CHAPTER - 1

INTRODUCTION
Kashmir is primarily an agricultural State and sheep treasure is an important source of earning to farming community. Sheep farming is an important source of livelihood for small and marginal farmers and landless labourers as it plays an important role in providing food, mutton, fibers, hides and manures. Mutton is consumed by a large fraction of total population of J&K, especially in Kashmir. These economically important animals graze in grasslands, meadows and other pasturelands viz., low and high altitudes. All these areas are bound by a number of small water bodies like ponds, ditches, wetlands etc. which form congenial habitat for different species of snails regarded to be intermediate host of Paramphistomes. It is at these sites that livestock is prone to get the infection of Paramphistomes by eating grass contaminated with metacercaria of said fluke.

Man with significant achievements in various fields of medical and veterinary sciences has yet to claim complete success in the battle against many diseases, while human diseases are getting more and more under control, the diseases of sheep and other livestocks particularly those caused by helminth infections, continue to remain comparatively unattended in many parts of the world. While large number of antihelmintics are known to cure helminth-born diseases, still the control of fresh infection among the livestock continue to be a serious problem requiring immediate attention in a developing country like ours. With the increasing human population there is an immediate need in increasing the population of sheep and other livestock (Plate 1).
In Jammu and Kashmir State, only a limited information is available on the parasitic fauna of local sheep. So far, little work has been undertaken on the incidence and intensity of infection of helminth parasites of sheep. Data pertaining to life cycle stages of nematode parasites, intermediate hosts involved in trematode and cestode parasites are also not fully known in Kashmir, which is both topographically and climatically different from the rest of India. The State of J&K is around 84 thousand square miles in area with a human population of about six million and an estimated sheep population of 1.5 million. Topographically and climatically, the State comprises of three zones (i) Arid zone of Ladakh (7,000 – 12,000 ft), (ii) Valley of Kashmir and districts of Doda (4000ft - 7000ft or above) and some part of Udhampur (above 4000ft), and (iii) Plains of Jammu, Kathua and Poonch districts (500-3000ft height) (Fig. 1).

The State has favourable topography for the development of sheep and wool both for summer and winter months. The stability of the State for this industry is further strengthened by the fact that there are large number of summer and winter pastures for grazing. Even the arid region of Ladakh is useful in its own way for the development of local sheep and goats for Wool and Pashmina Industry. Jammu region, though unfavourable for summer is well utilized for the migration of sheep during winter months for forage (Plate 2).

Sheep breeding is an ancient industry of the State, which has been feeding the famous cottage industries of Kashmir like Shawls, Ghabbas, Namdas, Carpets, Embroidery, Pattu and Blankets, all of which depend wholly on wool. Besides wool, improvements of mutton is equally important because of its ever increasing demand in Kashmir where the consumption of mutton is probably the highest when compared to other parts of India. This
demand is at present met with largely by importing sheep from Rajasthan and partly from the neighbouring areas of the State.

Major part of the sheep population of the State is owned by the migratory breeders who rear their sheep in high alpine pastures in summer while towards the approach of the winter, the breeders migrate their sheep to areas below snow line and to the plains. Two other systems prevalent in the State are semi-migratory and stationary type of the sheep farming. Although lately the State Government has concentrated its efforts towards the development of sheep breeding, particularly improvement of wool quality by cross breeding and this has started showing some favourable results. However, it is also found essential to devise a uniform type of sheep farming both in the private sector and the State sheep breeding farms. Due care is to be taken to control the helminth-born diseases and other diseases on the mass scale, after the required data are made available from various research centres which will have to be stationed in different selected locations of the State.

In our State ruminant rearing is so significant, that it is the only source of income to many tribes especially "Bakerwal". This is a nomadic tribe of our State migrating from Himalayan ranges in summer (Pirpunchal mountains) and low lying hills of Jammu in winter. Faizal (1999) reported 1/3rd growth retardation in ruminants due to helminth infections. The incidence of helminth infection varies with age, sex, season and agro-climatic conditions. The higher incidence of parasitic infection in domestic animals in a grazing system lowers productivity, leading to 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 (Skyes et al., 1992).
Fig. 1: Map showing location of the Valley of Kashmir
In the middle of spring and early summer these animals are sent to meadows, pastures and far away forests. These are reared by "Bakerwalas" and "Goojars" belonging to nomadic race. They return to warmer places in autumn. It is at these sites that the livestock get the infection of various diseases, one of them being paramphistomiasis which is caused by eating grass contaminated with metacercarial stage of different species of amphistomes.

Paramphistomiasis is world wide in distribution particularly reported from Africa, Newzealand, Egypt, India, Hungary and Balgaria (Boray, 1969; Dinik, 1964; Gupta, 1970; Sey and Grabber, 1980; and Georgiev et al., 1980).

