GENETIC RESISTANCE TO GASTRO-INTESTINAL NEMATODES IN GOATS

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DECLARATION

I hereby declare that this thesis, entitled **"GENETIC RESISTANCE TO GASTRO-INTESTINAL NEMATODES IN GOATS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis, entitled **"GENETIC RESISTANCE TO GASTRO-INTESTINAL NEMATODES IN GOATS"** is a record of research work done independently by **Dr. Aparna Shankar,** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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she gives the test first, the lesson afterwards.

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Dedicated To

My Beloved family

And

Respected Teachers

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Introduction

1. INTRODUCTION

Goat is a multi functional animal and plays a significant role in the economy and nutrition of small and marginal farmers in India. India's goat population is 124.5 million, which is around 17 per cent of global goat population (FAO, 2003). Goat rearing is an enterprise, which has been practiced by a large section of population in rural areas of the country. In pastoral and agricultural subsistence societies in India, they are kept as a source of additional income and as an insurance against disaster. In addition to this, goat has religious and ritualistic importance in many societies.

Goat population in Kerala is about 12,13,000 which accounts for six per cent of total livestock population (Livestock, poultry, agricultural machinery and implements and fishery statistics, 2003). Low availability of fodder, high cost of concentrate and reduction in grazing lands has resulted in more farmers drifting away from large animal rearing to rearing of small ruminants especially goats. One of the major problems faced by the farmers is the retarded growth in goats due to internal parasitism.

Parasitic infection results in 26.57 per cent of total economic loss caused by all diseases in goats of Kerala. Among these, Strongyle infection (74 per cent) forms the major part (Animal surveillance scheme, 2006). Current control methods focus on reducing the contamination of pastures through anthelmintic drug treatment and/or controlled/rotational grazing. The application and effectiveness of these methods are limited because of high costs of anthelmintic drugs, their uncertain availability and increasing frequency of drug resistance. In a thickly populated state like Kerala, with scarce grazing lands, there is very limited scope for controlled/rotational grazing. Thus it is the need of the hour to find an alternative method for controlling gastrointestinal nematodes (GIN) in goats.

An attractive, sustainable solution to control worm burden is to select goats which are resistant to gastrointestinal nematodes. Studies on genetic resistance of goats will act as the basis for this particular selection of animals against intestinal parasites in future, producing genetically superior stock.

The degree of resistance to GIN is usually assessed in terms of worm counts at necropsy or faecal egg counts (FEC). It is well documented that FECs are highly correlated with worm counts. It is important to assess the genetic and nongenetic factors affecting FEC, to learn about the scope for genetic selection against nematodes in goats. The packed cell volume (PCV) is an indication of the extent of worm infection and subsequent anemia. Hence correlation between FEC and PCV requires special attention and thus PCV might also aid in indirect selection for nematode resistance in goats.

When more characters are involved, selection becomes a difficult process and genetic progress made depends primarily on the heritability of these characteristics selected. Measures of heritability indicate the amount of genetic variance in the population and medium to high heritability for a trait indicates that there is immense scope for genetic progress through selection. Genetic variation in a population for FEC can be studied by estimating the heritability of this trait. Magnitude of heritability estimate determines the scope for utilizing FEC for selection and breeding of nematode resistant animals.

Marker Assisted Selection (MAS), by identifying loci associated with natural resistance, would facilitate selection of resistant animals and provide a rapid solution to helminth disease with potential long-term beneficial effects. One such marker associated with nematode resistance is the interferon gamma (IFNγ) gene. This gene is associated with the suppression of the TH_2 cell response, which in turn reduces the ability of the host to expel the nematodes (Male et al., 2006). Hence IFNγ gene is a candidate locus for exploring genetic resistance to GINs.

So the present study to explore the genetic resistance of goats in Kerala to GIN is undertaken with the following objectives.

1. Study the genetic and non genetic factors involved in GIN resistance in goats.
2. To find out the heritability of genetic resistance to GIN using FEC as indicator.
3. To find the correlation between FEC and PCV and
4. To assess the relationship between FEC and variations at intron 1 of the IFNγ gene.

Review of Literature

2. REVIEW OF LITERATURE

In recent years there has been a commendable increase in the research and application of disease genetics in sheep and goats. This has been due to a number of factors: a growing appreciation of the role that host genetics can play in disease control, an increase in the tools available to dissect host genetic variation in disease resistance, and growing pressures on breeders to select animals that are healthier and more resistant both to infectious and metabolic diseases, are some of these.

2.1 INCIDENCE OF GASTROINTESTINAL NEMATODOSIS

Gastro-Intestinal Nematode (GIN) infection has great impact on livestock sector. It affects health, production and economic gain from the sector.

Yadav and Tandon (1989) reported the overall infection rate due to various GINs to be 86.8 per cent in goats of India.

Miller *et al.* (1998) reported that sixty-two per cent of 5174 sheep producers surveyed in the United States (US) identified stomach/intestinal nematodes as a major concern.

Githigia *et al*. (2001) from their study on traits like Packed Cell Volume (PCV), Faecal Egg Count (FEC), body weight gain and mortality rates in goats of Kenya, concluded that GI helminths cause production losses, weight loss and mortalities in goats. A similar result was obtained by Khusroa *et al*. (2004) on Australian Merinos and they opined that selection against FEC would increase body weights.

According to Livestock, poultry, agricultural machinery and implements and fishery statistics (2003), parasitic infection resulted in 26.57 per cent of total

economic loss caused by all diseases in goats of Kerala. Among these, strongyle infection (74 per cent) formed the major part.

In the study conducted by Gadahi *et al*. (2009) on sheep and goat in and around Rawalpindi and Islamabad of Pakistan, 53.33 per cent of sheeps and 66.45 per cent of goats were found to be infected with GI parasites.

2.2 BREEDING FOR RESISTANCE AGAINST GIN

Gill (1991) found that in contrast to primary infection, resistant lambs had significantly lower FECs and worm burdens and higher antibody levels, mucosal mast cell hyperplasia and mucosal eosinophilia in response to a challenge infection when compared with random-bred lambs. This suggested that the genetic resistance of sheep to *Haemonchus contortus* resulted from the expression of an acquired immune response. The study showed that anti-parasite antibodies and mast cell-derived mediators may play an important role in genetically determined resistance of sheep to haemonchosis.

Douch *et al*. (1995) opinioned that ram selection based on serum antibody levels and FEC results in 51 to 67 per cent increase in the genetic gain for reducing FEC, than that could be achieved by considering FEC alone as selection criteria.

Gray (1997) firmly established that it is possible to exploit genetic variation in resistance to the nematode parasites of sheep by selection.

According to Waller (1997) and Coles (2001) alternative and sustainable control strategies like, quantification of genetic variation between animals in their resistance, search for genes or Quantitative Trait Loci (QTL) contributing to such differences and breeding for resistance are important in the face of growing anthelmintic resistance.

Reports by Miller *et al.* (1998) and Kaplan *et al.* (2004) shows that over-reliance on chemical dewormers will lead to development of anthelmintic resistance in GIN of sheep and goats, indicating need for an alternative method of worm control.

According to Bishop and Stear (2003), benefits from genetically improving nematode resistance include decreased anthelmintic requirements and/or reduced pasture contamination leading to decreased larval challenge and hence indirect benefits on health and performance.

Breeding sheep for worm resistance would aid in reducing reliance on anthelmintics to which most of the strains of GIN have developed resistance which in turn, would also help in maintaining the effective life of chemical drugs and also in reduction of production costs (Khusro *et al.,* 2004).

Vanimisetti *et al.* (2004) observed that 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. They inferred that selection for resistance to *H. contortus* was possible without adversely affecting growth of lambs and fertility of ewes.

In the study conducted by Mugambi *et al.* (2005), first cross (F1) Red Maasai×Dorper rams were mated to both Red Maasai (R) and Dorper (D) ewes to produce double backcross progeny. The difference between the backcrosses for both FEC and PCV was about a half of the difference between the straightbred R and D lambs indicating additive gene action.

Miller *et al.* (2006) studied the traits, FEC and PCV in Suffolk sheep (susceptible to infection), Gulf Coast Native sheep (more resistant to infection) and their F1 (first filial) and F2 (second filial) progenies. He observed the traits to be segregating in F2 progeny from susceptible and resistant parent breeds, as theoretically expected.

2.2.1 DETECTION OF GIN RESISTANT ANIMALS

Miller *et al.* (1998) stated that GIN infection can be evaluated indirectly by FEC and blood PCV, specifically for *H. contortus.* In the study, overall mean FEC and PCV were significantly higher and lower, respectively, for Suffolk lambs and native lambs.

Costa *et al*. (2000) studied patterns of Egg per Gram (EPG), Haemoglobin (Hb) and PCV in goats. The EPG rises coincided with drops in Hb. Similar trend was presented by PCV, but it was not as marked as that of EPG.

Mandonnet *et al*. (2001) used FEC and PCV to detect resistance against GINs in goats. They found that resistance to Strongyles, a heritable trait in Creole goats, had a good correlation between two adjacent measurements of FEC or PCV. Correlations between FEC and PCV fluctuated from -0.06 to -0.67.

Beh *et al.* (2002) conducted a genome scan to know the relation between certain chromosomal locations and FEC for *Trichostrongylus colubriformis*. He reported that a region on chromosome 6 had chromosome-wide significance and several other regions (on chromosomes 1, 3, 6, 11 and 12) had pointwise significance.

Chiejina *et al.* (2002) observed that, of the measures of infection used, namely FEC, circulating eosinophil (EOS) responses, PCV and body weight (BW), FEC and EOS responses exhibited marked individual variability, but only FEC (geometric mean of transformed counts) and PCV showed strong correlation with worm burden. The wide individual variability in FEC and its strong relationships to worm burden and PCV are pointers to its likely genetic basis.

In a study by Gauly *et al.* (2002), FEC of Rhön sheep was higher compared with Merinoland sheep ($P < 0.01$), whereas no differences in haematocrit, worm burden and IgG (Immunoglobulin G) antibody values could be found between the breeds.

Traits other than FEC viz., average worm size, number of eggs inutero in adult female worms, antibody responses and other indicators of immune response or pathogenesis (pepsinogen and fructosamine concentrations and eosinophil numbers) may also be used to assess resistance to nematodes or host response to infection. However, further investigation is probably required before such measures could be advocated as replacements for FEC (Bishop and Morris, 2007).

Rinaldi *et al*. (2009) reported that there is a good relationship (r = 0.6) between FEC and total GI strongyle worm burden in goats.

2.3 FEACAL EGG COUNT

Amarante *et al.* (1999) found high correlation between FEC and worm burden with a correlation coefficient of 0.7.

In a study conducted by Khusro *et al.* (2004) the uncorrected mean YFEC (Yearling FEC) of each flock year management group ranged from 2.2 to 19.50 on transformed scale.

In case of Merino sheep, most genetic correlations varied little over the age range with the exception of FEC and BW, which fell from zero at weaning to −0.63 at hogget age which is approximately 400 days of age. Host resistance to GI parasites could be assessed by FCS (Faecal Consistency Score) and DS (Dag Score) which may be good indicators of infection; but they are very different from FEC as an indicator for resistance (Pollott *et al.,* 2004).

