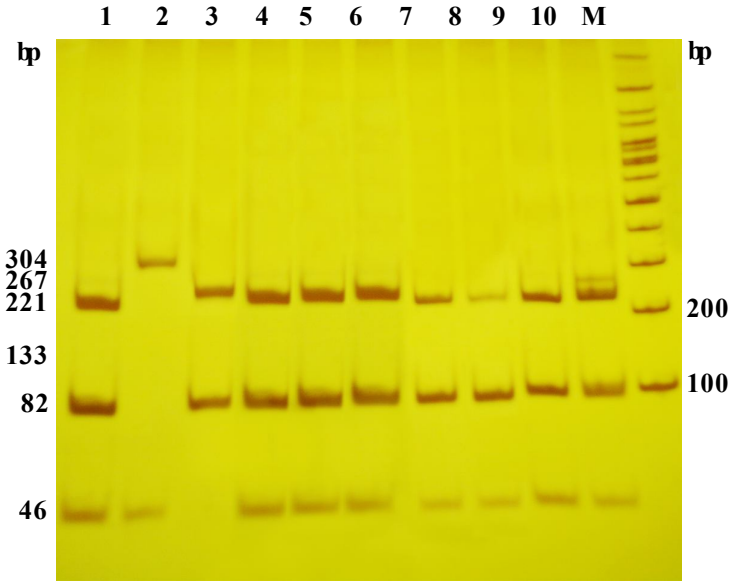


Fig. 4.7 : Restriction digestion of PCR product of DQAI exon2 gene with HinfI in Avikalin and Malpura breeds of sheep

Lane M : 100 bp plus DNA ladder
Lane 1&4-10 : Pattern "b" (221, 82 & 46 bp)
Lane 2 : Pattern "a" (304 & 45 bp)
Lane 3 : Pattern "c" (267 & 82 bp)

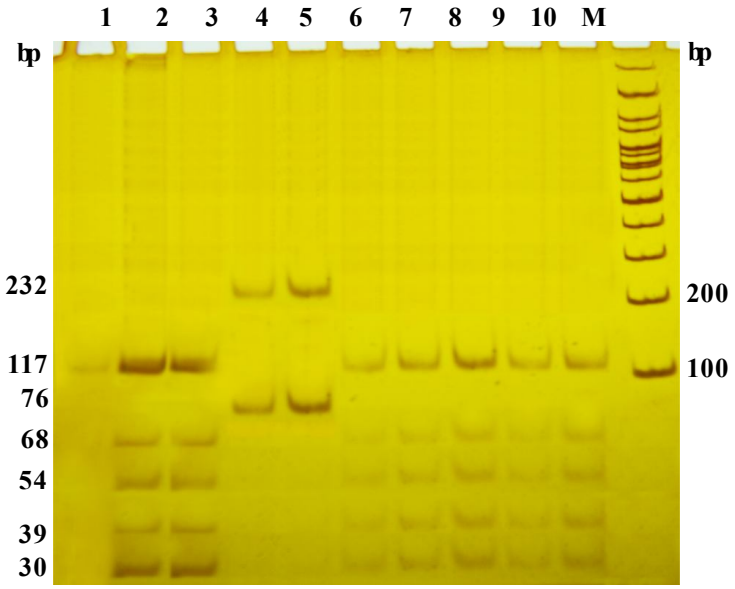


Fig. 4.8 : Restriction digestion of PCR product of DRBI exon2 gene with RsaI in Avikalin and Malpura breeds of sheep

Lane M : 100 bp plus DNA ladder
Lane 1-2,5-10 : Pattern "i" (117, 68 & 54, 39 & 30 bp)
Lane 3&4 : Pattern "j" (232 & 76 bp)

4.4.1 Allelic frequency in *DQA1.2* and *DRB1.2* in sheep population

Allelic frequencies for *Hinf PCR*-RFLP pattern of *DQA1.2* gene and *RsaI* PCR-RFLP pattern of *DRB1.2* gene for Avikalin and Malpura sheep are presented in Table 4.9. The frequency of *bi* and *bj* type allele in Avikalin were 66.67 and 33.33, respectively. The a*i* and *ci* allele were absent in Avikalin population. The frequency of *ai , bi, ci* and *bj* type allele in Malpura were 6.66, 80, 6.66 and 6.66 per cent, respectively(Table 4.9).

Table 4.9: Allelic frequency of *DQA1.2* and *DRB1.2* in Avikalin and Malpura

Allelic frequency (%)	Avikalin (n=30)	Malpura (n=30)
ai	0	6.6
bi	66.6	80
ci	0	6.6
bj	33.3	6.6

4.4.2 Distribution of PCR-RFLP alleles in *Haemonchus* resistant and susceptible groups of Avikalin sheep

The sheep flock maintained at CSWRI, Avikanagar was categorized into *Haemonchus* resistant and susceptible groups based on the EPG by the animal health division. Therefore, PCR-RFLP alleles were compared against the susceptible and resistant groups. In Avikalin, *bi* and *bj* PCR-RFLP allele showed a comparable distribution (P>0.05) between the two groups (Table 4.10).

