Haematological and Biochemical Studies of Helminth Infected Goats in South Kashmir

DISSERTATION

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By Masarat Nizam

Under the Joint Supervision of

Prof. M. Z. Chishti

Dr. Hidayattullah Tak

Supervisor Professor Emeritus Centre of Research for Development University of Kashmir, Sgr.

Co-Supervisor

Sr. Asst. Professor Deptt. of Zoology, University of Kashmir, Sgr.



Centre of Research for Development Faculty of Biological Science University of Kashmir Srinagar - 190006, Kashmir (NAAC Accredited Grade 'A' University) 2013

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Centre of Research for Development **University of Kashmir** Srinagar – 190 006, Kashmir

No: Date:

<u>Pertificate</u>

This is to certify that the dissertation entitled "Haematological and Biochemical Studies of Helminth Infected Goats in South Kashmir" submitted to the University of Kashmir for the award of the Degree Masters of Philosophy in Zoology, is the original research work of Ms. Masarat Nizam, a bonafide M. Phil. Research Scholar of the Centre, carried out under our joint supervision. The dissertation has not been submitted to this University or to some other University so far and is submitted for the first time. It is further certified that this dissertation is fit for submission for the degree of Masters of Philosophy (M. Phil.) in Zoology and the candidate has fulfilled all the statutory requirements for the completion of the M. Phil. Programme.

Prof. M.Z. Chishti

Supervisor Professor Emiretus Centre of Research for Development University of Kashmir

Dr. Hidayattullah Tak

Co-Supervisor Sr. Asst. Professor Department of Zoology University of Kashmir

Prof. (Dr.) Azra N.Kamili Director Centre of Research for Development Head P.G. Department of Environmental Science University of Kashmir, Sgr.

DEDICATED

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MY

BELOVED

PARENTS

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Abbreviations

Abbreviation	Full Form
FEC	Faecal Egg Count
EPG	Eggs Per Gram
Nem.	Nematodes
Ces.	Trematodes
Trem.	Cestodes
Hb	Hemoglobin concentration
PCV	Packed cell volume
ESR	Erythrocyte Sedimentation Rate
RBC	Red Blood Cell
WBC	White Blood Cell
TLC	Total Leukocyte Count
DLC	Differential Leukocyte Count
Fig.	Figure
SD	Standard Deviation
%	Percentage

$\underline{CHAPTER - 1}$

Introduction

omestic ruminants such a sheep, goats, cattle are among the first animals to be domesticated by man. Archaeological evidence suggests that sheep were being raised for wool production as long as 4000 B. C., while goat remains have been dated to between 6,000 – 7,000 B. C. The ancestor of modern goat is the Benzor from Asia Minor Middle East.

Goat (*Capra*), a member of the Bovidae family and subfamily Caprinae is one of the oldest domesticated species. For thousands of years they have been used for their milk, meat, hair and skin over much of the world. Female goats are referred to as does or nannies, intact males as bucks or billies; their offspring are known as kids. Goat is generally reared to procure meat, milk and skin. Goat is often regarded as poor man's cow. The milk of goat is quite similar to that of cow milk and it is more easily digested because of smaller globules. It is richer in milk content with a high amount of calcium, phosphorus and chlorine.

The state of Jammu and Kashmir is situated at 32.17° and 36.59° north latitude and 72.26° and 80.30° east of longitude with total area of 2,22,236 Sq. Kms. The climate is variable from subtropical (Jammu plains) to temperate (Kashmir Valley) to temperate cold but arid (Ladakh region). The soil topography, geoclimate, natural meadows and high land pastures of valley are naturally conducive for goat production.

Kashmir is primarily an agricultural state and animal treasure is one of the major sources of earning of farming community and goat farming is an important source of livelihood for small and marginal farmers and landless labourers as it plays an important role in providing food, fibre, manure etc. The major advantage of goats and sheep over other ruminants is to utilize pastures and wastelands to produce meat and wool. Goat dung is a natural source of organic fertilizer with nitrogen and potassium contents double than that of cattle dung, so goat manure is preferable for increasing the fertility of soil. The rearing of goat had the added advantage of filling an important ecological niche, being able to graze land on which sheep and cattle simply cannot thrive.

As per the Livestock Census of Jammu and Kashmir which was carried out in 2007, total sheep, goats, cattle and buffalo product in Jammu and Kashmir were 36.85, 20.63, 34.43 and 10.51 lakhs respectively in number. It has been estimated that livestock contributes about 11% to the economy of the state.

The 17th quinquennial livestock census posted 98.993 lakh livestock in the state which makes density of livestock to be 98 animals per sq. km. of area. When the indicator of livestock available per thousand of population is adopted, there are 926 animals per thousand of population in J&K state and this figure for all India is 457 animals. Average livestock per household (2001 census households) is 6 animals as against 3 animals for India. The species provides dependable source of income to 40% of the rural population which are below the poverty line in India. The goat meat (Chevron) production per year is 3,05,000 million tons (35% of the total meat production in the country). Its economy is of the order of Rs. 350 crores annually. The goat rearing farms are, therefore important part of rural as well as national economy of India.

In our state the local goats and sheep are generally reared in tablelands in spring and early summer months. In summer and early autumn the goats and sheep are taken to highland pastures for grazing (Plate 1). In our state ruminant rearing is so important that it is the only source of income to many tribes. Even name is given to a tribe on the basis of sheep called Bakerwal. This is a nomadic tribe of our state migrating from summer grazing Pirpanchal Mountains and low lying hills of Jammu in winter. Goat rearing is a tribal profession of nomads (Bakerwals, Gaddies) and many other farming communities in Jammu and Kashmir. Goats contribute to the subsistence of small holders and landless rural power. They also produce meat, milk, skins and manure and are also used for transport purposes especially in high altitudes. A Gaddi breed of goat is able to carry upto 10 kg load on much steep slopes.

There are various diseases which are a major setback to this industry. The various disease producing organisms are viruses, bacteria, protozoa, helminthes etc. There are other practices which contribute to low wool and meat production for this industry. The main contributing factors include large animal production with rapidly diminishing grazing areas and consequent overstocking, poor nutritional standard and traditional husbandry practices.

The viral and bacterial diseases are easily diagnosed by their clinical signs but parasitic infection when less in number or in early stages are without clinical signs and thus act as one of the major cause of production loss. Faizal (1999) reported $1/3^{rd}$ growth retardation in ruminants due to helminth infections. Herlich (1978) reported 5-10 % mortality and 10-20 % morbidity due to helminth parasites in small ruminants. The productivity of sheep (and goats) is constrained by parasitic infections (Dhar et al., 1982). Helminth infections remain one of the major constraints to small ruminant production in tropics (FAO, 1992). Surveys indicate that up to 95% sheep and goats in the tropics are infected with helminths and, Haemonchus and Trichostrogylus are the main genera involved (Rey, 1991). Mortality rates in herds may exceed 40% while weight losses 6-12 kg/year/animal may occur (IEMVT, 1980). However, insidious productivity losses through reduced feed intake and decreased efficiency in feed utilization, associated with subclinical or chronic conditions, are often the largest economic losses (Holmes, 1993; Gatongi, 1996). It is estimated that more than 300 species of helminthes parasitize livestock in India and new species are being frequently discovered (Singh et al., 1977).

The incidence of helminthes infection varies with age, sex, season and agroclimatic conditions. The higher incidence of parasitic infections in domestic animals in a grazing system lowers productivity, leading to important economic losses. The parasite infected animals increase their metabolic rate and reduce the amount of metabolic energy used for production, as the parasites use their nutrients, damage some vital organs and cause animals to become more susceptible to other pathogenic agents (Skykes *et al.*, 1992).

The helminth species which parasitize goats belong to three classes namely trematodes, cestodes and nematodes and pathogenicity of these helminth parasites also varies with different intensity. Goats are a treasure house of different helminth parasites like *Paramphistomum* spp., *Fasciola* spp., *Dicrocoelium* spp., *Haemonchus* spp., *Trichuris* spp., *Chabertia* spp., *Dictyocaulus* spp., *Moneizia* spp., and *Stilesia* spp.

Mature paramphistomes rarely produce clinical symptoms (Dube *et al.*, 2003), however immature migrating parasites have been reported causing serious diseases and even the death of their hosts by burying themselves in the submucosa of duodenum and feeding on the epithelial cells of brunners glands with result in anorexia, polydpsia, profuse diarrhoea, drop in plasma protein concentration and anemia (Buttler and Yeoman, 1962; Singh *et al.*, 1984).

Fascioliasis, is a liver fluke disease caused by several species. The two most important species in livestock are Fasciola hepatica and Fasciola gigantica. Fascioliasis causes pathological and necrotic lesions, which results from the parasitic migration through the liver parenchyma and the bile ducts causing hemorrhages (Plate 2). The flukes are also, haemophagous and infection results in anemia. Sinclair (1967) and Hammond and Sewell (1990) reported that *Fasciola gigantica* is more pathogenic and causes more production losses than Fasciola hepatica. In small ruminants, the disease causes severe economical losses because of reduced growth and productivity, immune suppression, reduced wool and milk production, condemnation of the livers as unfit for human consumption and sudden death of heavily infected animals (Boray, 1985; Ngategize et al., 1993 and Mulcahy et al., 1999). Since, liver is the main metabolic organ in the body, infection of hepatocytes is an essential feature of certain parasitic infections. In fascioliasis the metabolic process of the liver is gradually reduced (Fikry *et al.*, 1988). Hepatocytes are active in controlling levels of blood glucose, lipids and cholesterol and a number of plasma proteins, including albumin, fibrinogen and prothrombin.

Gastro-intestinal parasitism represents a severe health problem in small ruminant production system, especially sheep and goats and its consequences can be extensive ranging from reduced productivity to mortality (Skykes, 1994). It may also cause body composition changes and rendering the affected animals more susceptible to concurrent infections (Dominguez-Torano *et al.*, 2000). Gastrointestinal nematodiasis is a major threat and a primary constraint to sheep productivity, it endangers animal welfare worldwide (Tarig et al., 2010). The prevalence of GIN in tropical and sub-tropical areas has adversely affected the production potential of sheep and goats, leading to countless deaths and insidious economic losses in livestock sector (Al-Quaisy et al., 1987). One of the main culprits in ruminant nematodiasis is Haemonchus contortus which causes haemonchosis, anemia and parasitic gastroenteritis in goats, sheep and cattle (Leiper, 1957). They cause significant economic losses worldwide due to their feeding behaviour being haematophagous, *Haemonchus contortus* and Ostertagia ostertagi suck 0.05ml of blood/worm/day (Soulsby, 1986) (Plate 2). Trichostrongyle infection (Trichostrongylosis) is much more important as a veterinary problem and causes pathological conditions like anemia, weight loss, poor wool and milk production and bottle jaw. Oesophagostomum columbianum produces pathological conditions like diarrhoea, loss of appetite, emiaciation, weight loss and nodule formation, while infection of Bunostomum trigonocephalum is associated mainly with anemia and weight loss. Trichuris ovis, being less pathogenic but anemia, hemorrhage, necrosis, oedema of caecal mucosa and diarrhoea has been reported in severe infections. The filarial lungworm causes parasitic bronchitis in goat. In severe infection with *Dictyocaulus*, the bronchial epithelium is hyperplasticized and heavily infiltrated by eosinophils, which sometimes leads to parasitic pneumonia. In heavy infections the goats may die due to respiratory failure following the development of severe interstitial emphysema and pulmonary oedema. These nematode infections in general produce anorexia, reduced feed intake, loss of blood and plasma proteins into gastro-intestinal tract, alterations in protein metabolism, enteritis, diarrhoea resulting in reduced body weight gains and wool production and death due to secondary infections, thus resulting in great economic losses to goat farmers and goat industry.

Blood is an important and reliable medium for assessing the health status of individual animals (Oduye, 1976). Serum biochemistry and hematological analysis have been found to be important and reliable means for assessing an animal's health status and might give an indication of the degree of damage to host tissue as well as severity of infection (Otesile *et al.*, 1991).

Prevalence of helminthes in small ruminants being very high. They cause adverse effects on the host like haematological and biochemical disturbances (Rasool *et al.*, 1995; Iqbal *et al.*, 1998; Hayat *et al.*, 1996, Hayat *et al.*, 1999), loss of body weight (Khan *et al.*, 1988) and huge economic losses (Iqbal *et al.*, 1989, Iqbal *et al.*, 1993).

Detailed information about epidemiology, prevalence, etiology, biology and pathogenicity of helminthes in goats of Jammu & Kashmir is still not scanty. A lot of work has been carried out on different aspects of ruminant parasitology, but no substantial work has been conducted on the haemato-biochemical parameters of the goats, which is revealed by the absence of any reference from Kashmir. Hence the present work is aimed at to conduct haemato-biochemical studies of goats of south Kashmir and to correlate the results with the presence of helminth parasites in these hosts. A comprehensive work covering the dimensions of epidemiology and hematobiochemical parameters of goats for a period of one year from December 2011 to November 2012 under the title "Haematological and Biochemical Studies of Helminth Infected Goats in South Kashmir" was thus initiated with the following objectives.

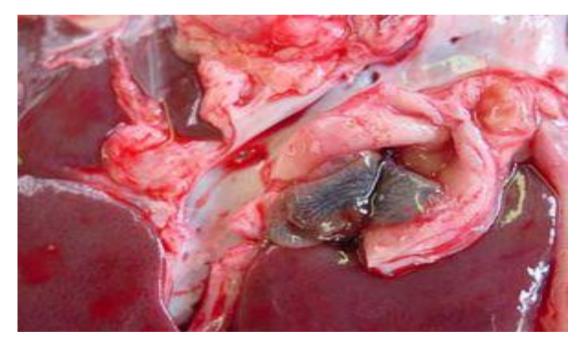
- 1) To study the epidemiology of Helminth parasites of goat in South Kashmir.
- To study the impact of Helminth parasites on haematology of goats of South Kashmir.
- To study the impact of Helminth parasites on blood biochemistry of goats of South Kashmir.



Plate 1: Goats grazing at pastures lands



Abomassum (Part of intestine) of goat infected with Haemonchus and Ostertagia sp.



Liver (bile ducts) of goat heavily infected with Fasciola sp.

Plate 2: Organs infected with Helminth Parasites

<u>CHAPTER – 2</u> **Review of Literature**

ince a lot of work has been done in the very important sector of veterinary parasitology, it is difficult to give a detailed account of the work done; therefore a brief account of literature available on related aspects of the present investigation has been critically reviewed and summarized below. The present study deals with the epidemiology and the haematobiochemistry, therefore the literature is presented separately under two headings.

2.1. Epidemiological review

2.2. Haemato-biochemical review

2.1. Epidemiological Review

Hsiang *et al.* (1990) studied 4534 faecal samples, collected in Taiwan over a three year period from randomly selected dairy goats for parasites. The most frequent parasites found were *Oesophagostomum* spp. (19%), *Haemonchus contortus* (17.3%), *Strongyloides papillosus* (8.5%), *Ostertagia ostertagi* (7.1%) and *Trichostrongylus colubriformis* (6.8%). Overall prevalence was observed greater in autumn and winter, and goats with access to pasture were more commonly infected than goats which fed indoors (penned goats).

Mattos (1991) reported gastro-intestinal nematodes parasitizing ruminants (cattle, sheep and goat) raised in Oriximina, Brazil. Eight species of parasites were reported namely, *Haemonchus contortus*, *Haemonchus similis*, *Trichostrongylus axei*, *Trichostrongylus columbriformis*, *Cooperia curticei*, *Cooperia punctata*, *Oesophagostomum venulosum and Bonostomum trigonocephalum*.

Lepojev *et al.* (1992) studied the gastrointestinal strongyles of goats. They counted the parasites of abomassum and intestine of 6 goats slaughtered in Radovid, Yugoslavia. Pal and Qayyum (1992) studied the distribution of gastrointestinal helminths of goats in Swat valley, Pakistan. 53 gastrointestinal tracts of 53 goats

from Swat valley were examined at abattoirs from September 1990 to January 1991. *Haemonchus contortus, Ostertagia ostertagi, Ostertagia circumcincta* and *Trichostrongylus axei* with prevalence of 94.35%, 81.13%, 66.03% and 50.94% respectively from abomassum and *Trichostrongylus colubriformis* (73.58%) from small intestine and *Oesophagostomum venulosum* (22.64%), *Trichuris ovis* (39.62%) and *Trichuris globulosa* (11.32%) from the large intestine. Thakur *et al.* (1992) reported that the prevalence of the parasitic infection was 100% in goats during the month of July in western Nepal whereas out of 32 samples collected from Manglapur VDC-2, 76.66% were positive for eggs of these parasites.

Frutschi *et al.* (1993) on autopsy of 104 small ruminants, 52 sheep and 52 goats, the following gastrointestinal nematodes were identified and counted in order of predominance. *Trichostrongylus* spp. (96%), *Oesophagostomum columbianum* (82%), *Haemonchus contortus* (67%), *Strongyloides papillosus* (55%), *Cooperia* spp. (47%) and *Trichuris ovis* (12%). Hoste and Chartier (1993) studied the impact of nematode parasitism of digestive tract on milk and milk quality in dairy goats. The study reported that the high producer goats had less resistance to infection associated with severe consequences on milk production. The reduction in milk production was reported with increase of worm load in the goats.

Dorny *et al.* (1995) studied the *Strongyle* infections in sheep and goats in Malaysia. Pattern of *Trichostrongyle* infections according to season, age, pregnancy and lactation was studied by faecal egg counts. *Haemonchus contortus* and *Trichostrongylus* spp. were reported to be most important strongyles in sheep and goats. Ndao *et al.* (1995) conducted epidemiological survey of gastrointestinal helminthes in 51 sheep and 51 goats in tree cropping region in Senegal from October 1990 to September 1991. They reported that all animals were infected with at least one helminth species.

Rafique and Hayat (1997) analysed faecal samples from the rectum of sheep and goats in the Quetta and Kalat area of Buluchistan, Pakistan for helminth eggs. 87.7% (50 of 57) prevalence was reported. *Nematodirus spathiger* was the most frequent parasites (72.7%) followed by *Trichuris globulosa* (27.3%), *Marshallagia marshalli* (6.3%) and *Strongylloides papillosa* (6.3%). Mixed infections were frequently reported. Vaughan *et al.* (1997) described two cases of infection with *Fasciola hepatica* in young farmed emus with sub-acute and chronic fascioliasis. The author reported gross lesion of necropsy and hepatic lesions in microscopic examination.

Gatongi *et al.* (1998) investigated the epidemiology of *Haemonchus contortus* infection of sheep (Red Maasai) and goats (Small East African Goat) in a semi-arid area of Kenya. Prevalence of *Haemonchus contortus* was over 90% in both sheep and goats and this species contributed to about 80% of the total worm burden. Only about 10% of the hypobiotic larvae were recovered from the mucosal digest whereas about 90% were recovered from the abomasal contents. Thamsborg *et al.* (1998) reported lungworm infection (*Dictyocaulus vivzarus*) on dairy cattle farms in tropical highlands of Tanzania.