2.3.1 Effect of sex of kid on FEC

Rege *et al.* (2002) inferred that in the LFEC (Logarithm-Transformed FEC) analyses, sex was significant (P<0.01) for 3 month LFEC and for yearling LFEC where male lambs had higher LFEC than female lambs.

At yearling age, males had lower FEC scores than females; but at hogget age, the effect was reversed and much larger, that is, males had much higher FEC scores than females (Khusro *et al.,* 2004).

Vanimisetti *et al.* (2004) reported that there was no difference for FEC between male and female prepubertal lambs. Miller *et al*. (2006) had a similar observation where they found no effect for sex on either FEC or PCV.

Good *et al* (2006) observed that male lambs had greater FEC than females ($P < 0.05$).

2.3.2 Effect of type of birth on FEC

In the LFEC analyses by Rege *et al*. (2002), birth type was significant ($P<0.001$) for the 2 and 3 month measurements, where multiple born lambs had higher LFEC than single born lambs, but birth type was not significant for yearling LFEC.

Khusro *et al.* (2004) reported that the animals born from multiple births, that is twin born and triplet born Merino lambs had significantly lower FEC values than singlets ($p<0.01$). Mugambi *et al.* (2005) also found the birth type to be significant ($P < 0.05$) in LFEC analysis for lambs sampled at 2 months of age.

The study by Miller *et al.* (2006) found no effect of birth type on FEC, but twinborn lambs had consistently lower PCV than single-born lambs.

2.3.3 Effect of age on FEC

In the LFEC analyses, age of lamb was significant ($P<0.001$) for 2 month LFEC with a negative regression coefficient for LFEC on lamb age, and for 3-month LFEC with a positive regression coefficient (Rege *et al.,* 2002).

Khusro *et al.* (2004) found that the effects of age at measurement (in days) on FEC was of significance (P<0.01). Genetic and phenotypic correlations between YFEC and HFEC were 0.27±0.13 and 0.37±0.04, respectively.

In the study by Mugambi *et al.* (2005), the effects of lamb age was significant (at least $P < 0.01$) for LFEC.

2.3.4 Effect of season on FEC

Miller *et al.* (1998) reported a seasonal influence on FEC where the FEC increased in the spring (periparturient rise) for most years and in the summer for all years studied.

In the LFEC analyses by Rege *et al*., (2002), season was not significant ($P<0.001$) for yearling LFEC.

Uriarte *et al.* (2003) observed three waves of GIN infection. The first wave of infection occurred between January and April, second wave of infection between June and July and the third wave of infection, between October and November. The highest number of worms was observed in July.

Mugambi *et al.* (2005) found that the effects of season was significant (at least $P < 0.01$) for LFEC at all sampling times.

Rinaldi *et al.* (2009) also found a significant effect of the month of faecal sample collection on GI strongyle FEC.

2.3.5 Effect of center on FEC

Khusro *et al.* (2004) conducted study on Australian Merinos in different commercial flocks across a range of geographical regions in Australia, and found that some resistance factor across regions does exist.

In an analysis by Pollott *et al*. (2004), the fixed effect site was found to be significant, which nearly always influenced FEC. The study was conducted on lambs at two different sites but of same sire group.

Good *et al.* (2006) reported that the effect of farm was highly significant ($P < 0.001$) in influencing FEC when data from six different farms were analysed.

2.3.6 Effect of breed on FEC

The results based on FEC and PCV, indicated that suckling native lambs were more resistant to strongylate nematode infection than suckling Suffolk lambs (Bahirathan *et al.,* 1996).

Romjali *et al.* (1996) reported that overall FEC at all sampling dates were not statistically different ($P > 0.05$) between four genotypes, S (Sumatra), H1 (50% Sumatra-50% Virgin Island), B1 (50% Sumatra-50% Barbados Blackbelly), El (50% Sumatra-50% Java Fat-tail), which were infected orally with a newly isolated strain of *H. contortus*.

Mugambi *et al*. (1997) studied the response of Red Maasai sheep to natural and artificial *H. contortus* infections and compared it with sheep of Blackheaded Somali, Dorper and Romney Marsh breeds. Significant breed differences in FEC, PCV and mortality rates showed that the Red Maasai sheep, which had lower egg output and a higher PCV, were more resistant to natural *H. contortus* infection than sheep from the other three breeds.

Miller *et al.* (1998) found significant breed (Suffolk Vs native) difference for FEC and PCV. Overall mean FEC and PCV were significantly higher and lower, respectively, for Suffolk lambs and for Native lambs. Native sheep were more resistant to infection (specifically *H. contortus)* than Suffolk sheep.

In the study conducted by Costa *et al.* (2000), the EPG, transformed as [log (EPG±75)], varied (P<0.01) between goats within breeds and between weeks of exposure, but not between goat breeds (P > 0.05).

Baker *et al.* (2001) observed that the Small East African kids were more resistant to GIN parasites than Galla kids as shown by their significantly lower FEC (P < 0·001) in the post-weaning period (8 to 14 month-old kids) and lower mortality from birth to 14 months of age (P < 0·05).

Rege *et al.* (2002) reported that the Menz lambs had significantly lower (P<0.001) LFEC and higher (P<0.001) PCV than Horro lambs. Menz lambs had significantly (P<0.001) lower cumulative mortality (37%) to 12 months of age than the Horro lambs (68%) which was attributed to better adaptation of the Menz to the study environment and not due to resistance to GIN parasites.

Relative resistance of mature Dorper crossbred ewes was comparable to that of Katahdin and St. Croix ewes and superior to that of Hampshire ewes Burke and Miller (2002).

Red Maasai were more resistant and resilient at post weaning, to infections with GINs than Dorper lambs as shown by their significantly lower FEC and their significantly higher PCV (Baker *et al.,* 2003).

Good *et al*. (2006) inferred that the Texel sheep are more adept at identifying parasite-specific antigen and hence stimulate stronger Th_2 (T helper cell-2) response. They observed significant breed differences (Suffolk lambs had a higher FEC than Texel lambs (P < 0.001)) in FEC irrespective of age suggesting that the greater resistance in Texel was either 'innate' or that they were able to generate a more rapid immune response.

Golding and Small (2009) assessed the relative resistance of Manx Loaghtan, Shetland and Southdown sheep to naturally acquired GIN infection

over a ten week period. Results of the study indicated that primitive Shetland and Manx Loaghtan sheep had greater resistance to gastrointestinal parasites.

Results from the study by Shakya *et al.* (2009) indicated that Gulf Coast native lambs had a more pronounced immune response to infection with *H. contortus* than Suffolk lambs and that may be responsible for the observed resistance to infection in native lambs

2.4 FEC- PCV CORRELATION

Costa *et al.* (2000) found a negative correlation between egg counts and blood values, (−0.45, P=0.0064, between log (EPG+75) and PCV; and −0.53, P=0.0009, between log (EPG+75) and Hb), suggesting breed differences in PCV and Hb were related to resistance to *H. contortus* infection and/or to its effects.

Baker *et al.* (2001) reported that the phenotypic and genetic correlation estimates between PCV and LFEC were moderately to highly negative and averaged –0·36 and –0·53, respectively. Chiejina *et al.* (2002) also reported a significant negative correlation between FEC and PCV.

Rege *et al.* (2002) observed no significant (P<0.001) phenotypic correlation between the live weight and LFEC (range -0.05 to 0.03) and between PCV and LFEC (-0.10 to -0.01).

Vanimisetti *et al.* (2004) found that yearling ewes were less resistant to infection than older ewes, with lower PCV (P < 0.05) and higher LFEC (P < 0.05). During infection, PCV was positively correlated with BW and negatively correlated with LFEC in both ewes and lambs.

2.5 HERITABILITY

According to Baker *et al.* (2001), heritability estimates for PCV and FEC at different sampling times were characterized by high standard errors and the

heritability estimates for records taken at 4·5 and 8 months of age from a repeated measure analysis were 0·18 ±0·08 for PCV and 0·13±0·07 for LFEC.

The heritability estimate for transformed fecal egg count was 0.37 ± 0.06 at weaning. During fattening, it increased from 0.14 ±0.05 at 4 month to 0.33 ± 0.06 at 10 month (Mandonnet *et al.,* 2001).

Gauly *et al.* (2002) reported that heritabilities for LFEC (±S.E.) were 0.35±0.14 and 0.17±0.07 in Rhön and Merinoland sheep respectively, ($P < 0.05$) while corresponding heritabilities for haematocrit were 0.08 ± 0.13 and 0.51 ± 0.27.

In case of LFEC, the additive genetic effect (h_a^2) estimates were low but significant at 2 (0.15±0.04) and 3 (0.13±0.04) months of age but not significant at 12 months of age (0.01±0.02). There were no significant differences between the two breeds or the two seasons in heritability estimates for LFEC at any age but at weaning there was a numerically higher heritability in Horro lambs (0.21±0.08) than Menz lambs (0.09±0.04) (Rege *et al.,* 2002).

In the study by Khusro *et al.* (2004), the heritability estimate for YFEC was 0.21 ± 0.02 and maternal heritabilities were generally small. The heritability estimate for HFEC was 0.38±0.03. Heritability for FEC was moderate, even when there was little uniformity across flocks, indicating that, across flock, sire effects on FEC were significant and some resistance factor across worm species and regions does exist. He also suggested that as the heritability estimates were moderate, animals can be selected on the basis of this indicator for parasite resistance.

Pollott *et al.* (2004) reported the heritability of FEC rises from 0.2 at weaning to 0.65 at 400 days of age. The rise in heritability was associated with increase in sire component of variance at that time.

Rout and Chauhan (2004) observed that there was moderate to high heritability for FEC and PCV which is important for study of disease resistance in response to nematode infection.

In lambs heritability was 0.39 ($P < 0.01$) for PCV, and 0.10 ($P < 0.05$) for LFEC. Heritability estimates for ewes was 0.15 ($P < 0.05$) for PCV and 0.31 ($P < 0.01$) for LFEC (Vanimisetti *et al.,* 2004).

Miller *et al.* (2006) observed that the heritabilities of FEC and PCV at three different infection periods (at weaning, after summer grazing period and experimental infection), respectively, were 0.15 and 0.29, 0.12 and 0.11, 0.22 and 0.12. Miller and Horohov (2006) stated that heritability estimates for FEC range from 0.22 to 0.63, which indicate that selection for resistance or selection against susceptibility using some measurement of FEC for nematode burden will be moderately successful.

2.6 IMMUNOLOGICAL SIGNIFICANCE OF IFNγ AND NEMATODE RESIATANCE

Urban *et al.* (1996) stated that intracellular parasites stimulate type 1 response where IFNγ is the predominant immune activator. At the molecular level, exogenous IL-12 (Interleukine-12) stimulates IFNγ production which elevates a protective type 1 response but converts the normally anti-worm type 2 response to a type I response that inappropriately regulates the infection.

In the study by Else and Finkelman (1998), it was found that, treatment of *Trichuris muris* resistant strains of mouse with IL12 allowed the establishment of a chronic infection. Here IL12 had its effects through the induction of IFNγ. IL12 has also been shown to prolong infection with *Nipostrongylus brasiliensis* and again the IL12 effects were dependent on IFNγ.

