ANTHELMINTIC RESISTANCE IN GASTROINTESTINAL NEMATODES OF GOATS

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DECLARATION

I hereby declare that this thesis, entitled **"ANTHELMINTIC RESISTANCE IN GASTROINTESTINAL NEMATODES OF 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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CERTIFICATE

Certified that the thesis entitled **"ANTHELMINTIC RESISTANCE IN GASTROINTESTINAL NEMATODES OF GOATS"** is a record of research work done independently by **Deepa .C.K.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Introduction

1. INTRODUCTION

Domestic ruminants, the precious possession of peasants next to land, play an important role in the rural economy of the Indian agricultural sector. But profitable animal husbandry is often impeded by different pathogens like bacteria, viruses and parasites. Among the parasitic diseases, nematodosis is a common disease entity in domestic ruminants. From the hoof to the brain and nose to the anus of an animal, occur different nematode parasites which adversely affect the production of milk, meat, wool and hide and even cause acute clinical diseases leading to death and heavy loss to the farming industry.

Nematode infection by ingestion of infective larvae from pastures and faecal pads is quite common. This type of infection is also termed manure cum pasture borne nematode infection. It is a serious problem in temperate climatic countries owing to the prevalence of ambient climate and season favoring the development of infective larvae in those countries. It leads to severe economic loss by way of loss of production and mortality in domestic ruminants.

The disease caused by gastrointestinal nematodes are long term and debilitating in their effects upon the hosts resulting in pathological alterations of the intestinal system and functions. Control of these parasites in India is undertaken using anthelmintics aiming primarily at a tactical treatment when found positive on faecal examination. This has led to indiscriminate use of anthelmintics resulting in the emergence of resistant strains of parasites. As the development of anthelmintic resistance in parasitic population continues its surge in small ruminants in most countries, it imposes serious limitations on the use of anthelmintics.

Anthelmintic resistance is initially suspected when a flock exhibit poor clinical response to anthelmintic treatments. Often resistance is not diagnosed and continued use of the same anthelmintic group increases the frequency of resistant

individuals until there is a major failure of control. High frequency of treatment of same chemical group to young animals and lower persistence of a low level therapeutic activity of the compound, account for the development of anthelmintic resistance (Prichard, 1994). Significant and widespread drug resistance has been reported from several countries in gastrointestinal nematodes of ruminants and horses (Boersema *et al.,* 1982; Taylor and Hunt, 1988; Sivaraj *et al.,* 1994; Borgsteede *et al.,* 1996; Borgsteede *et al.,* 1997).

In India, Varshney and Singh (1976) reported development of resistance in *Haemonchus contortus* against phenothiazine and thiabendazole in sheep. Ever since numerous reports on anthelmintic reisistance from the country are on record. (Yadav *et al.,* 1995; Swarnkar *et al.,* 1999; Jeyathilakan *et al.,* 2003; Dhanalakshmi *et al.,* 2003; Kumar *et al.,* 2004; Yadav and Garg, 2004).

Keeping the aforementioned facts in mind the present study has been undertaken to

1. understand the extent of parasitic burden and the species involved in goats
2. monitor the occurrence of anthelmintic resistance and to
3. suggest appropriate control strategies to prevent resistance development.

Review of Literature

2. REVIEW OF LITERATURE

2.1 PREVALENCE OF NEMATODES

Tripathi (1970) recorded the following species of nematodes from goats *viz.*, *Haemonchus* spp, *Strongyloides* spp, *Trichostrongylus* spp and *Oesophagostomum* spp at Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh.

Sathianesan and Peter (1971) studied the incidence of gastrointestinal nematode infections in goats in Kerala by examining the entrails of indigenous goats slaughtered in various parts of the state and by faecal examination and faecal culture in the case of farm bred goats. Eight hundred and sixty five entrails and 236 faecal samples were examined and 11 species of nematodes including two new species were identified. This study revealed that 89 per cent of the farm bred goats examined were positive for one or more species of gastrointestinal nematodes. In indigenous goats, the most commonly prevalent species was *Haemonchus contortus* (75 per cent) and the least common species was *Gaigeria pachyscelis* (9.1 per cent). In the farm bred lambs *Strongyloides papillosus* had the highest incidence (72.8 per cent) and *Trichostrongylus axei* the lowest (18.2 per cent). They did not notice any significant seasonal variation in the incidence.

Soulsby (1982) reported that neonatal immune unresponsiveness to gastrointestinal helminths was important and contributed to the high morbidity and mortality of young animals.

Craig (1986) reported that the most important parasite in small ruminants throughout the Southern United States was *H. contortus*.

Charies (1989) who conducted a survey from April 1979 to March 1982 in Brazil revealed the occurrence of *H.contortus, S.papillosus* and *Oesophagostomum columbianum* in goats. He also noted the seasonal occurrence

of the worm burdens. The acquisition of nematodes by tracer goats occurred mainly from mid rainy to early dry season.

Muraleedharan *et al.* (1990) studied the seasonal incidence of gastrointestinal parasites of cattle in Mysore and Mandya districts of Karnataka and found that *Haemonchus, Bunostomum* and *Oesophagostomum* were predominant there in hot season.

Jeyathilakan (1995) reported seven species of nematodes in goats by identifying the infective larvae obtained on coproculture from animals brought to the Veterinary hospitals and slaughter houses at Trichur and Mannuthy. They were *H.contortus, O.columbianum, O. asperum, Trichostrongylus colubriformis, T.axei, Bunostomum trigonocephalum* and *S.papillosus.*

A study conducted in a sheep flock maintained by the Department of Animal Science, Texas A and M University, *Trichostrongyles* were found to be the predominant nematode in Florida Native breeds at the end of natural infection period, while *Haemonchus* remained so in Rambouillet lambs (Amarante *et al.*, 1999a). Amarante *et al.* (1999b) later reported that the highest percentage of *Haemonchus* was found in faecal cultures of Rambouillet ewes (usually over 80 per cent) and the lowest in Florida Native ewes.

Bandyopadhyay (1999) upon coprological examination of sheep and goats at Composite State Animal Husbandry Farm, Salboni, West Bengal, reported the occurrence of eggs of *Haemonchus* spp, *Bunostomum* spp, *Trichuris* spp, *Strongyloides* spp, amphistomes and coccidial oocysts. Generally mixed infections were observed among the animals.

Valcarcel and CRomero (1999) reported that the seasonal pattern of prevalence of caprine trichostrongyles in a dry area of central Spain showed a real risk of infection all year round, with two peaks in the summer and autumn. Females were more frequently infected than males.

Dhanalakshmi *et al.* (2001) screened ten sheep farms located in six districts of Karnataka, and found that *Stongyle* infection was very common (82.8 per cent) followed by mixed infection (2.3 per cent) and coccidial infection alone (1.5 per cent). The faecal cultures showed the presence of *Haemonchus* spp in majority of the farms followed by *Oesophagostomum* spp, *Trichostrongylus* spp, *Cooperia* spp and *Nematodi rus* spp.

Tuteja *et al.* (2001) recorded *H. contortus* as the most prevalent nematode encountered in a flock of goats maintained at the Indian Grass Land and Fodder Research Institute, Jhansi, while Garg *et al.* (2003) examined 431 faecal samples and 321 abomasii of sheep and goats of the semiarid region of India and found out the occurrence of *H. contortus* in goats.

The prevalence study based on coprological examination in 2570 sheep in the district of Kathua, Jammu and Kashmir by Khajuria and Kapoor (2003) revealed infections of Strongyles (64.09 per cent), *Fasciola* spp (12.48 per cent), Trichuris (10.11 per cent), *Dicrocoelium* spp (4.18 per cent), *Eimeria* spp (2.85 per cent) and amphistomes (1.5 per cent). Screening of another 2040 goats showed that they were positive for parasites with corresponding figures as 67.46 per cent, 5.83 per cent, 8.11 per cent, 2.55 per cent and 3.01 per cent. Amphistome eggs were not detected in the faecal samples of goats.

A survey carried out by Pal and Bandyopadhyay (2004) in Sikkim revealed the presence of *H. contortus*, *Chabertia ovina*, *B. trigonocephalum*, *O. columbianum*, *T. colubriformis* , *S. papillosus*, *Nematodi rus* spp, *Trichuris globulosa and T. ovis* in goats. The seasonal distribution of infection indicated a higher percentage of infection in summer and autumn when compared to winter and spring.

Khalid *et al.* (2004) in Mymensingh, Bangladesh revealed the presence of *Strongyloides* spp (7.7 per cent), *Haemonchus* spp (18.18 per cent), *Trichuris* spp

(5.45 per cent), *Oesophagostomum* spp (9.09 per cent) and mixed infection (14.18 per cent) in goats.

Umur (2005) conducted a study in 50 goats in Burdeur region of Turkey and found out the occurrence of *O. circumcin ta* (78 per cent), *Marshallagia marshalli* (72 per cent), *Nematodi rus abnormalis* (66 per cent), *T. ovis* (60 per cent), *N. spathiger* (52 per cent), *T. skrjabini* (50 per cent) and *T. vitrinus* (40 per cent).

2.2 IDENTIFICATION OF INFECTIVE LARVAE

Niec (1968) suspected that the caudal extent of the larval sheath (*ie.*, the distance from the anus to the sheath extremity) was considered as a basic reference for identification. Presence or absence of buccal cavity, length of oesophagus and the nature of the intestinal cells were the other criteria for identification of nematode species.

Padmavathi *et al.* (1971) studied the morphology of the infective larvae of *H. contortus* and *H. bispinosa* of sheep.

The free living larval stages of *O. asperum* (Sathianesan and Peter, 1976), *H. contortus* (Sathianesan and Peter, 1977), *T. colubriformis* (Sathianesan and Peter, 1979) were studied in detail and described.

A detailed account of the measurements of infective larvae of nematodes has been given by Soulsby (1982).

Lancaster (1987) opined that *Trichostrongylus* spp could be differentiated by the presence of tubercles on the larval tails, 16 gut cells and tapered head. He further added that *Trichostrongylus* spp generally appeared narrower than that of ovine *Ostertagia* spp.

Bowman (1995) pointed out the presence of caudal tubercles in the tail of *Trichostrongylus* spp .

A simplified system was developed by Van Wyk *et al.* (2004) to facilitate identification of larvae of the common nematodes of cattle, sheep and goats. In that case, the mean length of tail sheath of *H. contortus* was 2 - 2.7x and that of *Oesophagostomum* spp from sheep was 4 - 7x, where x was assumed as the mean length of tail sheath of *Trichostrongylus* spp.

2.3 ANTHELMINTIC RESISTANCE

2.3.1 World

Drudge *et al.* (1957) reported resistance of *H. contortus* to phenothiazine in USA in sheep which formed the first report.

Sangster *et al.* (1979) reported that Merino and other crossbred sheep showed varying degrees of resistance to levamisole, morantel tartrate and thiabendazole.

Prichard *et al.* (1980) explained on the use of anthelmintics in livestock with respect to biochemical aspects of resistance, diagnosis of resistance, the state of resistance in the field of Australia, principles involved in the selection of resistance and advice which might be given to minimize the development of resistance.

Hall *et al.* (1981) reported resistance to albendazole, fenbendazole, levamisole, morantel, naphthalophos and phenothiazine from a commercial goat herd in Australia.

Kettle *et al.* (1983) conducted a survey on anthelmintic resistance against gastrointestinal nematodes of goats in 47 milking goat herds located throughout New Zealand. Resistance to benzimidazoles alone occurred in 36 per cent of farms, resistance to cell membrane depolarising drench (CMD) alone on four per cent and resistance to both on 38 per cent of farms.

Out of 52 commercial flocks in South East England thiabendazole resistance was found on seven farms and in each case *H. contortus* was the only species of nematodes involved (Cawthorne and Cheong, 1984).

Kerboeuf and Hubert (1985) reported benzimidazole resistance in field strains of nematodes from goats in France.

Van Wyk and Malan (1988) detected resistance in five strains of *H.contortus* to benzimidazoles, rafoxanide and closantel in South Africa, out of which four showed varying degrees of resistance to ivermectin.

Echevarria and Frindale (1989) reported a reduced efficacy against *H. contortus* after regular use of ivermectin for four to five years in a flock in Brazil.

Craig *et al.* (1992) tried moxidectin against ivermectin resistant strain of *H. contortus* in Texas and the efficacy of moxidectin against the resistant strain was 99.9 per cent and 100 per cent at 0.2 mg per kg and 0.4 mg per kg respectively.

Benzimidazole resistance was detected by Hong *et al.* (1992) who collected faecal samples from 209 randomly selected sheep farms in Southern England and by Jackson *et al.* (1992) in Cashmere goat herds in Scotland.

Craig (1993) opined that drug rotation, especially rapid rotation for less than one year induced multiple drug resistance.

Le Jambre (1993) reported ivermectin resistant *Haemonchus* in Australia while Sivaraj *et al.* (1994) found resistance of *H. contortus* against benzimidazoles and ivermectin on a sheep farm in Malaysia.

Prichard (1994) also reported benzimidazole, morantel and ivermectin resistance in nematodes of sheep and goats.

