6.1 Prevalence

The present study was undertaken to evaluate the prevalence of helminth parasites, their clinopathological alternations and immunological responses in different age groups of sheep in Kashmir valley. During the period of present investigation regular visits were made to all the districts of Kashmir valley to collect the faecal samples. In order to obtain adequate number of samples, attempts were made to collect a minimum of ten collections at each site. A total of 1325 faecal samples of sheep collected from different areas of Kashmir valley were examined and 1047 (79.01%) samples were found infected with different helminth parasites. Faecal examination revealed highest prevalence of nematodes (68%) followed by (31.01%) of cestodes and (28.98%) of trematodes. Among the trematodes, *Fasciola gigantica* was most prevalent (23.92%) followed by *Fasciola hepatica* (9.96%). *Dicrocoelium dendriticum* (4.45%) and *Paramphistomum cervi* (2.71%). The faecal examinations have revealed similar trend in the prevalence of parasites in different seasons. However, highest and lowest prevalence of trematode parasites was observed in summer and winter seasons respectively. During the course of study highest prevalence of trematode and cestode parasites was observed in above 4 years age group while as 0-2 year age group was found to have highest prevalence of nematode parasites.

During the survey period only three types of cestode eggs were observed. These were of *Moneizia* species, *Stilesia globipunctata* and *Avitellina woodlandi*. The overall prevalence of cestode parasites was (31.01%) and only (3.05%) of samples were found to have multiple infections. The most prevalent cestode parasite was *Moneizia* species followed by *Stilesia* and *Avitellina*.
Out of 1325 faecal samples screened for prevalence of nematode parasites, (68%) samples were found positive for nematode eggs. The present observations on the prevalence of nematode parasites revealed that *Haemonchus contortus* is most prevalent nematode parasite in the Kashmir valley followed by *Trichuris ovis*. Throughout the investigation period *Haemonchus contortus* parasite represented more than 30% of faecal egg counts. Seasonal variation was observed in nematode worm burden as varied faecal egg counts were observed which ranged from 350 to 6000. Two peak infections were observed during the course of study. First peak infection was observed in the month of May and second in the month of August. 0-2 year age group of sheep was found to be most susceptible to nematode parasites.

During the study period, exotic breed (Kashmir Merino) was found more susceptible to helminth parasites compared to native breeds. This was confirmed by high faecal egg counts and more alternations in haematobiochemical parameters in exotic breeds compared to local.

### 6.2 Histopathology

Tissues of naturally infected and uninfected animals were collected from abattoirs of Kashmir valley. High percentage of tissue samples were collected from exotic breeds compared to native breed, because of decreased population of native breeds. Freshly collected tissue samples were first washed in normal saline then immediately fixed in Bouin's fixative for 24 hours. After fixation the tissue samples were dehydrated, dealkoholised, infiltrated and then embedded in paraffin wax. 5-15 micron sections were cut, processed by normal histological procedure. Haematoxylin, Eosin and Mallory's triple stains were used for
staining purposes. Only the naturally infected and uninfected tissues of liver, stomach and intestines were subjected to histopathological examination.

Altered architecture of infected livers was observed. *Fasciola* species were found at different sites in infected livers. The adult *Fasciola* were mostly found in bile ducts and immature larvae were found embedded in the parenchyma. Swollen bile ducts and white granulated liver lobes were found in *Fasciola* infection. Lesions and hemorrhages were quite common in infected livers. In chronic and acute cases of fascioliasis, hyperplasia of bile ducts was quite common. Livers infected with *Dicrocelium dendriticum* infection were characterized by blackish brown colour with dilated bilts and focal depressions on liver capsule. No migratory tracts were seen in *Dicrocoelium dendriticum* infection as seen in *Fasciola* cases.

Abomasae infected with *Haemonchus contortus* had thickened walls and oedematous folds accompanied by hemorrhages on mucosal surface. There was minimal disruption and loss of epithelial cells at mucosal surface. Disruption of gastric glands and nodules were found in abomasae infected with *Ostertagia circumcincta*. Abomasal pH was measured in infected and uninfected animals with the help of pH sticks. Raised pH was observed in *Haemonchus contortus* and *Ostertagia circumcincta* infected sheep. The mean abomasal pH in infected sheep was $5.2 \pm 0.6$ compared to $2.4 \pm 0.2$ of uninfected control.

Mixed infections were observed in small intestines. Immature *Paramphistomum* were found to be embedded in the mucosae of small intestines. Histological examination revealed the presences of phagocytic cells. Migratory tracks associated with hemorrhages were found in small intestines infected with immature *Paramphistomum* larvae. Histopathological examination of small
intestines infected with *Moniezia* species revealed depressions, flat and shorting of intestinal villi. Abrasions of villi, hyperplasia of the crypts of liberkuhn and alterations in brush boarder epithelium was quite common in *Bunostomum* infected small intestines. In large intestines creamy nodules were noted towards the proximal end of colon. Inflammatory colons observed were due to infiltration of phagocytic cells.