Valcarceli and Romero (1999) examined 322 gastro-intestinal tracts of traditionally reared goats originating from a dry area of Central Spain. A large spectrum of gastrointestinal nematodes was observed with *Teladorsagia circumcinta*, *Teladorsagia trifurcate* being the most prevalent species, followed by *Trichostrongylus vitrinus* and *Nematodirus filicolis*.

Astiz *et al.* (2000) studied the seasonal distribution and larval shedding intensity of broncho-pulmonary parasites over two consecutive years using 285 faecal samples obtained from adult goats in Spain. A very high prevalence (81%) was reported and *Muellerius capillaris* was the predominant species (present in 98% of infections). They further reported that *Dictyocaulus* was not an important pathological infection in goats. Brunn *et al.* (2000) studied the cause for clinical symptoms like coughing, fever and weight loss in Saanen goats in Swiss Alps. On slaughtering 1^{st} stage larvae were reported from trachea. The larvae were identified that of *Muellaria, Capillaris* and *Protostrongylylus* spp.

Pathak and Pal (2000) collected 88 gastrointestinal tracts of goats from the slaughter house Supela, Bhilai and were also collected from the Veterinary College, of Drug district Chhattisgarh and were brought for the postmortem examination during November 1999 to October 2000. The percentage of overall prevalence of

parasitic infection *Paramphistomum* spp., *Cotylophoron* spp., *Moniezia* spp., *Avitellina* spp., *Haemonchus* spp., *Cooperia* spp., *Oesophagostomum* spp., *Bunostomum* spp., and *Trichuris* spp., were 80.68%, 45.45%, 17.04%, 3.40%, 26.13%, 5.68%, 3.40%, 30.68%, 5.68% and 27.27% respectively. In case of *Paramphistomum*, infection was highest in monsoon (91.8%) and lowest in winter (63.15%). The seasonal prevalence of gastrointestinal parasitic infection in goats showed that prevalence was highest in monsoon (94.60%), moderate in summer (87.50%) and lowest in winter (63.15%). Silvestre *et al.* (2000) investigated helminth infection, species diversity (proportion of each species in the community), species number, intensity of infection and antihelminthic resistance in 16 dairy-goat farms of south-western France. A total of 17 species of helminthes, among which 14 nematodes, one cestode (*Moniezia* spp.) and two trematodes (*Paramphistomum daubnevi* and *Dicrocoelium lanceolatum*) were recovered in the 26 necropised culled goats during the study.

Githigia *et al.* (2001) studied the impact of gastrointestinal nematodes on health and production of goats in Kenya. The faecal egg counts were found higher during short rainy season. In all the animals studied *Haemonchus contortus* was the nematode recovered during the study. It was concluded that gastrointestinal helminthes cause production losses, weight loss and mortalities in goats. Jithendran and Bhat (2001) studied the prevalence of gastrointestinal parasites in sheep and goats of Himachal Pradesh, India and found the prevalence in sheep and goats respectively as follows: *Fasciola* 9.6%, 8.8%; *Amphistomes* 3.8%, 2.5%; *Dicrocoelium* 7.2%, 2.5%; *Schistosoma* 1.2%, 0.6%; *Moniezia* 2.7%, 1.3%; *Strongyles* 91.6%, 100%; *Strongyloides* 4.8%, 5.1%; *Dictyocaulus* 1.2%, 1.3% and *Trichuris* 14.3%, 1.3%.

Sharkhuu (2001) performed the Post-mortem examinations of 236 goats from all provinces in Mongolia for the study of helminths in goats. Thirty-nine helminth species belonging to three classes, 14 families and 23 genera were found. The prevalence and intensity of helminth infections were reported for three age groups of goats in four seasons and three geographic zones in Mongolia. Common helminth infections of goats in all zones of Mongolia were infections of *Ostertagia*, *Marshallagia* and *Nematodirus*. The highest number of eggs per gram (EPG) of feces was counted in March (average 1335.3 ± 405.3) and the lowest count was in November (54 ± 18.6).

Magona and Musini (2002) studied the influence of age, grazing system, season and agroclimatic zone on the prevalence and intensity of gastrointestinal *Strongyles* in Uganda goats and reported that season and agroclimatic zones were the only significant factors which influenced intensity of nematodiasis in goats. Mazyzd and El-Nemr (2002) reported the endoparasites of sheep, goats and Shephered in North Sinai Governorate, Egypt. They revealed an overall infection of 12.7% with *Fasciola spp.*, 12.8% with *Moneiza expansa* and 4.59% with *Trichuris ovis*.

Love and Hutchinson (2003) observed pathology and diagnosis of internal parasites in ruminants. The purpose of their study was to overview the gross pathology and diagonosis of gastrointestinal and other parasites in ruminants, with particular emphasis on the economically important parasites of sheep, goat and cattle. Regasa *et al.* (2003-2004) conducted a study on epidemiology of gastrointestinal parasites of ruminants in Western Oromia, Ethiopia. The study showed the overall prevalence of gastro-intestinal parasites as 84.1% in goats. Nematodes of group *Strongyle* and *Eimeria* were most prevalent parasites encountered in this area.

Dhand *et al.* (2004) reported an outbreak of fasciolasis in sheep and goats in Punjab. 70 goats and 50 sheep of different age groups were affected and found that these animals were suffering from high fever with diarrhoea. Among these animals 5 goats and 40 sheep died before the investigation. *Fasciola gigantica* was recovered on postmortem examination. Mbae *et al.* (2004) studied 1106 sheep and goats in Kenya for nematode infections. Young animals were found more infected than older ones. The faecal egg counts were significantly higher in wet seasons in both sheep and goats. *Haemonchus contortus* was the most predominant nematode parasite encountered in the study. Sheikh *et al.* (2004) studied ovine fasciolasis in Kashmir valley. They examined 1150 faecal samples from endemic and non-endemic and hilly/ migratory groups collected directly from rectum. To compare the percent

prevalence, altogether 389 livers of locally reared sheep were examined for the presence of flukes. They found both immature and mature flukes.

Das *et al.* (2005) studied the effects of gastrointestinal nematodosis on the body weight and mortality in kids. Molina *et al.* (2005) studied the prevalence of infection with *Fasciola gigantica* and its relationship to carcass and liver weights, and flukes and egg counts in slaughter cattle and buffaloes in Southern Mindanao, Philippines. Muraleedharan (2005) observed the gastro-intestinal parasites of livestock in a central dry zone of Karnataka, India and reported the prevalence of gastro-intestinal parasites among cattle (18.22%), buffaloes (20.85%), sheep (39.44%) and goats (46.12%) of southern taluks of central dry zone of Karnataka during drought period. *Strongyles* were the most common nematode. *Fasciola, Amphistomes, Moniezia* and *Entamoeba* infections were low among livestock but *Fasciola* infection was not seen in sheep. *Eimeria* infection was found comparatively higher in sheep than goats. Ova of *Gongylonema* were recorded from one cattle and *Strongyloides* were observed only in sheep. Low incidence of *Trichuris* infection was noticed in cattle, sheep and goats. *Strongyle* infection in livestock was found higher during southwest monsoon.

Umur and Yukuri (2005) investigated the gastro-intestinal (GI) organs of 50 goats in Burdur region, Turkey for the prevalence of GI nematodes and the seasonal activity of the parasites. All the animals examined (100%) were found to be infected with GI nematodes. Twenty-two nematode species were identified and a total of 53,759 nematodes were collected from the infected goats. The number of parasites per goat ranged from 65 to 4811 (mean 1075.18), while the number of nematodes species per animal ranged from 1 to 12 (mean 6.34). The most frequently detected nematodes in the goats were *Ostertagia circumcinta* (78%), *Marshallagia marshalli* (72%), *Nematodirus abnormalis* (66%), *Trichuris ovis* (60%), *Nematodirus spathiger* (52%), *Trichuris skrjabini* (50%) and *Trichostrongylus vitrinus* (40%). The parasite counts in the goats increased in spring, declined in summer, reached maximum levels in autumn, and then tended to decline until winter, before increasing again in mid-winter.

Waruiru *et al.* (2005) conducted a study on gastro-intestinal parasitic infection of sheep and goats in semi-arid area of Machakos district, Kenya. The overall prevalence were *Strongyloides* (51.6%), *Fasciola* spp. (31.5%), *Coccidia* (28%), *Moniezia* (2.5%). *Haemonchus* (58%) was the most prevalent nematode followed by *Trichostrongylus* (29%) and *Oesophagostomum* (13%). Yadav *et al.* (2005) reported the highest incidence of gastro-intestinal nematodiasis in goats followed by buffalo and cattle in India. *Haemonchus*, *Trichostrongylus*, *Bunostomum*, *Oesophagostomum* and *Strongyloides* species were the main parasites recovered from the intestine of sheep, goats and buffaloes.

Di Gerbo et al. (2006) carried out a survey of parasites in goat farms in Bergamo province, north Italy from May 2005 to Jan, 2006. Fecal samples of 836 adult female goats from 31 dairy goat farms were examined. Strongyloides spp., showed higher values of prevalence in goats housed in summer while Nematodirus in winter in goats at pasture. Strongyloides occurred more frequently in autumn in stabled goats. Lima et al. (2006) studied the faecal samples collected from 20 goats in Paulista, Pernambuco, Brazil, from August 1998 to July 1999. They were subjected to eggs per gram faeces (EPG) determination and nematode larvae culture. It was shown that 82% of the samples were positive for helminths. Strongyloides, Moniezia and Trichuris spp. ova were obtained in 72.8%, 8.4% and 2.0% of the samples, respectively, while third of Haemonchus, *Trichostrongylus* stage larvae and Oesophagostomum spp. were obtained from 75.13%, 24.32% and 0.54% of the samples, respectively. The medium number of Haemonchus and Trichostrongylus spp. larvae per gram faeces was higher in the rainy months. There was a significant correlation between EPG and temperature, EPG and rainfall and EPG and the number of Haemonchus spp. larvae per gram faeces. Haemonchus spp. was present throughout the study period.

Mungube *et al.* (2006) estimated the prevalence and economic losses caused by *Fasciola gigantica* and *Fasciola hepatica* in the ruminant production systems of Taveta division of Kenyain a retrospective appraisal of the slaughter records on the total number of animals slaughtered and livers condemned over the period 1989 to 2004. Liver condemnation rates differed significantly between bovines, caprines and ovines ($p \le 0.05$) for *Fasciola gigantica* (26%, 6.6% and 5.2%, respectively) and for *Fasciola hepatica* (0.4%, 22% and 28%). Highest infection was observed with *Paramphistomum cervi* (65.28%) and lowest infection with *Cotylophoron cotylophoron* (36.11). Mixed infections with two or more species of amphistomes were found in 60.42%. The prevalence of amphistomes was very high all the year round and the rate of infection was 83.64%, 69.23% and 64.0% during monsoon, winter and summer season respectively.

Bal et al. (2007) studied the parasitic gastroenteritis in sheep and goats in Punjab state, India. Chaudary et al. (2007) investigated the prevalence and seasonal trend of the Haemonchus contortus in sheep and goats in the Potohar areas of northern Punjab, Pakistan from December 2004 to January 2006. Faecal samples collected from 968 sheep and 961 goats of different breeds were examined. Results revealed that the infection was significantly (P<0.05) higher in sheep compared to goats. The peak infection level was recorded during rainy season (July-October). On the other hand, low infection level was noted from December to May. Menkir (2007) carried out a two year epidemiology study of helminthes of small ruminants. The study involved the collection of viscera from 655 sheep and 632 goats from 4 abattoirs in eastern Ethiopia. A further more detailed epidemiology study of gastrointestinal nematode infections used the Haramaya University (HU) flock of 60 Black Head Ogaden sheep. The parasitological data included numbers of nematode eggs per gram of faeces (EPG), faecal culture L3 larvae, packed red cell volume (PCV), adult worm and early L4counts, and FAMACHA eye-colour score estimates, along with animal performance (bodyweight change). There were 13 species of nematodes and 4 species of flukes present in the sheep and goat, with Haemonchus contortus being the most prevalent (65–80%), followed by *Trichostrongylus* spp. The nematode infection levels of both sheep and goats followed the bi-modal annual rainfall pattern, with the highest worm burdens occurring during the two rain seasons (May and September).

Nwosu *et al.* (2007) carried out a survey to determine the prevalence and seasonal abundance of the egg and adult stages of nematode parasites of sheep and goats in the semi-arid zone of north-eastern Nigeria between January and December 2002. Faecal samples collected from 102 sheep and 147 goats and

examined by the modified McMaster technique revealed that 44(43.1%) and 82(55.8%) of the samples, respectively, contained atleast one nematode egg type. Three nematode egg types were recovered with Strongyle egg type (22.5% in sheep and 35.4% in goats) being the most prevalent followed respectively by Trichuris (5.9% in sheep and 4.1% in goats) and Strongyloides (4.9% in sheep and 4.1% in goats) egg types. Mean faecal egg counts were generally moderate in both sheep (1052±922 Strongyle, 1000±590 Strongyloides and 380±110 Trichuris eggs, respectively, per gm of faeces) and goats (2092±3475 Strongyle, 958±854 Strongyloides and 683±512 *Trichuris* eggs, respectively, per gm of faeces). The prevalence and counts of *Strongyle* nematode eggs showed a definite seasonal sequence that corresponded with the rainfall pattern in the study area during the period. In both sheep and goats, counts of Strongyle egg type increased with the rain and reached peak levels at about the peak of the rainy season in September. The other egg types encountered during the study did not show much variation with the season of the year.

Odoi *et al.* (2007) investigated the burden and risk factors of gastrointestinal nematode parasite infections in sheep and goats kept in smallholder mixed farms in the Kenyan Central Highlands. 370 small ruminants were sampled from 66 smallholder mixed farms in agro-ecological zones 1 (humid) and 3 (semi-humid) in the Kenyan Central highlands. Fecal samples were collected at each visit from each animal. Faecal egg counts (FEC) were performed using the modified McMaster technique. Study investigated the burden and risk factors of gastrointestinal nematode parasite infections in sheep and goats kept in smallholder mixed farms in the Kenyan Central Highlands. Parajuli (2007) studied intestinal nematode parasite of goats (*Capra hircus*) and found 181 (81.53%) positive samples among 222 total samples from Khasi bazaar of Kalanki, Kathmandu.

Raza *et al.* (2007) studied to determine the prevalence of gastrointestinal helminthiasis in ruminants in an irrigated area of lower Punjab (Pakistan). For this purpose, 100 faecal samples were collected from sheep, goats, cattle and buffaloes. The overall prevalence of helminthiasis was 51% in cattle, 47% in buffaloes, 62% in sheep and 52% in goats, with nematodes being the most common helminths. The

prevalence of helminths was higher in young animals compared with adults in cattle, buffaloes, sheep and goats. The prevalence of different species of helminths also varied in different age groups, with *Toxocara vitulorum* being higher in calves than adults both in cattle and buffaloes. Sex-wise prevalence of helminths was higher in males than females for buffaloes and sheep in contrast to cattle and goats.

Al-Shaibani et al. (2008) investigate epidemiological study on gastrointestinal nematodes of sheep was carried out in farms of small farmers in Hyderabad (Pakistan) district from May 2004 to April 2005. Faecal egg counts, pasture larval counts and worm counts from permanent grazing animals were recorded for 12 months. Haemonchus contortus (24.6%) was found to be predominant of gastrointestinal nematode parasites, Trichostrongylus spp. (18.0%) was the next most prevalent species, others, including: Ostertagia circumcincta, Spathiger papillosus, Trichuris ovis, Oesophagostomum columbianum and Chabertia ovina were found in varying percentages. The highest faecal egg counts (FEC) were recorded in September, whereas the lower FEC were in February. Chavhan et al. (2008) studied the prevalence of nematode parasites of ruminants in two villages, viz. Chicholi and Bodala of Nagpur district. Out of 615 animals examined 242 were positive (39.34%) for nematode infection. The infection rate in buffalo, cattle and goat was 41.63%, 32.18% and 51.94%, respectively. Higher infection was recorded during monsoon (63.07%) followed by winter (32.22%) and summer (21.33%). The percentage of animals infected with Haemonchus sp., Toxocara sp., Trichuris sp., Strongyloides sp. and mixed infection was found to be 38.01%, 27.68%, 14.87%, 11.98% and 7.43% respectively.

Gadre *et al.* (2008) examined the 2288 faecal samples collected from dairy animal of central zone of vidarbha region (Maharashtra) from July 2002 to June 2003 revealed 62.98% prevalence of helminthic infection. *Paramphistomum sp.* were predominant (12.28%) fallowed by *Toxocara* (10.97%). The percentage prevalence of *Monezia, Strongyloides, Haemonchus, Fasciola, Schistosoma, Trichuris, Oesophagostmum* and *Trichostongylus* species were 8.96%, 6.99%, 5.98%, 3.81%, 1.87%, 1.00% and 0.96% respectively. The overall prevalence of nematodes, trematodes, cestodes and mixed type of helminth infection was found to be 41.63%, 11.11%, 0.98% and 46.28% respectively. The helminth infection was most common encountered during and after rainy seasons. The infected animals showed significant reduction in Hb, PCV, TLC, TEC, neutrophils while lymphocytes and monocytes count does not show significant results. Ijaz *et al.* (2008) carried out a study to find out the infection rate of gastrointestinal tract (GIT) helminthes and its association with diarrhea in goats in Lahore, Pakistan. For this purpose, 300 faecal samples from goats suffering from diarrhea presented at the Outdoor Hospital, Department of Clinical Medicine and Surgery, Lahore and various private as well as government hospitals located in Lahore were examined coprologically for the presence of helminths. The result revealed that an overall infection rate of GIT helminthes was 63.33% in goats. When compared the classwise infection rate, highest infection rate of nematodes (42.67%) was observed, followed by trematodes (16.67%) and cestodes (4%).

Jani (2008) examined faecal samples of 40 Indian elephants (Elephas maximus) revealed 62.5 percent parasitic prevalence. Amongst the single infection of parasites, high prevalence of *Fasciolia* spp. (15.00%) was observed followed by prevalence of mixed infection. Elephants harbouring parasites were found clinically dull, depressed and lethargic. About 48 percent elephants manifested dehydration and loose faeces grossly along with a habit of soil licking. Mir et al. (2008a) investigated the parasitological examination of 1,325 faecal samples collected from naturally grazing sheep in Kashmir Valley, India, was conducted to assess the prevalence of trematodes. The level of parasitism varied among 28.98% of the sheep that had at least one infection. Fasciola gigantica (23.92%) and Fasciola hepatica (9.96%) were predominant, while Dicrocoelium dendriticum (4.45%) and Paramphistomum cervi (2.71%) were also found. Seasonal variations indicate that highest infections were recorded during the summer (13.94 %) followed by autumn (7.38%), spring (6.06%) and winter (1.41%). Highest (42.8%) prevalence of trematode parasites was observed in sheep that were more than 4 years old (42.8%) followed by 2-4 (37.7%) and 0-2 years (18.79%) of age groups respectively. The faecal examination indicated higher percentage of infection in exotic breed compared to native breed.