Table 4.10: Frequency distribution of PCR-RFLP alleles in Avikalin sheep with respect to EPG classes

	EPG classes	
PCR RFLP allele	**Resistant**	**Susceptible**
bi	5	5
bj	3	2

Chi square test significance was non-significant (P>0.05)

4.4.3. Distribution of PCR-RFLP alleles in *Haemonchus* resistant and susceptible groups of Malpura sheep

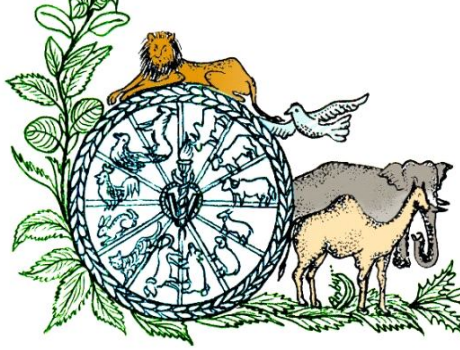
In Malpura breed, *ai* and *ci* type of PCR- RFLP allele was restricted exclusively to resistant group but not in the susceptible group. PCR-RFLP allele *bi* type showed a non-significant variation in the resistant and susceptible groups (P>0.05). PCR-RFLP allele *bj* type was confined to susceptible group (Table 4.11).

Table 4.11: Frequency distribution of PCR-RFLP alleles in Malpura sheep with respect to EPG classes

PCR-RFLP allele	EPG classes	
	Resistant	Susceptible
ai	1	0
bi	6	6
ci	1	0
bj	0	1

Chi square test significance was non-significant (P>0.05)

Discussion



5 Discussion

The livestock mainly ruminants either in an organized dairy herd or reared by marginal farmers in semi intensive system in India are affected by a variety of gastrointestinal (G.I.) nematodes. Parasitic gastroenteritis dominated by haemonchosis is one of the major constraints to profitable ruminants production in India. Gastrointestinal nematodes infection causes heavy economic losses to meat and wool industries in terms of decreased production, recurring overhead costs of treatment and/or prophylaxis and the direct losses due to death of infected animals through the voracious blood feeding activity of the parasites associated system dysfunction. GI parasitism is very common in tropical and subtropical countries due to temperature and humidity (Baker, 1999). These environmental conditions promote survival, rapid growth of nematodes and extended periods of transmission, which are making the parasites prevalent for most of the year (Kaplan, 2004). The major economically important nematodes are of the order Strongylida, which consists of oe*sophagostomum* Spp, *Chabertia ovina*, *Bunostomum* Spp, *Trichostrongylus* Spp, *Cooperia* Spp, *Ostertagia* Spp, *Teladorsagia* and *Haemonchus* spp. Among these parasites *Haemonchus contortus* is considered to be most pathogenic parasite of sheep and goats followed by *Oesophagostomum columbianum* and *Trichostrongylus* spp. The weight gain loss due to *H. contortus* infection alone in sheep and goat was estimated to the tune of 48.7 % and 32.2 %, respectively (Beriajaya and Copeman, 2006). It was estimated that the treatment cost for *Haemonchus contortus* alone $103m/ annum India (McLeod, 2004). In sheep, the estimate was up to 60% of all economic losses occurring due to GI nematode infection (Kloosterman *et al.* 1992).

The control of the nematode infestations in ruminants depends mainly on the proper organization of grazing and (or) use of anthelmintic agents. However, grazing management

systems are often impractical and expensive to implement particularly in developing countries including India, whereas frequent use of anthelmintic leads to problems such as rising resistance of parasites to drugs and increasing public concern about chemical residues in animal products and the environment. Some other control measures are also adopted such as biological control agents (Fungi) and botanical anthelmintics for control of these parasites. Due to these reasons, selection of animals based upon resistance is attractive approach. Selection of resistant animals can provide a long term solution for management of these parasites, moreover, resistance is transmitted from one generation to another generation of animals. These animals can be selected and used for future breeding programs. Selection and breeding for genetically nematode resistant animals are relatively simple and cheap method of reducing the effects of nematode infestation (Baker, 1999; Bishop and Stear, 1999). There are many reports on variation among breeds of sheep and cattle in resistance to common internal parasites, such as *H. contortus*, *Ostertagia* (*Teladorsagia*) spp., *Trichostrongylus* spp. (Stear *et al*., 1990; Gasbarre *et al*., 2000).

There are attempts at identifying QTL(s) or gene(s) of resistance to gastrointestinal nematode(s) allowing selection for resistance without expensive and wasteful of animals testing for nematode infestation. Moreover, such testing is unreliable for field data. Different parasite species may not be susceptible to the same immune responses and, therefore, identification of QTL(s) affecting resistance to specific parasite species requires extensive phenotypic study in a population structure suitable for statistical analysis. Such analyses require reference families of at least two, or much better three generations, preferably with pedigrees referring to common sires widely used in commercial populations.

A number of studies were performed to identify QTLs for resistance to gastrointestinal nematodes in ruminants. The relevant investigation was arranged in 1979 in New Zealand (Diez-Tascon *et al.,* 2002). Divergent sheep selection lines resistant and (or) susceptible to nematode parasites were used to find the respective QTLs. The QTL for resistance was localized in chromosome 3, and mapped to about a 5 cM region. The gene located to this region codes for the interferon gamma (IFNG) and is considered a putative candidate gene for resistance to nematode parasites. Results reported for naturally infected Soay sheep (Coltman *et al*., 2001) confirm the role of a gene conferring increased resistance to gastrointestinal nematodes being located at or near the *IFNG*.

