Chapter 1

Introduction
India is endowed with the largest livestock population in the world, which contributes nearly 7% towards its National income (Animal Health Year Book, FAO, 1988). According to Livestock Census 2003; there are about 61.47 million sheep, 124.36 million goats and 185.0 million cattle in the country. About five million households in the country are engaged in the rearing of small ruminants (sheep, goats and rabbits) and other allied activities. The production of wool was 44.50 million kg during 2004-2005 and rose to 50.0 million kg during 2005-06. According to FAO (1983), total mutton production is around 132 million kilograms, which is about 14% of the total meat production from all meat producing species reared in India.

Animal husbandry plays a prominent role in the rural economy in supplementing the income of rural households, particularly, the landless and small and marginal farmers. It also provides subsidiary occupation in semi-urban areas and more so for people living in hilly, tribal and drought prone areas where crop output may not sustain the family. According to Central Statistical Organization
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(CSO), the value of output from livestock and fisheries sectors is about 37.7 per cent of total agriculture and allied sectors. Livestock sector not only provides essential protein and nutritious human diet through milk, eggs, meat, etc; but also plays an important role in providing by-products such as wool, hides and skin, blood, bone, fat, etc.

In Jammu and Kashmir, animal husbandry constitutes a vital activity from the point of economic welfare of the farmers. About 13% of the gross domestic product (GDP) of the State is contributed by animal husbandry in the over all 33% contribution by the agriculture sector (The Annual Plan Document, 2006-07, Govt; of Jammu and Kashmir). The State has a precious wealth of livestock. Cattle, sheep and poultry, amongst all the livestock, are considered the most important tool for development of our overwhelming agrarian economy. Sheep husbandry is the core activity of rural areas of the State and plays a vital role in economic up-liftment of most under-privileged communities. In Kashmir valley, sheep and goat population rose from 13.89 lacs and 2.82 lacs in 1996-97 to 16.80 lacs and 4.47 lacs in 2005-06 respectively. Similarly mutton production has increased from 69.26 lacs kgs to 83.44 lacs kgs and wool production from 20.69 lacs kg to 92.00 lacs kg during the corresponding period (The Annual Plan Document, 2006-07, Govt; of Jammu and Kashmir).

The Jammu and Kashmir State has a sheep population of approximately 34 lacs at present and produces more than 58 lacs kgs of raw wool annually. The State is placed at the highest level with regard to production of fine type of raw wool in the country. About 14 lac people mainly comprising of Gujjar, Bakerwals, Gaddies, Chopans, Changpas and marginal farmers are involved in rearing sheep and goats in the State (The Annual Plan Document, 2006-07, Govt; of Jammu and Kashmir). The sheep and sheep rearing farms are, therefore, an important and crucial part of rural as well as national economy of our country.

Sheep (*Ovis aries*) suffer from many infectious diseases and heavy economic loss occurs due to obvious mortality as well as unseen morbidity. Sheep
being a close grazer is regarded as "museum" of parasites especially gastrointestinal protozoa and helminths. Economic losses due to helminth parasites in sheep throughout the world are considerable. Worldwide helminthiasis is an important cause of production losses and mortality in small ruminants (Karira and Kanyari, 2001). These losses include the direct effects of severe clinical signs such as anaemia and associated oedema, diarrhoea and anorexia. These signs can easily result in poor general performance, particularly in younger animals. More important are less visible sub-clinical losses such as decreased weight gain (Ploeger et al., 1990) and (Ploeger and Kloosterman, 1993), decreased milk yield (Gross et al., 1999), and decreased wool production (Liu et al., 2003).

A number of helminth parasites are found in sheep viz; Fasciola hepatica, Fasciola gigantica, Dicrocoelium dendriticum, Paramphistomum cervi, Cotylophoron cotylophorum, Moniezia spp., Avitellina spp., Stilesia spp., Thysanosoma spp., Haemonchus contortus, Trichuris ovis, Bunostomum spp., Oesophagostomum spp., Ostertagia spp., etc. In the present study histochemistry and scanning electron microscopy of some economically important helminths of sheep including Fasciola hepatica, Paramphistomum cervi and Moniezia expansa was studied thoroughly. A detailed description of these parasites including pathogenesis is given separately as under.