Cabaret *et al.* (1995) detected benzimidazole resistance in *T. colubriformis, H. contortus* and *O. venulosum* along with Kochapakdee *et al.* (1995) who first reported the resistance of benzimidazoles in Thailand.

Mwamachi (1995) reported resistance of gastrointestinal nematodes of sheep and goats in Coastal Kenya to ivermectin.

Borgsteede *et al.* (1996) found out benzimidazole resistant nematodes in goats in Netherlands.

Out of 182 sheep farms located in Brazil, 90 per cent farms recorded resistance to albendazole, 84 per cent to levamisole and 73 per cent to the combined product. Thirteen per cent of the farms recorded resistance to ivermectin (Echevarria *et al.*, 1996).

Eddi *et al.* (1996) detected resistance against benzimidazole (40 per cent), levamisole (22 per cent), ivermectin (6 per cent) and combination of drugs (11 per cent) in 65 sheep farms in Argentina.

Maciel *et al.* (1996) conducted a survey in Paraguay in Southern Latin America and detected a high level of resistance to benzimidazoles, levamisole and ivermectin.

Maingi *et al.* (1996) investigated the occurrence of anthelmintic resistance in 15 goat herds in Denmark against benzimidazoles, levamisole and ivermectin. Nematodes were found resistant to all the drugs used.

Twenty four out of 42 farms in Kenya showed resistance to atleast one anthelmintic group. Resistance to levamisole was found in nine out of 35 sheep farms tested and 12 out of 24 goat farms. Resistance to benzimidazole drug was found in 10 out of 28 sheep farms and six out of 20 goat farms (Wanyangu *et al.*, 1996).

According to Boersema and Pandey (1997) who conducted a survey on commercial sheep farms in the highveld of Zimbambwe concluded that fenbendazole resistance prevailed in all the farms.

Borgsteede *et al.* (1997) studied on the benzimidazole resistance in cyathostomes in horses in Ukraine.

Farias *et al.* (1997) reported albendazole resistance in 24 out of 25 sheep farms of Southern Brazil.

Fernandez *et al.* (1997) were the first to report on benzimidazole resistant nematodes from ruminants in Spain.

Waruiru *et al.* (1998) recorded simultaneous resistance of *H. contortus* against benzimidazoles, levamisole and rafoxanide and *T. colubriformis* and *Oesophagostomum* spp against levamisole in a farm in Kenya.

According to Chandrawathani *et al.* (1999) resistance to benzimidazole group was high in sheep and goat farms in Peninsular Malaysia.

Gopal *et al.* (1999) confirmed ivermectin resistance in a field strain of *T. colubriformis* in Northland, New Zealand.

Le Jambre *et al.* (1999) opined that host genetics and vaccination induced immunity were likely to reduce selection for anthelmintic resistance provided that the frequency of drug treatments were also reduced.

Papadopoulos *et al.* (2001) pointed out that drought and isolation were likely to be the major factors accounting for the development of anthelmintic resistance in nematodes in the island flocks in Greece.

According to Kaplan (2002) resistance in the cyathostomes to benzimidazoles and pyrantel showed a tendency to increase in horses.

Silvestre *et al.* (2002) opined that the most efficient way to limit the increase of anthelmintic resistance was by reducing selection pressure by drugs and by optimal timing.

Taylor (2002) suggested that the right advice given in relation to the anthelmintic usage was the best way to control anthelmintic resistance.

A survey conducted by Bartley *et al.* (2003) revealed thiabendazole resistance in sheep flocks in Scotland.

Mejta *et al.* (2003) thought that multispecies and multidrug resistance in Argentina was probably due to the intensive use of anthelmintics, the absence of refugia and frequent circulation of infected cattle.

Sargison (2004) highlighted the problem of anthelmintic resistance associated with parasitic gastroenteritis which was commonly encountered in lambs.

2.3.2 India

Varshney and Singh (1976) reported anthelmintic resistance in *H. contortus* to phenothiazine and thiabendazole in sheep of the Central Sheep and Wool Research Station, India for the first time.

A survey conducted by Kumar and Yadav (1994) between March and September 1992 in 32 traditionally managed rural flocks and 22 intensively managed flocks on three farms, showed no resistance to benzimidazole in traditionally managed flocks, but of the 22 intensively managed flocks, 15 had slight resistance (between 60 and 90 per cent) and four had severe resistance (less than 60 per cent reduction).

Yadav *et al.* (1995) reported the presence of multiple resistance to benzimidazoles, levamisole and morantel in a strain of *H. contortus* in a sheep farm in Haryana.

Gill (1996) surveyed five sheep farms in Ludhiana and detected resistance to albendazole and levamisole in all of them. Samples were negative for any kind of egg after treatment with ivermectin.

Studies conducted at the Centre of Advanced Studies, Department of Parasitology, Bangalore, on anthelmintic resistance, indicated presence of resistance to some of the commonly used anthelmintics in ten sheep farms (Jagannath *et al.*, 2000).

Swarnkar *et al.* (2001) revealed the emergence of resistance to benzimidazole and rafoxanide and a potential risk of development of levamisole or tetramisole resistance in an organized sheep farm in semiarid areas of Rajasthan.

An overview of anthelmintic resistance in gastrointestinal nematodes of livestock from an Indian perspective, with future prospects for control and research have been laid down by Singh *et al.* (2002).

Dhanalakshmi *et al.* (2003) detected resistant strains of *Trichostrongylus* spp, *Oesophagostomum* spp and *Haemonchus* spp to fenbendazole, albendazole and rafoxanide in two sheep farms of Bangalore.

Jeyathilakan *et al.* (2003) confirmed resistance to fenbendazole and suspected resistance to tetramisole in nematodes of sheep in Tamil Nadu.

Radha *et al.* (2004) indicated the development of anthelmintic resistance to albenazole in sheep in Mevalorkuppam village located near Chennai.

Sanyal (2004) suggested that the phenomenon of refugia played a much more important role in the selection of anthelmintic resistance than other phenomenon that were more frequently investigated, and recommended for counteracting it by reduced drenching, frequency of dosing, avoiding underdosing etc.

Yadav and Garg (2004) concluded that *H.contortus* in sheep from Haryana showed resistance to rafoxanide, levamisole, fenbendazole and morantel.

2.3.3 Detection of Anthelmintic Resistance by Faecal Egg Count Reduction Test (FECRT) and Egg Hatch Test (EHT)

Le Jambre (1976) used Egg Hatch Assay (EHA) as a widespread application to detect benzimidazole resistance.

Varshney and Singh (1976) used FECRT and Critical anthelmintic Test to detect phenothiazine and benzimidazole resistance in Uttar Pradesh.

Cawthorne and Cheong (1984) detected thiabendazole resistance in South East England using EHA and the ED_{50} value ranged from 0.065 to 0.032µg thiabendazole per ml.

Kerboeuf and Hubert (1985) reported benzimidazole resistance in France using EHA. A concentration of about 1.28µg of thiabendazole per ml was necessary for a 50 per cent reduction of hatching as compared with control in distilled water.

Taylor and Hunt (1988) used EHA for detecting benzimidazole resistance in a commercial farm in South East England whereas Craig and Miller (1990) used FECRT for detecting resistance in *H.contortus* to ivermectin in Angora goats.

Borgsteede *et al.* (1991) detected increased resistance in *H.contortus* to benzimidazoles since 1983 and used EHA for detecting resistance. The same test was carried out by Hong *et al.* (1992) to detect resistance to benzimidazoles in Southern England. In addition, Jackson *et al.* (1992) used both FECRT and EHA to detect benzimidazole resistance in Scotland.

Coles *et al.* (1992) described the methods for detection of anthelmintic resistance in strongylid nematodes of ruminants, horses and pigs, wherein they had recommended FECRT and EHT as the simplest and most effective tests that could be used in parasitological laboratories.

Swan *et al.* (1994) confirmed the presence of ivermectin resistance using FECRT in Australia.

FECRT and EHA were applied by Cabaret *et al.* (1995) to detect benzimidazole resistance in three farms located in Central France.

Kochapakdee *et al.* (1995) and Maciel *et al.* (1996) used FECRT to detect anthelmintic resistance in Thailand and Paraguay respectively.

Miller and Craig (1996) confirmed the prevalence of resistance in gastro intestinal nematodes to ivermectin, fenbendazole, levamisole and albendazole in Angora goats using FECRT.

Maingi *et al.* (1996) investigated the occurrence of anthelmintic resistance in Denmark using FECRT and EHA, while Wanyangu *et al.* (1996) reported anthelmintic resistance in Kenya using FECRT only. Meanwhile Boersema and Pandey (1997) applied both FECRT and EHA to detect benzimidazole resistance in Zimbabwae.

Fernandez *et al.* (1997) confirmed benzimidazole resistant nematodes from goats in Spain with ED_{50} value in EHA as 0.22µg thiabendazole per ml.

Maingi *et al.* (1998) detected resistance to benzimidazoles and levamisole on sheep farms in the Nyandaura district of Kenya using FECRT, EHA and Larval Developmental Assay (LDA). They concluded that these tests could be used to detect resistance with good degree of agreement.

Swarnkar *et al.* (1999) used FECRT and EHA to detect anthelmintic resistance in sheep from Rajasthan and the LC_{50} value on EHA was 0.74 ± 0.015µg thiabendazole per ml.

Varady and Corba (1999) recommended the use of EHA and LDA to monitor anthelmintic resistance in field along with FECRT.

Uppal (2000) reported that the main advantage of FECRT was that it was quick to undertake and had relatively low cost, since there was no requirement for highly skilled personnel, expensive and sophisticated equipment and other facilities.

Coles (2003) emphasized on the tests to detect resistance to anthelmintics in ruminants, such as FECRT, EHT, PCR and Microagar larval development test and put forth strategies to minimize resistance in nematodes of cattle, sheep and goats.

Materials and Methods

3. MATERIALS AND METHODS

3.1 PREVALENCE OF GASTROINTESTINAL NEMATODES OF GOATS

Investigation on the prevalence of gastrointestinal nematodes of goats in the Thrissur Corporation area was carried out by recognizing faecal eggs and larvae after coprological examination and coproculture respectively. A total of 320 animals were screened at monthly intervals during the period from June 2004 to May 2005 for this purpose. Studies related to the effect of age, season, breed, sex and management on the prevalence of gastrointestinal nematodes were also carried out.

3.1.1 Coprological Examination

The faecal samples for coprological examination were collected from animals brought to the University Veterinary hospitals, Kokkalai and Mannuthy, University Goat and Sheep farm, and nearby houses in Mannuthy.

The examination of faeces for eggs was done by the routine concentration technique, *ie.,* centrifugation cum sedimentation technique.

3.1.2 Coproculture

The modified Veglia's method (Sathianesan and Peter, 1970) was followed for coproculture (Fig.1). A clean dry glass bottle measuring 14cm of height and 5.5cm diameter was used for this purpose. It was rinsed with one per cent sodium carbonate to inhibit fungal growth. The faecal samples found positive for eggs were mixed, or powdered well by trituration and transferred into a bottle without soiling the sides of the bottle. Sufficient water was added to provide optimum moisture to the faeces. If the faeces was watery, excess water was blotted off or dry sand was added to the mass and the bottle was closed and kept at room temperature. The bottle cap was just loosened at daily intervals to let out the injurious gas produced as a result of fermentation. The culture was

examined at regular intervals to note the development of infective larvae. The larvae developed moved upwards from the faecal pad and were collected in the water droplets formed on the sides of the bottle, by condensation of vapour from the faeces as a result of fermentation heat. The presence of larvae were detected microscopically when the larval colonies were large, on account of their wriggling movement.

3.1.3 Recovery of Infective Larvae from Faecal Culture

The culture bottle containing the larvae was kept horizontally over a table and a small quantity of water was added. The bottle was then rolled over the table gently, in order to recover all the larvae in water. Care was taken to avoid the water touching the faecal pad. The water containing the larvae was then pipetted out into a cavity dish for identification.

3.1.4 Identification of Infective Larvae

The larvae were transferred to a glass slide and immobilized by gentle heating. They were then sealed with molten paraffin and examined microscopically. Larval species were identified by morphometry.

3.2 DETECTION OF ANTHELMINTIC RESISTANCE

Resistance to various anthelmintics by the nematodes in goats were detected by the methods namely Faecal Egg Count Reduction Test (FECRT) and Egg Hatch Test (EHT). These were done according to the methods described by Coles *et al.* (1992).

3.2.1 FECRT

Kids (3-6 months) maintained at the University Goat and Sheep Farm, Mannuthy, were screened for parasitic ova. Animals which were positive for nematode eggs were divided into four groups A, B, C and D of ten

each. Egg counts of all the 40 positive animals were taken and recorded as the pretreatment EPG. Group A was administered albendazole @ 7.5 mg per kg body weight orally, group B with ivermectin @ 0.2mg per kg body weight orally, group C was given morantel citrate @ 10mg per kg body weight orally, and group D remained as non medicated control. All the animals were housed and reared under similar conditions. Egg counts of the goats belonging to the four groups were recorded after 14 days as the post treatment EPG. All the data were then applied in the computer programme, for FECRT analysis, and the results recorded.

3.2.2 EHT

This test is for the detection of benzimidazole resistance. The procedure adopted was according to Coles *et al.* (1992) with some modifications. Faecal samples from 10 randomly selected kids aged six months maintained in the University Goat and Sheep Farm, were collected and brought to the laboratory within three hours.