Polymorphism in MHC gene is strongly influence the outcome of infection and lead to genetic resistance to infections. The main function of MHC molecule is the presentation of peptide antigen to T lymphocytes. Special attention is paid to the MHC class II molecules that induce the immune response in case of extracellular infection. The widest polymorphism among the MHC genes is found in *locus DRB*. This gene encodes the beta chain of the DR molecule, protein found in high concentrations on the surface of antigen-presenting cells. The most frequently investigated fragment of the *DRB* gene covers exon 2, which codes the binding site for a foreign protein (Charon, 2004).

Statistical analysis showed significant association between phenotypic parameters of resistance (faecal egg count) and the markers *OarCp73*, *DYMS1* and *BM1815*. The *DYA* gene belonging to the class IIb sub-region of the MHC, which is closely linked to the microsatellite *DYMS1*, is a possible candidate gene for resistance to *H. contortus* in sheep. In Scotland an ovine MHC class II antigen was identified (Schwaiger *et al*., 1995), being associated with 98% lower egg count in Scottish Blackface sheep naturally infested with *Ostertagia circumcincta*. A subsequent study showed that *DRB*1 class II antigen was associated with 10-fold reduction in faecal egg count. This result and results of others demonstrate the significant role of the MHC in ruminant resistance to parasites.

The present study was designed to study the role of MHC class II polymorphism (*DQA*1 and *DRB*1) of sheep and (*DQA*1 and *DRB*3) genes of goat and its association with G.I. nematode resistance particularly against *H. contortus* using Egg Per Gram/faecal egg count (EPG/FEC) as phenotypic indicator. Further, the EPG was correlated with Hb (haemoglobin) and Packed cell volume (PCV). EPG, PCV and changes in BWt were found to be reliable phenotypic markers and correlates of high responsiveness to infection (Behneke *et al*., 2006). In every case at least three distinct response phenotypes were consistently observed in the samples studied either sheep or goats, namely, a high EPG (>2000), medium EPG (500-2000) and low EPG (<500) phenotypes. FEC is viewed as a parameter most directly reflecting parasitological status of the sheep, and in young animal, can be well correlated with the burden of adult nematodes (Douch *et al.,* 1995, 1996).

There have been reports of genetic differences among breeds (Courtney *et al*., 1985; Baker *et al*., 2003) and within-breed variation in resistance to infection by gastrointestinal

helminthes. Moderate heritabilities have been reported for resistance to *H. contortus* in the Australian Merino (Woolaston and Piper, 1996), *T. colubriformis* in New Zealand Romneys (Bisset *et al*., 1992), and *O. ostertagi* in Scottish Blackface sheep (Bishop *et al*., 1996). There is no reports are available to set phenotypic markers for goats. The present study we developed the basic phenotypic criteria for study the resistance/resilience in goats. We divided the animals statistically in to three groups (<500, 500-2000 and >2000) based on EPG. Though the low EPG set for resilience/resistance in goats was <500, it is depends on species of strongyle infected due to high level susceptibility of goats to G.I. parasitism compared to sheep.

The normal Hb values in goats are above 8.0 with respect to low EPG (<500). The highly susceptible animals EPG was above 2000 and corresponding Hb was < 5.0. The mean Hb was 6.5, 6.8 and 7.2 in Jamunapari, Jhakhrana and Local hill goats, respectively. The mean PCV of goats was 28, 31 and 34 in Jamunapari, Jhakhrana and Local hill, respectively. The mean EPG was higher in Jamunapari (2326), followed by Jhakhrana (1940) and lowest in Local hill (1497). The local goats have more Hb and PCV value than Jamunapari and Jakhrana, it might be due the local goats adopted well in local environmental conditions. Further, the EPG also less in local goats compared to other two breeds, it indicated that the local native breeds are less susceptible to G.I. nematode infection. A number of indigenous "unimproved" breeds of sheep appeared to be significantly resistant or tolerant to parasites as compared with "improved" breeds (Charon, 2004).

Resistance is defined as the initiation and maintenance of responses provoked in the host to suppress the establishment of parasites and/or eliminate parasite burdens. Resilience (or tolerance) is defined as the ability of the host to survive and be productive in the face of parasite challenge (Clunies-Ross, 1932; Woolaston and Baker, 1996). The degree of resistance has usually been assessed in terms of worm counts at necropsy or faecal egg counts (FEC), the measure of resistance used in this study, during an infection period in live animals. In lambs it is well documented that faecal egg counts are highly correlated with worm counts (Woolaston and Baker, 1996). Resilience has been defined in terms of productivity (e.g. live-weight gain or wool production) under nematode challenge compared to productivity in non-infected animals (Albers *et al.*, 1987). When sheep are infected with the bloodsucking parasite *H. contortus*

they become anaemic and this is measured by PCV, which is a good indication of how the animal is managing to cope with the pathogenic effects of the parasite and survive when infected. However, other studies (Albers *et al.*, 1987) treated both FEC and PCV as two different measures of resistance.

EPG showed a negative correlation with both Hb and PCV that was highly significant ($P<0.01$) in present study. Therefore, it was concluded that if EPG would increase, the Hb and PCV value would decrease, or, otherwise. Earlier studies in sheep revealed PCV was positively correlated with body weight and negatively correlated with FEC in both ewes and lambs (Vanimisetti *et al*., 2004; Yadav *et al*., 2006). In lambs, heritabilities were 0.39 for PCV and 0.10 for FEC across all measurement. Heritability estimates for ewes were 0.15 for PCV and 0.31 for FEC. Correlations between dam and lamb records for FEC were generally low, suggesting different mechanisms of resistance in lambs and non lactating ewes. Ewes with higher genetic merit for growth as lambs were less resistant to infection as adults, but genetic merit for fertility and prolificacy were not related to parasite resistance. Lambs with higher genetic merit for body weight were more resistant to infection. Selection for resistance to *H. contortus* is therefore possible and should not adversely affect growth of lambs and fertility of ewes in this production environment (Vanimisetti *et al*., 2004).