Domeika *et al.* (2002) observed a marked synergism between porcine IL-12 and IL-18 in poPBMC (porcine peripheral blood mononuclear cells) for the

induction of IFNγ. The actual IFNγ producing cells were identified as probable NK (Natural Killer) cells (about 30%) and T lymphocytes (about 70%), using flow cytometry.

In a study by Obexer-Ruff *et al.* (2003) it was stated that the frequency differences for alleles 1 and 4 of the microsatellite linked to the IFNγ locus were not significant after correction of the *P*-value, due to the overall low frequency of these alleles. They also found high IFNγ levels to be phenotypically correlated with survival.

Sayers *et al.* (2005) reported that there was no association between IFNγ intron 1 haplotype and faecal egg count in Suffolks. In contrast, in the Texel breed, the B haplotype was associated with resistance to nematode infection (P = 0.02). These results suggest that intron 1 of the IFNγ gene has an important role in resistance to nematode infection in the Texel breed.

The experiments performed by Pintaric (2008) indicated a stimulatory role and strong synergistic effects of the investigated cytokines (immune regulatory cytokines IL-2, IL-12 and IL-18) in the activation of porcine NK cells in vitro, inducing IFNγ, perforin production and cytotoxicity against target cells.

In *Leishmania (Leishmania) chagasi* infection prescapular lymph nodes of asymptomatic dogs had the highest expression of IFNγ and TNF-α and low parasite burden, indicating that these cytokines play a role in protection against infection (Alves *et al.,* 2009).

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

One hundred and fifty goats of Malabari, Attapady Black and Malabari crossbreds formed the material for investigation. The centers selected for study were University Livestock Farm, Mannuthy and two field centers under All India Co-ordinated Research Project (AICRP) on goats, namely Tellichery in Kannur district (Centre 1) and Badagara in Kozhikode district (Centre 2).

Data on animals born during the period from December 2007 to December 2009 were utilized for the study.

3.2 DATA COLLECTION

Data on the following traits were collected.

- Sire of kid
- Sex of kid
- Type of birth - as singles, twins and triplets
- Season of sampling
- Age of kid
- Birth weight
- Center (area)
- Breed

3.3 COLLECTION OF BLOOD SAMPLES

Blood samples were collected by jugular venipuncture into EDTA vacutainer tubes. After collection of blood, tubes were shaken gently to facilitate thorough mixing of blood with anticoagulant. Then the samples were brought to

the laboratory without delay at 4 ºC, temperature being maintained with the aid of ice packs and stored at -20 ºC until processed for DNA extraction.

3.4 COLLECTION OF FAECAL SAMPLES

Faecal samples were collected directly from the rectum to estimate the faecal egg count (FEC). It was measured at two different ages, namely, at one month and around one year before deworming. FEC records measured for one year of age were available on 66 animals and for one month of age from 84 animals. These animals belonged to 16 different sires.

3.5 ESTIMATION OF FEC

FEC was assessed based on Modified McMaster Technique (Zajac and Conboy, 2006) with modifications to suit the McMaster counting chamber (J. A. Whitlock and Co., Australia; Figure 1) used. The procedure was as follows:

1. One gram of faecal material was combined with 29 ml of floatation (saturated sodium chloride) solution to yield a total volume of 30 ml.

2. Samples were mixed well using mortar and pestle and strained through cheese cloth to remove large pieces of debris.

3. Each chamber of the McMaster chamber was immediately filled with the sample mixture using a pasture pipette. The entire chamber was filled taking care to avoid air bubbles.

4. Allowed the slide to sit for at least 5 min before examining to allow the floatation process to occur.

5. Observed the slide through the 10X lens, focusing the lines of the grid of the chamber. Nematode eggs in each lane of the three chambers were counted.

To determine the number of parasite eggs per gram of faeces (epg), average of the counts from the three chambers were calculated. Since one chamber of the McMaster counting chamber was calibrated to accommodate 0.15 ml of faecal mixture, the average egg per chamber was multiplied by 200 to get FEC in epg.

3.6 STATISTICAL ANALYSIS OF FAECAL EGG COUNT

FEC were not distributed normally. Therefore before subjecting it to statistical analysis, the FEC was logarithmically transformed (LFEC) as ln(FEC+2000), i.e. after adding 2000 to the original data, natural logarithm of the resulting figure was taken (Vanimisetti *et al*., 2004).

3.6.1 Effect of Genetic and Non-genetic Factors on FEC

To find out the effect of genetic and non-genetic factors on FEC, logarithmically transformed data were subjected to least-squares analysis (Harvey, 1966).

The model used was

$$Y_{ijklmnop} = \mu + S_i + E_j + Z_k + A_l + M_m + X_n + V_o + e_{ijklmnop}$$

where,

$Y_{ijklmnop}$ - Observation of p^{th} kid of i^{th} sex, j^{th} type of birth, k^{th} age, l^{th} season of sampling, m^{th} birth weight, n^{th} center, o^{th} breed.

μ- Overall mean

S_i - Effect of i^{th} sex (i = 1,2)

E_j - Effect of j^{th} type of birth (j = 1,2,3)

Z_k - Effect of k^{th} age (k = 1,2)

A_l - Effect of l^{th} season of sampling (l = 1,2,3)

M_m - Effect of m^{th} birth weight (m = 1,2,3)

X_n - Effect of n^{th} center (n = 1,2,3)

V_o - Effect of o^{th} breed (o = 1,2,3)

$e_{ijklmnop}$ – Random error

The random errors were assumed to be normally and independently distributed with mean [$E(e_i)$] zero and variance σ^2_{ei}.

Duncan's multiple range test as modified by Kramer (1957) was used to compare means. After comparing, the least-squares means were back-transformed by taking the antilogarithms and subtracting 2000 from these antilogarithms. After determining the confidence intervals for least square means according to the formula (Olsson, 2005) given below, those were also back-transformed.

Lower or Upper limit of confidence interval = Mean ± (2.02 x Standard Error)

3.6.2 Estimation of Heritability

The calculations of heritability for FEC was done by SPAB 2.0- Software Statistical package for animal breeding (Sethi, 2002). The data adjusted for the effect of non-genetic factors were used for the estimation of heritability. The minimum numbers of progeny to qualify for inclusion was assumed to be four per sire. Heritability was estimated by paternal half sib correlation method (Becker, 1975). The model used was

$$Y_{ij} = \mu + S_i + e_{ij}$$

Where,

Y_{ij} - Observation of j^{th} progeny of i^{th} sire

μ - population mean

S_i – Effect due to i^{th} sire with $E(S_i)= 0$ and $V(S_i)= \sigma^2_{si}$ (i = 1,2,3….n)

e_{ij} – Random effect due to error with $E(e_i)= 0$ and $V(e_i)= \sigma^2_{ei}$

The analysis of variance (ANOVA) was

Source of variation	d.f.	Sum of squares	Mean sum of squares	Expected sum of squares
Between sires	s-1	BSS	MSs	$\sigma^2_e + k\sigma^2_s$
Within sires between progenies	n-s	WSS	MSe	σ^2_e

Where,

$$k = \frac{1}{s-1}\left(n - \frac{\sum n_i^2}{n}\right)$$

s = total number of sires

n = total number of progenies of all sires

n_i = total number of progenies of i^{th} sire

The heritability was then calculated as

$$h^2 = \frac{4\sigma^2 s}{\sigma^2 s + \sigma^2 e}$$

where,

σ^2_s = sire variance

$$= \frac{MSs - MSe}{K}$$

σ^2_e = Error variance or mean sum of squares due to error

The standard error of heritability was calculated by using the formula suggested by Swiger *et al.* (1964) as:

$$\text{S.E. } (h^2) = 4\sqrt{\frac{2(n-1)(1-t)^2[1+(k-1)t]^2}{k^2(n-s)(s-1)}}$$

Where,

t = intra- class correlation among paternal half sibs

s = total number of sires

n and k as described above

3.7 PACKED CELL VOLUME (PCV) ESTIMATION

PCV of the blood samples were determined within one hour of blood collection using microhematocrit method (Benjamin, 2001).

3.8 ESTIMATION OF CORRELATION BETWEEN LFEC AND PCV

Phenotypic correlation between LFEC and PCV was calculated by using the option available in Microsoft Excel, according to the following formula:

$$Correl(X,Y) = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2\sum(y-\bar{y})^2}}$$

Where,

X and Y are the traits (LFEC and PCV) for which correlation have to be estimated and $\bar{x}$ and $\bar{y}$ are the sample means.

3.9 ISOLATION OF DNA FROM WHOLE BLOOD

Genomic DNA was extracted from whole blood using the standard phenol-chloroform extraction procedure (Sambrook and Russel, 2001) with modifications. The procedure* was as follows:

1. To 5 ml blood, double the volume of ice cold RBC lysis buffer was added and mixed well. The entire mixture was incubated in ice with occasional shaking for 10 min for the complete lysis of erythrocytes.

2. The leukocytes were pelleted by centrifuging at 4000 rpm for 10 min and the supernatant containing lysed erythrocytes and haemoglobin was discarded.

3. The pellet was resuspended in ice-cold RBC lysis buffer and the above two steps were repeated till pellet was clear without any unlysed erythrocytes.

4. The pellet was washed twice with Tris buffered saline by vigorous vortexing, followed by centrifugation at 3000 rpm for 10 min.

5. The white blood cell pellet was resuspended by vortexing in 3 ml saline EDTA buffer so that no clumps remained. To this cell suspension, 25 µl of proteinase-K (20 mg/ml in water) and 0.25 ml of 20 per cent SDS were added and mixed gently and incubated at 50 ˚C in a water bath with occasional mixing for a period of at least three hours.

6. The digested samples were cooled to room temperature and 300 µl of 5 *M* sodium chloride solution was added and mixed well by vortexing. An equal volume of Tris saturated phenol (pH 7.8) was added and mixed gently by

**Composition and methods of preparation of reagents and buffers are provided in Annexure-I.*

inversion of the tubes for 10 min and centrifuged at 4500 rpm for 15 min.

7. The aqueous phase containing DNA was collected in fresh tubes, to which an equal volume of saturated phenol:chloroform:isoamyl alcohol (25:24:1) was added. The contents were mixed thoroughly by inversion of the tube and centrifuged at 4500 rpm for 15 min.

8. The aqueous phase was collected in fresh tubes, to which an equal volume of chloroform:isoamyl alcohol (24:1) was added, mixed and centrifuged at 4500 rpm for 10 min and this step was repeated.

9. The upper aqueous phase was carefully transferred to a sterile 50 ml beaker and one tenth volume of 3 *M* sodium acetate (pH 5.5) was added and mixed well.

10. An equal volume of chilled isopropyl alcohol was added and the precipitated DNA was either spooled out using a fresh micropipette tip with or without washing in 70 per cent ethanol, and air-dried or the mixture after overnight freezing was centrifuged at 14,000 rpm for 5 min in 1.5 ml microcentrifuge tubes, discarded the supernatant and the pelleted DNA was air dried.