3.2.2.1 Preparation of Eggs

1. Homogenised the faecal samples with a stirrer by placing the faeces in a measuring cylinder with 200 ml water, until all the pellets were broken.
2. Sieved and poured the filtrate into centrifuge tubes.
3. Centrifuged for two minutes at 3000 rpm and gently poured off the supernatant.
4. Agitated the tubes to loosen the sediment and then added saturated sodium chloride solution until a meniscus formed above the tube. Applied a cover slip and recentrifuged for two min at 1000 rpm.

5. Carefully removed the coverslips from the tubes and washed off the eggs into a conical centrifuge tube. Filled with water and centrifuged for two min again at 3000 rpm.

6. Removed the water, resuspended the eggs in water, estimated the number of eggs per milliliter and diluted to the required concentration.

3.2.2.2 Test Procedure

1. Placed two ml of fresh eggs containing about 25 eggs per ml from each of the 10 samples in each well of a 24 multiwell plate (Medox agencies, Chennai)

2. Added 10µl of serial concentrations of albendazole such as 0.05µg, 0.1µg, 0.15µg, 0.18µg and 0.20µg in aqueous hydrochloric acid to each well to find out the optimum dilution required to prevent 50 per cent of the visible eggs hatching (ED_{50}). Control wells received only solvent.

3. Incubated at 27 °C for 48 hours.

4. Counted the eggs and hatched out larvae at each anthelmintic concentration of each sample microscopically, after adding two drops of Lugol's iodine to stop further hatching. The mean per cent hatch inhibition was calculated from the mean per cent hatch of the respective albendazole concentration as per the formula given below.

   Mean % hatch inhibition =100-(Mean % hatch (respective albendazole concentration)/ (Mean % hatch (respective water control)x100

   The per cent of eggs failing to hatch at each concentration of albendazole were transferred to obtain probit values (Finney, 1971) from which the ED_{50} values were obtained using "Trend" in statistical analysis (Bauer, 2005). Animals in which the ED_{50} value was in excess of 0.1µg albendazole were considered to be carrying benzimidazole resistant strains of nematodes.

Results

4. RESULTS

4.1 PREVALENCE OF GASTROINTESTINAL NEMATODES OF GOATS

4.1.1 Coprological Examination

Screening of faecal samples of 320 goats during the period of study revealed 114 as positive (35.63 per cent). Gastrointestinal nematodes noted were *Strongyle* (95.61 per cent) and *Strongyloides* (4.38 per cent). The results are furnished in Table1. Monthwise prevalence of gastrointestinal nematodes showed maximum infection in May (48 per cent) while a lower prevalence in August (28 per cent). The monthwise prevalence is presented in Table 2 and depicted in Fig 2.

Seasonwise, the prevalence of gastrointestinal nematodes was found to be 30.77 per cent, 33.33 per cent and 39.13 percent during cold wet South West monsoon (heavy rainfall), warm wet North East monsoon (low rainfall) and dry season respectively (Table 3, Fig. 3).

The prevalence of gastrointestinal nematodes was observed to be higher in young goats below one year (42.10 per cent) and in females (85.96 per cent) than males (14.03 per cent) (Table 4, Fig. 4).

There was no significant variation between breeds and management since all the samples collected for this study were from crossbreds and houses respectively.

4.1.2 Coproculture

The prevalence of different species of *Strongyles* and *Strongyloides* in goats was studied by faecal culture. The results are presented in Table 5 and their specific identification is dealt with in the following pages.

The number of faecal cultures examined were 114. The species of nematodes encountered were *Haemonchus contortus* (55.26 per cent), *Oesophagostomum columbianum* (13.15 per cent), *Trichostrongylus colubriformis* (21.05 per cent), *Bunostomum trigonocephalum* (6.14 per cent) and *Strongyloides papillosus* (4.38 per cent).

4.1.3 Morphometry

The comparative biometry of various infective larvae of the commonly found nematodes are shown in Table 6.

4.1.3.1 Infective Larvae of Haemonchus contortus

It was easily identified by the presence of a sharp kink in the tail sheath just posterior to the end of tail proper. The larvae was of medium length with a gradually tapering head and measured 650 to 804 µm in length and 19 to 24 µm in width. The oesophagus was 40 to 55 µm long. The nerve ring and excretory pore were situated at 45.9 to 51.5 µm and 45.5 to 54.25 µm respectively from the anterior end. The intestinal cells were 16 in number. The length and width of the intestinal cells were 40 to 45 and 7 to 8.5 µm respectively. The genital primordium was located at 364.5 to 430 µm and anus at 650 to 720µm from the anterior end. The tail of the larvae proper was 38.25 to 55 µm long and tail sheath was 44.73 to 69.975 µm long (Plate 1.A, B & C)

4.1.3.2 Infective Larvae of Oesophagostomum columbianum

The intestinal cells were clearly demarcated, which was triangular in shape. The larvae measured 750 to 804 µm in length and 30 to 42 µm in width. The oesophagus was 145.35 to 172.125 µm long. The nerve ring and excretory pore were situated at 84.15 to 94.50 µm and 109.395 to 135.4 µm respectively from the anterior end. The intestinal cells were 16 in number. The length and width of the intestinal cells were 30.9 to 42.45 and 9.18 to 16.06 µm respectively. The genital primordium was located at 554.6 to 612 µm and anus at 625 to 645 µm from the

anterior end. The tail of the larvae proper was 70.38 to 80.325 µm long and tail sheath was 138.84 to 154.91 µm long (Plate 2.A, B & C).

4.1.3.3 Infective Larvae of Trichostrongylus colubriformis

The characteristic feature of this larvae was that it had two tubercles at the end of the tail. The tail sheath was short and stumpy. The larvae measured 624 to 699 µm in length and 21 to 30 µm in width. The oesophagus was 135 to 150 µm long. The nerve ring and excretory pore were situated at 85.29 to 94.47 µm and 97.155 to 113.22 µm respectively from the anterior end. The intestinal cells were 16 in number. The length and width of the intestinal cells were 41.6 to 45.135 and 9.56 to 13.38 µm respectively. The genital primordium was located at 344.42 to 367.2 µm and anus at 559.98 to 585.99 µm from the anterior end. The tail of the larvae proper was 50 to 62 µm long and tail sheath was 28.6 to 31.74 µm long (Plate 3.A, B & C).

4.1.3.4 Infective Larvae of Bunostomum trigonocephalum

It could be easily identified by its small size. The buccal cavity was very small. It measured 460 to 630 µm in length and 20 to 24 µm in width. The oesophagus was 13.5 to 147 µm long. The nerve ring and excretory pore were situated at 73.825 to 80.325 µm and 84.15 to 95.625 µm respectively from the anterior end. The intestinal cells were 16 in number. The length and width of the intestinal cells were 22.5 to 30 and 6.885 to 7.65 µm respectively. The genital primordium was located at 252.45 to 263.925 µm and anus at 330 to 35 µm from the anterior end. The tail of the larvae proper was 49 to 52 µm long and tail sheath was 80 to 94 µm long (Plate 4.A, B & C).

4.1.3.5 Infective Larvae of Strongyloides papillosus

It could be easily identified by the absence of sheath. The tail tip was forked and oesophagus long. It measured 549 to 555 µm in length and 13.5 to 22.5 µm in width. The oesophagus was 240 to 260 µm long. The nerve ring and excretory pore were situated at 76.5 to 80.32 µm and 80.325 to 87.975 µm

respectively from the anterior end. The intestinal cells were 16 in number. The length and width of the intestinal cells were 24.8 to 28.6 and 4.975 to 5.735 µm respectively. The genital primordium was located at 246.35 to 294 µm and anus at 516 to 531.6 µm from the anterior end. The tail of the larvae proper was 80.325 to 84.15 µm long (Plate 5.A, B & C).

4.2 ANTHELMINTIC RESISTANCE

4.2.1 Faecal Egg Count Reduction Test

Anthelmintics used in the study were albendazole, ivermectin and morantel citrate. Albendazole was the most frequently used drug in the University Goat and Sheep farm. Although, ivermectin injection was quite frequently in use, the oral preparation was introduced for the first time. Morantel citrate was occasionally used for treating goats. The present study revealed resistance to albendazole, ivermectin and morantel citrate in the gastrointestinal nematodes.

The results of the study are included in table 7.

Albendazole showed a percent worm reduction of 30 with 52 as lower 95 percent confidence limit.

Ivermectin revealed a per cent worm reduction of 53 and 34 as the lower 95 per cent confidence limit.

Morantel also revealed a percent worm reduction of 45 and 62 as the lower 95 percent confidence limit.

Though the efficacies of albendazole, ivermectin and morantel citrate were 47.3 percent, 46.1 percent and 50.7 percent respectively, the lower confidence limit attained in all the three drug trials was less than 90 percent, which indicated the development of anthelmintic resistance to all the three drugs in the present study.

4.2.2 Egg Hatch Test

The results of EHT are furnished in Table 8 and Fig.5. The ED_{50} value of albendazole (µg per ml) was found to be 0.211556 which exceeded the prescribed value of 0.1µg per ml of thiabendazole. Hence it was established in the present work, that the gastrointestinal nematodes were resistant to the drug albendazole.

Table 1. Results of coprological examination for eggs

Total examined	Total positive	Type of infection	Number positive	Per cent positive
320	114	Strongyle	109	95.61
		Strongyloides	5	4.38

Table 2. Month-wise prevalence of gastrointestinal nematodes in goats

Month	Number examined	Number positive	Per cent positive
June 2004	25	8	32
July 2004	28	9	32.14
August 2004	25	7	28
September 2004	29	10	34.48
October 2004	27	9	33.33
November 2004	25	8	32
December 2004	26	11	42.31
January 2005	29	13	44.83
February 2005	25	9	36
March 2005	28	9	32.14
April 2005	28	9	32.14
May 2005	25	12	48
Total	320	114	35.63

Table 3. Season wise prevalence of gastrointestinal nematodes

Season	Month	No. of samples examined	No. positive	Per cent
Cold-wet South west monsoon (heavy rainfall)	June to August	78	24	30.77
Warm wet North East monsoon (low rainfall)	September to November	81	27	33.33
Dry	December to May	161	63	39.13

Table 4. Sex wise and age wise prevalence of gastrointestinal nematodes

Age	No. of males positive	Per cent positive	No. of females positive	Per cent positive	Total number	Per cent
Below one year	13	27.08	35	72.9	48	42.10
One to two year	3	12	22	88	25	21.92
Above two year	-	-	41	100	41	35.96
Total	6	14.03	98	85.96	114	35.63

Table 5. Results of coproculture for nematode larvae

Type of larvae	Number positive	Per cent positive
Haemonchus contortus	63	55.26
Oesophagostomum columbianum	15	13.15
Trichostrongylus colubriformis	24	21.05
Bunostomum trigonocephalum	7	6.14
Strongyloides papillosus	5	4.38
TOTAL	114	35.63

Table 6. Comparative biometry of infective larvae of common nematodes of goats

Sl. No.	Infective larvae	Body		Oesophagus	Intestinal cell			Distance from anterior end				Tail (μ)	Tail sheath (μ)
		Length (μ)	Width (μ)	Length (μ)	No.	Length (μ)	Width (μ)	Nerve ring (μ)	Excretory pore (μ)	Genital primordium (μ)	Anus (μ)		
1.	*Haemonchus contortus*	650-804	19-24	81-50	16	40-55	7-8.5	45.9-51.25	45.5-54.25	364.5-430	650-720	38.25-55	44.75-69.975
a.		650-820	20-30	119-166	16	45-53	5-10	86-92	105-113	305-405	501-679	24-40	52-78
b.		675-810	21-25	135-157.5	16	45-	3.5-7	86-92	105-110	320-395	540-650	51-65	68-78
2.	*Oesophagostomum columbianum*	750-804	30-42	145.35-172.125	16	30.9-42.45	9.18-16.06	84.15-94.50	109.395-135.4	554.6-612	625	70.38-80.325	138.84-154.91
a.		715-840	30-32	140-170	24	30-35	10-12	75-95	92-110	370-422	595-615	70-90	137-153
b.		780-830	29.5-34	147-165	16-20	32-42	10.5-12	80-92	92-112	372-410	565-620	73-85	135-137
3.	*Trichostrongylus colubriformis*	624-699	21-30	135-150	16	41.6-45.135	9.56-13.38	85.29-94.47	97.155-113.22	344.2-367.2	559.98-585.99	53.93-55.84	28.6-31.74
a.		560-780	20-30	130-170	16	45-60	8-13	80-114	90-1222	325-400	559-680	50-62	25-40
b.		640-765	21-27	135-162	16	48-57	6.5-11.5	87-108	95-115	315-392	557.5-660	58-78	27-37
4.	*Bunostomum trigonocephalum*	450-549	22.5 27	130.5-147	16	22.5-30	6.885-7.65	73.825-80.325	84.15-95.625	252.45-263.925	330-350	49-52	80-94
a.		476-664	20-30	130- 170	16	30-32	7-10	74-87	82-105	250-300	341-550	50-67	80-102
b.		484-652	24.5-30	135- 160	16	23-35	5-9	76-85	84 -105	256-296	352-530	52.5-65	
5.	*Strongyloides papillosus*	549-555	13.5-22.5	260-240	16	24.8-28.6	4.975-5.735	76.5-80.32	80.325-87.975	246.35-294	516-531.6	80.325-84.15	Nil
a.		550-640	17-22	220-260	24	--	--	75-80	85-90	290-327	556-610	77-97	Nil
b.		570-630	17.5-21	225-256	16-24	25-35	5-8	76-80	87-94.5	290-320	520-580	80-98.5	Nil

a. Sathianesan (1968) b. Jeyathilakan (1995)