This disease inflicts heavy damage and mortality to the livestock. According to Bawa (1939) there is 80-90% mortality in sheep due to amphistomiasis. Olsen (1949) observed that paramphistomiasis killed 30-40% of herds of cattle. Roberts (1951) pointed out that these species of flukes might cause a serious disease in calves in Queensland. Infected animals may rapidly lose condition and oedema may appear beneath the jaws.

Ershove (1956) remarked that Paramphistomes inflict damage upon the rumen when attaching to it by means of their powerful acetabula. According to Whitlock (1960) the adult parasites in the fore stomach of sheep are practically non-pathogenic but on the other hand the immature parasites are responsible for marked irritation. Moreover, Lapage (1962) remarked that affected sheep and cattle become dull, weak and anaemic.

From the above remarks, it is clear that Paramphistomes cause tremendous damage to the livestock as has been reported earlier (Rudolphi, 1980; Fischeoder, 1901; Goldberger, 1910; Nasmark, 1937; Latif et al., 1970; Yamaguti, 1971 and Eduardo, 1981).
The stomach fluke has a complex life cycle which requires an intermediate host for completion. The intermediate hosts in India are planorbid snails. These are small, flat snails from 2-5 mm in diameter. The snails are found in permanent and temporary watercourses, irrigation channels, swamps, dam edges and depressions. They are normally found attached to vegetation in these habitats. Adult stomach fluke lives in the rumen and reticulum of sheep. The eggs are passed in manure, larvae then hatch in a wet environment and infect the intermediate host snail. Larval development is completed in the snail, and the next stage, the cercaria leave the snail and attaches to vegetation where it encysts and transforms into metacercariae. When the vegetation is grazed by ruminants, the immature fluke encysts and attach itself to the walls of the small intestine. It later migrates to the rumen and reticulum to become an egg producing adult. In light infection, young flukes migrate to the rumen within 4 to 6 weeks and normally no clinical symptoms occur. Egg production begins soon after the fluke enter the rumen. In heavy infections, development of the young flukes is retarded and they can stay in the small intestine for more than four months and cause severe diseases associated with destruction of the lining of the small intestine.

1.4. Objectives of the Study

The present study was aimed at studying the following parameters of Paramphistomes in Kashmir Valley.

1. Collection of gut and other visceral organs from the slaughtered sheep available in different abattoir and localities of Kashmir.

2. Isolation, Preservation and Identification of various species of Paramphistomes.

3. Maintenance of record of incidence / prevalence of Paramphistomes for a period of one year.
4. Isolation and Preservation of the various parasite infested organs of the sheep for histopathological study.

5. Development of suitable immunodiagonstics for comparative evaluation of reliability and specificity of the techniques in the diagnosis of paramphistomiasis:

(a) Immunodiffusion Test

(b) Electrophoresis and Immunoelectrophoresis.

(c) Indirect Haemagglutination test.

(d) ELISA (Enzyme - linked immunosorbent assay).

Sheep in Kashmir have the largest number of parasitic worms when compared to other domestic animals. Although exact data are not available with regard to actual losses suffered as a result of helminth infection among sheep in Kashmir but the estimates available show a heavy infection every year. Thousands die every year due to diseases caused by helminthes, bacteria, protozoans and viruses. Generally in private flocks, the rate of cumulative mortality ranges from 25-50% in a year. During unfavourable weather conditions the mortality rate due to helminth disease outbreaks was found to be as high as 75% or even more as recorded by State Sheep and Wool Development Department. Livestock census of the State according to annual census of 1992 is 29,46,922, constituting nearly 2 3rd of the total livestock population with 70% of the total population lives in rural areas (Digest of statistics, 2003-04). In our State the main occupation is agriculture farming and rearing of livestock. Its contribution to State economy according to preliminary estimates has been calculated to Rs. 1127 crores (1991-1992).

The immature flukes attach themselves to the mucosa and ingest it. Hence irritation is caused resulting in enteritis. Some flukes may bore through the peritoneum where haemorrhages may occur. In heavy infection there is
persistent foeted diarrhoea and the animal becomes exhausted and emaciated and may die in a few days. The clinical conditions caused by the immature parasites is known as immature amphistomiasis. The general symptoms of the disease include weakness, dull off feed and emaciation due to the offensive diarrhoea.

Diagnosis of paramphistomiasis during sub-clinical phase is of immense importance for early detection of the disease so that the mortalities can be reduced by timely intervention and treatment. However, early detection of the disease is difficult by applying coprological techniques so it is an urgent need for the development of a reliable diagnostic tests for early diagnosis of infection.

As the world population grows, the need for health care increases. Health care today not only means advances in therapy but also requires accurate sensitive, specific, quick and cost effective diagnostic systems. It is now a known fact that body defense system produces antibodies against a variety of pathogenic organisms namely bacteria, viruses, protozoans and helminths etc. Bio-assays utilizing antibodies as tools to perform such qualitative, quantitative or semi-quantitative determinations of either clinical or academic interest can be broadly grouped under the class of immuno-assays referred to as immuno-diagnostics, that include various techniques like diffusion, immuno-electrophoresis, agglutination, enzyme, immunoassays and other sensitive techniques based on antigen, antibody interactions.