6.3 Haematobiochemistry

Haematobiochemical profile was studied in naturally infected and uninfected sheep and the results were compared with animals experimentally infected with gastrointestinal nematodes. Blood samples of only those animals were selected whose faecal samples were examined for helminthiasis. The haematological parameters studied were: packed cell volume (PCV), erythrocyte sedimentation rate (ESR), haemoglobin (Hb), total RBC count (TRBC), total leucocyte count (TLC), differential leucocyte count (DLC), total protein, albumin, blood sugar, blood urea and alkaline phosphates, acid phosphatases, serum pepsinogen. Most of the haematobiochemical parameters were altered in naturally and experimentally infected animals. Raised ESR and decreased values of Hb, PCV, TRBC, TLC were observed in animals infected with helminths. Eosinophilia was observed as diagnostic phenomenon in case of fascioliasis. Decreased concentration of total protein and albumin was observed in helminthiasis particularly in *Haemonchus contortus, Bunostomum* and immature *Paramphistomum* cases. Alkaline phosphatases were elevated and acid phosphatases were decreased in nematodiasis. The concentration of blood sugar, blood urea and serum creatinine slightly decreased from their normal values in helminth infected sheep but the differences were not significant.
6.4 Immunological Response

*Haemonchus contortus* and *Fasciola hepatica* were collected from the infected organs, washed immediately in phosphate buffer saline. Their excretory-secretory products were collected by incubating them in 0.5M phosphate buffer saline pH 7.2 for 5 hours. After incubation parasites were removed and solution was centrifuged and the supernatant was stored at -20°C as excretory-secretory antigen. Crude tegument homogenate of *Fasciola hepatica* and *Haemonchus contortus* was also prepared by homogenizing the parasites in Triton X-100. Hyperimmune sera were raised in lambs and rabbits against the excretory-secretory antigens and crude tegumental homogenate. Excretory-secretory antigens and crude tegumental extract of *Fasciola hepatica* and *Haemonchus contortus* were fractionated in sphenex G-200 in order to find the best antigenic components. G-200 profile of excretory-secretory antigens of *Haemonchus contortus* showed four peaks designated as HEF_1, HEF_2, HEF_3, and HEF_4. Only HEF_2 appeared sharp and prominent peak and remaining peaks appeared as broader peaks. G-200 gel profile of *Haemonchus contortus* Triton X-100 crude homogenate showed six peaks, designated as HSF_1, HSF_2, HSF_3, HSF_4, HSF_5, and HSF_6. HSF_1 and HSF_5 appeared sharp peaks, HSF_2 and HSF_3 appeared distinct but short peaks, while as HSF_4 and HSF_6 were broader peaks. Several samples were loaded to the column and similar peaks were pooled then dialyzed against double distilled water in order to remove the excess salts.

G-200 gel profile of *Fasciola hepatica* crude Triton X-100 extract showed a total of six peaks, designated as FSF_1, FSF_2, FSF_3, FSF_4, FSF_5, and FSF_6 appeared sharp and high peaks, FSF_4, FSF_5, and FSF_6 appeared as short peaks and FSF_1 appeared as broad peaks. Eight peak fractions were observed in G-200 gel profile of *Fasciola hepatica* excretory-secretory homogenate. These
were designated as FEF₁, FEF₂, FEF₃, FEF₄, FEF₅, FEF₆, FEF₇ and FEF₈. Only FEF₇ appeared sharp peaks and remaining appeared as moderate peaks. SDS-PAGE profile of *Haemonchus contortus* tegumental as well as metabolic products revealed large number of polypeptides of molecular weight ranging between 29 KDa to 205 KDa. SDS-PAGE analysis showed some common antigens between excretory-secretory and crude tegumental homogenate. All the partially purified fractions of *Haemonchus contortus* were subjected to SDS-PAGE. The crude homogenate, excretory secretory products and partially purified antigens of *Haemonchus contortus* and *Fasciola hepatica* were tested in ELISA and Ouchterlony gel diffusion test. In order to evaluate the diagnostic value of excretory-secretory and crude somatic antigens of *Haemonchus contortus*, 432 *Haemonchus contortus* positive sera samples were collected from abattoirs. Out of 432 sera samples, (87.5%) and (72.22%) sensitivity was observed using excretory-secretory antigens and adult homogenate antigens respectively. Diagnostic specificity of ELISA with excretory secretory antigens was increased to (92.02%) from (76.81%) of crude somatic antigens. Among the partially purified antigens of adult homogenate of *Haemonchus contortus*, HSF₄ and HSF₆ appeared highly antigenic HSF₁ and HSF₄ were moderately antigenic and HSF₁ and HSF₂ were poorly antigenic. Only HSF₄, partially purified fractions of *Haemonchus contortus* excretory secretory antigens appeared highly antigenic, HEF₂ and HEF₃ fractions were moderately antigenic and HEF₂ fraction was observed as poorly antigenic. Ouchterlony gel diffusion test was performed on agar coated glass slides. The results of Ouchterlony gel diffusion test using adult worm crude antigens revealed three precipitation arches against homologous sheep hyperimmune sera and four precipitation lines against heterologous rabbit hyperimmune sera. The excretory-secretory antigens showed four precipitation arches in Ouchterlony gel diffusion test against both
homologous and heterologous sera. The diagnostic sensitivity of ELISA using excretory secretory antigens of *Fasciola hepatica* was (72.29%). Decreased cross-reactivity was observed in ELISA using excretory secretory antigens compared to adult tegument homogenate of *Fasciola hepatica*. The overall specificity of excretory-secretory antigens of *Fasciola hepatica* was (90.16%) and that of crude tegument homogenate was (73.55%). The Ouchterlony gel diffusion test performed for the diagnosis of *Fasciola hepatica* infection revealed 4 and 3 precipitation arches using excretory-secretory and crude homogenate respectively.