Table 7. Anthelmintic resistance by Faecal Egg Count Reduction Test

Anthelmintic	Day zero (pre treatment EPG)	Day 14 (post treatment EPG)	Per cent reduction	Lower 95 per cent confidence limit	Upper 95 per cent confidence limit	Result
Albendazole	323	170	30	52	-2	Resistant
Ivermectin	210	113	53	34	07	Resistant
Morantel citrate	270	133	45	62	20	Resistant
Control	210	243	--	--	--	--

Table 8 Anthelmintic resistance by Egg Hatch Test

Animal No.	Water control			Albendazole concentrations								
				0. 05			0.1			0.15		
	Number of eggs	Number of larvae	% hatch	Number of eggs	Number of larvae	% hatch	Number of eggs	Number of larvae	% hatch	Number of eggs	Number of larvae	% hatch
1	10	40	80	30	20	40	27	23	46	25	25	50
2	15	35	70	20	30	60	28	22	44	31	19	38
3	23	32	64	23	27	46	29	21	42	32	18	36
4	10	40	80	19	31	62	22	28	56	25	25	50
5	12	38	76	11	39	78	22	28	56	26	24	48
6	16	34	68	10	40	80	12	38	76	21	29	58
7	11	39	78	7	43	86	11	39	78	12	38	76
8	22	33	66	10	40	80	12	38	76	13	37	74
9	12	38	76	10	40	80	11	39	78	12	38	76
10	15	35	70	6	44	88	11	39	78	15	35	70

	Albendazole concentrations									
	0.18			0.20			Mean hatch		Mean hatch inhibition	Probit value
Animal No.	Number of eggs	Number of larvae	% hatch	Number of eggs	Number of larvae	% hatch				
1	35	15	30	40	10	20	Water	72.8		
2	35	15	30	40	10	20	0.05	70	3.9	3.2376
3	33	17	34	41	9	18	0.1	63	13.46	3.8969
4	28	22	44	21	19	38	0.15	57.6	20.88	4.1901
5	25	25	50	25	25	50	0.18	43	40.93	4.7699
6	26	24	48	29	21	42	0.20	42	42.31	4.8058
7	21	29	58	22	28	56				
8	15	35	70	20	30	60				
9	15	35	70	21	29	58				
10	17	33	66	21	29	58				