Pathak and Pal (2008) investigated the prevalence of gastrointestinal parasites in goats and revealed that the percentage of overall prevalence of infection was 85.22%. The prevalence of different parasites encountered were Paramphistomum spp. (80.68%), Cotylophoron spp. (45.45%), Moniezia spp. (17.04%), Avitellina spp. (3.40%), Haemonchus sp. (26.13%), Trichostrongylus spp. (5.68%), Cooperia spp. (3.40%), Oesophagostomum spp. (30.68%), Bunostomum sp. (5.68%) and Trichuris sp. (27.27%). Seasonal prevalence was highest in monsoon (94.60%), moderate in summer (87.50%) and lowest in winter (63.15%). Rajapakse et al. (2008) collected and examined the gastrointestinal tracts of 218 crossbred goats representing the dry zone of Sri Lanka during a year study period. 217 (more than 99%) of the animals examined were infected with one or more species of nematodes. Five species of nematodes were found in the abomasums and intestines. They were **Oesophogostomum** columbiamum (88%), Haemonchus contortus (81%), Trichostrongylus columbriformis (76%), Trichostrongylus axei (59%) and Trichuris ovis (59%).

Shirale *et al.* (2008) investigated 350 fecal samples of cattle from representative area of Western vidarbha region around Akola and examined for incidence of gastrointestinal helminth infestation. Out of total 232, positive sample 62.29% had single and 6.00% had mixed infection of *Haemonchus* and *Trichris* spp. Nine species of intestinal helminths i.e. *Strongyle* sp. (19.39%), *Strongyloides* sp. (11.14%), *Trichoderma* sp. (8.28%), *Haemonchus* sp. (6.57%), *Trichuris* sp. (5.42%), *Trichostrongylus* sp. (4.85%), *Moniezia* sp. (4.18%), *Facsiola* sp. (3.71%) and *Coccidia* sp. (3.14%) were encountered as a common helminths in cattle. Seasonal prevalence revealed higher in rainy season and lower in winter. *Strongylus* sp. was the predominant helminth infection in all the seasons. The infection was observed higher in nematodes followed by cestodes and trematodes.

Tariq *et al.* (2008) investigate the seasonal epidemiological prevalence of gastrointestinal tract (GIT) nematodes in different age groups, sexes and breeds (genotypes) of sheep through necropsy and faecal analysis over a period of 2 years in Kashmir valley, India. A total of 1533 sheep were examined. The overall prevalence of GIT nematodes in sheep in year 1 was 64.76% and 58.37% in year 2 (P = 0.04). The parasites in decreasing order of prevalence (%) in sheep were

Haemonchus contortus (59.6); Ostertagia circumcincta (38.0); Bunostomum trigonocephalum (37.7); Chabertia ovina (37.7); Trichostrongylus spp. (33.9); Nematodirus spathiger (29.4); Oesophagostomum columbianum (28.4); Trichuris ovis (23.5) and Marshallagia marshalli (22.1). The maximum nematode infection was observed in summer season and lowest in winter (P = 0.0005). Local Kashmiri breed was less infected as compared to other genotypes (P > 0.05). Lower age groups were more infected than adult animals. Prevalence was higher in rams (males) than eves (females) (P > 0.05).

Gadahi et al. (2009) analysed the 400 faecal samples comprising of 90 samples from sheep and 310 from goats of Rawalpindi and Islamabad to confirm the presence of gastrointestinal parasitic infection. 254 (63.50%) samples were found positive for endoparasites. Among the samples from sheep 48 (53.33%) and 206 (66.45%) from goats were detected positive for gastrointestinal parasites. Trichuris sp., Haemonchus sp., Coccidia sp., Nematodirus sp. and Fasciola sp., were found with prevalence of 40.00%, 28.88%, 27.77%, 11.11% and 4.44% respectively in sheep. In case of goat the incidence of Haemonchus sp., Coccidia sp., Trichuris sp., Nematodirus sp., Trichostrongylus sp., Strongyloides sp. and Fasciola sp., were 64.19%, 43.87%, 35.48%, 13.00%, 4.51%, 3.22% and 0.66% respectively. Qamar et al. (2009) observed epidemiological studies of Haemonchosis in sheep and goats at slaughterhouses, livestock farms and veterinary hospitals under the different climatic conditions existing in Punjab province (Pakistan). Infection rate of haemonchosis was 35.44%, 38.04% and 36.83%, respectively in slaughtered sheep and goats, sheep and goats at livestock farms and at veterinary hospitals. Overall the highest (43.69%) seasonal prevalence in all types of sheep and Goats was recorded during summer; followed by autumn (38.46%), spring (37.12%), while the lowest (28.79%) was recorded during winter. It was noticed that animals of either sex are equally affected. A higher infection rate was recorded in animals below 9 months than above 9 months of age.

Abouzeid *et al.* (2010) studied the prevalence of gastro-intestinal tract (GIT) parasites in 240 sheep was conducted in different area in the zoo garden and in Sinai district during the period of March 2009 to February 2010. The overall prevalence of infections with nematodes, *Fasciola* and coccidiosis in sheep in Sinai and zoo

garden were (27.5%); (10.0%) and (6.7%) respectively. Lower age group animals were more prone to infection than the adults. Serum calcium, inorganic phosphorus, magnesium, copper and iron levels were significantly decreased in all parasitic infested animals. Sutar *et al.* (2010) examined the helminth parasites of digestive system of goats in Ahmednagar District of Maharashtra during the period January 2009 to December 2009. For these 400 faecal samples of goats from different villages were collected. Out of 400 samples 251 were positive (62.75%). In rainy season, out of 150 faecal samples examined 116 were positive (77.33%), while in winter out of 120 samples examined 73 were positive (60.83%) and in summer out of 130 samples examined 67 were positive (51.53%). The seasonal prevalence of gastrointestinal parasites shows higher prevalence in monsoon season (77.33%) followed by winter (60.83%) and summer (51.53%). The percentage of animals with different gastrointestinal helminth parasite species viz., *Haemonchus* sp. (24.25%), *Trichuris* sp. (18%), *Strongyloides* sp. (21.25%), *Moniezia* sp. (5.50%), and *Fasciola* sp. (9.25%).

Tariq *et al.* (2010) investigate the seasonal epidemiological prevalence of gastro-intestinal nematodes (GINs) of goats with respect to sex and age of the host in the Kashmir valley. A total of 1267 goats were examined. The overall prevalence of GIN infection in these animals was 54.3%. The different parasites reported with their respective prevalences (%) were: *Haemonchus contortus* (48.3); *Bunostomum trigonocephalum* (30.1); *Chabertia ovina* (29.8); *Ostertagia circumcincta* (29.8); *Nematodirus spathiger* (25.2); *Trichostrongylus* spp. (25.1); *Oesophagostomum columbianum* (23.5); *Trichuris ovis* (19.0); and *Marshallagia marshalli* (16.6). Infection rate was found maximum in summer and lowest in winter. No significant changes were observed in the GIN infection in goats among male and female. With the increase in host age, prevalence of infection decreased significantly.

Godara *et al.* (2011) studied the efficacy of fenbendazole, levamisole and ivermectin was checked in comparison to untreated controls in twenty Jamunapari goats, naturally infected with gastrointestinal nematode parasites. Faecal examination at day 0 revealed an egg per gram of 930 ± 175.1 , 1350 ± 421.1 , 1060 ± 224.9 and 800 ± 279.7 in group A, B, C and D, respectively having five animals each. The results of larval culture examination revealed the presence of *Haemonchus*

spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Bunostomum* spp., and *Strongyloides* spp., in these animals. Faecal egg counts of the animals treated with fenbendazole (group A), levamisole (group B) and ivermectin (group C) were reduced by 23.66%, 63.70% and 98.11%, respectively on day 14 post-treatment. Lone *et al.* (2011) studied the prevalence of coccidia and gastrointestinal nematode infections in Goats of Baramullah District of Kashmir Valley. *Haemochus contortus* was found to be most prevalent as it showed prevalence of 60% followed by *Trichuris ovis* (51%), *Oesophagostomum* spp. (45%) and *Chabertia* spp. (1%).

Nabavi *et al.* (2011) observed that Gastro-intestinal nematodes of small ruminants are one of the major causes of productivity loss. This study was carried out to determine the correlation between the prevalence, seasonal incidence and geographical distribution of abomasal worm infection of native sheep in 3 different climatic zones of Iran, suitable for animal husbandry. The overall percentage of infection was 30.98% and *Haemonchus contortus*, *Teladorsagia circumcincta*, *Marshallagia marshalli*, *Ostertagia occidentalis*, *Ostertagia trifurcata* and *Parabronema skrjabini* were 6 species identified in all 3 studied areas. Although *Teladorsagia circumcincta* was the most prevalent and frequent worm species found.

Naem and Gorgani (2011) studied to determine parasitic infection of sheep with gastrointestinal helminthes in a slaughter house in Fereidoonkenar city, Iran. A total number of 50 sheep were examined and the results showed that 70% of examined animals were infected as follows: *Ostertagia circumcincta* (38%) and *Marshallagia marshalli* (38%), *Trichostrongylus colubriformis* (16%), *Nematodirus spathiger* (14%), *Skrjabinema ovis* (12%), *Haemonchus contortus* (10%), *Camelostrongylus mentolatus* (4%), and *Gongylonema pulchrum* (2%), *Cooperia punctata* (2%), *Bunostomum trigonocephalum* (2%), *Chabertia ovina* (2%). Among examined animals, 14% infected with *Moniezia expansa*, 10% with *Avitellina centripunctata* and 2% with *Helicometra giardi*. The infection rate in younger animals was higher than in adults.

Wadhwa *et al.* (2011) examined 200 faecal samples comprising of 100 samples each from cattle and buffaloes from different locations of Bikaner, Rajasthan were analyzed to confirm the presence of gastrointestinal parasitic

infection. Twenty four (12.00%) samples were found positive for *Strongyle* eggs. 11% cattle and 13% buffaloes were found to be positive for gastrointestinal helminthiasis. The prevalence in cattle varied from 9.09% to 12.50% in different locations. Prevalence range was slightly higher in buffaloes which ranged between 10.52% to 14.81%. The estimation of EPG count for *Strongyle* species in cattle ranged between 200-1000, with an average of 504.00+245.41. This range was 200-1400 with an average of 684.61+350.82 in buffaloes.

Farooq et al. (2012) carried out to assess the prevalence of gastrointestinal helminths infections among wild and domestic ruminants in Cholistan desert of Pakistan. For this purpose, 1010 faecal samples of different species of ruminants including cattle (n=300), sheep (n=250), goat (n=100), camel (n=200), chinkara (n=150) and blackbuck (n=10) were examined using standard parasitological procedures. The highest prevalence was recorded in cattle (44.7%) followed by sheep (43.6%), goats (39%), camels (37%), chinkara (26.7%) and black bucks (20%). Maximum number of the helminth species were recorded in sheep (n=14)followed by camels (n=13), cattle (n=09), goats (n=08), chinkara (n=07) and black bucks (n=02). Nematodes were the predominantly occurring (n=18) helminths followed by trematodes (n=6) and cestodes (n=3). *Haemonchus* and Trichostrongylus were the most frequently recorded genera. It was concluded that wild and domesticated ruminants of the Cholistan desert of Pakistan suffer with heavy infections of a variety of helminths including those of high economic significance. Lone et al. (2012) aim of the study was to compare a prevalence of infections with flukes, tape worms and nematodes parasitizing gastrointestinal tract in small ruminants from various regions of District Ganderbal Kashmir. Visceral examinations from 284 sheep and 318 goats indicated a marked variation in the level of parasitism in livestock raised in different geographic areas. It was found that the prevalence gastrointestinal helminthic infections were higher in goats than in sheep. The most common prevalent nematodes were *Haemonchus* (82%), *Trichuris* (74%), Nematodirus (60%), Trichostrongylus (58%), Chabertia (52%), Strongyloides (42%), Oesophagostomum (46%). Among the cestodes Moneiza (48%), Avitellina (42%), Thysenezia (28%) were reported. Among the trematodes Fasciola (60%), Dicrocoelium (52%), Paramphistomum (46%) were most prevalent. The study

indicates the prevalence of gastrointestinal helminthic infections varies in different seasons and in different age groups.

Singh *et al.* (2012) examined 862 cattle for both haematological and coprological investigations at Ludhiana, Punjab, India. Examination of Giemsastained peripheral blood smears exhibited that 22.9 % of cattle were infected with haematozoa comprising *Theileria annulata* (14.65 %), *Trypanosoma evansi* (0.28 %), *Babesia bigemina* (1.56 %) and *Anaplasma marginale* (8.53 %) while mixed infection appeared in 2.13 % animals. The prevalence of total haemoparasites and *Anaplasma marginale* infections were significantly higher in younger animals <1 year of age whereas, *Trypanosoma evansi* and *Anaplasma marginale* infections were significantly higher in males. Coprological examination revealed that the overall prevalence of gastrointestinal (GI) parasitic infection was 16.98 %.

2.2. Haemato-biochemical Review

Ahmad *et al.* (1990) studied the serum protein changes of lambs experimentally infected with *Haemonchus contortus* infection and reported a marked decrease in albumin, whereas α -globulin and β - globulin increased at peak of infection. Kassi *et al.* (1990) studied the relationship between haemoglobin genotype and the innate resistance to experimental haemonchosis, and the results were assessed by the help of FEC, worm count, PCV, Hb%, total protein and IgG, and suggested that the responsivenesss to nematode infection is under the control of genes. Rahman and Collins (1990) studied the change in live weight gain, blood constituents and worm egg output in goats artificially with a sheep-derived strain of *Haemonchus contortus*, and reported anemia and reduction in plasma protein level.

Abdel Ali (1992) conducted the hematological studies on naturally infected sheep with *Strongyloid* and reported normochromic anemia associated with eosinophilia in infected sheep. Mottelib *et al.* (1992) studied the effect of gastrointestinal parasites on blood picture in sheep and goats at Al-Gassim, and found that the clinical signs like anemia, emaciation, weight loss and diarrhea were caused by nematodiasis. They also reported that the decrease in RBC count, Hb%, PCV and lymphocytosis are directly proportional to the nematode infection.

Taimur *et al.* (1993) carried out haematological studies on cattle exposed to *Fasciola gigantica* infestation. They observed significant decline in total erythrocyte count, Hb level, packed cell volume (PCV) and mean corpuscular volume, Hb concentration and significant increase in erythrocyte sedimentation rate.

Chakarborty and Lodh (1994) studied the blood biochemical profiles in fascioliasis, haemonchosis and dictyocaulosis in goats and they recorded a decrease in the total serum protein and serum albumin and marked increase in serum globulin concentration in all infected goats.

Amarante *et al.* (1998) used the nematode egg count, PCV and body weight as parameters for identification of sheep resistance and susceptibility to gastrointestinal nematodes.

Maiti *et al.* (1999) have reported a decrease in the TEC, total plasma protein and and Hb%, but increase in the eosinophil number in the parasitic gastroenteritis in sheep. They recorded abnormal haemogram in highly infected sheep. Parangama *et al.* (1999) reported that the elevation in serum pepsinogen is a diagnostic index in haemonchosis of goats; they noticed that *Haemonchus contortus* burden was directly proportional to the serum pepsinogen levels in the blood. Hence it was concluded that serum pepsinogen concentration is a moderate sensitive marker of haemonchosis in goats and could be used as an adjunct in the diagnosis of the diseases. Stear *et al.* (1999) studied the relationship between the number and the size of nematodes in the abomasums and the concentration of pepsinogen in ovine plasma, and found that plasma pepsinogen level is related with the length of the *Ostertagia circumcincta*.

Egbe-Nwiyi *et al.* (2000) studied that the influence of age and sex on the heamatological values of goats and sheep studied in the arid zone of Borno State of Nigeria. Age and sex remarkable influence (P<0.05) on the red blood cell (RBC) counts of goats. Age influenced (P<0.05) the haemolglobin (Hb) and the packed cell volume (PCV) values. Age and sex greatly influenced (P<0.01) the mean corpuscular volume (MCV) values. Mean corpuscular haemoglobin concentration (MCHC) was influenced by age. Lymphocytes constituted more than 60% of the total white blood cell (WBC) counts in male and female goats. Neutrophill and eosinophil counts were influenced by sex and age. Sex influenced (P<0.05)

monocyte and lymphocyte values in goats. Abdel *et al.* (2002) made haematological estimations and faecal egg counts of 32 goats and 43 camel sampled from different wadis of St. Katherine Protectorate, Sinai, Egypt and showed significant differences in platelet counts, mean corpuscular haemoglobin concentrations, total leukocyte counts and percentage of eosinophils in goats. The prevalence of parasitic infection showed that 15% of the camels were infested with gastrointestinal helminths whereas 24% of goats were infested.

Natter *et al.* (2003) studied the response of wool sheep and hair sheep against experimental *Haemonchus contortus* infection. Body weight, FEC, PCV of hair sheep and wool sheep were compared over 8 weeks after experimental infection. Hair sheep were reported to have no ill effects of *Haemonchus contortus* even after establishment of *Haemonchus contortus* infection. They concluded by reporting that Caribbean Hair breeds of sheep may be able to contribute significantly to development of parasitic resistant sheep population.

Aatish et al. (2007) carried out to assess the prevalence of mange mite infestation in district Dera Ghazi Khan (D.G. Khan) and to investigate the effect of sheep mange on different blood and biochemical parameters. In mite infested animals, total erythrocyte count (TEC), hemoglobin (Hb) and packed cell volume (PCV) was found to be lower, while erythrocyte sedimentation rate (ESR) and total serum proteins were higher as compared to healthy animals. Additionally, eosinophilia was also observed in infested sheep. Akhtar et al. (2007) studied to determine the haematological and biochemical changes that occur in buffaloes with parturient haemoglobinuria (PHU). For this purpose, serum samples from 60 PHUaffected and 60 apparently healthy buffaloes were collected and analysed. Mean erythrocyte count $(3.6 \pm 1.0 \ 1012/l)$, haemoglobin concentration $(5.8 \pm 1.4 \ g/dl)$, and haematocrit (16.9 \pm 2.8%) of the PHU-affected buffaloes were lower (P < 0.001), while their erythrocyte sedimentation rate (104.1 \pm 36.2 mm/h) was higher (P < 0.001) in comparison to the healthy buffaloes. Neutrophils $(43.0 \pm 4.5\%)$, urea (49.7) \pm 7.8 mg/dl) and creatinine (2.1 \pm 0.4 mg/dl) concentrations were significantly higher in the PHU-affected buffaloes, while lymphocytes (48.7 \pm 2.9%) and erythrocytic glucose-6-phosphate dehydrogenase (G6PD) (92.3 \pm 13.2 mU/109 TECs) were lower than in the healthy buffaloes. It was concluded that PHU affected

buffaloes usually suffer from severe anaemia and hypophosphataemia, and erythrocytes with significantly reduced G6PD are prone to haemolysis, leading to haemoglobinuria in buffaloes.