For the sheep, the animals were phenotypically selected over eight years by CSWRI, Avikanagar. DNA was isolated from anticoagulant blood collected from phenotypically selected resistant and susceptible sheep (Avikalin and Malpura) of CSWRI, Avikanagar and goats (Jamunapari, Jhakhrana and local hill goats) of IVRI farm, Mukteswar.

PCR–RFLP of *DRB3.2* and *DQA1.2* gene in goats and its association with resistance

The amplification of Cahi – *DRB*3 exon 2 and *DQA1* exon 2 genes from three goat population using PCR generated a single 319 bp and 349 bp clear band, respectively. The amplified products contain exon 2 with flanked by introns in both side, so that the entire exon 2 will be obtained after sequence analysis. Restriction enzyme *Hae*III was used for *DRB*3 exon 2 and *Hinf*I was used for *DQA*1 exon 2, respectively. Three allelic patterns were observed using *Hae*III, 186/133 (A), 172/133/14 (B) and 172/81/52/14 (C) in all the three goat

population. However, *HinfI* yielded a single pattern, 221/82/46 (a) on *DQA1*. Several methods have been used to investigate the genetic polymorphism of MHC-DRB3 gene (Ledwidge *et al*., 2001). RFLP analysis of gene segments amplified by the PCR (Saiki *et al*., 1998) has been found useful for typing (Amills *et al*., 1996).

Li *et al*. (2006) studied the allelic variations in exon 2 of caprine MHC class II DRB3 gene in Chinese indigenous goats. Six alleles and 18 restriction digestion profiles were distinguished by digestion of PCR amplification product of DRB3 exon 2 with *Hae*III. The results also indicated that the potentially different ecological factors, such as climate, disease, topography, pasture conditions and pathogens might be account for the significantly different distribution of alleles and genotypes among the goat populations. The present study, the frequency of 'A' allelic pattern (13.30%) found only in Jamunapari not in other two breeds and the frequency of 'C' allelic pattern were observed maximum in all three breed of goats.

The effect of breed and PCR-*Hae*III-RFLP allele on EPG was statistically non-significant ($P>0.05$); however, there was a general trend that Jamunapari and Jhakhrana showed higher EPG than that of local hill goats. PCR-RFLP allele A and C were showing higher EPG while it was lower in RFLP-allele B. In Jamunapari breed of goat, the PCR-RFLP allele C was found in high EPG class (>2000) however, there is no difference among allelic pattern with respect to EPG in Jhakhrana and Local hill goats. The number of other studies (Outteridge *et al*., 1996; Paterson *et al*., 1998; Schwaiger *et al*., 1995; Van Haeringen *et al*., 1999) proved the evidence that MHC significantly associated with resistance to intestinal nematodes of ruminants. The polymorphism of the MHC complex increases the range of parasites recognized by the immune system. Amills *et al*. (1996) developed PCR-RFLP pattern for typing the second exon of the DRB gene and by this procedure they distinguished unequivocally 18 of the 22 caprine DRB alleles. Close associations have been found between RFLPs and amino acid substitutions at positions which are expected to be involved in the formation of the antigen-recognition site (ARS) of the DR molecule. These results suggest that PCR-RFLP may be a useful tool in typing the caprine *DRB* gene and in relating amino acid substitutions at the ARS of the DR molecule with disease resistance.

PCR–RFLP of *DQA*1 and gene *DRB*1in the sheep

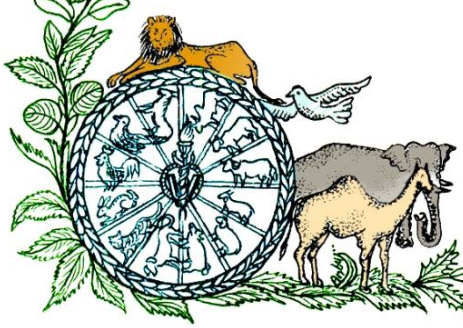
The present study, amplification of Ovar- *DQA1* exon 2 genes and Ovar – *DRB*1 exon 2 from two sheep breeds by PCR generated a single band of 349 bp and 308 bp, respectively. The amplified products contain exon 2 with flanked by introns in both side, so that the entire exon 2 will be obtained after sequence analysis. Restriction enzyme *HinfI* was used for *DQA1*.2 and *RsaI* was used for *DRB1.2* gene, respectively. *HinfI* PCR-RFLP revealed three patterns such as 304/45 (*a*), 221/82/46 (*b*) and 267/82 (*c*) while *RsaI* PCR-RFLP yielded two patterns such as 117/68/54/39/30 (*i*) and 232/76 (*j*). The allelic pattern analysis was made using both restriction patterns. The frequency of *bi* and *bj* type allele in Avikalin were 66.67 and 33.33, respectively. The a*i* and *ci* allele were absent in Avikalin population. The frequency of *ai , bi, ci* and *bj* type allele in Malpura were 6.66, 80, 6.66 and 6.66 per cent, respectively. In both breeds '*bi'* allele frequency were observed maximum.