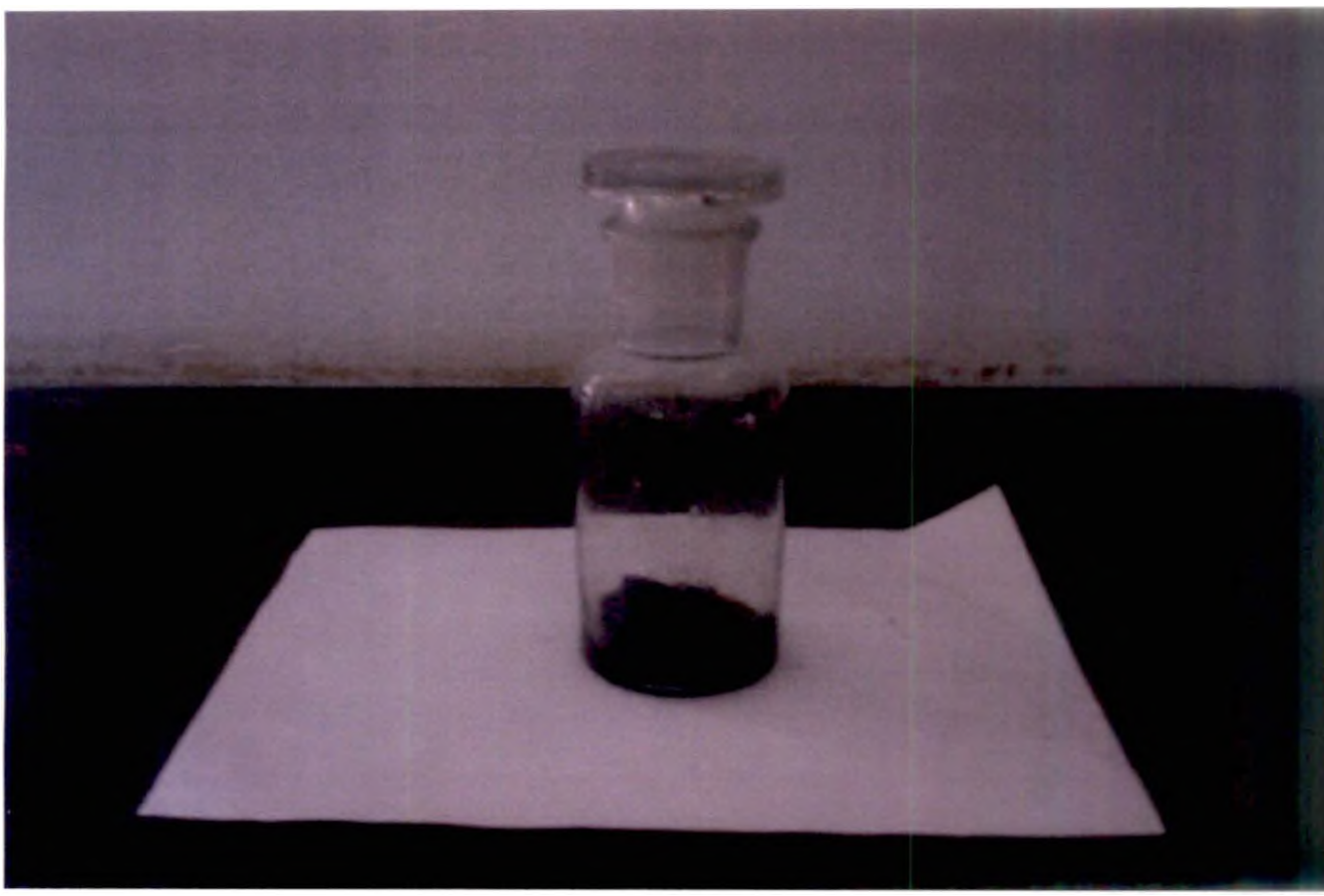


Fig. 1. Faecal culture

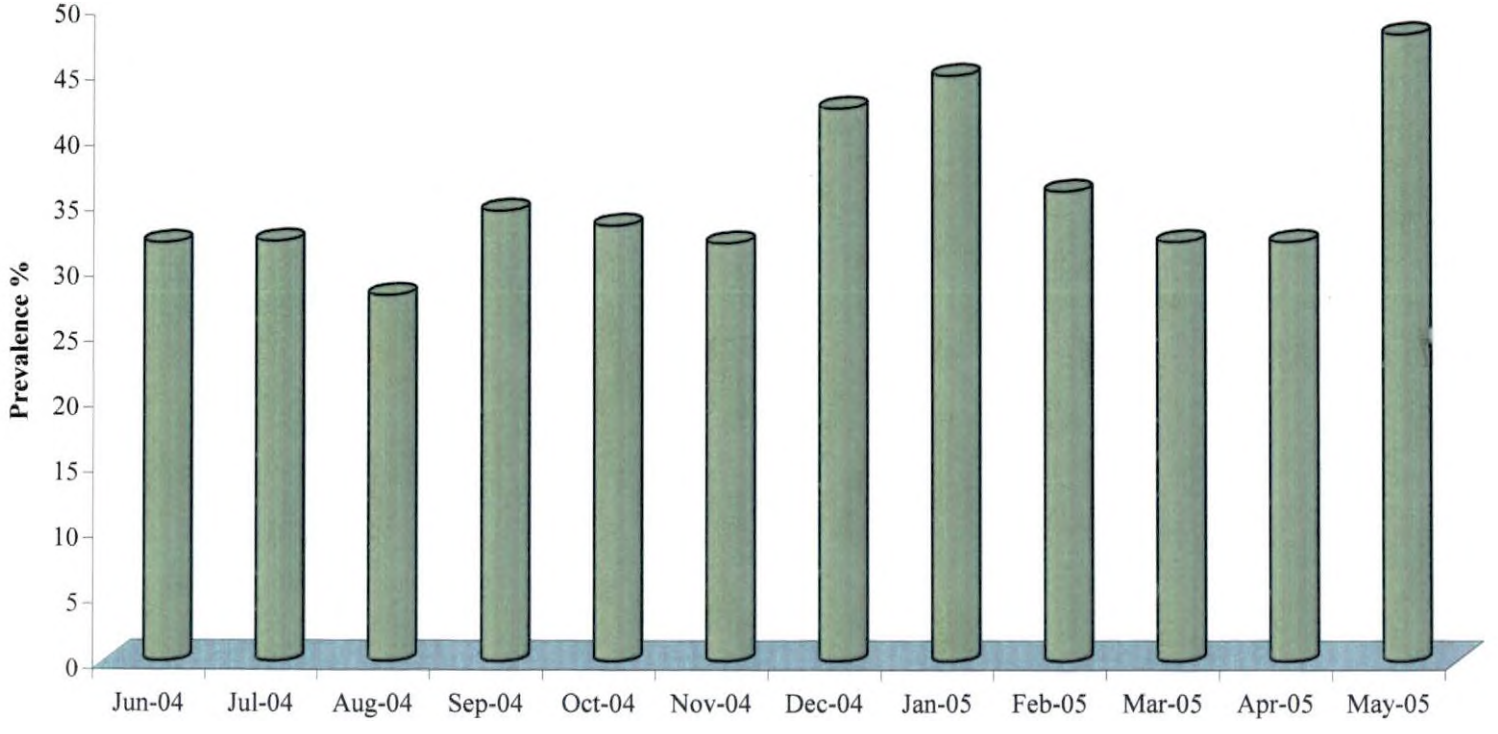


Fig. 2. Monthwise prevalence of gastrointestinal nematodes in goats

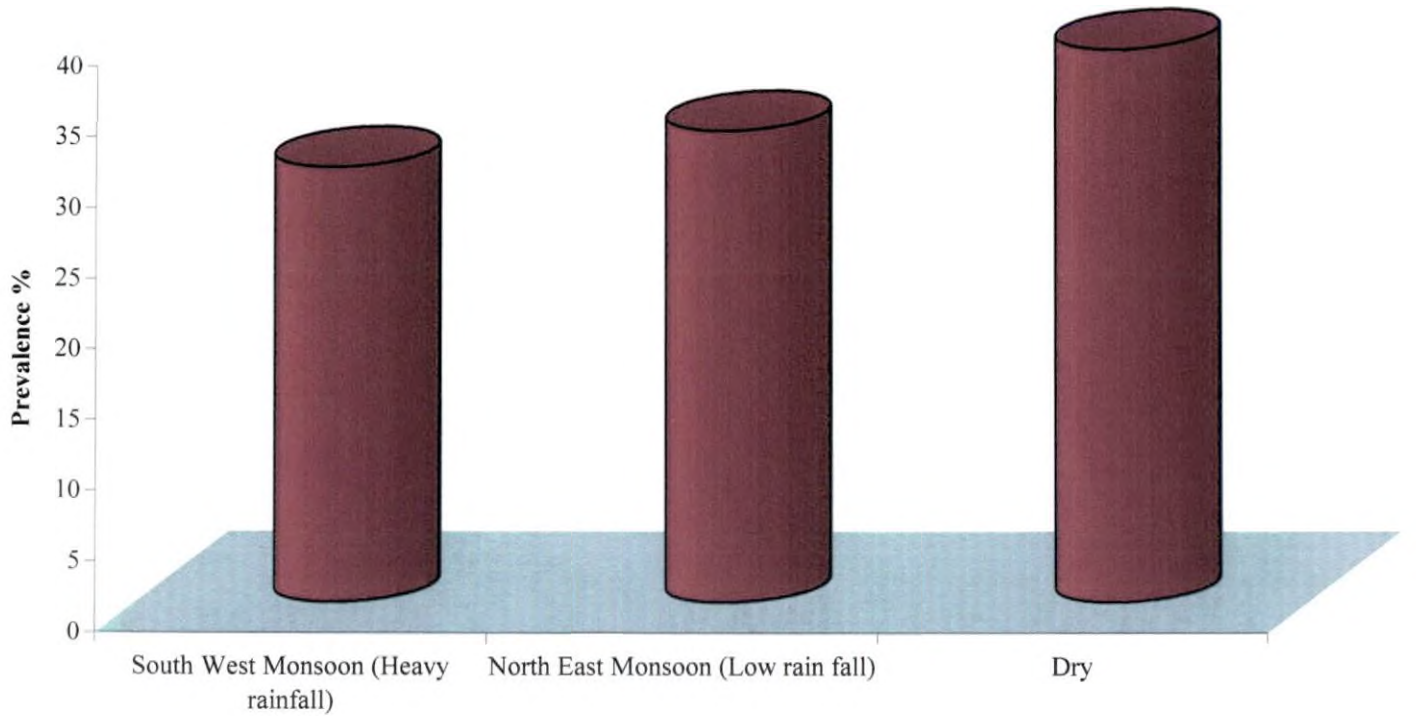


Fig. 3. Seasonwise prevalence of gastrointestinal nematodes in goats

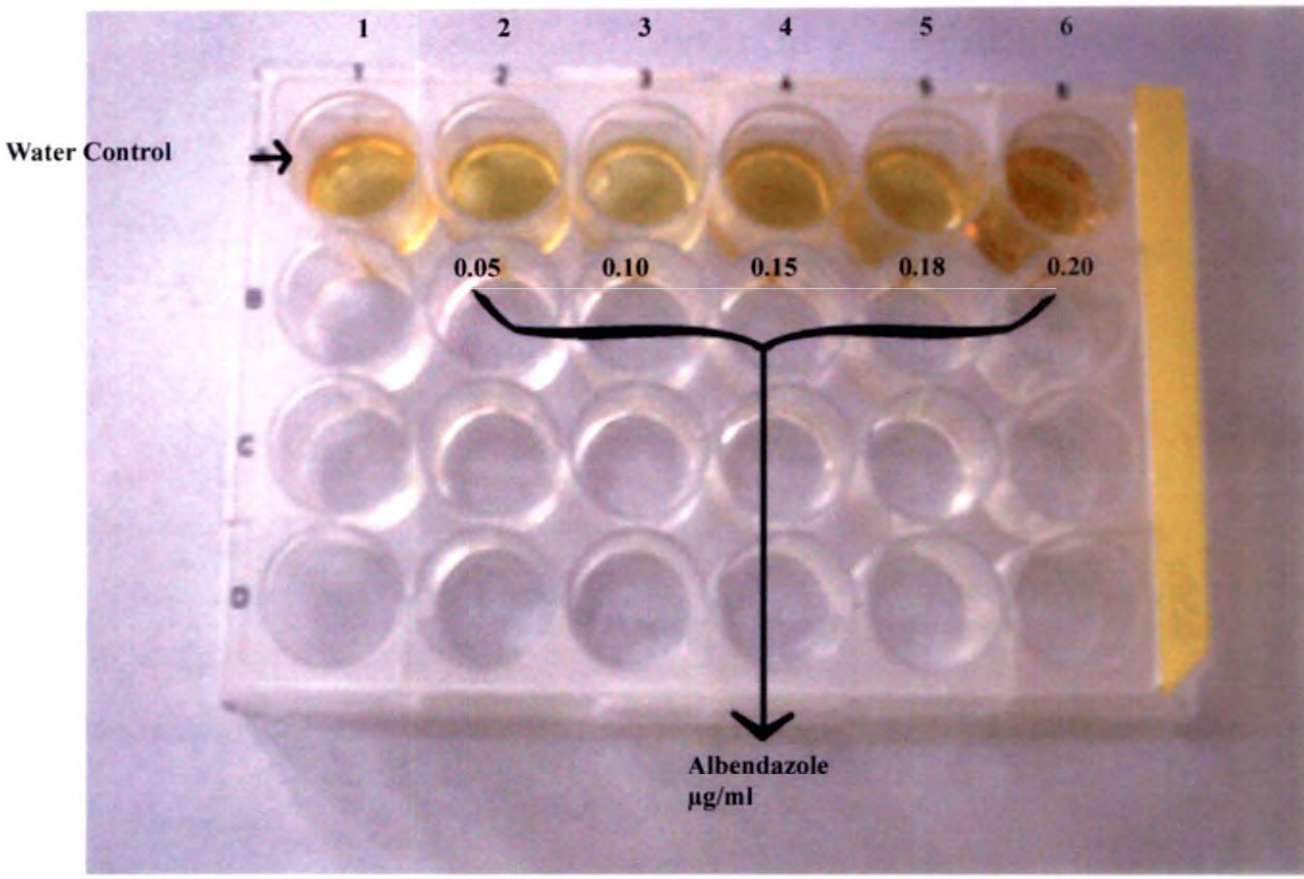


Fig. 5. Egg Hatch Test

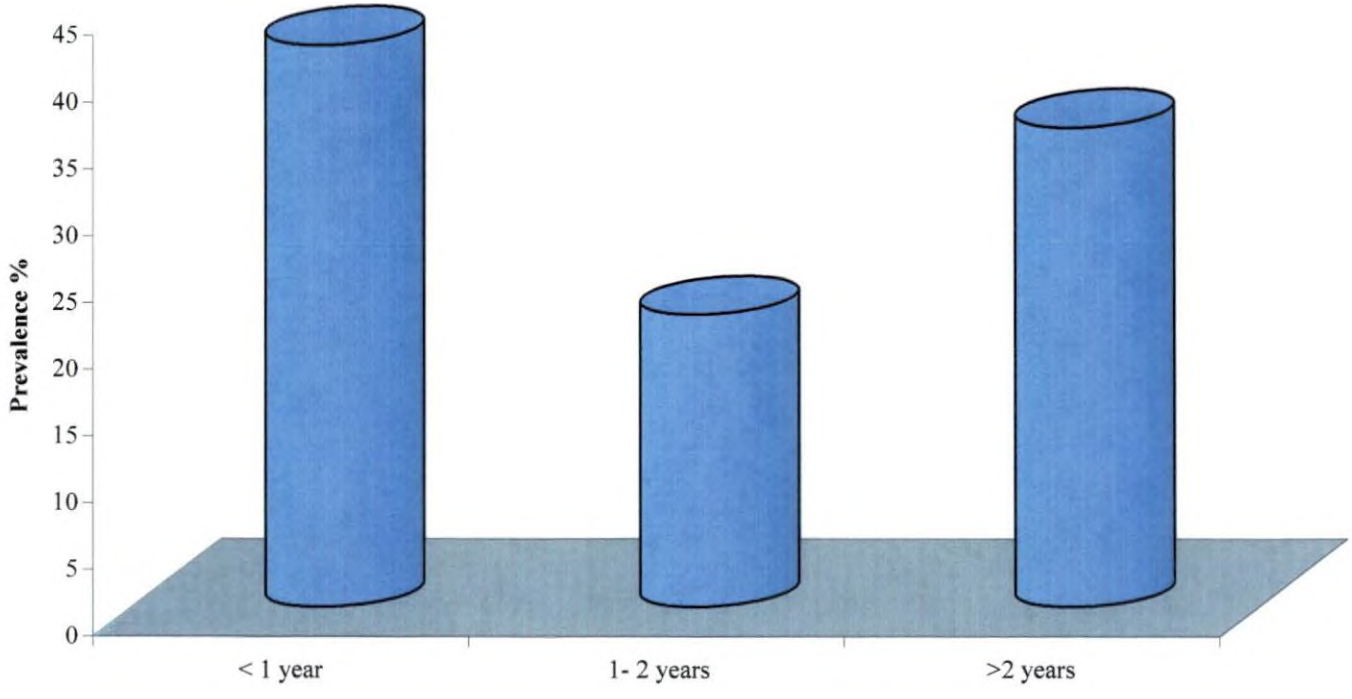


Fig. 4. Agewise prevalence of gastrointestinal nematodes in goats

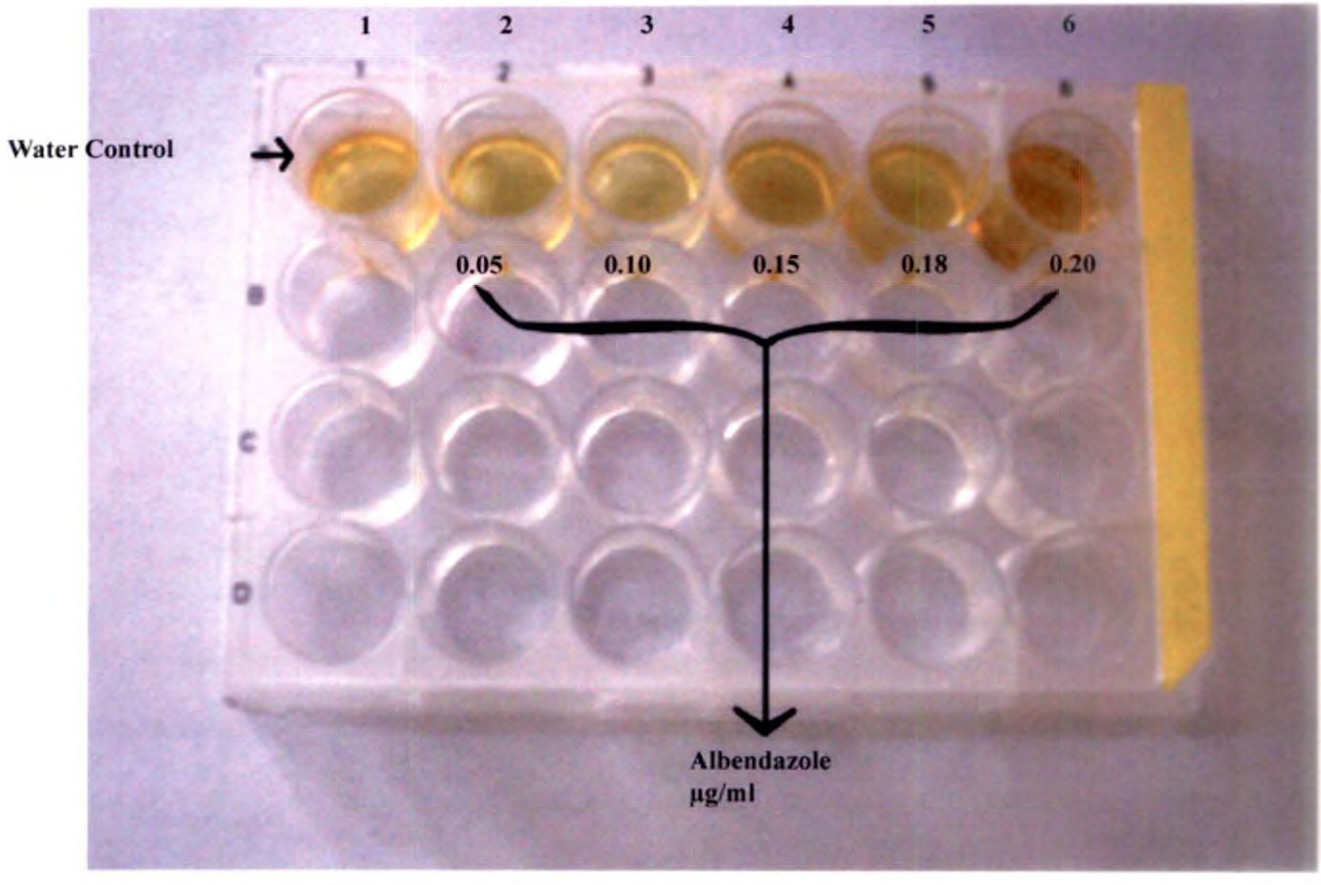
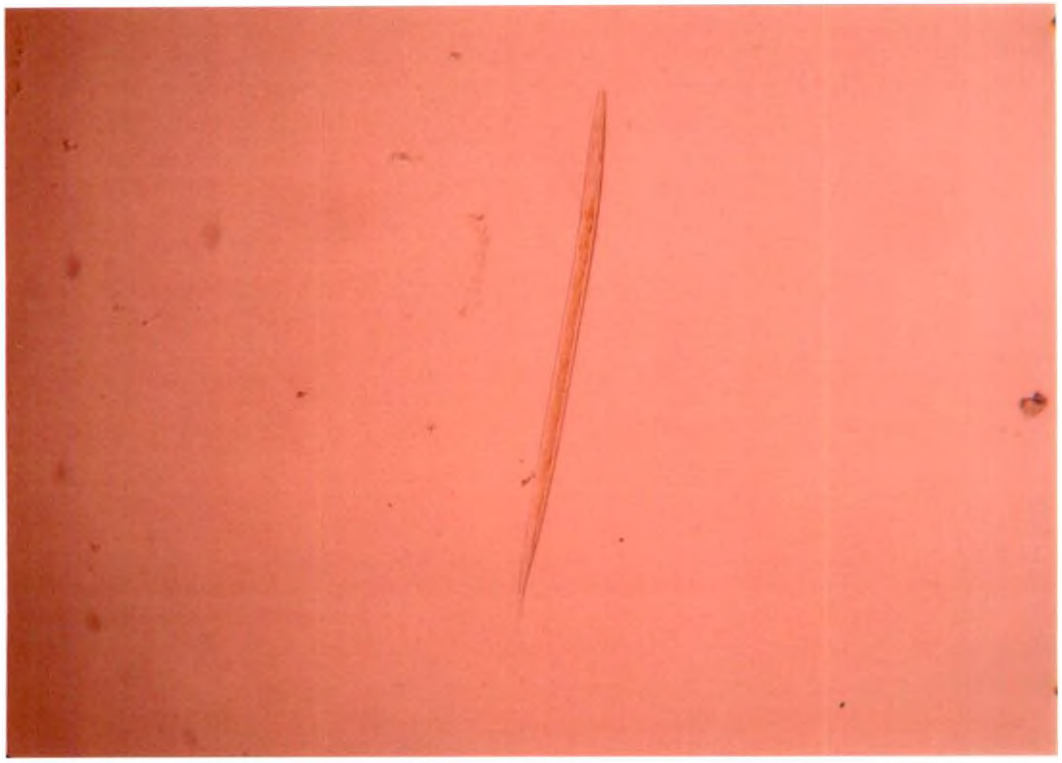
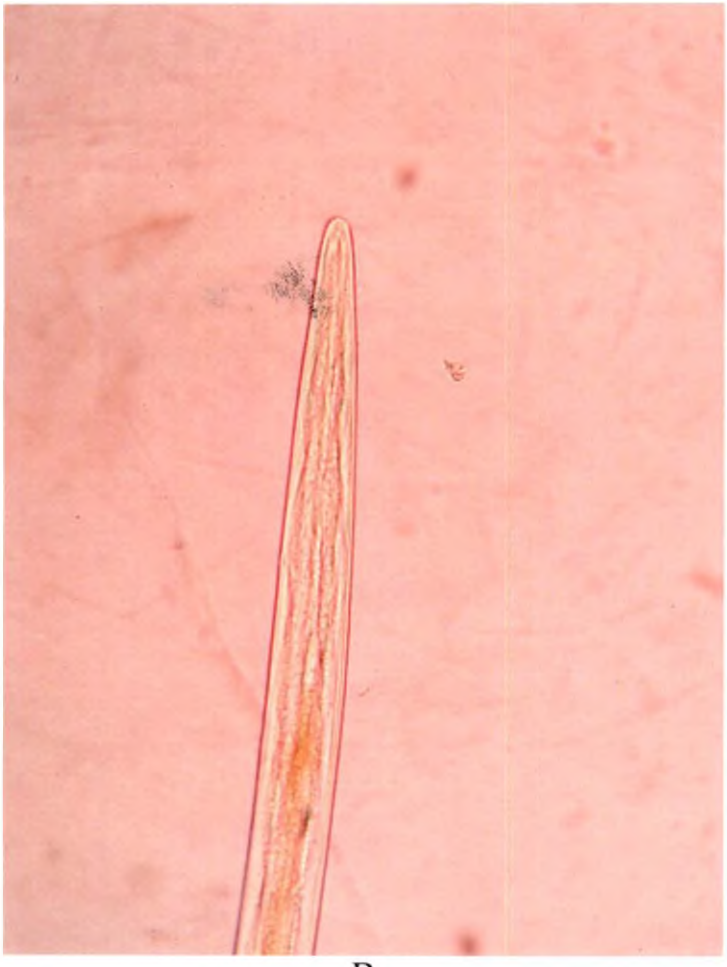