Teleb et al. (2007) investigated hematological, serum biochemical and histopathological changes in twenty five months old Farafra sheep. The hematological study showed a significant decrease (P < 0.05) in red blood cell (RBC) counts, hemoglobin (Hb) concentration, percentage of packed cell volume (PCV %) and monocytes counts in sheep infected with Fasciola gigantica compared to the control. Moreover, white blood cell (WBC) counts, eosinophil and neutrophil counts were significantly higher (P < 0.05) in infected groups than the control. In addition, the biochemical investigations revealed a significant decrease in serum total protein and albumin levels in infected sheep groups compared to control group. The significant hypoproteinaemia and hypoalbuminaemia recorded in the infected groups were accompanied with significant hyperglobulinaemia. Moreover, significant elevations in serum total bilirubin, activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT) were also observed in infected sheep comparing with control one. In addition, serum urea and creatinine levels were significantly higher in infected sheep than the control.

Jain (2008) investigate the haematological studies of elephants harbouring parasites and revealed mild anaemia and eosinophilia where as biochemical studies revealed non-significant hypoproteinemia on comparison with elephants that were not harbouring parasites. Mir *et al.* (2008b) studied the influence of *Haemonchus contortus* on haematological profile and occular mucus membrane colour of sheep from March 2005 to December 2005 under controlled condition. Eight local sheep were used for experiment and were divided in two groups. Group 1st animals were kept as control and group 2nd animals were infected orally by L₃ larvae of *Haemonchus contortus*. After the establishment of infection the faecal samples were regularly screened for nematode eggs and eyes were examined for mucous membrane colour. Blood samples were received from both the groups of animals for haematological studies. Lower haematocrit values and paler colour of eyes was observed in infected sheep compared to control. Raised ESR, decreased RBC count

and Hb values were observed in infected animals corresponding to control.

Nazifi *et al.* (2009) collected blood samples from 67 adult Iranian dromedary camels naturally infected with *Mycoplasma* spp, and a control group comprised 20 healthy dromedary camels. Haematological and serum biochemical parameters were measured using standard techniques. In Giemsa-stained peripheral blood smears, *Mycoplasma* appears attached to the surface of erythrocytes. In infected camels, the number of red blood cells, haemoglobin concentration and haematocrit (packed cell volume) significantly decreased (P<0.05). With regard to the values of mean corpuscular volume and mean corpuscular hemoglobin concentration, a normocytic and normochromic anaemia was observed in infected camels. In infected camels, the concentration of serum glucose was significantly lower as compared with controls (P<0.05).

Olayemi *et al.* (2009) studied the effect of management practices and sex on the hematological parameters of the West African Dwarf (WAD) goat. Hematological values of this breed of goat were evaluated under the intensive and extensive systems of management. The intensively managed animals had significantly higher (p < 0.01) erythrocyte, total white blood cell, lymphocyte and eosinophil counts than goats managed extensively. Similarly the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were significantly higher (p < 0.01) in the intensively managed goats. Both groups of animals however had similar monocyte counts, basophil counts, haemoglobin concentrations, packed cell volume and mean corpuscular haemoglobin concentration.

Abouzeid *et al.* (2010) studied the serum biochemical parameters of helminth infected sheep. The results showed that serum calcium, inorganic phosphorus, magnesium, copper and iron levels were significantly decreased in all parasitic infested animals. All treated sheep showed significant improvement. Addas *et al.* (2010) studied haematological studies of common indigenous goat breeds found in Mubi area kept under varying husbandary conditions and observed that haematological parameters were influenced by breed, sex and age. Significant (P<0.001) breed, sex and age differences were evident on packed cell volume (PCV). West African Dwarf (WAD) goat had highest (57.44±1.11) value, while

similar values were observed on other breeds: Sokoto red (SR) goat (31.31±0.87%), Kano brown (KB) goat (30.87±0.56%), Borno white (BW) goat (31.74±0.93%). Males had higher values than females on most parameters. Significant sex variation were recorded as male goats having highest values of PCV, Red blood cell count (RBC) and mean corpuscular volume (MCV).

Gwaze *et al.* (2010) studied the effect of season on faecal egg counts and biochemical profiles in indigenous Nguni goats of South Africa. Blood was analysed for packed cell volume (PCV), glucose, cholesterol, total protein, albumin, globulin, urea and creatinine. Significantly higher total protein and globulin values were recorded in the wet than the dry season. A significant positive correlation was recorded between body condition scores and albumin concentrations. Season had an effect on glucose, globulin, TP, creatinine, PCV and FEC of Nguni goats. Jain and Shani (2010) studied to assess the biochemical changes in goats treated with anthelmintic indigenous herbs. The analysis of data was done in 18 goats, irrespective of age, sex and breed. The experimental goats were randomly divided in six groups. The effect of crude powder and cold aqueous extract of *Nigella sativa*, *Swertia chirata* and *Piper longum* was studied on various biochemical parameters, i.e., Blood glucose, Total protein, Albumin and Globulin. Significant increase was noticed in the level of blood glucose, serum total protein and albumin.

Piccione et al. (2010)studied to determine haematological, haematochemical, and electrophoretic reference values for the Girgentana goat in order to form a basis for clinical interpretation. The study included 348 female Girgentana goats aged from 1 to 6 years. Red blood cell, white blood cell, neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts, and haemoglobin (Hb), packed cell volume percent (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW) were recorded. Additionally, the concentration of some haematochemical parameters, including alanine-aminotransferase (ALT), aspartate aminotransferase (AST), y-glutaminetransferase (γ -GT), creatinine, β -hydroxybutyrate, urea, nonesterified fatty acids (NEFA), potassium, sodium, and chloride, and the electrophoretic profile (albumin,

 α 1-globulins, α 2-globulins, β -globulins, γ -globulins, total proteins and albumin: globulins ratio) were determined. Roy et al. (2010) studied a total of 30 cows ranging between 3 to 9 years of age were selected for this study. Cows were divided into three groups, each consisting of 10 animals. The mean values of the different red blood cell count and white blood cell parameters showed significant changes during gestation period in Cows. RBC, PCV and Hb increased while total WBC and segmented neutrophils increased after the second gestation period in the second period. MCV showed significant difference in the first and second period of gestation. The numbers of total leucocytes and segmented neutrophils increased after the second period. An elevation in PCV appeared in the second gestation period, which was significantly different only compared to the third period. Hb concentration was also increased in the second period and differed significantly from the third period.

Sulaiman *et al.* (2010) examined the 175 native goats, 27 were infected with *Babesia ovis*, *Babesia motasi*, *Babesia foliata* and *Babesia taylori*, (recorded in Mosul for the first time) and 25 were clinically normal and served as control. Results indicated that the percentage of the infection with babesiosis was 15.42% and the percentage of parasitemia ranged between 3.5-10.4% with a mean 6.95%, infected goats showed signs of loss of appetite, weakness, pale mucous membranes, jaundice, fever, coughing, nasal discharge, recumbency, diarrhea and haemoglobinuria. Significant decrease was recorded in total red blood cells (RBC), haemoglobin concentration (Hb), packed cell volume (PCV) and platelets counts. Results of the biochemical tests indicated an increase in activity of alanin amino transferase (AST), aspartate amino transferase (ALT), total bilirubin, blood urea nitrogen and icterus index, with a significant decrease in total serum protein, albumin and globulin levels.

Bhat *et al.* (2011) investigated to carry out a comparative study to investigate nutrient deficiency, physiological and health status of Kashmiri goats in different climatic conditions. In this study haematological and biochemical parameters of Kashmiri goats were determined in 100 goats consisting of 50 adults (15 bucks male & 35 does female) and 50 young goats (15 buck-kids & 35 doe kids). The means for

packed cell volume (PCV), total white cell (TWC), red blood cell (RBC) and haemoglobin (Hb) were 29.4 \pm 0.8%, 13.5 \pm 0.8 \times 10³ ml, 11.5 \pm 0.4 \times 10⁶ ml and 9.8 \pm 0.3g/dl respectively. There are more lymphocytes (65.8 \pm 1.1%) than neutrophils (33.5 \pm 1.7%) in circulation. The values obtained for serum sodium, serum total protein and serum urea levels were 135.1 \pm 1.7mmol/L, 7.1 \pm 0.1g/100ml and 2.7 \pm 0.3mmol/L respectively. The values obtained for the serum transaminases; serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were 8.9 \pm 0.9IU/litre and 20.9 \pm 1.2IU/litre respectively; while alkaline phosphate (ALS) was 10.7 \pm 1.2IU/litre. There were significantly (p<0.05) higher Hb, red blood cell (RBC) and mean corpuscular haemoglobin concentration (MCHC) in adult goats. Lymphocytes percentage was higher (p<0.05) in male goats.

Igado et al. (2011) investigated the hematological and biochemical changes at different stages of gestation was conducted using ten intact adult female West African Dwarf goats (Capra hircus L). There were no statistically significant differences (p>0.05) between the mean values of the packed cell volume (%), hemoglobin (g/100 mL) and mean corpuscular hemoglobin concentration (g/mL) when the values before gestation (day 0) were compared with the values at day 50, day 100 of gestation and first day of parturition. Erythrocyte values at day zero differed significantly (p<0.05) from the values at day 100 and at parturition while the total leukocyte count at day zero differed significantly (p<0.05) from the values at day 50, 100 and day of parturition. Liver enzyme assay showed no significant difference (p>0.05) in the values obtained for alkaline phosphatase (ALP), while serum glutamic oxaloacetic transaminase (SGOT) on day zero(111.50±15.42 iu/L) was significantly higher (p<0.05) than day 50 (124.50±24.30 iu/L) and day 100 (86.50±18.14 iu/L); serum glutamic pyruvic transaminase (SGPT) value on day zero (17.00±4.32 iu/L) was significantly lower (p<0.05) than the value at parturition (50.50±15.78 iu/L). In conclusion, WAD goats may be susceptible to liver damage during gestation.

Bordoloi *et al.* (2012) studied changes in the haemato-biochemical pattern due to experimentally induced haemonchosis in Sahabadi sheep. Results show decrease in Hb concentration, PCV, TEC and serum protein. There was also a significant increase in serum enzymes level in infected sheep compared to uninfected control. Anemia and severe damage to abomasal mucosa occurs due to lower serum protein and higher enzyme activities. Esmaeilnejad et al. (2012) studied to evaluate the effect of babesiosis on some hematological and biochemical parameters in infected small ruminants with Babesia ovis. A total of 280 sheep and 122 goats from 40 herds were randomly examined for the presence of Babesia ovis in blood samples. Of 402 samples, 67 animals (16.7%) were positive for Babesia ovis of which 52 (18.5%) were sheep and 15 (12.2%) goats, respectively. The infected animals were divided into four subgroups according to parasitemia rates (<1%, 1%, 2%, and 3%). As a control group, 67 uninfected animals were also selected from the same farms. With increase in parasitemia rates, hemoglobin concentration (Hb), packed cell volume (PCV), red blood cells (RBCs), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) significantly decreased (P < 0.05), while, total leukocyte count, number of lymphocyte, monocyte, neutrophil and eosinophil showed a significant increase (P <0.05). Infected animals presented a significant elevation (P < 0.05) of total proteins and significantly lower level (P < 0.05) of albumin compared to non-infected animals. Significant elevation (P < 0.05) of creatinine, cholesterol and triglyceride level were found with parasitemia progression.

Opara *et al.* (2012) studied the blood chemistry and other haematological parameters of 130 West African Dwarf (WAD) goats consisting of 60 adults (30 bucks and 30 does) and 70 young ones (56 buck-kids and 14 doe-kids) were determined. Male WAD goats had significantly (p < 0.05) higher lymphocytes, neutrophil and WBC than the females, while other parameters were similar. There was significantly (p < 0.05) higher percentage of PCV, Hb and RBC in female WAD goats than the males. The WBC, MCV and MCH were significantly higher in the male WAD goats.

World	India	Kashmir
Hsiang et al. (1990);	Pathak and Pal (2000);	Sheikh et al. (2004);
Mattos (1991);	Jithendran and Bhat (2001);	Mir et al. (2008a);
Lepojev et al. (1992);	Dhand <i>et al.</i> (2004);	Tariq <i>et al</i> . (2008);
Pal and Qayyum (1992);	Das <i>et al.</i> (2005);	Tariq <i>et al</i> . (2010);
Thakur <i>et al</i> . (1992);	Muraleedharan (2005);	Lone <i>et al</i> . (2011);
Frutshchi et al. (1993);	Yadav et al. (2005);	Lone <i>et al.</i> (2012);
Hoste and Chartier (1993);	Bal <i>et al.</i> (2007);	
Dorny et al. (1995);	Chavhan <i>et al.</i> (2008);	
Ndao et al. (1995);	Gadre et al. (2008);	
Rafique and Hayat (1997);	Jain (2008);	
Vaughan et al. (1997);	Pathak and Pal (2008);	
Gatongi <i>et al.</i> (1998);	Sutar <i>et al.</i> (2010);	
Thamsborg et al. (1998);	Wadhua et al. (2011);	
Valcarceli and Romero (1999);	Singh <i>et al</i> . (2012);	
Astiz et al. (2000);		
Brunn et al. (2000);		
Silvestre et al. (2000);		
Vatta and Krecek (2000);		
Githigia et al. (2001);		
Sharkuu (2001);		
Magona and Musini (2002);		
Mazyzd and El-Nemr (2002);		
Love and Hutchinson (2003);		
Regasa et al. (2003-04);		
Mbae et al. (2004);		
Molina et al. (2005);		
Umur and Yukuri (2005);		
Waruiru et al. (2005);		
Di Gerbo et al. (2006);		
Lima et al. (2006);		

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Mungube <i>et al</i> . (2006);	
Menkir (2007);	
Chaudary <i>et al.</i> (2007);	
Nwosu et al. (2007);	
Odoi et al. (2007);	
Parajuli (2007);	
Raza et al. (2007);	
Al-Shaibani et al. (2008);	
Ijaz <i>et al.</i> (2008);	
Rajapakse et al. (2008);	
Shirale et al. (2008);	
Gadahi et al. (2009);	
Qamar et al. (2009);	
Abouzeid et al. (2010);	
Godara et al. (2011);	
Nabavi et al. (2011);	
Naem and Gorgani (2011);	
Farooq <i>et al</i> . (2012);	

World	India	Kashmir
Ahmad et al. (1990);	Chakerborty and Lodh	Mir <i>et al.</i> (2008b);
Kassi et al. (1990);	(1994);	Bhat <i>et al.</i> (2011);
Rahman and Collins (1990)	Jain (2008);	
Abdel Ali (1992);	Jain and Shani (2010);	
Mottelib et al. (1992);		
Taimur et al. (1993);		
Amarante et al. (1998);		
Maiti et al. (1999);		
Parangama et al. (1999);		
Stear et al. (1999);		
Egbe-Nwiyi et al. (2000);		
Abdel et al. (2000);		
Natter et al. (2003);		
Aatish et al. (2007);		
Akhtar et al. (2007);		
Teleb et al. (2007);		
Nazifi et al. (2009);		
Olayemi et al. (2009);		
Abouzeid et al. (2010);		
Addas et al. (2010);		
Gwaze et al. (2010);		
Piccione et al. (2010);		
Roy et al. (2010);		
Sulaiman et al. (2010);		
Igado et al. (2011);		
Bordoloi et al. (2012);		
Esmaeilnejad et al. (2012);		
Opara et al. (2012);		

Overview of the Literature of Haemato-biochemical Profile

<u>CHAPTER – 3</u> Material and Methods

he present study was aimed to carry out the epidemiological and haematobiochemical studies in goats of South Kashmir from December 2011 to November 2012. During the present study a total number of 340 local goats (uninfected and infected) of different age groups, gender were examined for the haemato-biochemical studies. Out of 340 samples, 198 (58.23%) were found infected with helminth infection. The methodology applied during current research work is given as below.

III. Collection and examination of faecal samples of goats for eggs/larvae

The accurate and the proper examination of faeces provide evidence for, or at times an accurate identification of most of the parasites that inhabit the host and discharge their eggs along with the faeces. The parasites of the respiratory tract were also diagnosed by faecal examination because the sputum that contains eggs and parasites is swallowed in most of animals.

a) Collection of faeces

The fresh faecal samples were collected from the rectum of goats for parasitological examinations. Suitable containers like screw-capped wide mouthed glass bottles were used for collection of faecal samples and transported to laboratory for further analysis. Sometimes plastic bottles and polythene bags were also used. These were made air tight as much as possible in order to prevent the rate of development and hatching of eggs. The samples collected from the remote areas were preserved in 4% formalin and then transported to the laboratory. 10 to 15 gms of faecal samples were collected each time and were kept in refrigerator before the further analysis.

b) Examination of faecal sample

1.1 Gross examination

The color, consistency, presence of blood, mucous, tapeworm segments and dead worms were looked before proper examination of faecal sample. Before examining the faecal samples microscopically, the macroscopic examination was done by naked eyes. The gross examination sometimes revealed adult parasites. This method was used as per Soulsby, 1982. Examination of faeces for helminth parasite eggs may vary from a simple direct smear to more complex methods involving centrifugation and the use of floatation techniques.

The faeces of the goats is a dark coloured with a peculiar smell and contains a lot of vegetable fibres, grains, plant hairs, spores and animal debris that resembles the parasitic forms (pseudoparasites), thus become difficult for the proper examination of the infection.

1.2 Examination of direct smears

This method was found useful only in the cases of heavy infections, in this method a small portion of faecal matter was directly placed on a grease free slide by a glass rod and a drop of saline was added to make a uniform suspension and then a cover slip was placed onto the smear and the slide was examined under low power microscope (X10).

1.3 Concentration methods

The concentration method was done in order to separate the parasitic objects from the bulk of the material in the specimen. For this purpose two methods were employed, the sedimentation and the floatation techniques.

1.3.1 Concentration by sedimentation

This method was found reliable for all types of parasitic eggs. 4 to 5 gms of faecal sample were taken and thoroughly mixed with 10 to 15 ml of water, the emulsion was strained through a sieve to remove all coarse particles. This filtrate was poured into a centrifuge tube. The centrifugation was done at the speed of 1500 rpm for five minutes. After centrifugation the supernatant was discarded and the

sediment was examined microscopically (10X) by placing a drop of sediment on slide and covered with a cover slip.