Two DQA genes, *DQA1* and *DQA2,* have been identified in sheep (Scott *et al*., 1991). Both genes are polymorphic. RFLP analyses have identified seven alleles plus a null allele at the DQA1 locus and 16 alleles at the DQA2 locus (Wright and Ballingall, 1994; Escayg *et al.,* 1996). Sequence analysis of some of these RFLP alleles and others has revealed nine DQA1 and 10 DQA2 sequences (Snibson *et al*., 1998; Zhou and Hickford, 2004). Lower levels of sequence variation may make some alleles difficult to differentiate, especially by RFLP analysis; hence, sequence polymorphism in the DQA genes may have been underestimated. The comparative analysis of sheep *DQA1* sequences with cattle revealed several clusters of ovine *DQA1* sequences, and some sheep alleles were more similar to cattle alleles than other sheep alleles. (Zhou and Hickford, 2004).

Distribution of PCR-RFLP alleles in *Haemonchus* resistant and susceptible groups of Malpura and Avikalin sheep was analysed. In Avikalin, *bi* and *bj* pattern showed a comparable distribution (P>0.05) between the two groups. However, in Malpura breed, *ai* and *ci* type of PCR- RFLP allele was restricted exclusively to resistant group but not in the susceptible group. PCR-RFLP allele *bi* type showed a non-significant variation in the resistant and susceptible groups (P>0.05). PCR-allele *bj* type was confined to susceptible group of Malpura sheep.

Ovar-*DRB1* is one of the most important response genes in the MHC class II region of sheep. Exon 2 of the *Ovar-DRB*1 gene codes for part of the MHC class II antigen binding cleft and over 160 alleles have been identified at this locus in sheep, two of which have been associated with decreased resistance (Konni *et al*., 2003; Sayers *et al*., 2005) and one with increased resistance to nematode infection (Sayers *et al*., 2005; Schwaiger *et al*., 1995). Diversity driven by pathogens implies a strong association between MHC alleles and patterns of resistance to specific autoimmune or infectious diseases. The polymorphism of the *Ovar-DRB*1 gene plays an important role in resistance to nematode infection in the Suffolk breed (Sayers *et al*., 2005). Suffolk sheep carrying the *DRB1**1101 (previously referred to as-DRB1*0203 or G2) allele have been reported to show increased resistance to natural Teladorsagia circumcincta infection compared to non-carriers (Hassan *et al*., 2011).

Summary and Conclusions



6 Summary and Conclusions

Parasitic gastroenteritis is one of the major impediments to profitable ruminant production. The haematophagous abomasal parasite, *Haemonchus contortus*; the small intestine parasite, *Trichostrongyle* colubriformis and *Oesophagostomum columbianum* are the dominant species in both small and large ruminants in India and the degree of infection is more in grazing livestock than tethered animals. The annual treatment cost of *H. contortus* in India was estimated at $103 million that excludes losses due to productivity. The weight loss in weaned lambs and kids due to *H. contortus* was estimated to the tune of 48.7 and 32.2 per cent, respectively. Current control methods for GI nematode parasites focus on reducing contamination of pastures through anthelmintic treatment and/or controlled grazing. In India, the use of these control methods has yielded limited success owing to the high cost of anthelmintics, their uncertain availability, increasing frequency of drug resistance and limited scope for rotational grazing due to communal pastoral systems. It appears unlikely that new broad-spectrum anthelmintics would be available in the near future because of the major costs associated with the development of new products. To date, no commercial vaccine is available to control GI nematode parasites. Alternative approaches to control endoparasites are therefore being considered; one such approach is the characterization and utilization of host genetic variation for resistance or resilience to endoparasites.

Polymorphism of MHC genes strongly influence the outcome of infection and lead to genetic resistance to infections. The main function of MHC molecule is the presentation of peptide antigen to T lymphocytes. Special attention is paid to the MHC class II molecules that induce the immune response to extracellular infection. The widest polymorphism among the MHC genes is found in *locus DRB* and *DQA*. These genes encode for the protein products of

the beta and alpha chains of the DR and DQ molecules that are found in high concentrations on the surface of antigen-presenting cells. The most frequently investigated fragment of the *DRB* and *DQA* gene covers exon 2, which codes the binding site for a foreign protein.

The present study was designed to explore the role of MHC class II polymorphism of *DQA1 exon2* and *DRB1 exon2* in Avikalin and Malpura breeds of sheep as well as *DQA1* exon2 and *DRB3 exon2* gene polymorphism in Jamunapari, Jakhrana and local hill goats with respect to its association with resistance to *H. contortus* using EPG as a phenotypic indicator. For the first time, we have set EPG as a phenotypic criterion for studying the resistance/ resilience in goats. The animals were divided in to low (<500), medium (500-2000) and high (>2000) EPG groups reflecting the severity of infection. The Hb content in goats was > 8.0 in low EPG group (<500) while it was < 5.0 in animals with high EPG (>2000). The mean Hb was 6.5, 6.8 and 7.2 in Jamunapari, Jhakhrana and Local hill goats, respectively. The mean PCV of goats was 28, 31 and 34 in Jamunapari, Jhakhrana and Local hill, respectively. The mean EPG was higher in Jamunapari (2326), followed by Jhakhrana (1940) and lowest in Local hill (1497). The local goats had more Hb and PCV value than Jamunapari and Jakhrana. Further, the EPG was also less in local hill goats as compared to other two breeds.