Fig. 5. Egg Hatch Test



A



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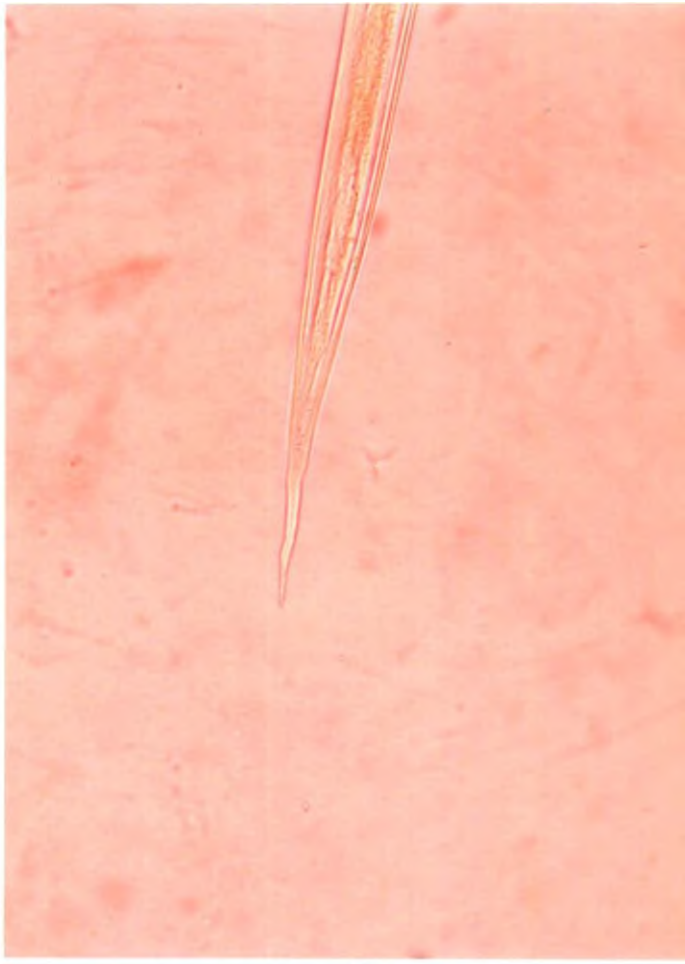


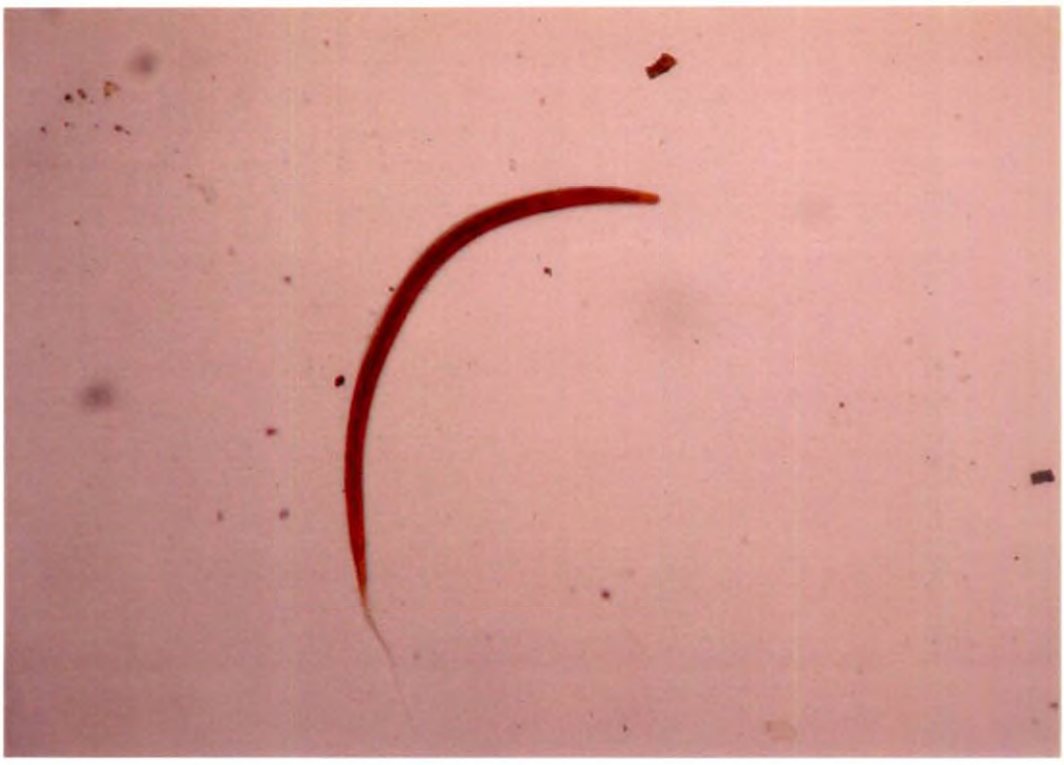
C

Plate 1. ***Haemonchus contortus***

A. Infective larva (x 100)

B. Head end (x 400)

C. Tail end (x 400)



A

B

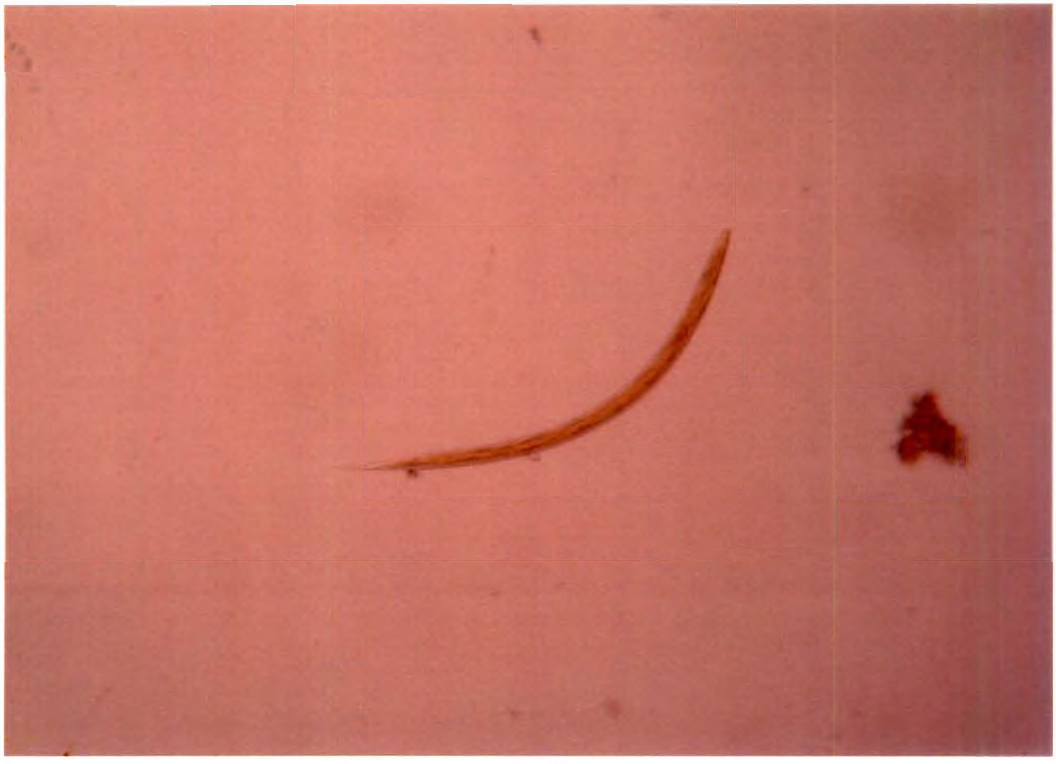
C

Plate 2. ***Oesophagostomum columbianum***

A. Infective larva (x100)

B. Head end (x 400)

C. Tail end (x400)



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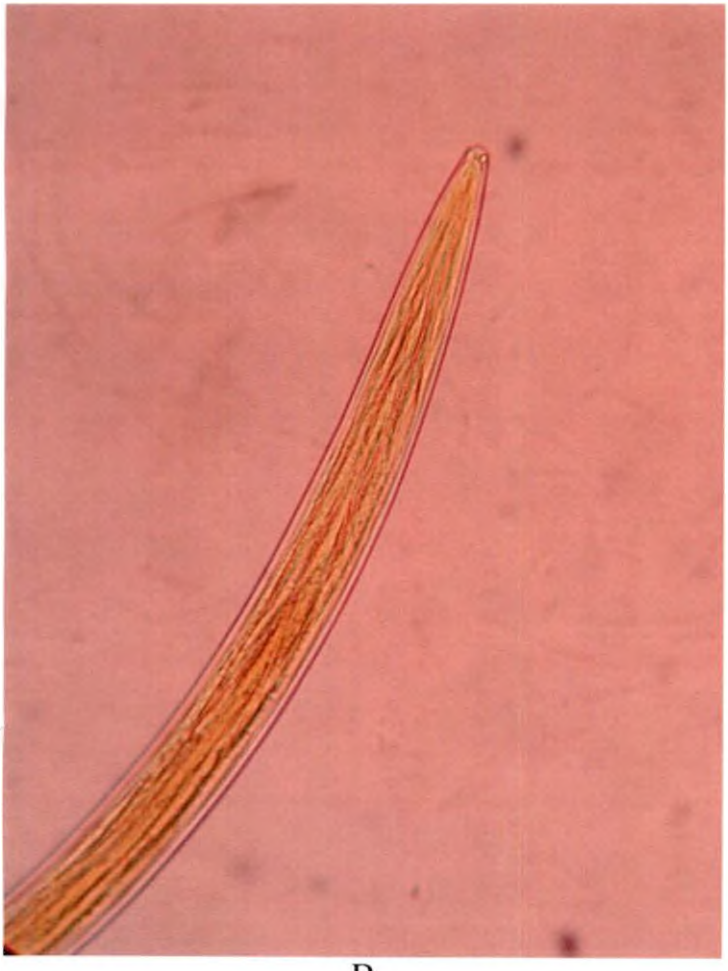
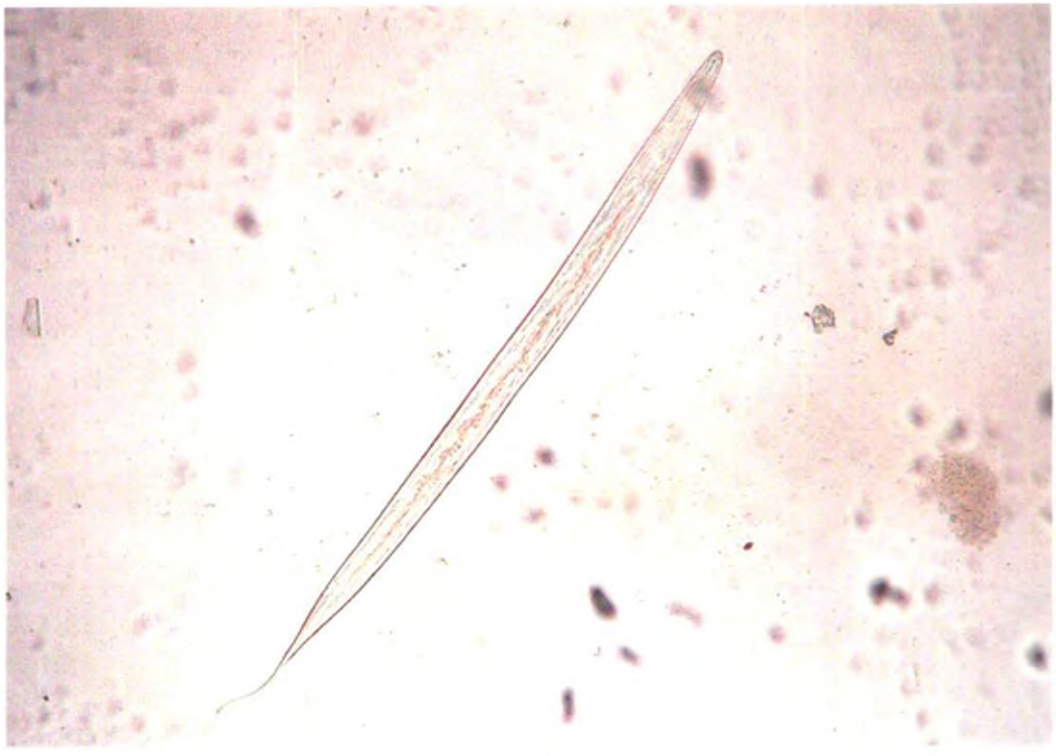