1.3.2 Concentration by floatation

This was done by simple test tube floatation method (Maplesstone and Bhaduri, 1940). This method was found very useful in the examination of Nematode infection. Light infection was invariably detected by this method. This method is based on the principle that lighter eggs float onto the surface of saturated solution and the eggs were easily skimmed out of the surface film. The most commonly used suspending media were:

- 1. Saturated solution of common salt
- 2. Zinc sulphate 32% solution
- 3. Saturated sugar solution
- Saturated solution of sodium nitrate The following techniques were employed.

Will's floatation technique

In this technique floatation was done in tubes which were filled upto one-third (1/3) with thick emulsion of faeces, rest of the tubes were filled upto the brim with the saturated solution of common salt till a convex surface was formed at the top. This floatation tube containing the faeces and the saturated solution was allowed to stand for half an hour undisturbed. A cover glass was placed onto the surface of fluid after this the cover glass carrying a drop of solution was placed on a slide and was examined for parasitic eggs under low power microscope (10x and 40x).

1.4 Faecal egg count

The faecal egg counts were of great importance in experimental and diagnostic work in which the comparison of counts in various goats provides a great information on worm burden, and only those animals having a high number of eggs per gram (EPG) of faeces were selected for hematological and biochemical studies. A number of methods have been suggested to determine eggs per grams of faeces but only McMaster's egg counting technique was employed for counting of eggs.

McMaster's egg counting technique

This was done by modified McMaster Counting Chamber as described by Urquhart *et al.* (1996). This technique is useful in the determination of number of eggs per gram (EPG) of faeces. In this technique 3 gms of faecal sample were taken and 42 ml of floatation solution is added to the sample and thoroughly mixed so that it forms a homogenous mixture. The solution is then transferred through a sieve in order to remove the coarse particles and filled in test tube and centrifuged at 2000 rpm for 2 minutes. The supernatant was poured off and the sediment was agitated again and filled the tube to the previous level with floatation solution. The tube was inverted many times and fluid solution was poured in both chambers of McMaster slide with the help of pipette and no fluid was left in the pipette, as the eggs would rise quickly in the floatation fluid. One chamber of slide was examined and numbers of eggs were multiplied by 100 to determine eggs per gram (EPG).

II. Collection of gut contents and various visceral organs from the slaughtered goats at various local abattoirs

The gut contents of the freshly slaughtered goats and the visceral organs from the local abattoir were collected and brought to the laboratory and observed minutely for various helminth parasites. The main sites of gut observed for various parasitic infections were abomassum, small intestines and large intestines. The other organs scanned for various helminth parasites were liver, lungs and brain. The parasites found were kept in different tubes. Various slaughtered houses were selected for collection of infected and uninfected gut material across the study area. The infected parts of gut were carried to the laboratory for further investigation. The whole gut content of the goats were observed for different helminth parasites. The nematode parasites were preserved in 70% alcohol (Mayer and Olsen, 1975).

Laboratory Investigation

The different organs collected were separately scanned.

Digestive tract

After removal, it was further cut into various portions such as oesophagus, stomach (rumen, reticulum, abomassum and ommassum), small intestines, colon, caecum, rectum etc. Before cutting each portion was tied with twine on either ends to prevent its contents to mix with those of other parts. Each portion was incised lengthwise and its contents were dislodged by flushing with water into the tray. Hold the cleaned portion against natural or artificial light (electric-lamp) to look for parasites embedded in the mucosa (e.g., *Strongyloides papillosus*) or in nodules. As a matter of routine, each part was scrapped with a scalpel down to the muscular layer and scrapings collected in the same tray with intestinal contents. Stir the suspension of intestinal contents and scrapings thoroughly and allow it to stand for a few minutes (such as 15 minutes). This practice was repeated by adding water and decanting the supernatant without disturbing the sediment. The parasites visible to naked eye were picked from the sediment with a hair brush or a crooked needle. Some suspension was poured into a petridish and examined under a dissecting microscope for the presence of small parasites.

Liver

It was incised first along the bileduct/vein, this will permit the parasite (e.g., *Fasciola* sp.) to escape out into the tray. Next it was incised or sliced in saline water and the suspension be examined under the dissection microscope or with a magnifying glass for small parasites (e.g., *Dicrocoelium* sp.).

Lungs

They were opened and examined along with trachea, bronchi and alveoli for lung worms.

III. Processing of Collected Material

(A) **Preparation of permanent slides of nematodes for study:** The nematodes after recovered from the infected organ were processed as per the following steps.

(i) Fixation: The nematodes on collection were transferred immediately to normal saline to free them of any foreign material or debris, if any kept in water for a long period, their cuticle is likely to burst and damage the specimen. They were then killed and fixed in hot 70% alcohol. Care was taken to prevent distortion of specimen during fixation. Hot 10% or 4% formalin can also be used for fixation of nematodes.

Large nematodes were dropped into acetic alcohol (1 part of glacial acetic acid and 3 parts of 95% alcohol), which result in a little less shrinkage than alcohol alone.

The heated solution causes worms to straighten instantly and die in that position, thus avoiding the curled and distorted specimens obtained when using cold fixatives.

(ii) **Preservation:** The nematodes were preserved in 70% alcohol or 4% formalin or 10% formalin. If the material is to be stored for sometime (month or longer) before being used, it is recommended that 70% alcohol to which 5% glycerine has been added.

Preservatives and fixatives used:

(a) 70% Alcohol			
Composition			
Absolute Alcohol	:	70 ml	
Distilled Water	:	30 ml	
(b) 4% formalin			
Composition			
Formalin (40% form	nald	ehyde) :	4 ml
Distilled Water		:	96ml

(iii) Storage: Each vial material should contain a note bearing the following information (a) scientific name of the host from which nematode was collected (b) locality from were host is collected (c) location of nematode parasite within host (d) fixative reagent used (e) date of autopsy (f) authors name. Specimens without complete accompanying data are worthless.

(iv) Clearing of nematodes: The almost impervious cuticle of adult nematodes makes it extremely difficult to prepare them as whole mounts by the methods used for flukes and tapeworms. The methods were generally studied microscopically almost entirely as cleared, unstained specimens in various media. Glycerin was found as good clearing agent for small parasites but its action was slow. The nematodes were generally cleared by putting them into lactophenol in a cavity block for 30 minutes to 2 hours. Lactophenol helps in quick clearing.

Composition of Lactophenol:

Distilled water	:	20 ml
Glycerine	:	40 ml
Lactic acid	:	20 ml
Phenol (melted crystals)	:	20ml

The solution was kept in a brown bottle or in a dark place, because exposure of light causes it to turn yellow.

Mounting of Nematodes

For semipermanent mounts of nematodes glycerine was used and for permanent mounts glycerine jelly was used.

Glycerine is an excellent mounting medium and is used extensively by nematologists. Nematodes were transferred directly from the fixative into a small amount of the following mixture: 2ml of 95% alcohol, 1ml of glycerine and 79ml of distilled water.

For permanent mounts glycerine jelly were used. The composition of which is given as below.

Composition

Gelatin	: 7gm
Distilled water	: 40ml
Phenol	: 1gm
Glycerine	: 50ml

Procedure

Soak 7gm of granulated gelatin in 40ml of distilled water for 30 minutes. Then melt in a warm water bath and filter through several layers of cheesecloth previously moistened with hot water. Finally, dissolve 1gm phenol in 50ml of glycerine and add to the gelatin. Stir until the mixture is homogenous.

Before mounting, the jelly was heated in a water bath. The nematodes are carefully placed in the medium and a cover slip was drawn over it. The jelly was allowed to coagulate and the slides were ringed with nail polish.

Glycerin Mounts

Glycerin was found best for temporary preparations, a 1:1 mixture of glycerin in water was generally used. The material was first gradually infiltered with glycerin before mounting to prevent distortion. Infiltration was accomplished by placing the material in a mixture of water and glycerin or alcohol and glycerin and water or alcohol was evaporated off. Evaporation was hastened by warming.

Following steps were used for mounting the material in glycerin.

- 1. A cell was prepared on the center of a clean slide. For temporary mounts, the cell wall was prepared with warm paraffin or glycerin jelly. Round cell were prepared by dipping the open end of the vial of a suitable size into the melted ringing solution and then transferred the ring of a solution to the slide. For permanent preparation the cell wall was prepared with varnish or balsam.
- 2. A small amount of glycerin was placed in the center of the cell. The material was placed in the cell.
- 3. A clean cover-glass filmed with moisture (by holding it over a beaker of steaming water) was lowered on it. The moisture film helped to prevent bubbles.
- 4. The preparation was sealed by a quick drying paint like nail polish.

Glycerin Jelly Mounts:

Glycerin jelly preparations were preferred over glycerin for permanent preparations. Following steps were used for mounting in glycerin jelly.

- 1. Placed a clean slide on a warming plate $(40^{\circ}-55^{\circ})$.
- 2. Some glycerin jelly was melted in a hot water-bath. Plenty of time was allowed for the solution to liquify so that bubbles if any to escape from the solution.
- 3. Air was expelled from the rubber bulbed pipette and inserted the pipette into glycerin jelly and some solutions from the bottom were sucked. This procedure was found suitable as it avoided introducing bubbles into the solution placed required amount of solution on the warmed slide.
- 4. Then transferred the object from glycerin: alcohol into glycerin jelly.
- 5. Lowered a cover-glass horizontally until it was just above the surface of warm glycerin jelly. Then the slide was lowered gently over the mountant. The moisture film helped to avoid bubbles.
- 6. Then the slide was kept aside to cool.
- 7. Excessive glycerin jelly was scrapped.
- 8. The slide was sealed and labeled properly.

For smaller worms, a different method of mounting was used.

Main steps followed were:

- 1. Placed a large round cover glass on a warming plate and at its center a small drop of glycerin jelly was put.
- 2. Then material was transferred from glycerin to glycerin jelly on the coverglass.
- 3. A small cover-glass was lowered on the bigger one so that it was centered on it and material was sandwiched between. The glycerin jelly was allowed to flow to the edge of the smaller cover-glass.
- 4. A drop of canadabalsam was placed on the smaller cover-glass and was brought to the center of a clean slide with the drop of mountant. The balsam followed to the edge of the large cover-glass, which was now on top.

The smaller cover-glass now attached to the slide and the preparation was automatically sealed by the ring of balsam between the outer edge of the small and large cover-glass. The prepared whole mounts were properly labeled on the left end with all the relevant information.

(B) Whole mount specimens or Preparation of permanent slides of Cestodes and Trematodes for study

Fixation is the first step to process a parasite for the permanent microscopic examination. Fixation means killing a parasite in a manner that it retains approximately the same shape and size (without distortion of tissues), as it had while alive. A fixing agent should penetrate the material rapidly and should kill the cell and prevent post-mortem disintegration. The processing of cestodes and trematodes was done by following steps.

(i) Fixation: The parasites were washed in the normal saline and cleaned the mucous prior to fixation. The parasitic worms (cestodes and trematodes) were removed from the saline solution and placed between the slides. Cornoy's fixative was allowed to run between the slides with the help of a dropper.

Composition of Cornoy's fixative.

Cornoy's fixative 1st:

Absolute Alcohol	:	60ml
Glacial Acetic acid	:	20ml

Cornoy's fixative 2nd:

Absolute Alcohol	:	60ml
Glacial Acetic acid	:	10ml
Chloroform	:	30ml

Formula 1st gave better results than the formula 2nd and hence was used more often. Of all fixatives Cornoy's fixative was used as a general purpose fixative and has good results with all materials. Formula 2nd helps in better penetration.

Some other fixative used in present study includes 10% Formalin which is having the following composition.

Formalin (40% Formaldehyde)	:	10ml
Distilled water	:	90ml

The formalin fixed organisms were washed several times in water for 15-30 minutes each to remove all traces of fixative. The material was then processed through 30%, 50% and 70% alcohol for 30-60 minutes in each. After fixing the worms were removed from the slide in a petridish containing distilled water. The material is then processed.

(ii) **Preservation:** The parasites after washing in distilled water were preserved in 70% alcohol or 4% formalin.

(iii) Staining: The purpose of staining is to stain the internal organs of a helminth parasite in a manner that its features and topography are easily visible. However, staining is resorted for trematodes and cestodes only and not for nematodes. After traces of fixing reagent/ preservative were removed prior to staining by washing the specimen in distilled water. Following stains were used to study the specimens for detailed microscopic examination.

(a) Borax Carmine (Grenachers alcoholic borax carmi

Composition:

Carmine	:	3gms
Borax	:	4gms
Distilled water	:	100ml
70% alcohol	:	100ml

Carmine and the borax were added in the water and boiled until carmine is dissolved (30 minutes or more), allow the mixture to stand for 2 or 3 days, with an occasional stirring, until this occurs; then alcohol was added. Allow the solution to stand for a few days to filter.

(b) Aceto-alum Carmine (Bulloughs Aceto-alum Carmine):

Composition:

Potash alum	:	5gms
Carmine powder	:	5gms
Glacial Acetic acid	:	5ml
Distilled water	:	100ml

5gms of carmine powder, 5ml of glacial acetic acid and 100ml of distilled water were boiled till carmine dissolved fully then 5gms of potash-alum and 100ml of distilled water were added to the above solution and boiled it again. The solution was cooled and filtered. Thymol crystals were added as preservative. The stain was diluted in the ratio 1:1 or as required.

However, aceto-alum carmine gave better results. The specimens were kept for half an hour in dilute aceto-alum carmine (1 part of aceto-alum carmine and 10 parts of distilled water). However, if the specimen were under stained they were further stained and if over stained they were destained by acid alcohol.

The composition of acid alcohol is as below:

2% Acid Alcohol

70% Alcohol	:	98ml
Hydrochloric acid	:	2ml

(iv) Dehydration: Dehydration is usually carried out by passing material through increasing strengths of alcohol. Dehydration is the process of replacing water specimen with an anhydrous solution. The stained specimens were passed through various grades of ethanol in an ascending order i.e., 30%, 50%, 70%, 90% and 100%. However the specimens were given two washes in 100% alcohol. The time required in the different grades of alcohols for dehydration depends upon the specimens and the permeability of its integument.

(v) **Dealcoholization and Clearing:** All traces of water have been removed from the specimens; it is then transferred to a clearing reagent (xylene) which renders it transparent and miscible with a resinous mountant. After complete dehydration parasites were transferred into xylene for clearance. Two washes in xylene were given to ensure complete dealcoholization.

(vi) Mounting: The specimens cleared in xylene were mounted in Canada balsam or DPX (Dextrin Plasticized Xylene). The specimens were properly placed on the slide and a sufficient amount of mountant was added. Then a proper sized coverglass was lowered on the specimen to avoid displacement of specimen or formation of air bubble.

After mounting, cover-glass on the slides were allowed to dry thoroughly before final cleaning. The excess of the mountant was removed from the slide with a razor blade. The remaining resin film was wiped away with a cloth moistened in xylol.

(vii) Labelling: Slides were labelled on the left end with all the information of the material.

(viii) Storing Slides: The prepared slides were stored in wooden boxes, protected from dust, dirt and sunlight. If stored in open boxes or trays, the stains may fade very rapidly. The slides were kept flat during storage by standing the boxes on end to avoid the gradual drifting of the material through the mountant.

Microscopy

Microscopy was conducted under research microscope with lens combination of 5X, 6X, 7X, 8X, 10X, 15X eye pieces and 4X, 10X and 40X objectives. The slides were carefully studied and various morphological peculiarities were examined which helped in the identification of various helminth parasites.

Photomicrography

Photographs of whole mounts or important parts were taken with the help of Digital Olympus Camera mounted on a Research Microscope.

V. Collection and Examination of Blood Samples

Blood samples were collected from different slaughter houses and also from the live goats through juglar vein with the help of syringe in the vails containing EDTA and were carried to laboratory in a ice-cabinet for further analysis. Blood smears were prepared from the fresh blood (i.e. EDTA free blood) for the differential leukocyte count. Serum was collected from the blood by centrifugation at 3000rpm for 10-15 minutes.

A). Hematological Parameters

(1) Estimation of Haemoglobin concentration:

The haemoglobin was estimated by Cyanomethemoglobin method (ICSH, 1973). In this method, Ferricyanide present in the Drabkins solution converts ferrous (Fe2+) iron of haemoglobin to the ferric (Fe3+) state to form methemoglobin. Methemoglobin reacts with potassium cyanide to form Cyanomethemoglobin. The colour developed was measured spectrophotometrically at 540 nm (Wharton and McCarty, 1972; Van Assendelf, 1974).

Drabkin's solution used was prepared by mixing the following reagents in the given proportion:

Sodium bicarbonate	:	1.0 gm.
Potassium cyanide	:	0.05 gm
Potassium ferricyanide	e:	0.2 gm.
Distilled water	:	1000 cc.

Calculation:

Hb (g/100ml) = A540 test sample x15.06 (Std.conc.as stamped on the vail x 0.251) A540 standard

(2) Total erythrocyte count:

Red blood cell count estimates the total number of red blood cells in a cubic millimetre of blood. An improved Neubaurs chamber was used for counting RBC (Baker and Silverson, 1982). The Hayem's dilution fluid which was used had following composition:

Mercuric chloride (HgCl2) : 0.5gm Sodium Chloride (Nacl) : 1.0gm

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Sodium sulphate (Na2SO4) : 5.0gm Distilled water (H2O) : 200ml

Blood was drawn upto the 0.5mark in the RBC Pippette. The tip of the pipette was cleared and RBC dilution fluid was drawn up to 101mark. The resulting solution was shaken for 3 minutes. The first few drops of the solution were discarded and then chamber was loaded by one or two drops of blood solution. RBC was counted by using 40X, after counting the RBC's in smallest 80 squares, calculations were made:

Calculation:

RBC count = Number of cells counted x dilution factor x depth of chamber Area counted

Where dilution factor is one in 200, depth is 1/10mm and area counted =

$$RBC \text{ count} = \frac{\text{Number of cells counted x 200 x 10}}{\frac{1}{5}}$$

RBC /cu.mm = number of cells counted x 10,000

(3) Total leukocyte count

A white cell count (TLC) estimates the total number of white cells in a cubic millimeter of blood. WBC diluting fluid or Turk' fluid contains a weak acid to lyse the red blood cells and Gentian violet stain for staining the nucleus of White blood cells. This was done in the same manner as the RBC count was done. For leukocyte classification the nomenclature of England and Bain (1976) was followed. Turk's WBC dilution fluid was used which had the following composition:

Glacial acetic acid (CH ₃ COOH)	: 1.5 ml
1% Aqueous solution of Gentian violet	: 1.0ml
Distilled water	: 100ml

This fluid contains two things, weak acid which lyse the RBC cells and stain which gives colour to the nucleus of WBC.