EPG showed a negative correlation with both Hb and PCV that was highly significant ($P<0.01$) in present study. Therefore, it was concluded that if EPG would increase, the Hb and PCV value would decrease, or, otherwise.

The amplification of Cahi – *DRB*3 exon 2 and *DQA1* exon 2 genes from three goat population using PCR generated a single band of 319 bp and 349 bp, respectively. The amplified products contained exon 2 flanked by introns on either side, so that the entire exon 2 was obtained after sequence analysis. Restriction enzyme *Hae*III was used for *DRB*3.2 and *Hinf*I was used for *DQA*1.2. Three allelic patterns were observed using *Hae*III, 186/133 (A), 172/133/14 (B) and 172/81/52/14 (C) in all the three goat population. However, *HinfI* yielded a single pattern, 221/82/46 (a) on *DQA1*.

In the present study, the frequency of 'A' allelic pattern (13.30%) was restricted exclusively to Jamunapari but not in other two breeds and the frequency of 'C' allelic pattern was observed maximum in all three breed of goats.

The effect of breed and PCR-*Hae*III-RFLP allele on EPG was statistically non-significant (P>0.05); however, there was a general trend that Jamunapari and Jhakhrana showed higher EPG than that of local hill goats. RFLP- allele A and C showed higher EPG while it was lower in RFLP-allele B. In Jamunapari breed of goat, the RFLP-allele C was found in high EPG class (>2000); however, there was no statistical difference among allelic pattern with respect to EPG in Jhakhrana and Local hill goats.

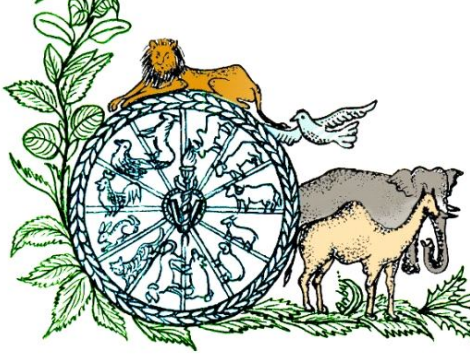
Based on eight years of EPG data, the sheep flock was categorized into *Haemonchus* resistant and susceptible groups by the animal health division, CSWRI, Avikanagar. In the present study, amplification of Ovar- *DQA1* exon 2 genes and Ovar – *DRB*1 exon 2 from two sheep breeds by PCR generated a single band of 349 bp and 308 bp, respectively. The amplified products contained exon 2 flanked by introns on either side, so that the entire exon 2 was obtained after sequence analysis. Restriction enzyme *HinfI* was used for *DQA1*.2 and *RsaI* was used for *DRB1.2* gene. *HinfI* PCR-RFLP revealed three patterns such as 304/45 (*a*), 221/82/46 (*b*) and 267/82 (*c*) while *RsaI* PCR-RFLP yielded two patterns such as 117/68/54/39/30 (*i*) and 232/76 (*j)*. The allelic pattern analysis was made using both restriction patterns. The frequency of *bi* and *bj* type allele in Avikalin was 66.67 and 33.33, respectively. The a*i* and *ci* allele were absent in Avikalin population. The frequency of *ai , bi, ci* and *bj* type allele in Malpura were 6.66, 80, 6.66 and 6.66 per cent, respectively. In both breeds, '*bi'* allele frequency was observed maximum.

Distribution of PCR-RFLP alleles in *Haemonchus* resistant and susceptible groups of Malpura and Avikalin sheep was analysed. In Avikalin, *bi* and *bj* pattern showed a comparable distribution (P>0.05) between the two groups. However, in Malpura breed, *ai* and *ci* type of PCR- RFLP allele was restricted exclusively to resistant group but not in the susceptible group. PCR-RFLP allele *bi* type showed a non-significant variation in the resistant and susceptible groups (P>0.05). PCR-allele *bj* type was confined to susceptible group of Malpura sheep.

Future Perspectives:

- The results of the present study need to be corroborated with large sample size in a controlled experimental set up to avoid guilt by association.
- More number of restriction enzymes and its combination should be analyzed for different allelic pattern and its possible association with EPG for resistance and along with MHC markers, other immunological parameters such as Th1 and Th2 responses need to be studied.