11. Dried DNA was resuspended in 0.5 ml of Tris EDTA buffer and stored at -20°C.

3.10 QUANTIFICATION AND QUALITY CHECK OF DNA BY AGAROSE GEL ELECTROPHORESIS

The quality and molecular weight of DNA were measured electrophoretically using 0.7 per cent agarose in 1 X TAE buffer in a horizontal submarine electrophoresis unit. An appropriately sized gel tray was cleaned and sealed. The tray was placed on a leveled surface and comb kept in proper position in the tray. Agarose in 1X TAE buffer was heated in a microwave oven until it was a clear solution, cooled to 50 °C and 1.0 µl of ethidium bromide (10 mg/ml)

was added into the molten agarose and mixed well. The mixture was cooled to hand bearable temperature and carefully poured into the gel tray avoiding air bubbles. After polymerization, the comb and sealing were removed gently and the tray was immersed in the buffer tank (Amersham Pharmacia Biotech., USA) containing 1X TAE buffer with the wells towards the negatively charged electrode.

One microliter of each DNA sample was mixed with a tiny drop of 6X gel loading dye and loaded into the wells. Electrophoresis unit was set at 80 V for 2 h and the gel was visualized and documented after the electrophoresis under the gel documentation system (BioRad, Gel Doc 2000^{TM}).

3.11 PCR-RFLP ANALYSIS

3.11.1 Amplification of Intron 1 of Interferon Gamma (IFNγ) Gene

3.11.1.1 Template DNA Preparation for PCR

Template DNAs for PCR were prepared by diluting the DNA stock solution with sterile triple distilled water to a final concentration of 50 ng/µl and were stored at -20°C. For every 15µl PCR reaction, 1.5 microlitres of this working solution was used.

3.11.1.2 Design of Primers

The DNA sequence information on intron 1 of IFNγ gene of goats is not available in literature. Therefore the primers were designed on the basis of the ovine sequence (Accession # Z73273; Schmidt *et al*., 1996) downloaded from the National Centre for Biotechnology Information (NCBI) site (*http://www.ncbi.nlm.nih.gov*). Forward and reverse primers were designed based on this DNA sequence information using the software Primer3 (v.0.4.0) available at the site *http://frodo.wi.mit.edu.*

Forward primer : 5′ TGA TTG ACT TGA TGT TTG ATG TTG 3′

Reverse primer : 5′ CCC ATC TTT TGG AGG ATT CA 3′

3.11.1.3 Synthesis and Dilution of the Primers

The primers were custom synthesized commercially (Sigma Aldrich Chemicals Pvt. Ltd., Bangalore) and obtained in lyophilized form. The tubes were centrifuged prior to opening for preventing the loss of pelleted oligonucleotide. They were reconstituted in sterile de-ionized triple distilled water to make a stock solution of 200 p*M*/µl concentration. The solutions were incubated at room temperature for one hour and then stored at -20°C. Working solutions of each primer were prepared by diluting from the stocks as 10 p*M*/µl.

3.11.1.4 Setting up of PCR

The PCR assay was performed in 200 µl reaction tubes with a total volume of 15 µl, containing 1.5 µl of DNA template, 1.5 µl 10X PCR buffer, 1.875 µl $MgCl_2$, 0.5 µl of each primer, 1.2 µl of each dNTP and 1unit Taq DNA polymerase. A negative control, containing all reaction components except template DNA was also set up to check contamination, if any. Mastermix containing distilled water, 10X PCR buffer, $MgCl_2$, dNTPs, primers and Taq DNA polymerase was prepared and 13.5 µl of the mix was distributed to 1.5 µl of DNA template taken in separate tubes.

The tubes were spun briefly and placed in the thermal cycler (MyGene (MG25+), Longgene Scientific Instruments Co. Ltd., China). The peltier thermal cycler was pre-programmed for temperature and cycling conditions. The cycling conditions for DNA amplification included a touchdown protocol with an initial annealing temperature of 57 °C and ending at an annealing temperature of 52 °C. Initials cycles were repeated 5 times while final cycle was repeated 20 times, followed by final extension at 72 °C for 10 min and keeping at 10°C for 10 min. The PCR products were stored at –20 ºC till further analysis.

3.11.1.5 Agarose Gel Electrophoresis of Amplified DNA

The amplification was checked by agarose gel electrophoresis following the procedure described earlier in this section. Three and a half microliter of the product was mixed with a small drop of 6 X gel loading buffer and loaded into the wells carefully and electrophoresed using 2 per cent agarose gel in 1X TAE buffer. As a molecular size standard, pUC19/ *Msp*I digest (Bangalore Genei Pvt. Ltd.) was also mixed with gel loading buffer and loaded into one of the wells.

Electrophoresis was carried out at 2V/cm until the bromophenol blue dye migrated more than 4 cm length of the gel. The gel was visualized and documented in a gel documentation system (BioRad, Gel Doc 2000^{TM}) and checked for amplification of target DNA.

3.11.2 Restriction digestion of amplified products

In the present PCR-RFLP analysis, restriction digestion pattern of intron 1 of IFNγ gene, using restriction endonuclease *BspH*I was investigated.

Seven microliter of the amplified product was digested with 2.5U of restriction enzyme *Bsp*H1 at 37 ºC for two hours in a dry bath. The composition of reaction mixture in a final volume of 10 µl was as follows:

PCR product	-	7.0 µl
10X assay buffer	-	1.0 µl
*Bsp*HI (10U/µl)	-	0.25 µl
Distilled water	-	1.75 µl
Total	-	10 µl

Mastermix containing the enzyme, 10X assay buffer and distilled water was prepared and 3 µl of the mix was distributed to 7 µl of amplified products taken in separate tubes.

After the digestion, enzyme was inactivated by incubating the tubes at 65ºC for 20 minutes. The digested products were stored at -20 ºC till analysed.

3.11.2.1 Agarose Gel Electrophoresis of restriction digested products

Visualization and documentation of digested products were done using agarose gel electrophoresis as discussed earlier. In this case 2 per cent agarose gel was used and the electrophoresis was carried out at 2 V/cm until the bromophenol blue dye migrated more than 8 cm length of the gel.

3.12 DNA SEQUENCING

Sequencing was carried out commercially (Bioserve Hyderabad, Pvt. Ltd.) by the dideoxynucleotide sequencing method using an automated DNA sequencer (Applied Biosystems, USA).

3.13 SEQUENCE ANALYSIS

3.13.1 DNA Sequence Analysis

The sequence obtained through sequencing was analysed for homology using BLASTn programme at NCBI site (http://www.ncbi.nlm.nih.gov/BLAST). The BLASTn programme compares a nucleotide query sequence against nucleotide sequences available in the database. The 202 bp nucleotide sequence of the intron1 of IFNγ gene was submitted as query sequence and selected the BLASTn programme for searching similar sequences from the DNA database.

3.13.2 Multiple Sequence Analysis of DNA

The multiple sequence alignment was done by the computer programme EBI tool ClustalW (http://align.genome.jp/). This programme is available from European Bioinformatics Institute (EBI) ftp server. The input sequences were loaded in FASTA format. Using this programme, the intron1 IFNγ sequence was compared simultaneously with sequences of other species to highlight the areas of homology and divergence.

3.13.3 Submission of the Sequence to GenBank

The sequence of IFNγ intron1 was submitted to the GenBank using BankIt option (http://www.ncbi.nlm.nih.gov/BankIt/) at the NCBI site to obtain the unique accession number for the new sequence.

Results

4. RESULTS

4.1 FECAL EGG COUNT

4.1.1 EXAMINATION OF FAECAL SAMPLE

On examination of the faecal samples, nematode eggs of Strongyle (Figure 2) and *Strongyloides* sp. were observed. Coccidial oocysts were also seen. Fecal egg count (FEC) is expressed as eggs per gram (epg).

4.1.2 Factors Affecting FEC

Average nematode FEC of faecal samples studied was 764.39 epg and the values ranged from 0.00 to 16700 epg. Effects of various nongenetic and genetic factors on FEC were analysed after logarithmically transforming FEC as ln(FEC+2000). Least-squares means according to sex, type of birth, age, season of sampling, birth weight, centre, and breed with their confidence intervals, after back-transforming are presented in table 4.1.

4.1.2.1 Effect of Sex of Kid

Least-squares means of FEC according to sex is presented in table 4.1. Effect of sex was not significant for changes in FEC. The least squares means of FEC for females and males were 722.40 and 807.03 respectively.

4.1.2.2 Effect of Type of birth

Least-squares means of FEC according to type of birth revealed no significant effect of type of birth on FEC. The Least-squares mean of FEC for singles was 968.46. The corresponding values for twins and triplets were 650.62 and 684.84 respectively.

4.1.2.3 Effect of Age

According to least square analysis, age did not have any significant effect over FEC. Least-squares means of FEC with their confidence interval, for 1 month and 1 year of age are given in the table 4.1.

4.1.2.4 Effect of Season of Sampling

Presented in tables 4.1 are the least-squares means of FEC in accordance with season of sampling. Season of sampling had no significant effect on FEC.

4.1.2.5 Effect of Birth weight

Least-squares means in accordance with birth weight are presented in table 4.1. Birth weight had no significant effect on FEC.

4.1.2.6 Effect of Center (area)

Presented in table 4.1 are the least-squares means of FEC along with their confidence interval, according to the center where the kids are reared. Center had significant effect on FEC. The mean FEC for Tellichery, Badagara and farm reared kids were 1297.53, 1122.87 and 51.42 epg respectively. Thus kids reared in farm had lowest FEC followed by Badagara and Tellichery kids.

4.1.2.7 Effect of breed

According to the least squares analysis (LSA), breed had no significant effect on FEC. The least-squares means of FEC in animals belonging to different breeds are given in table 4.1.

4.1.3 HERITABILITY

Heritability for FEC was estimated by paternal half sib method and the heritability estimate was 0.39 ± 0.3630.

4.1.4. CORRELATION BETWEEN LFEC AND PCV

The value of PCV was assessed through micro-haematocrit method. The value of PCV ranged between 19 and 48 per cent. This data was used to find out the correlation between LFEC and PCV. A negative phenotypic correlation of -0.1791 was observed between the two.