B

C

Plate 3. ***Trichostrongylus colubriformis***
A. Infective larva (x100)
B. Head end (x 400)
C. Tail end (x 400)



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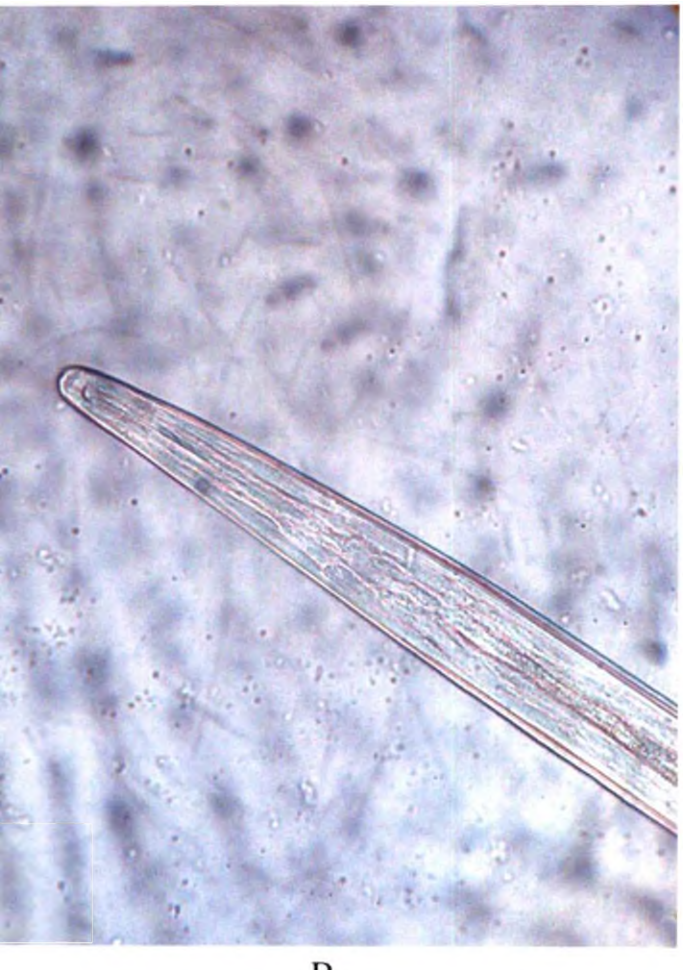
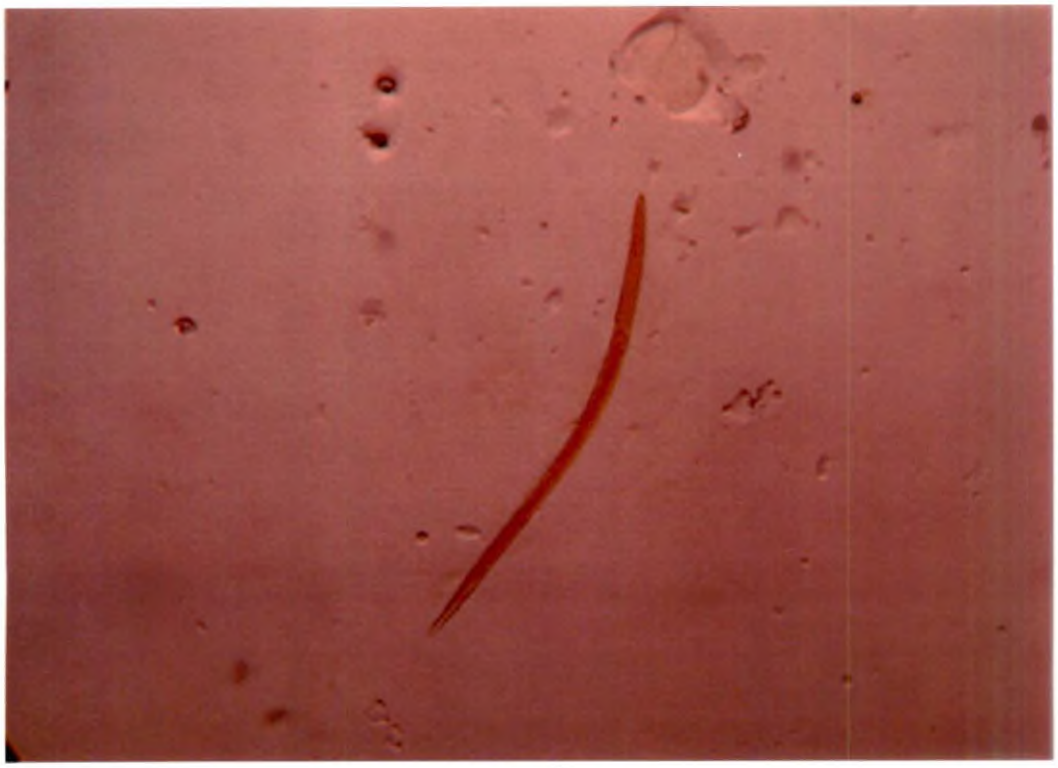


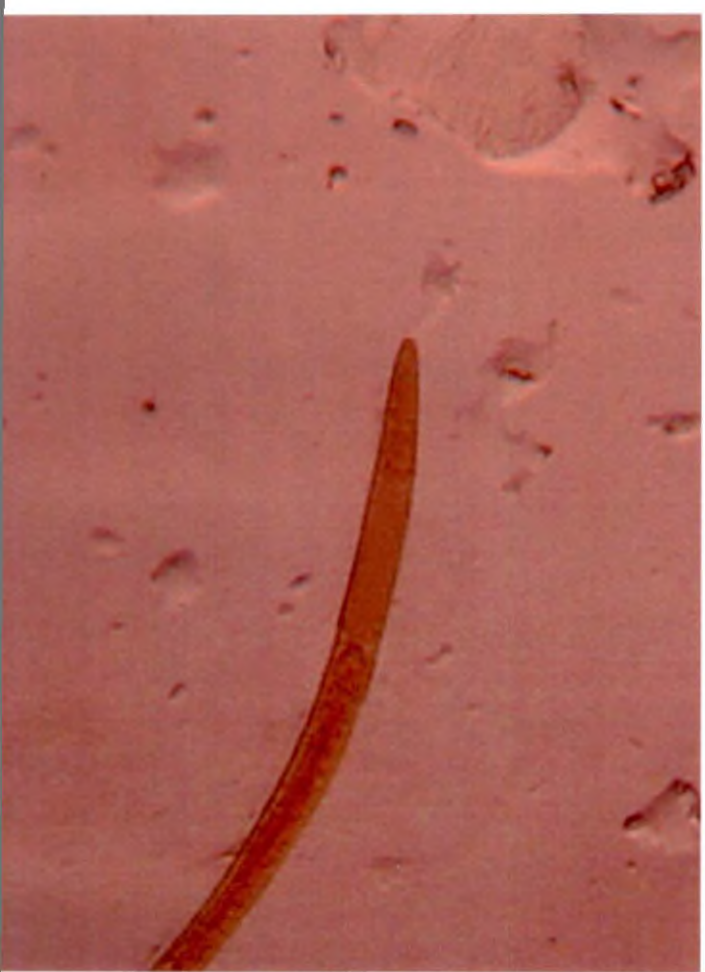
B

C

Plate 4. ***Bunostomum trigonocephalum***
A. Infective larva (x100)
B. Head end (x 400)
C. Tail end (x 400)



A



B

C

Plate 5. ***Strongyloides papillosus***
A. Infective larva (x100)
B. Head end (x 400)
C. Tail end (x 400)

Discussion

5. DISCUSSION

5.1 PREVALENCE OF GASTROINTESTINAL NEMATODES OF GOATS

5.1.1 Coprological Examination

The prevalence of gastrointestinal nematodes of goats was determined by coproexamination for eggs and by faecal culture for larvae.

The prevalence of *Strongyles* was found to be higher(95.61 per cent)in the present study. This finding was almost comparable with those of Dhanalakshmi *et al.* (2001) and Khajuria and Kapoor (2003) who recorded higher prevalence of *Strongyles* in goats from Karnataka and Jammu and Kashmir respectively. Monthwise prevalence of gastrointestinal nematodes showed maximum infection in May (48 per cent) and season wise during dry season *ie* from December to May (39.13 per cent). This was in accordance with that of Charies (1989) and Muraleedharan *et al.* (1990) who reported the higher incidence of nematode infection in goats in early dry season from Brazil and in cattle in hot season from Karnataka. The prevalence of gastrointestinal nematodes was found to be higher in kids (below one year). This may be due to reduced immunity to *Strongyles* in young ones (Soulsby, 1982). Females were more infected than males which concurred with that of Valcarcel and CRomero (1999) from Spain who also reported severe infection in females.

The prevalence of *Haemonchus contortus* was found to be the highest (54.8 per cent) in the present study as reported by Sathianesan and Peter (1971), Jeyathilakan (1995), Dhanalakshmi *et al.* (2001) and Garg *et al.* (2003).

The other species prevalent in goats in Thrissur Corporation area included *Trichostrongylus* spp, *Bunostomum* spp, *Oesophagostomum* spp and *Strongyloides* spp. These species were also reported by Jeyathilakan (1995) in goats in Trichur.

5.1.2 Identification of Infective Larvae

Five species of larvae were identified in the present study, although Sathianesan and Peter (1976) and Jeyathilakan (1995) were able to identify more number. The details of morphology and measurements of the larvae recorded were more or less similar to those identified and speciated by the previous workers namely Sathianesan (1968), Padmavathi *et al.* (1971), Jeyathilakan (1995) and Bowman (1995).

5.2 Anthelmintic Resistance

The results from the FECRT and EHT provided evidence for the presence of albendazole, ivermectin and morantel citrate resistant strains of nematodes in goats from the University Goat and Sheep Farm, Mannuthy. This is the first report on the existence of anthelmintic resistance in goats from Kerala.

The method of World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.), which utilizes post treatment EPG of a treated and untreated control group appears least conservative and detects resistance when it is at a low level compared with other methods. This may be the most appropriate method to use in surveys, to monitor the resistance and to inform the farmers of any change in the efficacy of anthelmintics.

The *invitro* test, Egg Hatch Assay is based on the fact that benzimidazoles will inhibit the embryonation and hatching of nematode eggs. The assay is based

on the ovicidal properties of benzimidazoles and ability of eggs from resistant strains to develop and hatch at higher concentrations of drug than that of susceptible strains. This test is more sensitive than FECRT.

Benzimidazole group

In the present study, albendazole was chosen as the drug. From the benzimidazole group, the animal showed albendazole resistance with a per cent worm reduction of 30 with less than 90 lower 95 per cent confidence limit *i.e.*, 52 which suggested that nematodes developed resistance against albendazole. The lower efficacy and resistance to albendazole might be attributed to their continuous and prolonged use in controlling gastrointestinal nematodes in goats.

Benzimidazole resistance is known to be inherited as a polygenic trait. The benzimidazoles act by attaching to tubulin dimers, preventing their polymerization to microtubules and by causing disassembly of existing cytoplasmic microtubule structure and cause energy deprivation. Resistant worms have the ability to reduce their energy demands, to switch to alternative energy pathways or reduce their uptake of anthelmintics (*Jagannath et al.*, 2000).