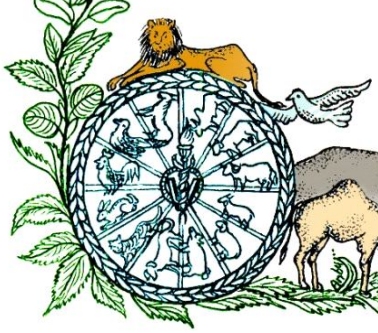
Mini Abstract



7 Mini Abstract

The present study was designed to study the role of MHC class II polymorphism of *DQA1* exon2 and *DRB1*exon2 genes of sheep and *DQA1* exon2 and *DRB3* exon2 genes of goat with respect to *Haemonchus contortus* infection using Egg Per Gram/faecal egg count as a phenotypic indicator. A total of 75 animals including 45 goat (Jamunapari, Jakhrana and Local hill breed) and 30 sheep (Avikalin and Malpura) were used for the study. The goats were divided into three groups (<500, 500-2000 and >2000) based on EPG and correlated with Hb and PCV. The local hill goats had higher Hb and PCV than that of Jamunapari and Jakhrana breeds. Further, the EPG was less in local goats as compared to other two breeds. EPG showed a negative correlation with both Hb and PCV that was highly significant (P<0.01). Restriction enzyme *Hae*III was used for *DRB*3 exon 2 and *Hinf*I was used for *DQA*1 exon 2 gene of goats. Three allelic patterns were observed using *Hae*III, 186/133 (A), 172/133/14 (B) and 172/81/52/14 (C) in all the three goat breed population. However, *HinfI* yielded a single pattern, 221/82/46 (a) on *DQA1*. The frequency of 'A' allelic pattern (13.30%) found only in Jamunapari but not in other two breeds and the frequency of 'C' allelic pattern was observed maximum in all three breed of goats. In Jamunapari goat, the PCR-RFLP allele C was found exclusively in high EPG class (>2000); however, there was no difference among allelic pattern with respect to EPG in Jakharana and Local hill goats (P>0.05). The sheep flock was categorized into *Haemonchus* resistant and susceptible groups based on the EPG over the period of eight years by CSWRI, Avikanagar. Restriction enzyme *HinfI* was used for *DQA1*.2 and *RsaI* was used for *DRB1.2* genes of sheep. *HinfI* PCR-RFLP revealed three patterns such as 304/45 (*a*), 221/82/46 (*b*) and 267/82 (*c*) while *RsaI* PCR-RFLP yielded two patterns such as 117/68/54/39/30 (*i*) and 232/76 (*j)*. The a*i* and *ci* allele were absent in Avikalin population. In Avikalin, *bi* and *bj* pattern showed a comparable distribution (P>0.05) between the two groups. However, in Malpura breed, *ai* and *ci* type of PCR- RFLP allele was restricted exclusively to resistant group but not in the susceptible group. PCR-RFLP allele *bj* type was confined to susceptible group of Malpura sheep. It is concluded that EPG was negatively correlated with Hb and PCV in goats. Local hill goats appeared to have significantly low EPG indicating innate resistance as compared to Jamunapari and Jakhrana breeds. In Jamunapari, C type allele was associated with high EPG. PCR-RFLP alleles, *ai* and *ci* were restricted to *Haemonchus* resistant Malpura group while bj allele was associated with susceptible Malpura group.

लघु सारांश



8 लघु सारांश

वर्तमान अध्ययन MHC कक्षा 2 की बहुरूपिता में भेड़ के DQA1 exon 2 और DRB1 exon 2 एवम् बकरी के DQA1 exon 2 और DRB3 exon 2 जीन Haemonchus contortus संक्रमण के संबंध में अण्डा प्रति ग्राम/मल अण्डा संख्या को प्रारूपी सूचक मानकर किया गया था। कुल 75 पशुओं जिसमें 45 बकरी (जमुनापारी, जखराना एवं देशी पहाड़ी नस्ल) और 30 भेड़ (अविकालिन एवं मालपुरा) को अध्ययन में सम्मिलित किया गया था। बकरीयों को अण्डा प्रति ग्रम के आधार पर तीन समूहों में बाटा गया (<500, 500-2000, >2000) और उनका सहसंबंद्ध हीमोग्लोबिन और PCV से किया गया था देशी पहाड़ी बकरीयों में हीमोग्लोबिन तथा PCV जमुनापारी और जखराना की तुलना में ज्यादा पाया गया। देशी पहाड़ी बकरीयों में अण्डा प्रति ग्राम दूसरी दो नस्लों की तुलना में कम था। अण्डा प्रति ग्राम दोनों हीमोग्लोबिन तथा PCV के साथ नकारात्मक सहसंबंध में पाया गया (P<0.0.1.1) बकरीयों में प्रतिबध एजाइम HaeIII DRB3 exon1 के लिए और HinfI DQA1 के लिए उपयोग किया गया। HaeIII के उपयोग से तीन एलील पैर्टन 186/133(A), 172/133/14 (B) और 172/81/92/14 (C) देखे गये। जबकि Hinf I से DQA1 exon 2 में एक पैर्ट्रन 221/82/46 (a) मिला। 'A' ऐलीलीक पैर्ट्रन की आवृत्ति सिर्फ जमुनापारी (13.30%) में पायी गयी लेकिन ऐलीलीक पैर्ट्रन की आवृत्ति सभी तीन नस्लों में अधिकता में पाई गयी। जमुनापारी बकरी में PCR-RFLP एलील 'C' विशेष रूप से उच्च अण्डा प्रति ग्राम वर्ग में पाया गया। तथापि जखराना और देशी पहाड़ी बकरी में अण्डा प्रति ग्राम से संबंधित एलीए पैर्ट्रन में कोई अंतर नहीं पाया गया CSWRI अवीकानगर द्वारा भैड़ झुड़े को आठ साल में Haemonchus प्रतिरोधी और अति संवेदनशील वर्गों में बाटा गया। प्रतिबंध एजाइंम HinfI, DQA1 exon 2 के लिए एवं RsaI DRB1 exon 2 के लिए उपयोग किया गया। PCR-RFLP Hinf I से तीन पैर्ट्रन 304/45(a), 201/82/46 (b), 267/82 (c), जबकि RsaI PCR-RFLP से दो पैर्ट्रन 117/68/54 136/30 (i) और 232/76 (j) पाये गये। एलील 'a' और 'ci' अविकालीन भेड़ में अनुपस्थित थे। अविकालीन भेड़ में एलील 'bi' और 'bj' का दो वर्गों के बीच एक तुलनीय वितरण पाया गया जबकि मालपुरा भेड़ में 'ai' और 'ci' एलील विशेष रूप से प्रतिरोधी वर्ग में ही सीमित था। इस अध्ययन का निष्कर्ष यह निकाला गया है कि अण्डा प्रति ग्राम हीमोग्लोबिन और PCV के साथ नकारात्मक सहसंबंद्ध में पाया गया। देशी पहाड़ी बकरीयों में जमुनापारी और जखराना की तुलना में अण्डा प्रति ग्राम कम पाया गया जोकि उनकी सहज प्रतिरोध क्षमता का संकेत देता है। जमुनापारी बकरी में 'c' एलील उच्च अण्डा प्रति ग्राम वर्ग के साथ जुड़े थे मालपुरा भेड़ में PCR-RFLP एलील 'ai' तता 'ci' Haemonchus प्रतिरोधी वर्ग के साथ जुड़े थे और एलील 'bj' Haemonchus अति संवेदनशील वर्ग के साथ जुड़ा था।