Table 4.1 Results of LSA of Genetic and Non-genetic Factors on FEC

(Least square means expressed in epg with their confidence interval)

Effect	**Mean FEC**	**Lower limit Of Confidence interval**	**Upper limit Of Confidence interval**
Sex			
Female	722.40[a] *	218.00	1341.51
Male	807.03[a]	275.38	1462.89
Type of Birth			
Single	968.46[a]	358.77	1735.73
Twin	650.62[a]	127.19	1302.85
Triplet	684.84[a]	-126.67	1847.90

Table 4.1 Continued…….			
Age			
1 month	924.27[a]	323.68	1680.09
1 year	613.25[a]	86.23	1273.40
Season			
Winter	887.14[a]	64.02	2038.51
Summer	1008.02[a]	234.56	2049.19
Pre-monsoon	445.21[a]	15.61	966.38
Birth weight			
Low	763.31[a]	224.54	1432.59
Medium	717.15[a]	60.22	1583.54
High	813.55[a]	76.52	1812.18
Center			
Farm	51.42[a]	-281.08	448.24
Thalassery	1297.53[b]	442.15	2452.51
Badagara	1122.87[b]	304.09	2232.62
Breed			
Crossbred	507.95[a]	-41.97	1212.31
Malabari	545.69[a]	68.60	1132.81
Attapady Black	1308.82[a]	272.73	2817.24

*The categories of each effect differing among them are given different superscripts

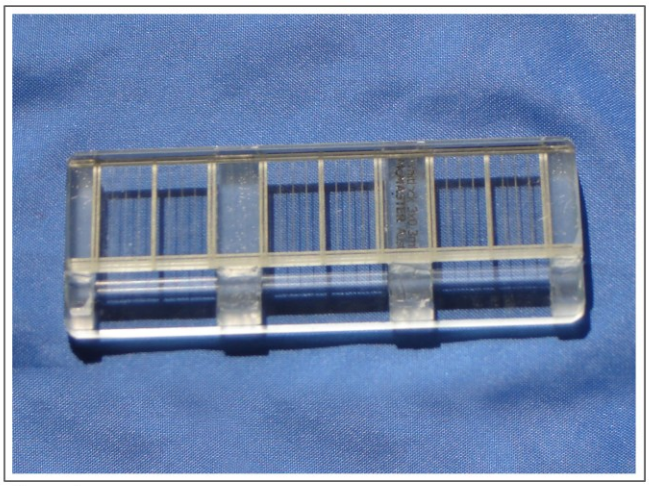

Figure: 1
Mc Master worm egg counting chamber

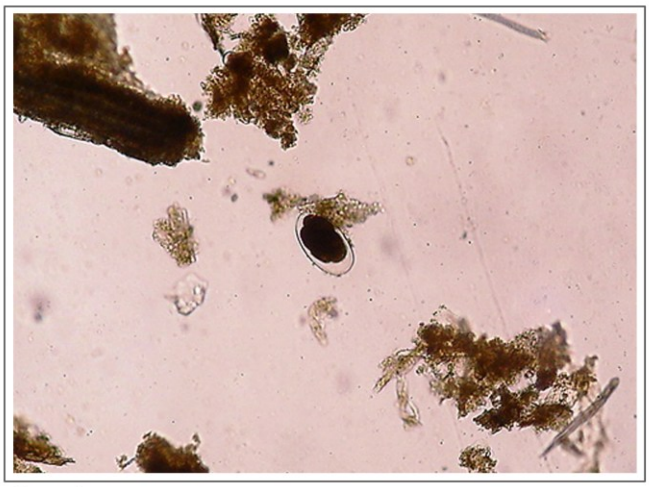

Figure: 2
A typical Strongyle Ova

4.2 FAECAL EGG COUNT AND INTERFERON GAMMA INTRON1

4.2.1 ISOLATION OF GENOMIC DNA

DNA samples were obtained from the whole blood samples of Malabari, Attappady Black and crossbred goats using phenol chloroform extraction procedure.

4.2.1.1 Quality of DNA

The DNA samples, checked for quality by agarose gel electrophoresis, appeared as single, clear band without sheared fragments indicating good quality high molecular weight DNA.

4.2.2 PCR AMPLIFICATION OF INTRON 1 OF THE IFN γ GENE

The primers for amplification of intron 1 of the IFN γ gene were designed on the basis of the ovine sequence (Accession # Z 73273; Schmidt *et. al*., 1996). The primers were custom synthesized for PCR amplification of the samples. PCR amplification was attempted on all the samples using the primer pair.

4.2.2.1 Optimized PCR protocol for IFN γ gene

The composition of PCR, temperature and time of cycles were optimized for efficient amplification of product using particular set of primers. To determine the correct magnesium chloride concentration, annealing temperature and Taq DNA polymerase, it took around 30 different PCR assays. The reaction components optimized for the above PCR are presented in Table 4.2. A $MgCl_2$ concentration of 1.25 m*M* in the reaction mixture resulted in robust amplification of the product without any non-specific products. Other $MgCl_2$ concentrations like, 1 m*M* and 1.5 m*M* gave lesser and 0.75 m*M* and 1.75 m*M* gave no amplification. The amplifications obtained from conventional PCRs were nil or suboptimal. Adoption

of touchdown PCR protocol gave favorable results. The standardized temperature and cycling parameters are presented in Table 4.3.

4.2.2.2 Agarose gel electrophoresis of PCR products

The PCR products were checked for amplification by electrophoresis on 2 per cent agarose gels using pUC19/*Msp*I digest as the size standard. The amplified products were found to be 202 bp as expected. The PCR products obtained were without any major non-specific amplified products (Figure 3). There was no amplification in the negative control.

4.2.3 RFLP ANALYSIS OF INTRON 1 OF THE IFN γ GENE

Digestion of PCR product with restriction enzyme, *BspH*I and further electrophoresis revealed one restriction digestion pattern with the identification of one allele namely B (Figure 4). The enzyme had cut PCR product into two fragments of about 180 bp and 22 bp. The 180 bp fragment was visible on gel electrophoresis.

4.2.4 INTRON1 IFNγ/*BspH*I POLYMORPHISM AND FEC

The association study between FEC and IFNγ intron1/ *BspH*I polymorphism could not be carried out as the amplified products obtained for the portion of gene were monomorphic.

4.2.5 DNA SEQUENCING

PCR amplified product was gel eluted, cloned and sequenced commercially. Sequencing was done using M13 universal reverse primer. The sequence obtained was containing reverse primer sequence therefore the reverse complementary sequence was taken. The sequence obtained is given in Figure 5. The sequence included a tetra nucleotide repeat, $(GT_4)_5$ and an Adenine present at a position, 49 bp downstream of this microsatellite. The presence of this Adenine was creating the

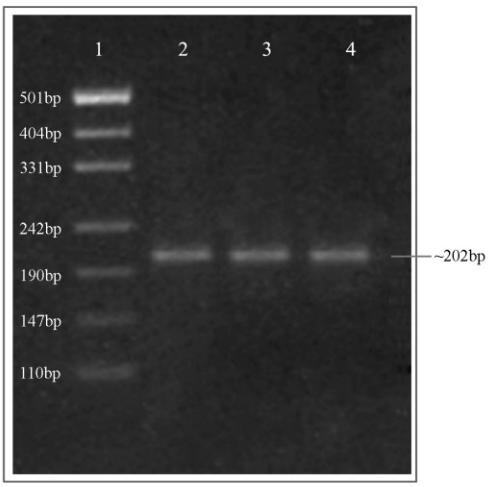

Figure: 3
The 202 bp, PCR amplified fragment of IFNγ intron 1 in goats (2% agarose)
Lane 1: pUC19/Msp1 digest as DNA Marker
Lane 2-4: 202 bp, PCR amplified products

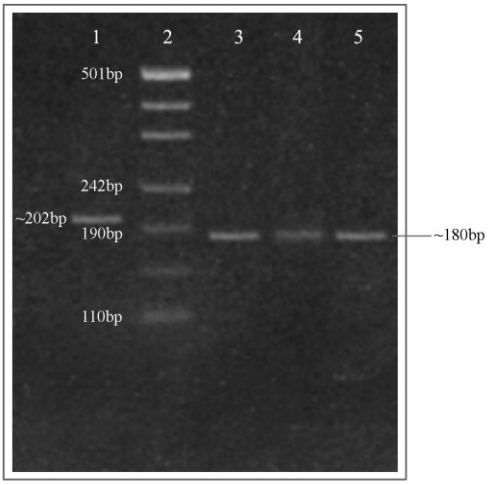

Figure: 4
BspHI restriction pattern of IFNγ intron 1 in goats (2% agarose)
Lane 1: 202 bp, PCR amplified product
Lane 2: pUC19/Msp1 digest as DNA Marker
Lane 3-5: BspHI restriction digested fragments of IFNγ intron 1 in goats (180bp)

restriction endonuclease recognition sequence (5'…T▼CATGA… 3') for *BspH* I enzyme.

4.2.6 SEQUENCE ANALYSIS

4.2.6.1 DNA Sequence Analysis

The result of BLAST analysis of the sequence is presented in Table 4.4. The analysis of the nucleotide sequence of the intron1 of IFNγ gene of goats obtained through sequencing revealed 100 per cent similarity with that of sheep (*Ovis aries*; Accession # Z73273.1). The sequence was also found to be having 89 per cent identity with that of European cattle (*Bos taurus*; Accession # Z54144.2) and 73 per cent with that of pig (*Sus scorfa*; Accession # X53085.1). The sequence had 70 per cent identity with human IFNγ gene (*Homo sapiens;* Accessions # NG_015840.1).

The results of ClustalW multiple nucleotide sequence alignment of the complete sequence showed a high degree of homology with a score of 100 with sheep, 92 with European cattle, 73 with pig and 74 with human.

The complete alignment of the corresponding sequences of IFNγ intron1 of goat and other species is shown in Figure 6.

4.2.6.2 GenBank Accession Number

The new nucleotide sequence data of goat-IFNγ intron1 was submitted to the GenBank DNA database and obtained a unique Accession Number: HM047072.

Figure 5. Goat IFNγ intron1 nucleotide sequence showing a microsatellite (violet) and the recognition sequence of *BspH* I (red)

```
  1    TGATTGACTTGATGTTTGATGTTGACTTGGAATTCTATTTGTGATGGGCTCTCATCTCTA 60

 61    GTCTTCAGTCATTTTGAGAAGACTTGGTGTTATTGTGACTGTTGGCTAGCTGTGTTTGTT 120

121    TGTTTGTTTGTTTGACTAAACGATCTCTGCTCAGTTTGCTACAGAGATTTAGGAGGGATT 180

181    CATGAATCCTCCAAAAGATGGG                                        202
```

Figure 6. CLUSTALW multiple nucleotide sequence alignment of Goat IFNγ Intron1 with corresponding regions of other species

```
SeqA Name    Len(nt)   SeqB Name    Len(nt)   Score

================================================
1    Goat     202       2    ovine   202       100
1    Goat     202       3    bos     210       92
1    Goat     202       4    sus     200       73
1    Goat     202       5    homo    175       74
2    ovine    202       3    bos     210       92
2    ovine    202       4    sus     200       73
2    ovine    202       5    homo    175       74
3    bos      210       4    sus     200       76
3    bos      210       5    homo    175       76
4    sus      200       5    homo    175       75
================================================
```