Neubaur's haemocytometer (Baker and Silverson, 1982) was used for counting leucocytes. The blood was sucked up in the WBC Pipettes upto the 0.5mark and then WBC dilution fluid was drawn upto the 11 mark of pipette. Solution was mixed gently and bubbling was avoided. The Neubaur's chamber was charged by the resulting mixture. The cells were counted under 40x objective lens.

 $TLC = \frac{\text{Cells counted x blood dilution x chamber depth}}{\text{Area of chamber}}$ $TLC = \frac{\text{Cells counted x 20 x10}}{4}$

TLC/cu.mm.= Cells counted x 50

(4) Differential Leucocytes count:

A thin blood film was made by spreading a blood drop evenly on clean grease free slide using smooth edged spreader. Modification of Romanowsky's stain (Marshal *et al.*, 1975) namely Leishman's stain was used. For Giemsa's staining the air dried blood smears were prefixed with acetone free methanol for 5 minutes (Conn and Darrow, 1960). The DLC results obtained were compared with the estimated normal values of Simmons *et al.* (1974) and Schalm *et al.* (1975).The stains used had following composition.

Giemsa's stain:

Giemsa powder	: 0.3gms
Glycerine	: 25.0ml
Acetone free methyl alcohol	: 25.0 ml

Leishman's stain:

Powdered Leishman's stain : 0.15 gm Acetone fee methyl alcohol : 133ml

(5) Estimation of ESR

The estimation of ESR was done by Wintrobe's method (Dacie and Levis, 1975). The wintrobe's tube is about 11 cm long with a bore diameter 2.5mm and the 10cm of the tube is graduated. The graduations are from zero (top) to hundred (bottom) for ESR and Zero (bottom) to hundred (top) for PCV. Blood containing EDTA was used for the estimation of ESR. Wintrobe's tube was filled upto zero mark on top and the tube was kept vertically in ESR stand for one hour. This is the rate at which erythrocytes sediment by their own weight when blood containing anticoagulant is held in a vertical column. It is expressed as the fall of RBC's in mm at the end of first hour as described by Bottiger and Svedberg (1967). After an hour reading was taken from the tube directly.

(6) Hematocrit or Packed cell volume

This was obtained by centrifuging blood (containing 5mg/ml EDTA) in a graduated tube until corpuscles were packed down to a constant volume. The volume of packed cell was then expressed as a percentage of the original volume of blood. With the aid of capillary pipette a Wintrobe's haematocrit tube was filled to the 100 mark with the anticoagulated blood and centrifuged for 5-10 min at ~ 7,000 RPM. As the original column of blood in the tube is 100 mm long, the volume of packed cell is read directly as percentage. The analysis was done according to England and Walford (1972).

B). Biochemical parameters

(1) Estimation of Total Protein: Total protein content of the serum of goat was estimated by Biuret method which is based on the principle that proteins and peptides containing at least two adjacent peptides bonds which react with cupric ions in alkaline solution forming violet coloured complex having absorption maximum at 550nm.

Reagent	Blank	Test	Standard
Total Protein Reagent	1 ml	1 ml	1 ml
Distilled Water	0.02 ml	-	-
Serum (sample)	-	0.02 ml	-
Standard	-	-	0.02 ml

Table: 3.1: Estimation of Total Protein

The tubes labelled as Standard, Blank and Test were mixed well and incubated at 37°C for 10 minutes and then the absorbance of all the tubes was measured at 540 nm against the blank.

Calculation:

Total protein in g/dl = (absorbance of test /absorbance of standard) x 8 gm/dl.

(2) Albumin estimation

The serum albumin was estimated by BCG (Bromo Cresol Green) method which is based on the principle that serum albumin binds with certain dyes such as bromocresol green, forming coloured complex. The blue green complex has maximum absorption at 630nm. The concentration of albumin in serum is estimated by comparing the colour intensity of test to the known albumin at 630nm.

Reagent	Blank	Test	Standard	
Albumin Reagent	1.0 ml	1.0 ml	1.0 ml	
Distilled water	0.01 ml	-	-	
Serum (sample)	-	0.01 ml	-	
Standard	-	-	0.01 ml	

Table: 3.2: Estimation of Albumin

The tubes were mixed well and then allowed to stand for five minutes at room temperature. The absorption of all the test tubes was measured at 630nm against blank.

Calculation:

Serum albumin = (absorption of test/absorption of standard) x 4gm/dl.

(3) Globulin estimation

Globulin was estimated by subtracting the value of total albumin from the value of total protein as:

Globulin in g/dl = (Total Protein in g/dl - Albumin in g/dl).

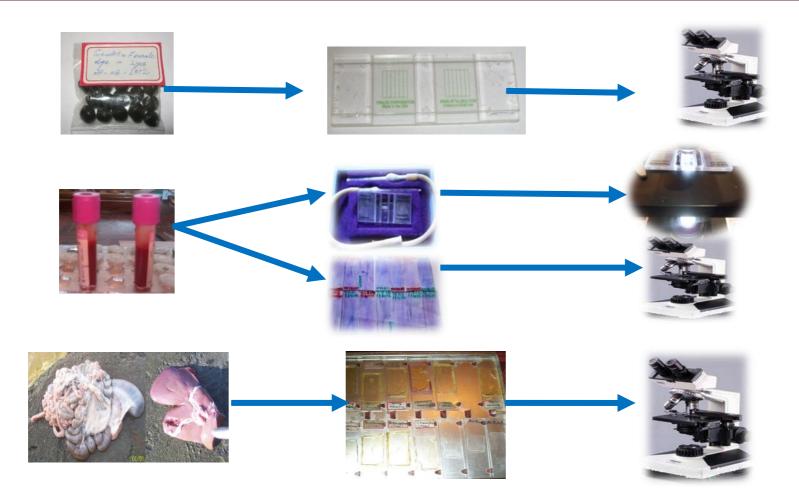


Plate 3: Overview of the Material and Methods

<u>CHAPTER – 4</u>

Results and Discussion

The present study was aimed at studying the epidemiology and hematobiochemical parameters of helminth infected goats in South Kashmir. For this purpose goats were examined from December 2011 to November 2012 for the period of twelve months. For the proper understanding, the observations are divided into two main parts. The first part dealing with epidemiology of GIT parasites of goats and second part with their impact on Hemato-biochemical parameters. The agroclimatic and geographical conditions of Jammu & Kashmir state are conducive for goat rearing. Further, the hilly terrians and highland pastures are suitable for goat farming. However, losses due to infectious disease are not uncommon. Three groups of helminth parasites *viz.*, nematodes, trematodes, and cestodes were encountered during present study. For a clear understanding, the observations have been divided into following headings dealing with various aspects of the study.

4.1. Epidemiology of helminth parasites of goats

4.1.1. Overall Prevalence

- 4.1.2. Seasonal Prevalence
- 4.1.3. Age wise prevalence
- 4.1.4. Gender wise prevalence

4.2. Haematology and blood biochemistry of goats

- 4.2.1. Hemoglobin (g/dl)
- 4.2.2. Packed Cell Volume (PCV) (%)
- 4.2.3. Erythrocyte Sedimentation Rate (ESR) (mm/hr)

4.2.4. RBC Count (10⁶/mm³)

4.2.5. WBC Count (10³/mm³)

4.2.6. Differential Leukocyte Count (DLC) (%)

4.2.7. Total Protein (g/dl)

4.2.8. Albumin (g/dl)

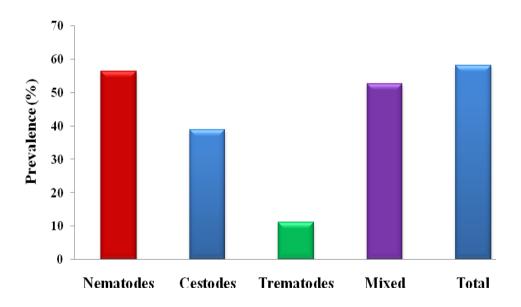
4.2.9. Globulin (g/dl)

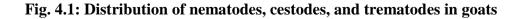
4.1. EPIDEMIOLOGY OF HELMINTH PARASITES OF GOATS

The epidemiology and hemato-biochemical parameters of the helminth infected goats in South Kashmir has been studied taking into consideration the overall prevalence, seasonal prevalence, age wise prevalence and the associated risk factors.

4.1.1. Overall Prevalence

The present study which was carried out from December 2011 – November 2012, 340 goats were examined through faecal and gut examination for helminth infections. Out of 340 goats examined, 198 (58.23%) were found infected with helminths. Out of 340 goats; 192 (56.47%) were found infected with nematodes, 132 goats (38.82%) with Cestodes, 38 goats (11.17%) with Trematodes and 179 (52.64%) showed mixed infections (more than one parasitic infection).

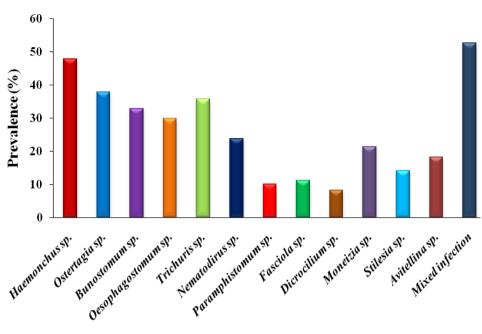




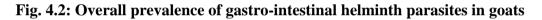
The faecal and gut examination of goats from different areas of South Kashmir, revealed the presence of variety of gastrointestinal helminth parasites in goats. The parasites observed during the present study were: *Haemonchus* sp. (47.94%), *Ostertagia* sp. (37.94%), *Bunostomum* sp. (32.94%), *Oesophagostomum* sp. (30.0%), *Trichuris* sp. (35.88%), *Nematodirus* sp. (23.82%), *Paramphistomum* sp. (10.0%), *Fasciola* sp. (11.17%), *Dicrocoelium* sp. (8.23%), *Moneizia* sp. (21.47%), *Stilesia* sp. (14.11%), *Avitellina* sp. (18.23%) and Mixed infection (52.64%) i.e., infected with more than one parasite. (Table 4.1) (Plates 4, 5 and 6).

Parasites	No. of infected hosts	Percentage
Nematodes	192	56.47
Haemonchus sp.	163	47.94
Ostertagia sp.	129	37.94
Bunostomum sp.	112	32.94
Oesophagostomum sp.	102	30.00
Trichuris sp.	122	35.88
Nematodirus sp.	81	23.82
Trematodes	38	11.17
Fasciola sp.	38	11.17
Dricocoelium sp.	28	8.23
Paramphistomum sp.	34	10.00
Cestodes	132	38.82
<i>Moneizia</i> sp.	73	21.47
Stilesia sp.	48	14.11
Avitellina sp.	62	18.23

 Table 4.1: Overall prevalence of gastro-intestinal helminth parasites in goats (n=340)



Helminth Parasites



In the present study the prevalence of nematodes was found to be 56.47%. Among nematodes, *Haemonchus* sp. showed the highest prevalence (47.94%) and *Nematodirus* sp. showed lowest (23.82%). The difference in prevalence of nematode parasites is dependent on local, geographical, climatic factors, management and husbandary strategies.

The prevalence of cestodes in the present study was found to be 38.82%. Among cestodes, *Monezia* sp. showed the highest prevalence (21.47%) whereas the *Stilesia* sp. showed the lowest prevalence (14.11%). The moderate prevalence of cestodes in goats was due to adequate non-availability of intermediate host i.e., mite (Soulsby, 1986). This may also be due to the climatic conditions in the valley.

The prevalence of trematodes in the present study was found to be 11.17%. Among trematodes, *Fasciola* sp. showed the highest prevalence (11.17%) whereas the *Dricocoelium* sp. showed the lowest (8.23%). The low prevalence of trematodes in goats in the present study may be attributed to the low frequency of intermediate hosts.

The percentage of infection found in present study are in accordance with the researchers which are given as below in table. This may be attributed due to same geographical and similar climatic conditions in the Kashmir valley.

Parasites	Name of researchers			
	Tariq <i>et al.</i> (2009)	Lone <i>et al</i> . (2011)	Lone <i>et al</i> . (2012)	
Nematodes				
Haemonchus sp.	48.3%	60%	48.45%	
Ostertagia sp.	29.8%			
Bonostomum sp.	30.1%			
Oesophagostomum sp.	23.5%	45%	26.0%	
Trichuris sp.	19.0%	51%	41.80%	
Nematodirus sp.	25.2%		32.4%	
Trichostrongylus sp.	25.1%		32.6%	
Cestodes				
<i>Moneizia</i> sp.			18.0%	
Avitellina sp.			24.25%	
Trematodes				
Fasciola sp.			30.65%	
Dicrocoelium sp.			32.0%	
Paramphistomum sp.			22.45%	

Various other researchers outside the state showed different results. Rajapakse *et al.* (2008) reported the overall prevalence of *Oesophagostomum columbianum* (88%) and *Haemonchus contortus* (81%) respectively. Umar and Yukuri (2005) in Turkey reported the prevalence of *Ostertagia circumcinta* (78%). Sutar *et al.* (2010) reported the prevalence of *Monezia* spp. (0.94%) in goats. This disparity may be due to different geographical, topological and climatic conditions.

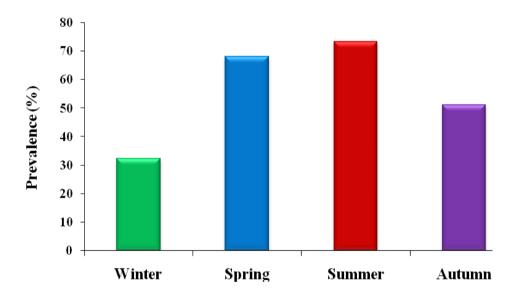
4.1.2. Seasonal Prevalence

After pooling of data, the seasonal prevalence of helminth infection revealed the highest infection in summer and lowest in winter (Table 4.2). Out of 340 goats

examined, 198 (58.23%) were positive for helminth infection. The maximum helminth infection (73.46%) was found in summer followed by spring (68.18%) and autumn (51.16%) and lowest (32.35%) in winter (P = 0.02; $\chi^2 = 9.53$).

Seasons	No. Examined	Positive	Percentage	P-Value
Winter	68	22	32.35	
Spring	88	60	68.18	
Summer	98	72	73.46	0.02
Autumn	86	44	51.16	
Total	340	198	58.23	

Table 4. 2: Seasonal prevalence of gastrointestinal helminth parasites in goats





Our findings are in agreement with others who have reported the prevalence of helminth parasites from other parts of the world. El-Azazy (1995) in Saudi Arabia reported the overall worm counts and infection rates lowest in winter season in sheep and goats. Makhdoomi *et al.* (1995) also observed highest infection in the summer season in Kashmir valley. Khajuria and Kapoor (2003) in sheep and goats from Kuthua region of the J & K state reported highest infection (70.07-94.25%) in summer. Nasreen

et al. (2005) observed the highest infection (33.18%) in summer and lowest (15.25%) in winter. Ahmad and Ansari (1987) have also reported almost similar findings.

The highest incidence of helminth infection during summer and spring may be correlated with the seasonal/climatic pattern and conditions. These seasons provide optimum conditions for the herbage growth in this region and the required moisture is maintained on the grasslands. As a consequence, the incidence starts showing upward trend leading to crest of infection in the months of summer. Relatively the lowest rate of infection and worm burden in respect of all helminths during winter months may be because of minimum temperature and dry conditions which might have inhibited the development of eggs and larvae. Besides weather conditions, self cure phenomenon (Stewart, 1953) may also be the reason for the decrease in infection during colder months.

4.1.3: Age wise prevalence

The age wise incidence of helminth parasites in various groups is shown in Table 4.3. It is evident from the table, that with increase in age the infection level decreases. The animals in lower age group are more prone to infection than the higher age groups. It was observed that prevalence was higher in age group of<1 year and lower in higher age groups. In the present study the maximum nematode infection (80.48%) was observed in age group of <1 year and lowest (23.80%) in age group of > 4 years. The maximum cestode infection (51.21%) was observed in age group of <1 year and lowest (19.04%) in age group of >4 years. Similarly the maximum trematode infection (15.85%) was observed in age group of <1 year and lowest (4.76%) in age group of >4 years. (*P*=0.001; χ^2 =20.30).

			P value		
Age Group	No. Exam.				
		Nem. (%)	Ces. (%)	Trem. (%)	
<1	82	66 (80.48)	42 (51.21)	13 (15.85)	
1-2	78	54 (69.23)	34 (43.58)	10 (12.82)	
2-3	72	38 (52.77)	28 (38.88)	8 (11.11)	0.001
3-4	66	24 (36.36)	20 (30.30)	5 (7.57)	
>4	42	10 (23.80)	8 (19.04)	2 (4.76)	
Total	340	192 (56.47)	132 (38.82)	38 (11.17)	

Table 4.3: Age wise prevalence of gastro-intestinal helminth parasites in goats

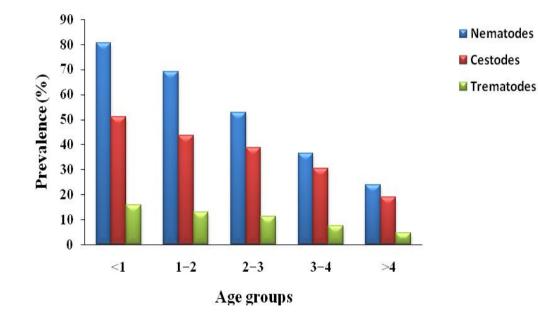


Fig. 4.4: Age wise prevalence of gastro-intestinal helminth parasites in goats

The results are in accordance with those of Patel *et al.* (2001) who reported that animals <1 year showed greater susceptibility (61.90%) to *Paramphistomum* than animals >1 year of age (49.36%). Magona and Musini (2002) also reported that young

goats are at more risk of infections than adult goats. Lateef *et al.* (2005) reported higher infection of helminth parasites in young sheep in Pakistan. Lone *et al.* (2011) also reported that the prevalence of helminth infection is more in <1 year age group (58%) as compared to >1 year (34%). Tariq *et al.* (2008) also reported that younger animals are more prone to infection than adults.

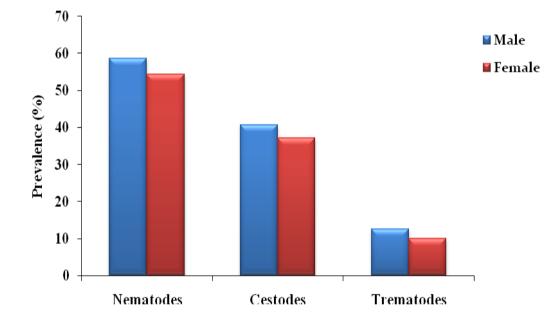
The lower age groups of animals found to be more infected with gastrointestinal helminth parasites in goats is because of the high susceptibility and low resistance in young animals. Thus age is an important factor in the onset of infection because immunity plays a great role in the establishment of parasites in the host body. The low level of parasitism reported in adult animals is due to the development of significant immunity, which is initially low but increases with the intensity and duration of exposure of infection. When the animals cross the one year of age the major part of their infection is eliminated because of development of self-cure phenomenon and tend to remain relatively resistant to reinfection; however, constant exposure of some level of infection is required to maintain their resistant status (Vlasoff *et al*, 2001).