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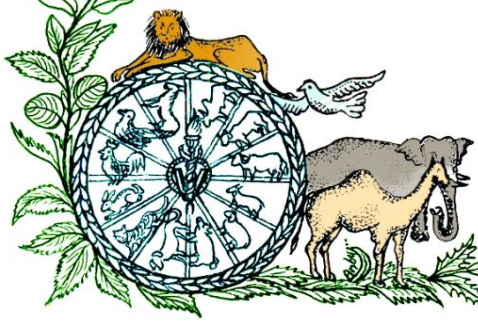

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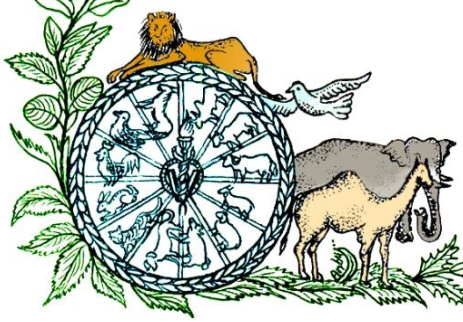
Appendix



Appendix-1

TAE (10X) (Tris Acetate EDTA)

Tris base	48.4 gm
Glacial acetic acid	11.42 ml
EDTA (0.5M pH 8.0)	100 ml
Distilled Water	upto 1 liter

Gel Loading Dye (6x)

Bromophenol blue	1.0%
Sucrose in water	40%

Acrylamide: Bis-acrylamide (30%)

Acrylamide	30 g
Bis-acrylamide	1 g
Distilled water (pre-heated to 37°C)	100 ml

Filtered and stored in dark coloured bottle at 4^0 C.

Ethidium bromide (10 mg/ml)

Ethidium bromide	10 mg
Distilled water	1 ml

Store at 4°C.

Loading dye (6X)

Tris-HCl (pH 7.6)	10 mM
Bromophenol blue	0.03%
Xylene cyanol FF	0.03%
Glycerol	60%
EDTA	60 mM

95% ethanol

Ethanol	95 ml
Distilled water	5 ml

5x TBE

Tris base	54gm
Boric acid	27.5gm
0.5M EDTA	20 gm
Autoclave distilled water	1000ml

Sterilize by autoclave and stored at room temperature

Taq DNA polymerase enzyme

Taq DNA polymerase	5 U/µl
Strore at - 20° C	

Restriction enzyme

*Hinf*I (Fermentas)	10 U/µl
HaeIII (Fermentas)	10 U/µl
RsaI (Fermentas)	10 U/µl

Ammonium Persulphate (10%)

Ammonium Persulphate	1gm
DDW	upto 10ml

Stored at 4^0 C APS decays slowly in solution, so replace stock solution every 2-3 weeks.

Fixative for PAGE

Glacial acetic acid	500 µl
Ethanol	10 ml
DDW	Up to 100ml

Stain for silver staining

Silver Nitrate	0.040g
DDW	Up to 100 ml

Developing solution

Sodium hydroxide	3 gm
Formaldehyde	750 µl
DDW	Up to 100 ml

Stop solution

Glacial acetic acid	5 ml
DDW	Up to 100 ml

10 X *Taq* DNA polymerase buffer

Tris HCI (pH 8.8)	100 mM
KCI	500mM
$MgCl_2$	15 mM
Gelatin	0.01%

Stored at –20°C.

20% Proteinase K

Proteinase-K	20 mg
Autoclaved double distilled water (up to)	1 ml

Store at –20°C

Primer

Working solution concentration	10 ng/µl

Store at –20°C.

dNTPs solution (pH 7.0)

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

Store at –20°C

Preparation of dNTPs

Ten µl of each dATP, dGTP, dCTP and dTTP are taken and 60 µl of sterile TDW mixed to make100µl mixture of dNTPs. Each of these dNTPs contains 100mM in 250µlvial. The required concentration of dNTPs is 200µM of each dNTPs in PCR. The 100µl of this dNTP mix contains 10mM of each dNTP. So, the volume of this mixture to be taken to provide 200µM of each dNTP in a 25 µl PCR reaction is 0.5µl.

100 bp DNA Ladder

It contains 10 bands of double stranded DNA fragments ranging from 100 to 1000 bp.

Vitae

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