Goat = *Capra hircus;* ovine = *Ovis aries;* bos = *Bos taurus;* sus = *Sus scorfa;* homo = *Homo sapiens*

```
CLUSTAL 2.0.12 multiple sequence alignment

Goat        TGATTGACTTGATGTTTGATGTTGACTTGGAATTCTATTTGTGATGGGCTCTCATCTCTA 60
ovine       TGATTGACTTGATGTTTGATGTTGACTTGGAATTCTATTTGTGATGGGCTCTCATCTCTA 60
bos         ----TGACTTGATGTTTGATGTTGACTTGGAATTCTATCTATGATGGGCTCTCATCTCTA 56
sus         ----------------TGATGCTGAATTGGAATTGTGTCTATGATGGACTCTCGTCTCTA 44
homo        -------------------------------------------TGATAGGCTGTCATCTCTT 19
                                                       **** * ** ** *****

Goat        GTCTTCA-GTCATTTTGAGAAGACTTGGTGTTATTGTG----ACTGTTGGCTAGCTGTGT 115
ovine       GTCTTCA-GTCATTTTGAGAAGACTTGGTGTTATTGTG----ACTGTTGGCTAGCTGTGT 115
bos         GTCTTCA-GTCATTTTGAGAAGACTTGCTGTTATTGTG----ACTGTTAGCTAGATGTGT 111
sus         ATCCACAAGTCATCTTGAGAAGACTTGGTGTTATGGTGACTGTTTGTTGGCTGGATGAGT 104
homo        GTAGGCA-GTCATTTTGAGAT---TTGGTGTTATTTTG-TTAATTATTGACTAGATGAGT 74
             *   ** ***** ******    *** ******  **      * **  ** * ** **

Goat        TTGTTTGTTTG---------TTTGTTTG----ACTAAACGATCTCTGCTCAGTTTGCTAC 162
ovine       TTGTTTGTTTG---------TTTGTTTG----ACTAAACGATCTCTGCTCAGTTTGCTAC 162
bos         TTGTTTGTTTGGTTTTTTT-TTTTTTTTTCTGACTAAACAATCTCTGCTCAGTTTGCTAT 170
sus         TTTTTTGACTAGATGAGCTAGATATTTATTGTTTTAACCC---TCTGTTCAATTTGCTAT 161
homo        TCCTT-GACTAAATAATCTAGATATTGTTT----TAACCT---TCTGCTCAGTTTG-TAT 125
            *  ** *  *            * **        *** *    **** *** **** **

Goat                          AGAGATTTAGGAGGGA-TTCATGAATCCTCCAAAAGATGGG 202
ovine                         AGAGATTTAGGAGGGA-TTCATGAATCCTCCAAAAGATGGG 202
bos                           AGAGATTTAGAAGGGA-TTCATGAATCTTCCAAAAGATGGG 210
sus                           AGACATTTAAGGGAGAGTTCATGAATCCTCCAAAAGATG-- 200
homo                          AGAGACTTAAAAGGGA-TTTATGAATTTTCCAAAAGATGGG 165
                              *** * ***   * ** **  *****  ***********
```

Table 4.2 Standardized quantities of reagents for the PCR assay to amplify the intron1 of IFN-γ gene

Sl. No.	**Parameter**	**Quantity taken**
1.	Template DNA (50ng/µl)	1.5 µl
2.	$MgCl_2$ (10 m*M*)	1.875 µl
3.	10X Reaction Buffer	1.5 µl
4.	dNTPs (2.5m*M*)	1.2 µl
5.	Forward Primer (10 p*M*/µl)	0.5 µl
6.	Reverse Primer (10 p*M*/µl)	0.5 µl
7.	Taq DNA Polymerase (3U/ µl)	0.33 µl
8.	Distilled water	7.595 µl
9.	Reaction Volume	15 µl

Table 4.3 Standardized temperature and cycling conditions for the PCR

Sl. No.	Parameter	Temperature/Time
1.	Initial Denaturation	94.0 ^{0}C for 2 min
2.	Denaturation	94.0 ^{0}C for 45 sec
3.	Annealing	57^{0}C for 1 min
4.	Extension	72.0^{0}C for 1 min
5.	No. of Cycles	5
6.	Denaturation	94.0 ^{0}C for 45 sec
7.	Annealing	55 ^{0}C for 1 min
8.	Extension	72 °C for 1 min
9.	No. of cycles	5
10.	Denaturation	94.0 ^{0}C for 45 sec
11.	Annealing	53 ^{0}C for 1 min
12.	Extension	72 °C for 1 min
13.	No. of cycles	5
14.	Denaturation	94.0 ^{0}C for 45 sec
15.	Annealing	52 ^{0}C for 1 min
16.	Extension	72 °C for 1 min
17.	No. of cycles	20
18.	Final extension	72.0 ^{0}C for 10 min
19.	Hold	10 ^{0}C 10 min

.

Table 4.4 Results of BLASTn of the nucleotide sequence of intron1 of IFNγ gene in goat, showing the percentage of identity with other species

Accession	Description	Maximum score	Total score	Query coverage (per cent)	Maximum identity (per cent)
# Z73273.1	*Ovis aries* IFNγ gene (intron1)	365	365	99	100
# Z54144.2	Bos taurus IFNγ gene for interferon gamma peptide	343	343	97	89
# X53085.1	*Sus sp.* DNA for interferon-gamma	118	118	90	73
# NG_015840.1	*Homo sapiens* Interferon- gamma (IFNG) on chromosome 12	71.6	71.6	79	70

Discussion

5. DISCUSSION

Goat is an important species of livestock in India. They contribute greatly to the agrarian economy, especially in areas where crop and dairy farming are not economical. They definitely play an important role in the livelihood of a large proportion of small and marginal farmers and landless labourers. In goats mortality is quite high due to internal parasitic infections and it causes large morbidity and economic loss. High cost of anthelmintics and development of resistance to the drugs has lead to the search for animals resistant for parasitic infections. Detection of Gastrointestinal Nematode (GIN) resistant animals using Faecal Egg Count (FEC) as an indicator and use of molecular markers related to FEC will give a basic idea about development of goats resistant to parasitic diseases.

5.1 FAECAL EGG COUNT

5.1.1 EXAMINATION OF FAECAL SAMPLE

According to animal surveillance scheme: annual report (2006) of Animal Husbandry Department of Kerala, Strongyle infection (74 per cent) formed major part of the parasitic infections. GIN encountered during the present study also belonged to Strongyle and *Strongyloides* sp. Overall average of nematode eggs counted was 764.39 eggs per gram of the faeces (epg) and the FECs belonging to GIN species ranged from 0.00 to 16700 epg. This indicates that the FEC ranged between non-infective and highly infected levels. Animal is said to be moderately infected with nematodes if the unspeciated GIN count is between 1000 to 2000 epg (Kassai and Heinemann, 1999).

5.1.2 Factors Affecting FEC

Effect of different factors namely sex, type of birth, season of sampling, age, birth weight, centre and breed, on FEC was studied. Logarithmic transformation of FEC as ln(FEC+2000) was done before analysis, as the data available on FEC was not normally distributed. This non normality of data and logarithmic transformation of FEC was also observed in the study by Vanimisetti *et al.* (2004). Back transformation was done before expressing the least-squares means and their confidence intervals.

5.1.2.1 Effect of Sex of Kid

Least square analysis (LSA) revealed that sex of the kid had no significant effect on FEC. This result was comparable with the result obtained by Vanimisetti *et al.* (2004) and Miller *et al*. (2006). On the contrary Rege *et al.* (2002), Khusro *et al.* (2004) and Good *et al.* (2006) found a significant effect of sex on FEC. The non significant effect of sex could be due to the similarity in managemental conditions given to kids of both the sexes.

5.1.2.2 Effect of Type of birth

Type of birth had no significant effect on FEC. This finding goes in line with that of Miller *et al.* (2006) in sheep. According to Valderrábano *et al.* (2002), Kahn *et al.* (2003), Torres-Acosta *et al.* (2004), Hoste *et al*. (2005), Burke and Miller (2008), Arsenos *et al*. (2009) and McClure (2009), nutrition affected resistance to GIN in animals. In this study, type of birth had non significant effect due to the reason that majority of kids available for study was either singlets or twins. Also, managemental conditions in the University farm assure adequate milk to all the kids either directly from dam or through pail feeding. Equal availability of nutrients to all birth type kids with similar environmental, managemental and climatic conditions might be the reason for non significant effect of birth type on FEC.

Rege *et al*. (2002), Khusro *et al.* (2004) and Mugambi *et al.* (2005) reported a different observation that is, type of birth influenced FEC in lambs. This effect of birth type on FEC might be due to the reason that these studies were done in large flocks where individual care for kids is not possible.

5.1.2.3 Effect of Age

LSA revealed no significant effect of age on FEC. Rege *et al.* (2002) observed that the age of lambs influenced FEC. Mugambi *et al.* (2005) and Khusro *et al.* (2004) also reported age related changes in FEC. In the present study the non significant effect of age could be due to the reason that only two age groups were selected and faecal samples were collected before deworming in both the groups.

5.1.2.4 Effect of Season of sampling

Season of sampling did not have any significant effect on FEC. Studies by Miller *et al.* (1998), Rege *et al*. (2002) and Mugambi *et al.* (2005) revealed a significant effect of season on FEC at different ages. The seasonal influence reported by these authors on FEC could be due to the occurrence of clearly defined seasons in their area of study and the difference in availability of favorable or unfavorable environmental conditions for the survival and development of the parasite during each season. Though categorizations of seasons were made during the period of study, clearly defined demarcations between seasons could not be appreciated. During summer and pre-monsoon periods, Kerala encountered showers and temperature recorded during winter was not as low as North Indian states. This might be the reason for the non significant effect of season on FEC.

5.1.2.5 Effect of Birth weight

Birth weight did not have significant effect on FEC. This shows that though there is difference in weight at birth in kids, if similar nutrition and other managemental conditions were given, they do not show difference in parasite resistance status.

5.1.2.6 Effect of Center (area)

Center had significant effect on FEC with University farm showing a least square mean of 51.42, Thalassery showing 1297.53 and Badagara 1122.87 epg. This statistically significant difference might be due to the improved health status of animals in the farm, which can be accounted to careful selection among breeding stock leading to better germplasm. The significant effect of different centers may also be attributed to the regional differences as they are located in three different districts of Kerala and due to the differences in the herd management and resources. These might be the reasons for significantly reduced FEC (51.42 epg) in farm stock. This result was comparable with those obtained by Khusro *et al.* (2004), Pollott *et al*. (2004) and Good *et al.* (2006) in different sheep breeds.

5.1.2.7 *Effect of Breed*

The mean FEC did not vary between breeds, which was in accordance with the results of Costa *et al.* (2000) where FEC varied within goat breeds but not between goat breeds. Non significant effect of breed might be due to the reason that Malabari bucks were used for breeding Malabari purebred and Malabari crossbred females and hence the level of Malabari inheritance in selected kids was more than 50 per cent in both the genetic groups. Also data regarding the third genetic group i.e., Attapady Black kids were relatively less in this study.

This observation was in contrary to those by Bahirathan *et al.* (1996), Romjali *et al.* (1996), Mugambi *et al*. (1997), Miller *et al.* (1998), Rege *et al.* (2002), Baker *et al.* (2003) and Good *et al*. (2006) in different breeds of sheep and by Baker *et al*. (2001) in Galla and Small East African goat breeds, where they found significant breed differences for FEC.

The results on FEC and factors affecting FEC have shown that the FEC of goats under farm conditions was significantly lower than that under field conditions. This might be due to the improved immune status of the animal, which can be accounted to careful selection among breeding stock leading to better germplasm. Another factor is the enhanced general health of animals, along with individual care and proper management given in the farm. The non-significant effect of age, sex, birth weight and type of birth might be due to the uniform managemental conditions existing especially under farm conditions which do not favour GIN prevalence. The reason for failure of season influencing FEC could be due to the fact that no clear cut demarcation existed between the seasons in Kerala, during the period of study.