4.1.4: Gender wise prevalence

After arranging the whole data, sex wise observations in the present study revealed that the males were more infected with gastrointestinal helminth parasites than the females in goats (Table 4.4). In the present study the maximum nematode infection (58.75%) was observed in males as compared to females (54.44%). The maximum cestode infection (40.62%) was observed in males as compared to females (37.22%). Similarly the maximum trematode infection (12.50%) was observed in males as compared to females (10.00%). (*P*=1.000; χ^2 =0.576).

			Infected			
Gender	No. Exam.	Nem. (%)	Ces. (%)	Trem. (%)		
Male	160	94 (58.75)	65 (40.62)	20 (12.50)		
Female	180	98 (54.44)	67 (37.22)	18 (10.00)	1.000	
Total	340	192 (56.47)	132 (38.82)	38 (11.17)		

 Table 4. 4: Gender wise distribution of gastro-intestinal helminth parasites in goats





The results are in accordance with those of Courteny *et al.* (1985) who reported that males (Ram) were found to be more infected (72.5%) as compared to females (64.07%) with *Haemonchus contortus*. However many studies have reported females more infected than males (Patel *et al.* (2001); and Shahiduzzamam *et al.* (2003).

Thus from the present study and the work of other researchers it seems that although sex plays a significant role in the preponderance of helminth infection but environmental, managements and climatic conditions have a greater role to play on the onset of helminth infection in goats despite the gender differences reported by several authors. The influence of sex on the susceptibility of animals to infections could be attributed to genetic predisposition and differential susceptibility owing to hormonal control.

4.2. HEMATOLOGY AND BIOCHEMISTRY

4.2.1. Hemoglobin (g/dl)

The mean hemoglobin value was found to be 12.33 ± 1.03 , 12.96 ± 0.46 , 11.90 ± 0.35 and 12.36 ± 1.02 in the uninfected goats in different season's viz., winter, spring, summer and autumn respectively (Table 4.5). The mean hemoglobin value for the helminth infected goats in different season's viz., winter, spring, summer and autumn was found to be 11.0 ± 1.41 , 8.83 ± 1.16 , 7.83 ± 1.16 and 9.66 ± 1.21 respectively (Table 4.6). The results show slight decrease in hemoglobin concentration in the uninfected goats during summer as compared to other seasons (*P*=0.167). The results showed significant decrease in hemoglobin concentration in helminth infected goats and the effect was most prominent in summer season followed by spring and less in winter (*P*=0.002).

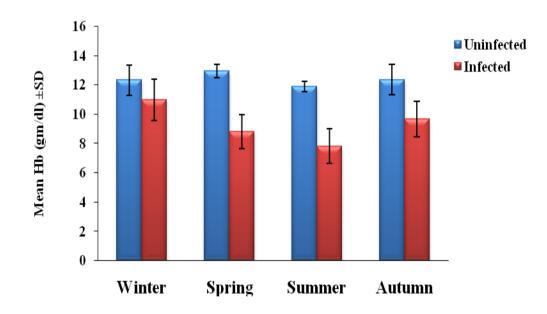


Fig. 4.6: Seasonal variation in Hemoglobin of helminth infected and uninfected goats

The results of the uninfected goats are in accordance with those of Appleman and Delouche (1958); Vaidya *et al.* (1970); Mehrotra *et al.* (1954); Kaushish *et al.*

(1976). Abdelatif *et al.* (2009) reported the decrease in haemoglobin concentration in summer as compared to spring and winter in the uninfected goats. The results of the helminth infected goats are in accordance with Teleb *et al.* (2007); El-Aziz *et al.* (2002). Bordoloi *et al.* (2012) reported the decrease in hemoglobin concentration in sheep experimentally infected with *Haemonchus*. Sulaiman *et al.* (2010) also reported decrease in Hb concentration in goats infected with *Babesia ovis*. Waweru *et al.* (1999); Ahmad *et al.* (2006) and Matanovic *et al.* (2007) also showed decrease in Hb concentration in helminth infected ruminants.

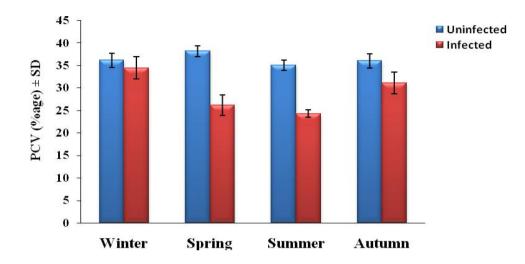
In the uninfected goats the decrease in haemoglobin concentration in the summer season may be due to increase in ambient temperature (Appleman and Delouche (1958); Bianca, 1968; Prasetyo *et al.* 1984), and haemodilution. The decline is also attributed to depression of thyroid secreation which is associated with decreased erythopoises. Thyroid hormones increase the proliferation rate of erythroid progenitors (Popovic *et al.* 1977; Dainiak *et al.* 1978) and enhance the production of erythropoietic growth factors (Dainiak *et al.*1986; Fandrey *et al.* 1994). The relatively higher values in spring may be associated with the nutritional status of the goat and the climatic conditions (Payne *et al.* 1990).

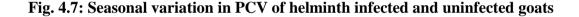
The reduction in Hb concentration in the helminth infected goats may be attributed to the acute loss of blood by sucking activity of various parasites especially *Haemonchus contortus* and *Ostertagia ostertagi* which suck 0.05ml blood/worm/day (Soulsby, 1986). Coles (1986); Kaneko *et al.* (1997); Kramer (2000); El-Sayed *et al.* (2003); Lotfy *et al.* (2003); Omran and El-Kholany (2003) reported that the severe anemia may be due to a chronic liver inflammation, which causes depression of erythrogenesis.

4.2.2. Packed Cell Volume (PCV) (%)

The mean value for packed cell volume in the uninfected goats was found to be 36.16 ± 1.60 , 38.25 ± 1.20 , 35.10 ± 1.13 and 36.06 ± 1.59 in different seasons viz., winter, spring, summer and autumn respectively (Table 4.5). The mean PCV value of the helminth infected goats in different seasons viz., winter, spring, summer and autumn was found to be 34.5 ± 2.42 , 26.16 ± 2.31 , 24.33 ± 0.81 and 31.16 ± 2.40 respectively (Table 4.6). The results show decrease in packed cell volume percentage in uninfected goats during summer as compared to other seasons (*P*=0.007). The results show significant

decrease in packed cell volume percentage in helminth infected goats and the effect was most prominent in summer and spring season as compared to winter season (P=0.001).





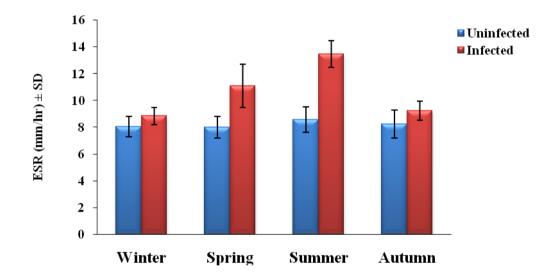
The results of the uninfected goats are in accordance with those of Appleman and Delouche (1958). Abdelatif *et al.* (2009) reported the decrease in packed cell volume percentage in summer as compared to spring and winter in the uninfected goats. Similar results in the helminth infected goats have been reported by Teleb *et al.* (2007); Eguale and Abie (2003); Bordoloi *et al.* (2012). Sulaiman *et al.* (2010) reported decrease in PCV in goats infected with *Babesia ovis.* Mottelib *et al.* (1992) also reported decrease in PCV in nematode infected goats and sheep.

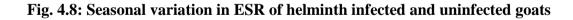
In the uninfected goats the decrease in haemoglobin concentration in the summer season may be due to increase in ambient temperature (Appleman and Delouche (1958); Bianca, 1968; Prasetyo *et al.* 1984), and haemodilution. The decline is also attributed to depression of thyroid secreation which is associated with decreased erythopoises. Thyroid hormones increase the proliferation rate of erythroid progenitors (Popovic *et al.* 1977; Dainiak *et al.* 1978) and enhance the production of erythropoietic growth factors. The relatively higher values in spring may be associated with the nutritional status of the goat and the climatic conditions (Payne *et al.* 1970).

The reduction in PCV in the helminth infected goats may be attributed to the acute loss of blood by sucking activity of various parasites especially *Haemonchus contortus* and *Ostertagia ostertagi* which suck 0.05ml blood/worm/day (Soulsby, 1986).

4.2.3: Erythrocyte Sedimentation Rate (ESR) (mm/hr)

The ESR in the uninfected goats was found to be 8.08 ± 0.75 , 8.0 ± 0.80 , 8.6 ± 0.95 and 8.25 ± 1.05 in different seasons viz., winter, spring, summer and autumn respectively (Table 4.5). The mean ESR value of the helminth infected goats in different seasons viz., winter, spring, summer and autumn was found to be 8.85 ± 0.65 , 11.11 ± 1.60 , 13.48 ± 0.98 and 9.25 ± 0.69 respectively (Table 4.6). The results show significant increase in ESR in helminth infected goats and the effect was most prominent in summer season and less in winter (*P*=0.001).





The results of uninfected goats showed slight variation in ESR in the different seasons (P=0.667). The results of the helminth infected goats are in accordance with Taimur *et al.* (1993) who reported increase in ESR in cattle experimentally infected with *Fasciola gigantica*. At ish *et al.* (2007) also reported the increase in ESR by the mange mite infestation in sheep in Pakistan.

Increase in ESR may be attributed to increased level of gamma globulins because of the increased helminth infection.

4.2.4: RBC Count (10⁶/mm³)

The mean RBC count was found to be 13.83 ± 0.75 , 14.48 ± 0.46 , 12.88 ± 0.27 and 13.96 ± 0.63 in different seasons viz., winter, spring, summer and autumn respectively in the uninfected goats (Table 4.5). The mean RBC values for the helminth infected goats was found to be 12.83 ± 1.47 , 9.5 ± 0.54 , 8.16 ± 1.16 and 11.0 ± 0.89 in different seasons viz., winter, spring, summer and autumn respectively (Table 4.6). The results show decrease in RBC count in the uninfected goats during summer as compared to other seasons (*P*=0.001). The results show significant decrease in RBC count in helminth infected goats and the effect was most prominent in summer and spring season and less in winter (*P*=0.001) (Plate 7).

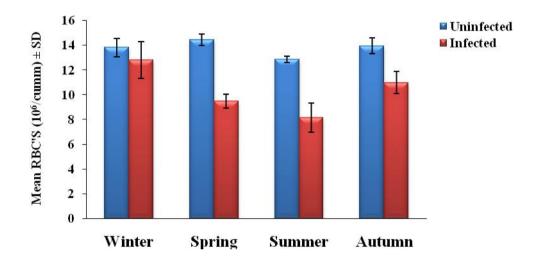


Fig. 4.9: Seasonal variation in RBC count of helminth infected and uninfected goats

The results of the uninfected goats are in accordance with those of Appleman and Delouche (1958); Vaidya *et al.* (1970); Mehrotra *et al.* (1954); Kaushish *et al.* (1976). Abdelatif *et al.* (2009) reported the decrease in RBC count in summer as compared to spring and winter in the uninfected goats. The results of the helminth infected goats are in accordance with those of Sulaiman *et al.* (2010); Teleb *et al.* (2007); El-Aziz *et al.* (2002); Ahmed *et al.* (2006) and Matanović *et al.* (2007). Mottelib *et al.* (1992) reported decrease in RBC count in nematode infected sheep and goats. Esmaeilnejad *et al.* (2012) reported decrease in Red Blood Cells in goats and sheep infected with *Babesia ovis.* In the uninfected goats the decrease in RBC count in the summer season may be due to increase in ambient temperature (Appleman and Delouche (1958); Bianca, 1968; Prasetyo *et al.* 1984), and haemodilution. The decline is also attributed to depression of thyroid secreation which is associated with decreased erythopoises. Thyroid hormones increase the proliferation rate of erythroid progenitors (Popovic *et al.* 1977; Dainiak *et al.* 1978) and enhance the production of erythropoietic growth factors (Dainiak *et al.* 1986; Fandrey *et al.* 1994). The relatively higher values in spring may be associated with the nutritional status of the goat and the climatic conditions (Payne *et al.* 1970).

The reduction in RBC count in the helminth infected goats may be attributed to the acute loss of blood by sucking activity of various parasites especially *Haemonchus contortus* and *Ostertagia ostertagi* which suck 0.05ml blood/worm/day (Soulsby, 1986). The reduction is comparatively much higher compared to the environmental changes. Coles (1986); Kaneko *et al.* (1997); Kramer (2000); El-Sayed *et al.* (2003); Lotfy *et al.* (2003); Omran and El-Kholany (2003) reported that the severe anemia may be due to a chronic liver inflammation, which causes depression of erythrogenesis.

4.2.5. WBC Count (10³/mm³)

The mean WBC count was found to be 9.0 ± 0.89 , 9.21 ± 0.90 , 8.08 ± 0.57 and 9.06 ± 0.92 goats in different seasons viz., winter, spring, summer and autumn respectively in the uninfected goats (Table 4.5). The mean WBC count value for the helminth infected goats in different seasons viz., winter, spring, summer and autumn was found to be 9.16 ± 1.16 , 10.83 ± 1.16 , 11.83 ± 0.75 and 9.66 ± 0.81 respectively (Table 4.6). The results showed no marked variation but slight decrease in WBC count in uninfected goats during summer (P=0.11). The results show significant increase in WBC count in helminth infected goats and the effect was most prominent in summer season and less in winter (P=0.001).

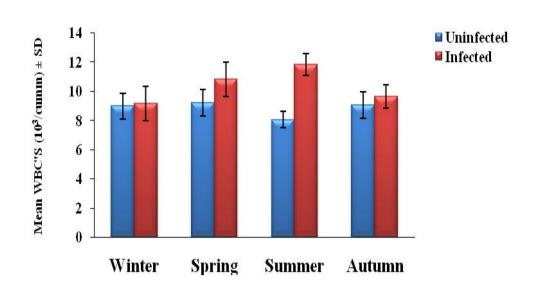


Fig. 4.10: Seasonal variation in WBC count of helminth infected and uninfected goats

In the uninfected goats similar results have been reported by (Vaidya *et al.* 1970; Vergula *et al.* 1985) in goats and (Hassan *et al.* 1966; Hassan *et al.* 1987) in sheep. The results of the helminth infected goats are in accordance with those of Teleb *et al.* (2007); Waweru *et al.* (1999); El-Sayed *et al.* (2003); Lotfy *et al.* (2003) and Omranand El-Kholany (2003). Mottelib *et al.* (1992) reported increase in WBC count in nematode infected goats and sheep. Esmaeilnejad *et al.* (2012) also reported increase in WBC count in goats and sheep infected with *Babesia ovis.*

The reduction in the WBC count in the summer season in the uninfected goats could be associated with physiological responses to hot climate which include decrease in food take and expansion of plasma volume resulting in haemodilution. Increase in WBC count in the helminth infected goats may be attributed to the immune response of body against the helminth parasites as a means of self defense.

4.2.6: Differential Leukocyte Count (DLC) (%)

The mean percentage value for neutrophils in the uninfected goats in different seasons viz., winter, spring, summer and autumn were found to be 31.16 ± 0.75 , 31.99 ± 0.23 , 32.40 ± 0.49 and 31.35 ± 1.34 respectively (*P*=0.05) (Table 4.7). The mean percentage value for lymphocytes in the uninfected goats in different seasons viz., winter, spring, summer and autumn were found to be 50.66 ± 0.81 , 51.20 ± 0.90 , 51.61 ± 0.97 and 50.76 ± 1.03 respectively (*P*=0.29) (Table 4.7). The mean percentage

value for eosinophils in the uninfected goats in different seasons viz., winter, spring, summer and autumn were found to be 3.0±0.89, 3.3±0.30, 3.7±0.47 and 3.2±0.49 respectively (P=0.23) (Table 4.7). The mean percentage value for monocytes in the uninfected goats in different seasons viz., winter, spring, summer and autumn were found to be 3.0 ± 0.63 , 2.86 ± 0.30 , 2.83 ± 0.26 and 2.9 ± 0.42 respectively (P=0.90) (Table 4.7). The mean percentage value for basophils in the uninfected goats in different seasons viz., winter, spring, summer and autumn were found to be 2.0 ± 0.89 , 2.11 ± 0.24 , 2.83 ± 0.57 and 2.05 ± 0.26 respectively (P=0.05) (Table 4.7). The mean percentage value for neutrophil in the helminth infected goats in different seasons viz., winter, spring, summer and autumn were found to be 31.83±0.75, 37.66±1.03, 41.83±0.75 and 33.66 ± 1.03 respectively (P=0.001) (Table 4.8). The mean percentage value for lymphocyte in the helminth infected goats in different seasons viz., winter, spring, summer and autumn were found to be 51.5±1.04, 64.16±1.16, 71.83±1.16 and 54±0.89 respectively (P=0.001) (Table 4.8). The mean percentage value for eosinophil in the helminth infected goats in different seasons viz., winter, spring, summer and autumn were found to be 3.16±0.75, 5.0±0.63, 6.16±0.75 and 3.83±0.75 respectively (P=0.001) (Table 4.8). The mean percentage value for monocyte in the helminth infected goats in different seasons viz., winter, spring, summer and autumn were found to be 2.83±0.40, 2.16±0.25, 1.16±0.75 and 2.5±0.54 respectively (P=0.001) (Table 4.8). The mean percentage value for basophil in the helminth infected goats in different seasons viz., winter, spring, summer and autumn were found to be 2.0 ± 0.63 , 3.0 ± 0.63 , 3.16 ± 0.75 and 2.66 ± 0.81 respectively (P=0.04) (Table 4.8). The results showed not much variation in the DLC in the uninfected goats. The results showed a significant increase in neutrophils and lymphocytes and slight increase in eosinophils and basophils whereas monocytes decrease slightly in the helminth infected goats. (Plate 7).