Immunodiagnostic techniques are not only important in medical diagnosis but also have several applications of academic interest in biological sciences. Though immuno-diagnostics is an inter-disciplinary science, its basis is immunology, and a thorough understanding of the molecules that participate in immune system is obligatory before one proceeds to study its applications. The techniques in immuno-diagnostics can be broadly classified
unlabelled immuno-assays e.g., immuno-precipitation, agglutination and complement fixation and sophisticated labelled immuno-assays e.g., Enzyme Linked Immunosorbent Assay (ELISA) and Radio Immuno Assay (RIA).

The science of immunology has been growing rapidly in therefore, its application in the form of immuno-diagnostics has replaced or is replacing conventional and cumbersome techniques of bacteriology, parasitology or serum biochemistry and haematology.

1.2. ELISA (Enzyme-Linked Immunosorbent Assay)

Effres et al. (1985) for the first time independently introduced the idea for using enzymes conjugated with antibody or antigen to detect and measure antigen or antibody respectively. As its name implies, this technique utilizes enzyme as a marker and either the antibody or the antigen is "adsorbed" on a solid surface (e.g., polystyrene microhemagglutination plate) providing the immunosorbent. The ability of the immunosorbent to recognize and fix the corresponding antigen-antibody to it and the ability of the enzyme to produce or destroy a colour while acting upon its substrate leads to a 'colour or no colour' type visual test for the analyte (the substance that is being detected). A conjugate of enzyme-labelled antiglobulin (e.g., alkaline phosphatase conjugated to rabbit anti-human immunoglobulin-G) and its corresponding enzyme substrate (e.g., paranitrophenyl phosphate) are generally used in ELISA. Among numerous variations of ELISA techniques, two are particularly popular, the Sandwich ELISA and the competitive ELISA. There are innumerable applications of ELISA in such divergent fields as human and veterinary medicine, food technology, serodiagnosis of bacterial, viral, protozoan and helminthic infections. It is the test of choice in routine diagnosis of AIDS. ELISA is reliable, specific, simple economical and sensitive technique and has been in wide use since 1974.
1.3. Electrophoresis and Immunoelectrophoresis

The great advances in our knowledge of macromolecular chemistry and physics are partly due to the development of numerous electrophoretic techniques. As a medium for electrophoresis, a gel offers several advantages. Gordon (1965) used an agar gel for simple electrophoresis without involvement of immunological reactions. Simple electrophoresis is an ordinary technique notably used for separation, analysis and identification of serum-constituents or biological mixtures.

Grabar and Burtin (1964) developed immunoelectrophoresis for studying immune serum. As compared with simple electrophoresis, immunoelectrophoretic analysis presents the advantage of differentiating constituents even though their mobilities are identical, since as a consequence of the specificity of the precipitation reaction these substances produce independent arcs. Since, the detection of the constituents of a mixture is based on the specific precipitation reaction, only those constituents that are capable of yielding a precipitate with antibodies can be revealed. Immunoelectrophoretic analysis (IEA) has made it possible to study more precisely the abnormalities of serum and urinary proteins and their interrelations. It also helps in studying the abnormalities of serum proteins, especially globulins. Immunoelectrophoretic analysis (IEA) presents a considerable advantage in the investigation of systemic lupus erythematosus (SLE) with nephrotic syndrome and chronic lymphocytic leukemia. Ever since the beginning of immunoelectrophoretic analysis (IEA) efforts have been made to apply this method to secure diagnostic information about the hepatic diseases (hepatitis, cirrhosis), the C-reactive protein (CRP) and rheumatoid factor (inflammatory disorders). Since the C-reactive protein is often increased in rheumatic diseases, its electrophoretic migration velocity could be determined accurately by IEA. In summary, it would appear that immunoelectrophoresis is
most useful in the evaluation of many pathological conditions and immunological
deficiencies (hypogammaglobulinemia).

1.4. Passive Haemagglutination Test (IHA)

Indirect haemagglutination test employing erythrocytes coated with antigen from other sources to be specifically clumped by antibodies against the coated antigens. Erythrocytes serve merely as a matrix for coating and an indicator system for detection of the antibodies in the serum samples. Equal volumes of antigen coated erythrocytes and two fold serially diluted antiserum are mixed in the wells of microtitre plates/test tubes and incubated at room temperatures for about 2 hours. The results are read by inspection of shield/mat in the positive wells and compact red button in the negative wells. The highest dilution of the antiserum that gives a clear positive reaction in the end point dilution and its reciprocal is taken as the titre of the antiserum.