5.1.3 HERITABILITY

The heritability estimate for FEC was 0.39 ± 0.3630. Heritability estimate in the studies by Baker *et al.* (2001), Mandonnet *et al.* (2001), Gauly *et al.* (2002), Rege *et al.* (2002), Khusro *et al.* (2004), Vanimisetti *et al.* (2004) and Miller *et al.* (2006) were low to moderate. While the heritability observations by Rout and Chauhan (2004) and Miller and Horohov (2006) were moderate to high. Overall heritability estimates for FEC from various studies ranged between 0.14 and 0.63. But majority of researchers reported a heritability of around 0.30 for FEC and the present study agrees with these reports.

The cause of high standard deviation for heritability estimate could be due to the availability of reduced number of progeny per sire. A more elaborate study with higher sample size will definitely reduce the standard error. Moderate heritability estimate for FEC, denotes that there is significant genetic variation within the population for the trait, which makes selection for GIN resistance possible.

5.1.4 CORRELATION BETWEEN LFEC AND PCV

The PCV values assessed in the study ranged between 19 and 48 per cent. PCV values obtained were compared with the normal PCV values of goats, i.e. 22 per cent to 38 per cent (Schalm *et al.*, 1975). Goats with PCV values below 22 per cent could be considered as anaemic while with values more than 38 per cent as dehydrate (Chandrawathani *et al*., 2009).

Negative correlation was observed between PCV and LFEC. The correlation coefficient observed in this study (-0.1791), was comparable to the reported values of Costa *et al.* (2000), Baker *et al.* (2001) and Chiejina *et al.* (2002). The results imply that, when infection rate with GIN increases, PCV reduces.

The correlation coefficient between PCV and LFEC suggest that their combinations could provide an estimate for predicting resistance to GIN in goats. Further studies are needed to assess the genetic correlation between LFEC and PCV. Correlation between PCV and LFEC may be utilised as a selection criterion in obtaining nematode resistant animals, as supported by the statements of Miller *et al.* (1998) and Mandonnet *et al*. (2001). Thus the negative, phenotypic correlation existing between traits studied are favorable for reducing FEC and increasing nematode resistance. This correlation can thus be taken as advantage in breeding programmes.

5.2 FECAL EGG COUNT AND INTERFERON GAMMA INTRON1

5.2.1 OPTIMIZATION OF PCR

The amplification obtained from conventional type of PCRs were nil or with low intensity initially. Adoption of the particular touchdown (TD) PCR protocol gave favorable results. The amplified products obtained through TD PCR were of good quality.

According to Viljoen *et al.* (2005), optimal PCR conditions can be very different for different assays, as influenced by the unique template, conditions and primer sets, other biochemical variables and the specific type of cycler used. Optimization of the PCR has to be carried out with respect to many factors like, template, Mg2+ concentration, primer concentrations, annealing temperatures, enzyme concentration, optimal reaction pH and any other variable that may influence the reaction. These were found to be true during the study and it took about 30 different PCRs to standardize the conditions.

As conventional PCR protocol failed to give good quality product, a modified touchdown PCR was followed which gave good quality products. This observation was in line with that of Don *et al.* (1991), Roux (1995), Hecker and Roux (1996) and Viljoen *et al.* (2005) where they opined that TD PCR eliminate lengthy adjustment procedures for temperature or $MgCl_2$ concentration and inconsistency of well temperatures within or between thermal cycling machines. It also offers simple one-step optimization of PCR reactions that are expected to be sub-optimal with regard to primer/template homology. In the current study, primers based on sheep template DNA were used for amplification of IFN γ intron1 of goat DNA.

5.2.2 IFN γ INTRON1/BspH1 POLYMORPHISM

Enzyme digestion of PCR products with *BspH*I endonuclease gave only single type of band pattern on agarose electrophoresis. The PCR amplified product was cut by the enzyme *BspH*I, into two fragments of sizes about 180 bp and 22 bp, of which 180 bp fragment was visible on gel. The finding confirmed the presence of restriction site for *BspH*I endonuclease in the sequence. This result could be compared to that obtained by Sayers *et al.* (2005), where they reported an allele named B for IFN γ intron1 which was cut by endonuclease *BspH*I. In their study four haplotypes were identified, where as in this research study only one genotype was detected.

Sayers *et al.* (2005) also reported an association of IFN γ intron1 polymorphism with nematode resistance in Texel breed of sheep. In the present study the association between FEC and IFN γ intron1 allelic variation could not be carried out as all the animals tested were of the same genotype. It is recommended that the study can be conducted in a larger population with wider genetic base, which might reveal polymorphism and thus aid in marker assisted selection for nematode resistance in goats.

5.2.3 DNA SEQUENCING

One of the good quality PCR products was gel purified, cloned and sequenced at the DNA Sequencing Facility, Bioserve, Biotechnologies (India), Pvt. Ltd. Hyderabad. The nucleotide sequence was determined by the dideoxy-chain termination method using M13 reverse primer.

5.2.3.1 Structure of IFN γ intron1

The sequence data confirmed the PCR product to be 202 bp in size. The sequence contained a tetra-nucleotide repeat, repeated five times, $(GT_4)_5$. This observation was in 100 per cent agreement with the findings by Schmidt *et al.,* 1996 (GenBank Accession No.Z73273).

As per the sequence available for B allele in sheep, the sequence data for goat IFN γ intron1 also contained an Adenine at position 49 bp downstream of the microsatellite. This is contained in the site where the restriction enzyme *BspH*I cuts the DNA strand. This finding explained the reason for band pattern obtained after restriction enzyme digestion in this study and was in line with the report by Sayers *et al.* (2005) where the particular enzyme cut at the same position.

5.2.3.2 Comparison with sequences from other species

Characterization of sequence homology between goat and other animal species by using BLAST2 sequence alignment programme demonstrated a high degree of homology with IFN γ intron of other species. The nucleotide sequence data obtained for goat IFN γ intron1 was found to be having 100 per cent identity with that of sheep (Schmidt *et al.,* 1996; GenBank Accession No.Z73273).

The review of available literature did not reveal any reference on DNA sequence information on IFN γ intron1 in goats. This work seems to be the first of its kind in establishing the DNA sequence data and structural peculiarities of IFN γ intron1 in goats.

Summary

6 SUMMARY

Goats play a significant role in the economy and nutrition of small and marginal farmers in India and goat farming is gaining momentum among rural poor of Kerala. Retarded growth rate and problems relating to internal parasitic infections are the major setbacks which prevent goat farmers from flourishing in this animal husbandry sector. As parasitic infection produces greater economic loss than any other disease, it has to be efficiently controlled for profitable goat rearing. With scarce grazing lands, there is very limited scope for controlled/ rotational grazing and over usage of drugs has led to anthelmintic resistance in animals.

At this juncture, breeding animals for parasitic resistance forms a major thrust area and studies on host genetic resistance of goats will act as the basis for selection of nematode resistant goats.

One hundred and fifty goats of different breeds namely, Malabari, Attappady Black and Malabari crossbreds formed the material for investigation. The centers selected were University Livestock Farm, Mannuthy and two field centers under All India Co-ordinated Research Project (AICRP) on goats, namely Tellichery in Kannur district (Centre 1) and Badagara in Kozhikode district (Centre 2).

Feacal samples of goats were collected before deworming and Faecal Egg Count (FEC) was determined using modified McMaster Technique. The values for FEC ranged from 0.00 to 16700 eggs per gram of the faeces (epg). The average FEC was 764.39 epg. This average count was much closer to that could be considered as moderately infected (1000 to 2000 epg) with mixed nematode species.

Data on sex, season of sampling, age, birth weight, breed, type of birth and center were collected and subjected to statistical analysis. Least square

analysis was performed on logarithmically transformed FEC to study the effect of above said factors on FEC.

Sex, type of birth, age, season of sampling, birth weight and breed had no significant effect on FEC. Center had significant effect on FEC. University Goat & Sheep farm, Mannuthy recorded the least FEC, which might be due to the improved immune status of the animal that can be accounted to careful selection among breeding stock leading to better germplasm. The significant effect of different centers may also be attributed to the regional differences and due to the differences in the herd management and resources.

The non-significant influence of sex, type of birth and birth weight, might be due to uniform managemental conditions given to kids irrespective of sex, type of birth or birth weight, which did not favour GIN prevalence. The reason for failure of season in influencing FEC could be due to the fact that, much demarcation was not observed between seasons during the study. The genetic similarity between Malabari purebreds and Malabari crossbreds, and the lesser sample size for Attapady Black breed might be the reasons for non significant effect of breed on FEC.

The Packed cell volume (PCV) values assessed in the study ranged between 19 and 48 per cent and on analysis, a negative phenotypic correlation of -0.1791 was estimated between LFEC and PCV. The data adjusted for non genetic effects were used for the estimation of heritability of FEC by paternal half sib correlation method. The heritability estimate of FEC was 0.39 ± 0.3630.

Genomic DNA was isolated from blood samples of 150 goats maintained in the farm and two field centers of Kerala using phenol chloroform extraction procedure. All DNA samples obtained were having good quality as read from agarose gel electrophoresis.

A 202 bp fragment of intron1 of interferon gamma (IFNγ) region was amplified by PCR using specific primers designed on the basis of IFNγ intron 1 sequences of sheep. The PCR assay was performed in 200 µl reaction tubes with a total volume of 15 µl, containing 1.5 µl of DNA template, 1.5 µl 10X PCR buffer, 1.875 µl $MgCl_2$, 0.5 µl of each primer, 1.2 µl of each dNTP and 1unit Taq DNA polymerase.

The cycling conditions for DNA amplification included a modified touchdown protocol with an initial annealing temperature of 57 °C and ending at an annealing temperature of 52 °C. Initial cycles were repeated 5 times while final cycle was repeated 20 times, followed by final extension at 72 °C for 10 min and keeping at 10°C for 10 min.

The PCR products were digested with restriction enzyme, *Bsp*HI and only one restriction digestion pattern was observed with the identification of one allele namely B (180 bp and 22 bp fragments). All digested products gave same restriction digestion pattern with *Bsp*HI.

The PCR product was gel purified, cloned and sequenced. The sequence was 202 bp long which included a tetra nucleotide repeat, $(GT_4)_5$ and an Adenine present at position 49 bp downstream of this microsatellite. The presence of this Adenine was creating the restriction endonuclease recognition sequence (5'…T▼CATGA… 3') for *BspH* I enzyme.

The review of available literature did not reveal any reference on DNA sequence information of IFNγ intron 1 region in goats. As per available literature, this work seems to be the first of its kind in establishing the nucleotide sequence IFNγ intron 1 of goats. The new nucleotide sequence data of goat-IFNγ intron1

was submitted to the GenBank DNA database and obtained an Accession Number: HM047072.

The analysis of sequence data revealed 100 per cent similarity with that of sheep, 89 per cent with that of European cattle, 73 per cent with that of pig and 70 per cent identity with human IFNγ gene. The results of ClustalW multiple nucleotide sequence alignment of the complete sequence showed a high degree of homology with a score of 100 with sheep, 92 with European cattle, 73 with pig and 74 with human.