1.1. Fasciola hepatica

Fasciola hepatica belongs to Phylum Platyhelminthes, Class Trematoda and Subclass Digenia. This economically most important trematode, commonly known as liver fluke, occurs in the bile ducts of the sheep, goats and cattle, although a wide range of other unusual hosts viz; pig, rabbit, elephant, dog, cat, kangaroo, horse and man have been reported. In the unusual hosts the fluke may be found in aberrant sites such as in the lungs and subcutaneously.

The fluke is cosmopolitan in distribution and is the cause of fascioliasis or 'liver rot' disease especially in sheep and cattle. Fasciola hepatica is leaf-shaped.
broader anteriorly than posteriorly, with an anterior cone-shaped projection which is followed by a pair of broad shoulders. The ventral sucker is situated at the level of the shoulders and is about as large as the oral. The tegument is well armed with backwardly directed sharp spines, which together with the suckers serve as an effective mechanism to maintain the position of the parasite in the bile ducts. The intestinal caeca have numerous branches and extend far back. The testes are much branched, filling the median field in about the second and third quarters of the body. There is well-developed cirrus and the cirrus sac also encloses the prostate and seminal vesicle. The ovary is situated to the right of the middle anterior to the testes, and is branched. The vitelline glands consist of fine follicles filling the lateral fields and the ducts of the follicles unite to form two transverse ducts, which pass inwards to open into a median yolk reservoir, from which a duct passes to the ootype. The uterus lies anterior to the testes.

*Fasciola hepatica* holds a prominent place in parasitology because its life cycle was the first digenetic trematode cycle to be completely worked out (Thomas, 1883). The typical eggs are large and operculate. These eggs are laid before complete development of the enclosed miracidium and pass into the alimentary tract via the bile ducts. They eventually pass out of the host in faeces. After being in water for 4 to 15 days at 22°C, the miracidium within the shell is completely developed and escapes. Lytic enzymes secreted into the cavity of the cup facilitate penetration.

Upon entering the snail host, each miracidium metamorphoses into a sporocyst, which in turn gives rise to rediae. The rediae give rise to daughter rediae. The germ balls in the brood chambers of daughter rediae develop into cercariae, which escape from the snail and become free swimming. When the cercariae reach aquatic vegetation, they lose their tails and encyst as metacercariae. Metacercariae swallowed by the definitive host excyst once they reach the duodenum. They then penetrate the intestinal wall and are found in the coelomic cavity. From the coelomic cavity, they penetrate the liver, migrate through the parenchyma and
become established in the bile ducts, where they mature. During migration through the liver, the young adults actively feed on the host's liver cells. The rate and extent of development of *Fasciola hepatica* within the snail host depend on the degree of infection and on the availability of nutrients within the snail, primarily those stored in the digestive gland.

Fascioliasis or 'liver rot' in sheep and cattle is a serious economic problem. It is an economically important helminth disease responsible for heavy economic losses to livestock industry in India (Bhalerao, 1973). Fascioliasis is characterized by the destruction of the host's liver tissue, damage to liver ducts, atrophy of the portal vessels, and secondary pathologic conditions. In many cases the disease leads to the death of the host. Essentially the disease entity can be divided into an acute form and a chronic form.

Acute fascioliasis is less common than the chronic entity and is almost invariably seen in sheep. It is essentially a traumatic hepatitis produced by the simultaneous migration of large numbers of immature flukes. The most damaging stages are those six to eight weeks of age, causing extensive destruction of the liver parenchyma and marked haemorrhage. If numbers are excessive, rupture of the liver capsule may occur with haemorrhage into the peritoneal cavity. Animals may die within a few days of the onset of clinical signs.

Chronic fascioliasis is the most common form of the infection in sheep, cattle and other animals including man and the major consequence of the infection with *F. hepatica* is hepatic fibrosis. The pathology may be divided into hepatic fibrosis and hyperplastic cholangitis (Dargie et al., 1974; Rushton and Murray, 1977). Migration of immature flukes in the liver produces migratory tracts within which there is traumatic destruction of the liver parenchyma, haemorrhage and necrosis. Migration of the flukes also results in thrombus formation in the hepatic veins and liver sinusoids and subsequent obstruction to the blood flow by these thrombi causes an ischemic, coagulative necrosis in the liver parenchyma. Healing
and regeneration of these lesions begins approximately four to six weeks after infection, collagen is laid down and fibrosis occurs.