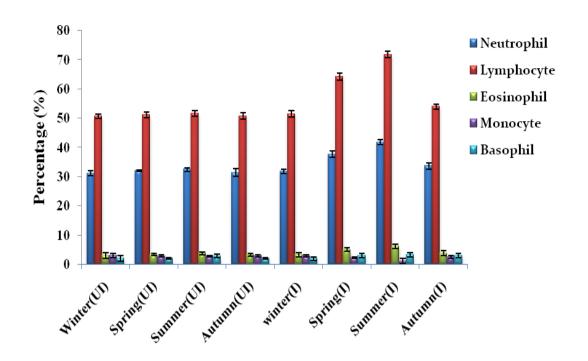


Fig.4.11: Seasonal variation in DLC count of helminth infected (I) and uninfected goats (UI)

The results are in accordance with those of Waweru *et al.* (1999); El-Sayed *et al.* (2003); Lotfy *et al.* (2003) and Omranand El-Kholany (2003). Teleb *et al* (2007) reported increase in neutrophils, lymphocytes, eosinophils and basophils and also reported decrease in monocytes. Moreover, eosinophilia has been reported to be proportional to the degree of antigenic stimulation or parasitic burden in helminthes infections (Ackerman *et al.*, 1981). This is normally linked to antigen antibody reaction which occurred when the sensitivity to the protein of the parasites has developed or when the secretory products were released within the blood (Jain, 1993); associated with cellular- mediated immunity (Duffus *et al.*, 1980). Leukocytosis and eosinophilia detected in the present study were similar to those previously reported by Sykes *et al.* (1980); Zhang *et al.* (2005) and Ahmed *et al.* (2006).

Parameters	D		(N	(Iean ± SD)))		
	Range	Winter	Spring	Summer	Autumn	P value	
Hb (g/dl)	8-13	12.33±1.03	12.96±0.46	11.90±0.35	12.36±1.02	0.167	
RBC (10 ⁶ /ul)	8-17	13.83±0.75	14.48±0.46	12.88±0.27	13.96±0.63	0.001	
WBC (10 ³ /ul)	4-12	9.0±0.89	9.21±0.90	8.08±0.57	9.06±0.92	0.111	
PCV (%)	23-39	36.16±1.60	38.25±1.20	35.10±1.13	36.06±1.59	0.007	
ESR (mm/hr)	5-15	8.08±0.75	8.0±0.80	8.60±0.95	8.25±1.05	0.667	

 Table 4.5: Haematological parameters of uninfected goats

Table 4.6: Haematological parameters of helminth infected goats

Parameters	Range	(Mean ± SD)					
		Winter	Spring	Summer	Autumn	P value	
Hb (g/dl)	8-13	11.0±1.41	8.83±1.16	7.83±1.16	9.66±1.21	0.002	
RBC (10 ⁶ /ul)	8-17	12.83±1.47	9.50±0.54	8.16±1.16	11.00±0.89	0.001	
WBC (10 ³ /ul)	4-12	9.16±1.16	10.83±1.16	11.83±0.75	9.66±0.81	0.001	
PCV (%)	23-39	34.50±2.42	26.16±2.31	24.33±0.81	31.16±2.40	0.001	
ESR (mm/hr)	5-15	8.85±0.65	11.11±1.60	13.48±0.98	9.25±0.69	0.001	

Cells	Range	Mean±SD					
	Kange	Winter	Spring	Summer	Autumn	P value	
Neutrophil	20-45	31.16±0.75	31.99±0.23	32.40±0.49	31.35±1.34	0.05	
Lymphocyte	40-75	50.66±0.81	51.20±0.90	51.61±0.97	50.76±1.03	0.29	
Eosinophil	0-7	3.0±0.89	3.30±0.30	3.70±0.47	3.20±0.49	0.23	
Monocyte	0-4	3.0±0.63	2.86±0.30	2.83±0.26	2.90±0.42	0.90	
Basophil	0-4	2.0±0.89	2.11±0.24	2.83±0.57	2.05±0.26	0.05	

Table 4.7: Differential Leukocyte Count of uninfected goats

Table 4.8: Differential Leukocyte Count of helminth infected goats

Cells	Range		Mean±SD					
		Winter	Spring	Summer	Autumn	P value		
Neutrophil	20-45	31.83±0.75	37.66±1.03	41.83±0.75	33.66±1.03	0.001		
Lymphocyte	40-75	51.50±1.04	64.16±1.16	71.83±1.16	54.0±0.89	0.001		
Eosinophil	0-7	3.16±0.75	5.0±0.63	6.16±0.75	3.83±0.75	0.001		
Monocyte	0-4	2.83±0.40	2.16±0.25	1.16±0.75	2.50±0.54	0.001		
Basophil	0-4	2.0±0.63	3.0±0.63	3.16±0.75	2.66±0.81	0.048		

4.2.7. Total Protein (g/dl)

The mean total protein was found to be 7.06 ± 0.28 , 7.02 ± 0.35 , 7.04 ± 0.46 and 7.04 ± 0.48 in different seasons viz., winter, spring, summer and autumn respectively in the uninfected goats (Table 4.9). The mean total protein value for the helminth infected goats was found to be 7.06 ± 0.29 , 7.0 ± 0.20 , 6.83 ± 0.42 and 7.03 ± 0.27 in different seasons viz., winter, spring, summer and autumn respectively (Table 4.10). The results showed no significant changes in total protein content in the uninfected goats (*P*=0.99). The results showed the decrease in total protein content in the helminth infected goats and was most prevalent in the summer season and less in winter (*P*=0.57).

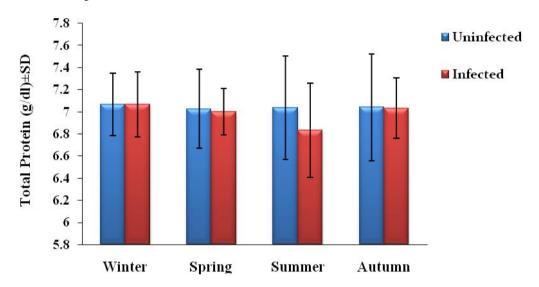


Fig. 4.12: Seasonal variation in Total Protein content of helminth infected and uninfected goats

The results of the helminth infected goats are in accordance with that of Knox *et al.* (1993) who reported decrease in the total protein content in the small ruminants infected with nematode parasites. Rehman and Collins (1990) also reported the decrease in total protein content in goats. Teleb *et al.* (2007) reported the decrease in total protein content in serum of Farafrasheep experimentally infected with *Fasciola gigantica*.

The decrease in total protein is due to haematophagous parasites especially *Haemonchus contortus* and *Ostertagia ostertagi* which suck 0.05ml of blood/worm/day (Soulsby, 1986). The infection of liver and destruction of liver parenchyma also resulted in alteration in protein values (Ismail *et al.* (1990); Mohamed (2000) and Matanovic *et al.* (2007).

4.2.8. Albumin (g/dl)

The mean albumin was found to be 2.35 ± 0.20 , 2.40 ± 0.30 , 1.93 ± 0.33 and 2.31 ± 0.31 in different seasons viz., winter, spring, summer and autumn respectively in the uninfected goats (Table 4.9). The mean albumin value of the helminth infected goats was found to be 2.34 ± 0.20 , 2.24 ± 0.18 , 1.90 ± 0.20 and 2.30 ± 0.18 in different seasons viz., winter, spring, summer and autumn respectively (Table 4.10). The results showed the decrease in albumin content in the uninfected goats in the summer season as compared to other seasons (*P*=0.04). The results showed the decrease in albumin content in the negative s

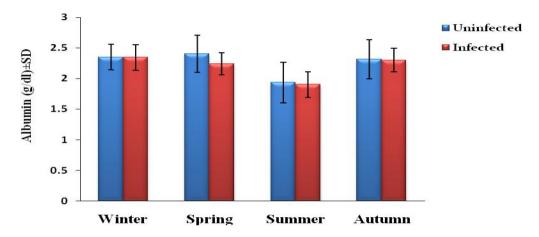


Fig. 4.13: Seasonal variation in Albumin content of helminth infected and uninfected goats

In the uninfected goats similar results have been reported by Hassan *et al.* (1966); Abdelatif *et al.* (2009). Similar results have been reported in helminth infected goats by Ahmad *et al.* (1990), who studied the serum protein changes of lambs experimentally infected with *Haemonchus contortus* and reported a marked decrease in albumin content. Teleb *et al.* (2007) reported the decrease in albumin protein content in serum of Farafrasheep experimentally infected with *Fasciola gigantica*. However, Ahmad *et al.* (2006) detected no significant change in albumin levels in sheep infected with *Fasciola gigantica*.

The decrease in albumin level in the summer in the uninfected goats could be attributed in part to expansion of plasma volume resulting in haemodilution. The plasma volume increases by 17% during tropical summer in Merino sheep (Macfarlane *et al.* 1966).

The decrease in albumin levels in the helminth infected goats may be attributed to reduced albumin synthesis caused by liver damage and haematophagous feeding behavior of various parasites.

4.2.9. Globulin (g/dl)

The mean globulin was found to be 4.71 ± 0.34 , 4.62 ± 0.42 , 5.11 ± 0.16 and 4.73 ± 0.38 in different seasons viz., winter, spring, summer and autumn respectively in the uninfected goats (Table 4.9). The mean globulin value of the helminth infected goats in different seasons viz., winter, spring, summer and autumn was found to be 4.72 ± 0.35 , 4.76 ± 0.39 , 4.92 ± 0.41 and 4.73 ± 0.43 respectively (Table 4.10). The results showed the increase in globulin content in the uninfected goats in the summer and spring as compared to other seasons (P=0.09). The results showed the slight increase in globulin content in the helminth infected goats and was most prevalent in summer and less in winter (P=0.80).

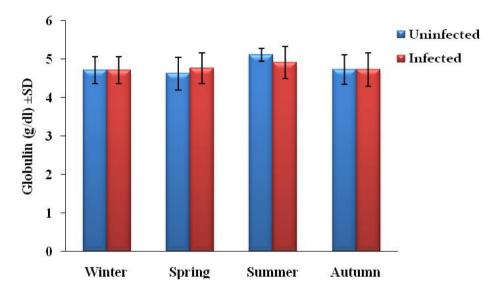


Fig. 4.14: Seasonal variation in Globulin content of helminth infected and uninfected goats

The results of uninfected goats are in accordance with those of Al-Busaidi *et al.* (2008); Johnson *et al.* (1995). The results of the helminth infected goats are in accordance with the Rehman and Collins (1990) who reported the increase in globulin fraction of plasma proteins in goats. Ahmad *et al.* (1990) also reported the increase in α - globulin and β - globulin in the lambs experimentally infected with *Haemonchus contortus* and was found highest at the peak of infection. Teleb *et al.* (2007) also showed the increase in globulin protein in experimentally infected sheep with *Fasciola gigantica*. Amer *et al.* (2002) and Matanovic *et al.* (2007) also reported the increase in globulin levels in helminth infections.

The increase in globulin levels in serum in the uninfected goats may be due to their potentiated immunity (Al-Busaidi *et al.* 2008). The increase in globulin levels in serum in the helminth infected goats may be attributed to the result of immune response

to infection (Matanovic *et al.* 2007) and due to the increase in $\alpha \& \beta$ globulin production (Duncan *et al.* 1994).

Parameters	Range	(Mean ± SD)					
		Winter	Spring	Summer	Autumn	P value	
Total Protein (g/dl)	6.0-7.5	7.06±0.28	7.02±0.35	7.04±0.46	7.04±0.48	0.999	
Albumin (g/dl)	1.9-2.4	2.35±0.20	2.40±0.30	1.93±0.33	2.31±0.31	0.041	
Globulin (g/dl)	4.1-5.1	4.71±0.34	4.62±0.42	5.11±0.16	4.73±0.38	0.09	

Table 4.9: Biochemical parameters of uninfected goats

Table 4.10: Biochemical parameters of helminth infected goats

Parameters	Range	(Mean ± SD)				
		Winter	Spring	Summer	Autumn	P value
Total Protein	6.0-7.5	7.06±0.29	7.0±0.20	6.83±0.42	7.03±0.27	0.574
(g/dl)						
Albumin	1.9-2.4	2.34±0.20	2.24±0.18	1.91±0.20	2.3±0.18	0.003
(g/dl)						
Globulin	4.1-5.1	4.72±0.35	4.76±0.39	4.92±0.41	4.73±0.43	0.80
(g/dl)						

The whole Data was fed into a Microsoft Excel 2010. Primer software was used for data analysis, chi-square test and t-test were applied. The data was presented as mean \pm standard deviation (SD).



Haemonchus egg



Ostertagia egg



Trichuris egg



Nematodirus egg



Dicrocoelium egg



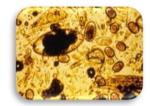
Fasciola egg



Moneiza egg



Paramphistomum egg

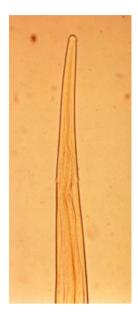


Mixed Parasitic Infection

Plate 4: Eggs of various Helminth Parasites



Haemonchus sp.



Ostertagia sp.



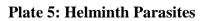
Nematodirus sp.



Bunostomum sp.



Fasciola sp.





Dricocelium sp.



Scolex of Moneiza sp.



Scolex of Avitellina sp.



Scolex of Stilesia sp.



Proglottid of Moneiza sp.

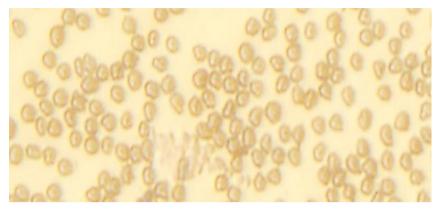


Gravid segment of Avitellina sp.

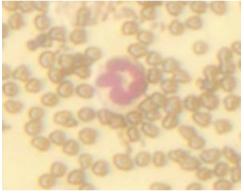


Mature segment of Stilesia sp.

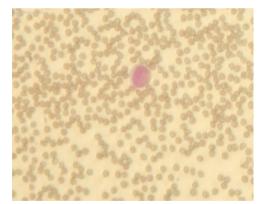
Plate 6: Helminth Parasites



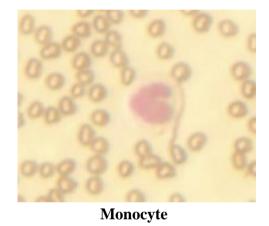
RBC'S

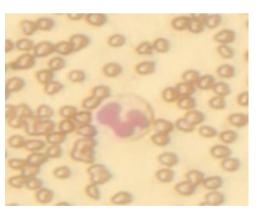


Neutrophil



Lymphocyte





Basophil



CHAPTER – 5

Summary, Conclusion and Recommendations

SUMMARY

The present study deals with the epidemiology and hemato-biochemical investigations of goats naturally infected with helminth parasites from December 2011 to November 2012 in South Kashmir.

- The present study revealed that the infection status by the various species of helminth parasites is very high. This means that geographical and climatic conditions of this region are suitable for helminth infection.
- ✤ The overall percentage of helminth infection was found to be 58.23%.
- Out of 340 goats, 192 (56.47%) were found infected with nematodes, 132 goats (38.82%) with Cestode infection, 38 goats (11.17%) with Trematode infection and 179 (52.64%) were having mixed infection (i.e. more than one parasite).
- The gastrointestinal helminth parasites reported in the infected goats were Haemonchus sp. (47.94%), Ostertagia sp. (37.94%), Bunostomum sp. (32.94%), Oesophagostomum sp. (30.0%), Trichuris sp. (35.88%), Nematodirus sp. (23.82%), Paramphistomum sp. (10.0%), Fasciola sp. (11.17%), Dicrocoelium sp. (8.23%), Moneizia sp. (21.47%), Stilesia sp. (14.11%), and Avitellina sp. (18.23%).
- This study showed the seasonal pattern of helminth infestation, with high infection rate in summer (73.46%) followed by spring (68.18%), autumn (51.16%) and lowest in winter (32.35%).
- The study also showed that the young animals are more prone to infection than the adult ones. Therefore young animals need special attention because of their

high susceptibility to infection. They should be included in deworming programming.

- The study also revealed that maximum helminth infection was observed in males as compared to females. The influence of sex on the susceptibility of animals to infections could be attributed to genetic predisposition and differential susceptibility owing to hormonal control.
- Hematological studies revealed significant decrease in hemoglobin concentration, packed cell volume and total erythrocyte count in the helminth infected goats. Whereas erythrocyte sedimentation rate and total leukocyte count showed significant increase in the helminth infected goats.
- Among biochemical parameters, albumin showed the significant decrease and globulin showed slight increase in the helminth infected goats where as total protein showed slight decrease in helminth infected goats.

CONCLUSION

It should be concluded that gastro-intestinal parasitism represents a severe health problem in small ruminant production system, and its consequences can be extensive ranging from reduced productivity to mortality. Gastro-intestinal nematodiasis is a major threat and a primary constraint to goat productivity, it endangers animal welfare worldwide. The main culprit in ruminant nematodiasis is *Haemonchus contortus and Ostertagia ostertagi* which causes haemonchosis, anemia and parasitic gastroenteritis in goats. They cause significant economic losses worldwide due to their feeding behaviour being haematophagous. It could also be concluded that changes in the hematological and biochemical parameters in goats infected with helminth parasites reflects a veterinary problem and causes pathological conditions like anemia, weight loss, poor wool and milk production. Fascioliasis cause pathological and necrotic lesions of liver parenchyma and the bile ducts causing hemorrhage, so modem diagnostic techniques need to be incorporated to estimate the degree of infection.

The present observation may suggest in planning chemotherapeutic and prophylactic strategies for the helminth control in the region. With the early diagnosis of helminthiasis in animals, a treatment schedule could be designed to avoid more infection and animal losses on the farm level and in turn economical losses. Control of helminth infection requires knowledge of the epidemiology and ecology of the parasites. This knowledge is needed not only for planning better strategies of parasitic control but also provide insight into the natural processes of controlling parasite population. Present results can be useful to future researchers in this field and can lead to proper control of parasitic infections in goats of Kashmir Valley.

RECOMMENDATIONS

On the basis of the present study following recommendations are given as under.

- Regular screening of animals for the presence of helminth infections should be carried out to understand the disease potential.
- Overcrowding at farms should be avoided and good sanitary conditions should be provided.
- Based upon epidemiological principles, *Three Check Points* to manage and control helminthiasis in ruminants in our state are suggested and recommended.
 (1) *Pre grazing*: Deworming early spring before the animals are sent to pastures for grazing.
 (2) *During grazing*: Deworming during the middle of grazing months (somewhere between July- August).
 (3) *Post grazing*: Deworming before the onset of winter.
- Animals should be provided with balanced feed containing more than 3% protein.
- Alternatives to chemical control also have viable effect in the treatment of helminthes, so they must be incorporated alternatively to avoid resistance.
- The intiative should be taken at government level to develop and update quality and quantity of pastures and for animals grazing along with the supplementary food. Recent studies have indicated that improvement in animal nutrition especially metabolizable protein can enhance the host response against primary and secondary infections and to reduce the patho-physiological changes associated with helminthes.
- Further studies are needed on life cycle and larval ecology of these parasites so as to provide a basis for developing sustainable helminth control strategies for the temperate climate of the Kashmir Valley. Sustainable and novel control strategies (manipulation of diet, vaccines, biological control etc.) need to be employed to control helminthiasis in Jammu and Kashmir.

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