The present study revealed that there is scope for selection of GIN resistant animals as there is significant genetic variation within the population for the trait. Selection of superior individuals incorporating results of FEC will increase the rate of genetic progress favoring resistance to GIN. DNA sequence data of intron1 of IFN γ gene in goats was established, but polymorphism could not be identified. Research on this region polymorphism should be extended to sizeable goat population of different geographical regions of the state to confirm the influence of this marker on nematode resistance. This study helped in establishing possibility of selection for parasitic resistance in goats in the population.

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Annexures

ANNEXURE – I

COMPOSITION OF REAGENTS AND BUFFERS USED IN THE STUDY

Agarose (0.7%)

Weighed 0.7 g of agarose powder and mixed with 100 ml of 1X TAE buffer in a conical flask. Solution was heated in a microwave oven until boiling and cooled slowly.

Agarose (2%)

Weighed 2 g of agarose powder and mixed with 100 ml of 1X TAE buffer in a conical flask. Solution was heated in a microwave oven until boiling and cooled slowly.

EDTA (0.5*M*, pH 8.3)

Dissolved 18.61 g of EDTA (disodium, dihydrate) in 80 ml of distilled water by bringing the pH to 8.3 with NaOH solution and volume made upto 100 ml. Stored at room temperature after filtration and autoclaving.

Ethidium Bromide (10 mg/ml)

Dissolved 100 mg of Ethidium bromide in 10 ml of distilled water. Solution stored at 4 °C in a dark coloured bottle.

Gel loading buffer

Bromophenol blue	0.25 %	50 mg
Xylene cyanol	0.25 %	50 mg
Sucrose	40 %	8 g

Components were mixed and stirred well in 20 ml distilled water and stored at 4 °C.

Phenol (Saturated, pH 7.8)

Commercially available crystalline phenol was melted at 65 °C in a water bath. Hydroxyquinolone was added to a final concentration of 0.1 percent. To the molten phenol, 0.5 *M* Tris HCl (pH 8.0) was added in equal volume and stirred for 30 min in a magnetic stirrer. The contents were transferred into a separating funnel. Lower phenolic phase was collected and mixed with equal volume of 0.1 *M* Tris HCl (pH 8.0) and stirred again for 30 min. The phenolic phase was collected and extraction repeated with 0.1*M* Tris HCl (pH 8.0) until the pH of phenolic phase was more than 7.8. Finally 0.1 volume of 0.01 *M* Tris HCl (pH 8.0) added and stored in a dark bottle at 4 °C.

RBC lysis buffer

Ammonium chloride	150 mM	8.0235 g
Potassium chloride	10 mM	0.7455 g
EDTA	0.1 mM	0.0372 g

Dissolved the contents in distilled water and volume made up to 1000 ml. Stored at 4 °C after filtration and autoclaving.

Sodium acetate

Dissolved 40.824 g of Sodium acetate in 70 ml of distilled water and pH adjusted to 5.5 with glacial acetic acid. Volume was made up to 100 ml, autoclaved and stored at room temperature.

Sodium chloride (5 M)

Dissolved 29.22 g of sodium chloride in 80 ml distilled water and volume made up to 100 ml. Solution filtered and stored at room temperature.

Sodium chloride- EDTA (SE) buffer (pH, 8.0)

Sodium chloride	75 mM	4.383 g
EDTA	35 mM	9.306 g

Dissolved the contents in 900 ml distilled water and pH adjusted to 8.0. Made up the volume to 1000 ml, filtered, autoclaved and stored at 4°C.

Sodium dodecyl sulphate (SDS) 20 %

SDS	20 g
Distilled water make up to	100 ml

Stirred, filtered and stored at room temperature.

Tris Acetate EDTA (TAE) buffer (50X)

Tris base	48.4 g
Glacial acetic acid	11.42 ml
0.5 M EDTA (pH 8.0)	20 ml
Distilled water up to	1000 ml

Autoclaved and stored at room temperature.

Tris Buffered Saline (TBS) pH 7.4

Sodium chloride	140 mM	8.18 g
Potassium chloride	0.5 mM	0.0373 g

Tris base	0.25 mM	0.0303 g

Dissolved in 900 ml of distilled water and pH adjusted to 7.4. Made up the volume to 1000 ml, filtered, autoclaved and stored at 4 °C.

Tris EDTA (TE) buffer (pH 8.0)

Tris base	10 mM	1.2114 g
EDTA	0.1 mM	0.3722 g

Dissolved in 900 ml of distilled water and adjusted the pH to 8.0. Made up the volume to 1000 ml, filtered, autoclaved and stored at 4 °C.

Tris 1*M* (pH 8.0)

Tris base	121.14 g

Added distilled water up to 1000 ml, pH adjusted to 8.0, filtered and stored at room temperature.

ANNEXURE – II

SOURCES OF IMPORTANT CHEMICALS AND ENZYMES USED IN THIS STUDY

(A) CHEMICALS

Agarose (Low EED)	- Bangalore Genei Pvt. Ltd.
Ammonium chloride	- SRL, Bombay
Boric acid	- SRL, Bombay
Chloroform	- Merck
Crystalline phenol	- Merck
dNTPs	- Bangalore Genei Pvt. Ltd.
EDTA	- SRL, Bombay
Ethanol	- Merck
Ethidium bromide	- BDH lab, England
6 X gel loading buffer	- Bangalore Genei Pvt. Ltd.
Glacial acetic acid	- BDH-E, Merck (India) Ltd.
Hydroxy quinolone	- Qualigens Chemicals, Mumbai
Isoamyl alcohol	- Merck
Isopropyl alcohol	- SRL, Bombay
Methanol	- SRL, Bombay
Potassium chloride	- SRL, Bombay
Sodium acetate	- SRL, Bombay
Sodium chloride	- SRL, Bombay

Sodium dodecyl sulphate (SDS)	- SRL, Bombay
Tris base	- SRL, Bombay

(B) PRIMERS

Integrated DNA Technologies, Inc.
1710 Commercial Park, Coralville, IA (www.idtdna.com)

(C) MOLECULAR MARKER

pUC19/*Msp*I digest	- Bangalore Genei Pvt. Ltd.

(D) ENZYMES

Taq DNA polymerase	- Bangalore Genei Pvt. Ltd.
Proteinase-K	- Bangalore Genei Pvt. Ltd.
Restriction endonuclease, *BspH*I	- New England Biolabs.

ANNEXURE – III

ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
BLASTn	Nucleotide BLAST
bp	base pair
cm	centimeter
DNA	Deoxyribo Nucleic Acid
dNTP.	Deoxyribo Nucleotide Triphosphate
EDTA	Ethylene Diamine Tetraacetic Acid
FAO	Food and Agricultural Organization
FEC	Faecal Egg Count
g	gram
i.e.	that is
L	litre
M	moles per litre
MAS	Marker Assisted Selection
mg	milligram
$MgCl_2$	Magnesium chloride
m*M*	millimolar
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
NH_4Cl	Ammonium Chloride
ng	nano gram
PCR	Polymerase Chain Reaction
p*M*	Pico moles
QTL	Quantitative Trait Loci
RBC	Red Blood Cell
RFLP	Restriction Fragment Length Polymorphism

rpm	Revolutions per minute
s	second
SDS	Sodium Dodecyl Sulphate
sp.	species
WBC	white blood cell
µg	microgram
µl	microlitre
µm	micrometer
°C	degree Celsius

GENETIC RESISTANCE TO GASTRO-INTESTINAL NEMATODES IN GOATS

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ABSTRACT

The present study aimed at exploring the possibility of incorporating Gastro Intestinal Nematode (GIN) resistance as a criterion for selection in goat breeding. Here Faecal Egg Count (FEC) was taken as an indicator of host resistance. Heritability estimate for FEC and correlation of FEC with Packed Cell Volume (PCV) were calculated. While assessing resistance at phenotypic level using FEC as indicator, the possibility of using PCV as an alternative to FEC was also considered. Using IFNγ intron 1 as the locus of study, an attempt was made to identify a genetic marker for GIN resistance in goats.

University Livestock Farm, Mannuthy and two field centers under All India Co-ordinated Research Project (AICRP), namely - Tellichery and Badagara, formed the venue for study. In the present investigation, data from three genetic groups - Malabari, Attappady Black and Malabari crossbreds were used.

Representative population in the study comprised of 150 goats. The nematode eggs encountered during the study belonged to Strongyle and *Strongyloides* sp. Mean FEC was 764.39 eggs per gram of the faeces (epg) with values ranging from 0.00 to 16700 epg. The value of PCV estimated ranged between 19 and 48 per cent.

Effect of non-genetic factors on FEC was analyzed after logarithmically transforming the FEC. University Goat & Sheep farm, Mannuthy recorded the least FEC. This might be due to the improved immune status of the animal through careful selection along with scientific managemental practices followed in the University farm. The non-significant influence of sex, birth weight and type of birth might be due to uniform managemental conditions given to kids irrespective of sex, birth weight or type of birth, which do not favour GIN prevalence. The reason for failure of season in influencing FEC could be due to the fact that, not much demarcation was observed between seasons during the study.

The genetic similarity between Malabari purebreds and Malabari crossbreds, lesser sample size for Attapady Black breed and similar management given to the breeds led to non significant effect of breed on FEC.

A negative phenotypic correlation estimate of -0.1791 was observed between LFEC and PCV. The heritability estimated for FEC was 0.39 ± 0.3630 as per paternal half sib correlation method and it was moderate. This moderate heritability estimate for FEC shows that there is significant genetic variation within the population for the trait, which makes selection for nematode resistance possible.

Genomic DNA was isolated from blood samples of 150 goats maintained in the farm and two field centers of Kerala using phenol chloroform extraction procedure. Through PCR, a 202bp product of IFN γ intron 1 was amplified. On restriction digestion of the amplified product, the restriction-endonuclease, *BspH*I cut PCR product into two fragments of 180 bp and 22 bp sizes. Monomorphic pattern was observed on the particular RFLP analysis.

The 202bp PCR product was sequenced by the dideoxynucleotide sequencing method with M13 universal reverse primer using an automated DNA sequencer. The sequence included a tetra nucleotide repeat, $(GT_4)_5$ and an Adenine present at a position, 49 bp downstream of this microsatellite.

The sequence had 100 per cent homology with sheep, 89 per cent with European cattle, 73 per cent with pig and 70 per cent with human. On submission of the nucleotide sequence data of goat-IFNγ intron1 to the GenBank DNA database, an Accession Number: HM047072 was obtained. As per available literature, this work seems to be the first of its kind in establishing the DNA sequence data of intron1 of IFN γ gene in goats. It is recommended that study of this gene in a larger population with greater genetic base might bring out the polymorphism and help in marker assisted selection for GIN resistance in goats.

Overall, the study aided in establishing nucleotide sequence of IFN γ intron1 in goats and showed that there is immense scope for selection of animals for nematode resistance. This study emphasizes the fact that along with other production traits, host genetic resistance should also be included as a criterion for selecting goats, producing genetically superior stock.