Analysis of helminth populations in the livestock over the period of investigation has shown that age of the host has remarkable influence on the parasite burden. Survey of helminth parasites in Kashmir valley has shown that both the numbers and the structures of parasite populations fluctuate throughout the year. Longevity of infective stages and seasonal development of new populations of those infective stages are the two important factors which have major influence on seasonal changes of parasitic burden. The survival of infective stages varies according to the species of helminths and the nature of the infective stages e.g. egg, larvae or cyst and the existing climatic conditions. In most of the trichostrongyles and strongyles the third larval stage is the infective one and in the temperate zones they are capable of survival in significant numbers for periods of upto one year. They survive particularly well under conditions of moderate temperatures (10-18°C) and are also capable of resisting periods of sub-zero temperatures. Obviously where susceptible sheep are grazed throughout the winter, they will be continuously infected until mortality of the over wintered infection on the herbage occurs. The geographical areas where sheep are housed during the winter, the date on which they are brought to the
fields in the spring will influence the level of infections acquired from the overwintering infections on the herbage. In geographical area with a prolonged cold winter and good snow cover such as Kashmir, survival of infective larval stages on pastures can be surprisingly good, this probably represents an adaptation for survival by the helminths. The winter survival rates in some parasites such as *Nematodirus battus* is high because the infective larval stages are enclosed within the egg membranes.

The infective stages of the trematode and cestode parasites appear to have similar requirements for survival to those of nematode infective stages. The infective helminth larvae, eggs or cysts are capable of surviving for a sufficient period through conditions adverse to the development of free living stages and so infect the next batch of susceptible young lambs. The later are usually born when climatic conditions are suitable for the growth of pasture and development of free living stages of helminth.

Observations, in the present study on haematobiochemical changes caused by naturally and experimentally infected animals carry importance as they indicate the extent of damage and help in better understanding of the pathogenesis especially in the absence of other possible factors which may influence these changes. Some of the biochemical parameters can be used as diagnostic tools as their levels in the serum are altered in specific helminth infections. In the present observation the serum pepsinogen concentration was abnormally raised in abomasal infections. This can be exploited as the diagnostic aid even before the parasite becomes sexually mature.

The faecal egg counts, worm burden and haematobiochemical observations suggest that exotic breeds are more susceptible to helminth
infections compared to Native breeds. Although the present observations are not sufficient to confirm that Native breeds are more resistant to helminth infections than exotic breeds, some of the data pointing in this direction is inadequate and deserves further investigation. Among the phenotypic parameters analyzed, the number of eggs per gram considered to be one of the most indicative markers for resistance, was significantly low in the animals of Native breeds. Further studies are necessary to determine the genetic resistance of native breeds to helminth parasites which will be important to obtain Native flocks whose genomes possess these genes at high frequency for crossings.

The immunological studies suggest that excretory secretory antigens of *Fasciola hepatica* and *Haemonchus contortus* are highly antigenic and could be exploited for mounting the protective immune response in sheep. This is due to the fact that excretory secretory products of helminths usually display a relatively simple antigenic composition compared to somatic worm antigens. The results of the present study suggest that low molecular weight antigens are highly immunogenic in nature. Thus low molecular weight antigens of *Haemonchus contortus* and *Fasciola hepatica* deserve further investigation as this is the preliminary study first of its kind in this part of the country.