A hyperplastic cholangitis is caused by the presence of the adult flukes in the bile ducts. At first the epithelium of the bile ducts is hyperplastic and numerous eosinophils and mononuclear cells infiltrate the lamina propria. The spines and suckers of the flukes subsequently denude the bile duct epithelium and organization of the inflammatory reaction results in fibrosis of the lamina propria of the bile duct and surrounding tissues.

1.1.1. Human fascioliasis

Sporadic cases of human fascioliasis have been reported in many countries of the world including Europe and the USA (Facey and Marsden. 1960). The eating of raw or poorly cooked watercress contaminated with metacercariae appears to be common source of human infection (Jones et al., 1977; Marsden and Warren, 1984). Adult *F. hepatica* can be found in aberrant sites such as lungs and subcutaneously. Here the flukes are found in cysts containing brownish purulent material. They may be removed surgically. In man the presence of adult *F. hepatica* in the bile ducts causes a variety of symptoms: malaise, intermittent fever, weight loss, pain under the right costal margin. In addition, urticaria, dermatographia, mild jaundice and anaemia may be seen.

The infection is diagnosed by the identification of eggs of *F. hepatica* in the faeces. Serological tests, particularly immunodiffusion and fluorescent antibody tests using *F. hepatica* antigen, are also done. The infection is treated surgically, but the treatment of choice is biothionol.

It is of interest to note that if raw bovine liver harbouring *Fasciola* is eaten by humans, young flukes may become attached to the buccal or pharyngeal membranes, causing pain, irritation, hoarseness and coughing. This condition is known as halzoun.
1.2. *Paramphistomum cervi*

*Paramphistomum cervi* belongs to Phylum Platyhelminthes, Class Trematoda and Subclass Digenia. This amphistome is found in the rumen and reticulum of sheep, goats and cattle. The flukes are conical, pear-shaped, slightly concave ventrally and convex dorsally with a large posterior (ventral) subterminal sucker. The genital pore is situated at the end of the anterior third of the body. The testes are slightly lobed and tandem, anterior to the ovary. The vitellaria are in compact groups between the pharynx and the posterior sucker. The uterus runs forwards on the dorsal part of the body and is coiled. The tegument is spineless.

The operculate eggs passed through faeces hatch into miracidia. The developmental period varies with the temperature and is approximately 12-21 days. Liberated miracidia swim in the surrounding water and enter water snail, viz; *Planorbis*, *Bulinus*, and *Lymnaea* etc. Following penetration of the mantle cavity, the miracidia lose their ciliated covering and by 12 hours an elongate sporocyst is present. Growth during the next few days is marked and the sporocysts are mature and contain a maximum of rediae each. The rediae are liberated on the 10th to 11th day of infection. They undergo marked growth and contain 15-30 cercariae.

Cercariae are released from the rediae in an immature state and require a period of maturation in the snail tissues before being shed. Liberated cercariae are readily recognized as amphistome because of the presence of anterior and posterior suckers. They are active for several hours, and then encyst on herbage or other objects in water. Encystment is complete in about ten minutes and the new metacercariae gradually darken to an almost black colour. These stages remain viable for about three months.

The final host is infected by ingestion of the metacercariae with herbage. Excystation occurs in the intestine where the immature paramphistomes spend the first part of their vertebrate developmental cycle. Immature paramphistomes attach themselves to the mucosa of duodenum and after six to eight weeks they migrate.
forward through the reticulum to the rumen, where they attain sexual maturity. In extremely heavy infections there is retardation in the size of the flukes in the anterior part of the small intestine and migration to the rumen is delayed in these heavily infected animals (Horak, 1967).

Paramphistomiasis is prevalent in ruminants causing serious morbidity and heavy mortality in tropical and sub tropical areas. In India numerous outbreaks of acute paramphistomiasis associated with high mortality among young sheep, goats, buffaloes and cattle have been recorded (Katiyar and Varshney, 1963; Panda and Misra, 1980).