Similarly, resistance to albendazole in gastrointestinal nematodes has been widely reported from different countries by Gill (1996), Boersema and Pandey (1997), Waruiru *et al.* (1998), Maingi *et al.* (1998) and Dhanalakshmi *et al.* (2003) from India, Zimbambwe, Kenya, Denmark and Bangalore respectively.

Taylor *et al.* (2002) found that regular use of any chemical to control infective organisms possess the risk of resistant population development. Hence this may be the reason for the development of albendazole resistance since this drug has been in use for a prolonged period. If there is resistance to one benzimidazole compound, side resistance to other benzimidazoles occurs even if

those compounds have not been used on those animals. (Sivaraj *et al.*, 1994). So there is a chance for resistance to develop to other drugs also in the benzimidazole group.

Morantel citrate

The present study revealed morantel citrate resistance with a per cent worm reduction of 45. The lower 95 per cent confidence limit was 62. The per cent worm reduction was a little high when compared to albendazole since this drug was not used as regularly as albendazole in the animals selected.

Morantel act as cholinergic agonists which cause depolarization of muscle membranes. Resistance to morantel is due to the reduction in the affinity of cholinergic receptors (Craig, 1993).

The resistance detected against morantel citrate may be also due to multiple resistance. Multiple resistance is a situation where animals become resistant to two or more anthelmintic groups because of either selection by each group independently or by side resistance as interpreted by (Singh *et al.*, 2002).

The resistance in gastrointestinal nematodes of goats against morantel citrate has also been reported by Sangster *et al.* (1979), Prichard (1994) and Yadav and Garg (2004).

Ivermectin

The present study revealed ivermectin resistance with a per cent worm reduction of 53. The lower 95 per cent confidence limit was 34. The drug was more effective than the other two as the drug is of recent introduction in the treatment of goats.

Ivermectin is known to paralyse the somatic musculature of nematodes there by inhibiting feeding in adult worms. Ivermectin resistant nematodes were found to be less sensitive to ivermectin when compared to susceptible worms, indicating that pharynx formed an important target of this class of drugs (Singh *et al.*, 2002). Resistance to ivermectin may also be due to the multiple resistance developed by the goats.

Le Jambre (1993), Sivaraj *et al.* (1994), Mwamachi (1995), Maciel *et al.* (1996) and Gopal *et al.* (1999) also reported on the resistance of nematodes in goats to ivermectin.

5.2.1 Faecal Egg Count Reduction Test (FECRT)

Although several tests have been used for detection of anthelmintic resistance in nematodes of veterinary importance, the faecal egg count reduction test is still the simplest and most effective *in vivo* test that can be used with all types of anthelmintics (Coles *et al.*, 1992). Detection of FECRT was based on the calculation of arithmetic mean, percentage reduction and 95 per cent confidence limits. Resistance to an anthelmintic was presumed when either the percentage reduction in egg counts was less than 95 per cent or when 95 per cent confidence level was less than 90 per cent.

The present study employed FECRT as the *in vivo* method for the detection of anthelmintic resistance. The test is of choice especially in surveys of resistance because it uses only few resources, is easily performed and is applicable in the evaluation of the performance of any anthelmintic under field conditions. This test involved the treatment of naturally infected animals and can be used with ruminants, horses and pigs, with all types of anthelmintics, with all species of nematodes in which eggs are shed in the faeces (Coles *et al.*, 1992). This test in the present study revealed resistance to albendazole, ivermectin and

morantel citrate. The same test has been used by different workers like Jackson *et al.* (1992), Kochapakdee *et al.* (1995), Maciel *et al.* (1996) and Maingi *et al.* (1998).

5.2.2 Egg Hatch Test (EHT)

The EHT is well validated and can detect resistance reliably *ie.,* it provides a more quantitative estimate of the level of resistance Thiabendazole is generally used in the EHT in field surveys, but other benzimidazoles may be used for research purposes (Coles *et al.*, 1992). The present study chose albendazole instead of thiabendazole for detecting benzimidazole resistance. Egg Hatch Test in this study revealed albendazole resistance in goats with an ED_{50} value of 0.211556 µg albendazole per ml. These findings concur with those of Cawthorne and Cheong (1984), Taylor and Hunt (1988) and Swarnkar *et al.* (1999).

Fernandez *et al.* (1997) confirmed benzimidazole resistant nematodes from goats in Spain with an ED_{50} value of 0.22 µg thiabendazole per ml in EHT which almost matches with that in the present work.

A study conducted by Yadav *et al* (1995) revealed that parasitic strain possessed side resistance to other benzimidazole group and cross resistance to levamisole and morantel. It was also understood that multiple resistance could occur with frequent use of different anthelmintics. This was in accordance with our study *ie.,* the present study revealed resistance to chemically unrelated drugs like albendazole, ivermectin and morantel citrate. Sargison (2004) also reported multiple resistance to benzimidazole, levamisole and ivermectin by the nematode *Teladorsagia circumcinata* in lambs in Scotland.

Presence of anthelmintic resistance may be the result of selection of resistant genotypes within the parasite population. The selection pressure is

influenced by the proportion of the total parasite population exposed to the drug. The total population of genotypes include stages on pastures as well as those in animals and usually the majority are on the pasture. As a result only minority of the population is exposed to selection pressure. In genetic sense, only one or two nematode generations are completed under field conditions in each year. These factors slow the rate of increase of resistant genotypes. Therefore the prevalence of subclinical resistance occurs. The emergence of clinical resistance problem is the culmination of long selection process which usually goes unnoticed. The selection of resistance is enhanced by the high biotic potential of the gastrointestinal nematodes especially *H. contortus*. Their fecundity can produce large numbers of generations in a short time, especially if the climate is favourable for the free living stages (Craig, 1993). This is an adding factor for the development of resistance in our farm because one of the common species encountered in this farm is *H.contortus*. The resistant worms could be carried to the farm by contamination such as importation of faecal particles from the areas with anthelmintic resistance on inanimate objects and living creatures. (Borgsteede *et al.*, 1996)

It is questionable whether such investigations serve any useful purpose. Resistance is brought about by the selection of resistant individuals already present in a population at low frequencies. Using an anthelmintics, will only lead to an increase in the frequency of these resistant worms in the population. Hence our aim is to check the multiplication of resistant strains (Cawthorne and Cheong, 1984).

5.3 CONTROL OF ANTHELMINTIC RESISTANCE

As the worm population with anthelmintic resistance multiplies, one of the most valuable weapon in the battle to conserve susceptibility in nematode population is the ability to detect resistance while it is still at a low level.

Anthelmintic resistance is best controlled in the ground level by the use of correct type of anthelmintics at the correct dose against nematodes in goats. Annual rotation of the class of anthelmintics can also prevent the development of resistance. To control resistance, studies on non chemotherapeutic options such as genetic selection for resistant hosts and biological control should be adopted in future.

Summary

6. SUMMARY

Investigation on the prevalence of gastrointestinal nematodes of goats in Thrissur Corporation area was carried out by detecting faecal eggs by coprology and larvae by coproculture. The faecal samples for coprological examination were collected from animals brought to the University Veterinary hospitals, Kokkalai and Mannuthy, University Goat and Sheep farm and from nearby houses in Mannuthy. Screening of faecal samples of 320 goats during the period of study from June 2004 to May 2005, revealed 114 as positive (35.63 per cent). The type of positive infection noted were *Strongyle* (95.61 per cent) and *Strongyloides* (4.38 per cent) infection.

Monthwise prevalence of gastrointestinal nematodes showed maximum infection in May (48 per cent) while a lower prevalence in August (28 per cent). Seasonwise, the prevalence of gastrointestinal nematodes was found to be 30.77 per cent, 33.33 per cent and 39.135 per cent during cold wet South West monsoon (heavy rainfall), warm wet North East monsoon (low rainfall) and dry season respectively. The prevalence of gastrointestinal nematodes was observed to be higher in young goats below one year (42.10 per cent) and in females (85.96 per cent) than males (14.03 per cent). There was no significant variation between breeds and management since all the samples collected for this study was from crossbreds and houses respectively.

The modified Veglia's method was adopted for coproculture. The species of nematodes encountered were *Haemonchus contortus* (55.26 per cent), *Oesophagostomum columbianum* (13.15 per cent), *Trichostrongylus colubriformis* (21.05per cent), *Bunostomum trigonocephalum* (6.14 per cent) and *Strongyloides papillosus* (4.38 per cent). The comparative biometry of various infective larvae of the commonly found nematodes were done.

Resistance to various anthelmintics by the nematodes in goats were detected by the methods namely Faecal Egg Count Reduction Test (FECRT) and Egg Hatch Test (EHT). Forty kids belonging to the University Goat and Sheep Farm, Mannuthy, aged between three to six months were used for this purpose. Animals positive for nematodes were divided into four groups. Each of them were administered albendazole @ 7.5 mg per kg body weight orally, ivermectin @ 0.2mg per kg body weight orally and morantel citrate @ 10mg per kg body weight orally while the fourth group remained as non medicated control. Results recorded revealed that FECRT detected resistance to albendazole, ivermectin and morantel citrate by the gastrointestinal nematodes in goats. Albendazole showed a per cent worm reduction of 30 with 52 as lower 95 per cent confidence limit. Ivermectin revealed a per cent worm reduction of 53 and 34 as the lower 95 per cent confidence limit. Morantel also revealed a per cent worm reduction of 45 and 62 as the lower 95 per cent confidence limit.

Egg Hatch Test was particularly used to detect the specific resistance to the drug albendazole. The ED_{50} value of albendazole (µg per ml) in EHT was found to be 0.211556 which further established resistance to albendazole by the gastrointestinal nematodes in goats.

The lower efficacy and resistance to albendazole might be attributed to their continuous and prolonged use in controlling gastrointestinal nematodes in the farm. The resistance detected against morantel citrate and ivermectin may be ascribed to the development of multiple resistance.

Presence of anthelmintic resistance may be the result of selection of resistant genotypes within a parasite population. The emergence of clinical resistance problem is the culmination of such long selection process. These resistant worms could be carried to the farm by contamination such as importation of faecal particles from the areas with anthelmintic resistance on

inanimate objects and living creatures. The development of anthelmintic resistance of gastrointestinal nematodes among the goats of the University Goat and Sheep farm implies the possibility of such contamination.

Anthelmintic resistance is best controlled in the ground level by the use of correct type of anthelmintics at the correct dose against nematodes in goats. Annual rotation of various classes of anthelmintics can also prevent the development of resistance.

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ANTHELMINTIC RESISTANCE IN GASTROINTESTINAL NEMATODES OF GOATS

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ABSTRACT

Investigation on the prevalence of gastrointestinal nematodes of goats in Thrissur Corporation area was carried out by detecting faecal eggs and larvae after coprological examination and coproculture respectively. The faecal samples for coprological examination were collected from animals brought to the University Veterinary hospitals, Kokkalai and Mannuthy, University Goat and Sheep farm and from nearby houses around Mannuthy.

Screening of faecal samples of 320 goats during the period of study from June 2004 to May 2005 revealed 114 as positive (35.63 per cent). The type of positive infection noted were *Strongyle* (95.61 per cent) and *Strongyloides* (4.38 per cent) infection. Monthwise prevalence of gastrointestinal nematodes showed maximum infection in May (48 per cent) while a lower prevalence in August (28 per cent). Seasonwise, the prevalence of gastrointestinal nematodes was found to be 30.7 per cent, 33.33 per cent and 39.13 per cent during cold wet South West monsoon (heavy rainfall), warm wet North East monsoon (low rainfall) and dry season respectively. The prevalence of gastrointestinal nematodes was observed to be higher in young goats below one year (42.10 per cent) and in females (85.96 per cent) than males (14.03 per cent). There was no significant variation between breeds and management .

The species of nematodes encountered were *Haemonchus contortus* (55.26 per cent), *Oesophagostomum columbianum* (13.15per cent), *Trichostrongylus colubriformis* (21.05 per cent), *Bunostomum trigonocephalum* (6.14 per cent) and *Strongyloides papillosus* (4.38 per cent). The comparative biometry of various infective larvae of the commonly found nematodes were noted.

Resistance to various anthelmintics by the nematodes in goats of the University Goat and Sheep farm, Mannuthy were detected by the methods namely Faecal Egg Count Reduction Test (FECRT) and Egg Hatch Test (EHT). Forty kids aged between three to six months were used for this purpose.

Faecal Egg Count Reduction Test revealed resistance to albendazole, ivermectin and morantel citrate by the gastrointestinal nematodes. The drugs namely albendazole, ivermectin and morantel citrate showed a per cent worm reductions of 30, 53, 45 with 52, 34 and 62 as lower 95 per cent confidence limits.

Specific resistance to benzimidazole group by EHT showed the ED_{50} value of albendazole (µg per ml) in EHT to be 0.211556 which further established resistance to albendazole by the gastrointestinal nematodes of goats.

Anthelmintic resistance is best controlled in the ground level by the use of correct type of anthelmintics at the correct dose against nematodes in goats. Breeding for disease resistance, development and use of vaccines and biological control using nematophagous fungi are the prospective methods for the control of anthelmintic resistance.