The adult worms in the forestomach are essentially non-pathogenic even though large numbers may be present. The immature stages of the *P. cervi* in the duodenum and upper ileum are responsible for several pathological changes. These are embedded in the mucosa and are plug feeders, drawing pieces of the mucosa into the suckers which pinch them off causing necrosis and haemorrhage. In heavy infections haemorrhagic duodenitis may be produced with immature flukes embedded in the mucosa. Histologically there is extensive catarrhal and haemorrhagic inflammation of the duodenum and jejunum with destruction of the intestinal glands, degeneration of the associated lymph nodes and other organs. Associated with these lesions is an anaemia, hypoproteinaemia, oedema and emaciation. Clinical signs consist of profuse fluid foetid diarrhea, marked weakness and frequently death.

### 1.3. *Moniezia expansa*

*Moniezia expansa* belongs to Phylum Platyhelminthes, Class Cestoda and Order Cyclophyllidea. This economically important tapeworm occurs in the small intestine of sheep, goats, cattle and several other ruminants in most parts of the world. It may reach a length of 600 cm and a width of 1.6 cm. The scolex is 0.36-0.8 mm wide, with prominent suckers. There is neither rostellum nor hooks. The segments are broader than long and each contains two sets of genital organs with
marginal genital pores. The ovaries and the vitelline glands form a ring on either side, median to the longitudinal excretory canals, while the testes are distributed throughout the central field. At the posterior border of each proglottid there is a row of rosette-like interproglottidal glands which extend almost across the width of the proglottid. The uterus becomes sac-like when filled with eggs. The eggs are somewhat triangular in shape, containing a well-developed pyriform apparatus.

Proglottids and eggs are passed in the faeces and may be eaten by birds which therefore, may disseminate the infection. Cysticercoids develop in oribatid mites of the genera Galumna, Oribatula, Peloribates, Scheloribates and others. Infective stages are produced in approximately four months. Ruminants are infected by the ingestion of infected mites with herbage. There is marked seasonal occurrence of Moniezia infection due to mites overwintered on pastures. Lambs become infected very early in life and may pass ripe proglottids when they are six weeks old.

Monieziasis is an important disease affecting sheep, goats and cattle and only lambs, kids and calves under six months of age are substantially infected. In heavy infections the intestine may be virtually a solid mass of tapeworms and cause diarrhea and unthriftiness. Obstruction of the intestine has been recorded. In the USSR Moniezia infections are considered to be highly pathogenic in lambs causing depressed wool and meat production and are associated with many deaths. A high incidence of enterotoxaemia has been associated with Moniezia infections in lambs in the USSR (Vibe, 1976).

1.4. Background

As per the review of literature considerable studies on the histochemistry of helminth parasites of sheep are scanty. In India very little work has been carried out on this important aspect of helminthology. The histochemistry of helminth parasites of sheep has been studied by Rogers (1947), Yamao (1952), Erasmus (1956), Brand and Mercado (1960), Pantelouris (1964), Halton (1967), Threadgold (1968),

In Jammu and Kashmir no reference is available on the histochemistry of sheep helminths. Therefore, it was of utmost importance to conduct the present research work so as to study the histochemistry of helminth parasites of sheep thoroughly because histochemistry forms the basis for studying the overall biology of the parasites. An understanding of the histochemistry of various tissues and organs of helminths helps to solve various questions of its biology and is useful in a search for effective anthelmintics. During the present study detailed histochemical and scanning electron microscopic studies on some helminth parasites of sheep including Fasciola hepatica, Paramphistomum cervi and Moniezia expansa were carried out. The scanning electron microscopy enables us to study the surface microtopographical and ultrastructural features of the helminths revealing novel microanatomical features of the host-parasite relationship. Scanning electron microscopy also provides valuable information in helminth taxonomy and in assessing the efficacy of test substances in drug screens. The distribution of various biochemicals viz; lipids, proteins, glycogen and some enzymes including acid phosphatase, alkaline phosphatase, glucose-6-phosphatase and ATPase has been studied thoroughly by means of different histochemical techniques allowing exact localization of the biomolecules and enzymes in various tissues and organs of the concerned helminths and thus providing possible clues to their biological significance.